Abdi İbrahim der ki:
Doktorculuk oynamayın.
Bilinçsiz ilaç kullanmayın!

Book of Abstracts
Ankara University Faculty of Pharmacy Publication No: 112
June 2015 Ankara, Turkey

JSOPE-11 HAS BEEN GENEROUSLY SUPPORTED BY

1. REPUBLIC OF TURKEY, MINISTRY OF CULTURE AND TOURISM
2. ANKARA UNIVERSITY
3. THE SCIENTIFIC AND TECHNOLOGICAL RESEARCH COUNCIL OF TURKEY (TÜBİTAK)
4. TURKISH COOPERATION AND COORDINATION AGENCY (TIKA)
5. TURKISH PHARMACISTS’ ASSOCIATION (TEB)
6. PHARMACISTS’ CHAMBER OF ANKARA
7. NOVAGENTEK LABORATUVAR ÜRÜNLERİ VE TEKNOLOJİLERİ SAN. LTD.ŞTİ.
8. LUNDBECK İLAÇ TİC. LTD. ŞTİ.
9. KOÇAK FARMA İLAÇ VE KİMYA SAN. A.Ş.
10. SEM LABORATUVAR CİHAZLARI PAZARLAMA SAN. VE TİC. A.Ş.
11. PFIZER İLAÇLARI LTD. ŞTİ.
12. ATABAY İLAÇ FABRİKASI A.Ş.
13. Tüm ECZA KOOPERATİFLERİ BİRLİĞİ
14. BERKO İLAÇ VE KİMYA SAN. A.Ş.
15. LEICA TEKNOLOJİ ÇÖZÜMLERİ VE DAĞITIM
16. ABDİ İBRAHİM İLAÇ SAN. VE TİC. A.Ş.
17. NOBEL İLAÇ SAN. VE TİC. A.Ş.
18. ABBVIE LAB. LTD.ŞTİ.
19. BAYER TÜRK KİMYA SAN. VE TİC. A.Ş.
20. DEVA HOLDİNG A.Ş.
21. MUSTAFA NEVZAT İLAÇ SAN. A.Ş.
22. AMGEN İLAÇ TİC. LTD. ŞTİ.
23. DEVA DESTEK HİZMETLERİ KİM. SAĞ. GİD. İTH. İHR. TİC. LTD. ŞTİ.
24. İNO ENDÜSTRİ BİLİMSEL TEKNİK CİHAZLAR PAZARLAMA SAN. VE TİC. LTD. ŞTİ
25. GNC
26. SERVİER İLAÇ VE ARAS. A.Ş.
27. BİLİM İLAÇ SAN. VE TİC. A.Ş.
28. NEVZAT ECZA DEPOSU TİC. VE SAN. A.Ş.
29. HELVACİZADE GIDA İLAÇ KİMYA SAN. VE TİC. A.Ş.
30. NATURMED İLAÇ KİMYA VE KOZMETİK SAN. VE TİC. LTD.ŞTİ.
31. PHYTOPHARMA İLAÇ VE BİTKİSEL SAĞLIK ÜRÜNLERİ SAN. VE TİC.LTD. ŞTİ.
32. AYES GRUP
33. ASTRA ZENECĂ İLAÇ SAN. VE TİC. LTD. ŞTİ.
34. JOHNSON & JOHNSON SIHHİ MALZ. SAN. VE TİC. LTD. ŞTİ.
35. SANDOZ İLAÇ SAN. VE TİC. A.Ş.
36. SANTA FARMA İLAÇ SAN. A.Ş.
37. BRISTOL-MYERS SQUIBB İLAÇLARI INC.
38. BOERHİNGER INGELHEIM İLAÇLARI A.Ş.
39. HERKÜL ECZA DEPOSU MEDIKAL İNŞAAT A.Ş.
40. ANKYRA TEMEL VE ENDÜSTRİYEL LABORATUVAR CİHAZLARI SAN. VE TİC. LTD ŞTİ.
41. NOVARTİS ÜRÜNLERİ
42. MIHENK HALİ
43. ÇİTAĞ GÜMÜŞ
44. KURU KAHVEKİ MEHMETEFENDİ
45. KOCATEPE KAHVE EVİ
Dear Participants and Guests,

We would like to thank all the participants of 11th International Symposium on Pharmaceutical Sciences for their valuable contributions. You are cordially welcome to the charming country that shelters the oldest and the greatest cultures and civilizations of the world.

The development of Symposiums on Pharmaceutical Sciences, which is held in our faculty, is getting more expanded through the 1989. This symposium was organized biaanually until 1997 and then every three years. The previous 10 symposiums were held as;

1st International Symposium on Pharmaceutical Sciences 21-23 June 1989
2nd International Symposium on Pharmaceutical Sciences 11-14 June 1991
3rd International Symposium on Pharmaceutical Sciences 15-18 June 1993
5th International Symposium on Pharmaceutical Sciences 24-27 June 1997
6th International Symposium on Pharmaceutical Sciences 27-29 June 2000
7th International Symposium on Pharmaceutical Sciences 24-27 June 2003
8th International Symposium on Pharmaceutical Sciences 13-16 June 2006
9th International Symposium on Pharmaceutical Sciences 23-26 June 2009
10th International Symposium on Pharmaceutical Sciences 26-29 June 2012

The second institution in pharmacy education, Faculty of Pharmacy of Ankara University was founded in 1960 and started education in 1961-1962 semester. The length of pharmacy education had been 4 years until 2005 and increased to 5 years starting by that date. The new 5-year educational program has been updated according to the suggestions of the Advisory Committee on Pharmaceutical Training. This new program covers the basic courses such as mathematics, physics, chemistry as well as the basics in pharmacy education. Fifth year consists of some elective courses and the preparation of a graduation project. During the 5 years, students have to complete the 6-month training program mandatory in pharmacy/hospital or optionally in the industry. Our faculty has 5470 graduates since the established and the current number of students is 967.

Present educational and scientific resources allow a total of 125 faculty members, 54 professors, 13 associate professors, 12 assistant professors, 46 research assistants. Moreover, 76 administrative staff members and other personnel are working at different offices.

The mission of 11th International Symposium of Pharmaceutical Sciences will be to perform a broad scientific perspective by the invitation of distinguished scientists having national / international reputation in their areas, so most recent advances will be discussed interactively and empower the knowledge-based drug research development and multidiciplinary collaborations. It is our intention to make this symposium a memorable event, both scientifically and socially for the attendees.

We are pleased to announce that around 650 scientists are registered to ISOPS-11 in which 459 oral/poster presentations will be participating as well as 33 distinguished lecturers are invited from several countries.

In addition to general sessions and the posters, the exhibitors of some companies from drug industry that will introduce their equipments and products.

Panel contains the topics of Traditional and Complementary Medicine Practices: Phytotherapy and The Products Used in Phytotherapy. The lecturers are: Prof. Dr. Ahmet Basaran, Prof. Dr. Gulcin Saltan, Prof. Dr. Haluk Deda, Assoc. Prof. Dr. Aységul Karatas, Dr. Pharm. Aslı Can Agca and MD Sencar Tepe.

Free internet and computer service are available in computer room on the second floor. Please do not hesitate to ask for any other technical and social services from registration desk.

On behalf of the Organizing Committee, we would like to mention our gratitude to the President of Ankara University who gave the whole support for the Symposium Organization. We would like
to thank Turkish Ministry of Culture, The Scientific and Technological Research Council of Turkey (TUBITAK), Turkish Pharmacist’s Association, Pharmacist’s Chamber of Ankara, valuable presenters of the pharmaceutical industry for their financial supports and pharmaceutical companies for their valuable sponsorship. We congratulate the organizing committee and all the other committees with all our heart and also all academic and managing personnel because of their extensive work.

Prof. Dr. Maksut COŞKUN  
Chair of ISOPS-11

Prof. Dr. Gülbin ÖZÇELİKAY  
Dean of Faculty of Pharmacy,  
Ankara University
Honorary President of the symposium
Prof. Dr. Erkan İbiş
President of Ankara University

ORGANIZING COMMITTEE

Maksut COŞKUN (Chair)
Gülbin ÖZÇELİKAY (Treasurer)
Sinan SÜZEN
Nilüfer YÜKSEL
Betül SEVER YILMAZ
Arzu ONAY BEŞİKCİ

Tansel ÇOMOĞLU
Sinem ASLAN ERDEM (Secretariat)
Meltem CEYLAN ÜNLÜSOY
Müjde YÜCE ERYILMAZ
Arzu Zeynep KARABAY
Burcu DOĞAN TOPAL
İşil ÖZAKCA

SOCIAL COMMITTEE

Selen ALP
Ebru AROĞLU İNAN
İlker ATEŞ
Filiz BAKAR
Kayhan BOLELLİ
Derya ÇİÇEK POLAT
Merve DEMİR’BÜGEN
Zehra Ceren ERTEKİN
Ayşe Mine G. ÖZKAN
Alper GÖKBULUT

Gizem GÜLPINAR
Mehmet GÜMÜŞTAŞ
Berna GÜVEN
M. Mesud HÜRKUL
Özge İNAL
Melek KARACAOĞLU
Banu KAŞKATEPE
Ecem KAYA
Gizem KAYKI MUTLU
Merve E. B. KIYMACI

Dilan KONYAR
Sevinç KURBANOĞLU
Aslıhan H. ALGAN
Umut Can ÖZ
Selman ŞABANOĞLU
Tangül ŞEN
Mehmet Barlas ÜZUN
Gülderen YILMAZ
Serap YILMAZ

SCIENTIFIC COMMITTEE

Nurten ALTANLAR (Turkey)
M. Levent ALTUN (Turkey)
Zeynep ATEŞ ALAGÖZ (Turkey)
Tunca Gül ALTUNTAŞ (Turkey)
Asuman BOZKIR (Turkey)
Zeliha BÜYÜKBİNGÖL (Turkey)
Benay CAN EKE (Turkey)
Nina CHANISHVILI (Georgia)
Tülay ÇOBAN (Turkey)
Tuncer DEĞİM (Turkey)
Bilgehan DOĞRU (Turkey)
Bülent GÜMÜŞEL (Turkey)
Christoph HIEMKE (Germany)
Ayşegül KARATAŞ (Turkey)

Ceyda S. KILIÇ (Turkey)
Marie-Aleth LACAILLE-DUBOIS (France)
Henk LINGEMAN (The Netherlands)
A. Tanju ÖZÇELİKAY (Turkey)
Gülbin ÖZÇELİKAY (Turkey)
Ayşe Mine ÖZKAN (Turkey)
Keykavous PARANG (USA)
Gülçin SALTAN (Turkey)
Sevgi ŞAR (Turkey)
Istvan TOOTH (Australia)
Bengi USLU (Turkey)
Sulhiye YILDIZ (Turkey)
PLENARY LECTURES

PL-1: IS BORIC ACID TOXIC TO REPRODUCTION IN HUMANS? ASSESSMENT OF THE ANIMAL REPRODUCTIVE TOXICITY DATA AND EPIDEMIOLOGICAL STUDY RESULTS

PL-2: NANODIAGNOSTICS WITH SIMPLE PAPER/PLASTIC-BASED PLATFORMS
A. Merkoci

PL-3: USE OF NANO-ENTITIES AS SEPARATION MEDIA IN MODERN LIQUID PHASE SEPARATION TECHNIQUES
Z. El Rassi, S. Akanthar, C. Aydogan, N. Ganewatta

PL-4: NEWEST STRATEGIES IN THE SEARCH FOR BIOACTIVE SAPONINS FROM THE TROPICAL PLANT BIODIVERSITY
M. A. Lacaille Dubois

PL-5: CHEMISTRY AND SOME INTERESTING BIOLOGICAL ACTIVITIES OF SECONDARY METABOLITES FROM MARINE-DERIVED FUNGI
A. Kijoia

PL-6: CARDIOPROTECTIVE EFFECTS OF BETA-BLOCKERS MEDIATED BY SCAVENGING REACTIVE OXYGEN AND NITROGEN SPECIES IN DIABETES
B. Turan

PL-7: STEM CELL-BASED THERAPY: A DOUBLE-EDGED SWORD?
A. B. Abdel Mageed

PL-8: RECENT DEVELOPMENTS IN ENANTIOSEPARATION OF CHIRAL DRUGS
B. Chankvetadze

PL-9: CAPILLARY NANO-LIQUID CHROMATOGRAPHY: PRINCIPLES AND APPLICATIONS IN DRUG ANALYSIS
S. Fanalli

PL-10: LIPOPHILIC VACCINE DELIVERY SYSTEMS
I. Toth

PL-11: INSIDE THE KINGDOM OF COMPLEMENT: THE TALE OF NANOPARTICLE AFFAIRS
S. Moien Moghimi

PL-12: CLINICAL CHEMISTRY: IMMUNOLOGICAL VERSUS CHEMICAL ASSAYS
H. Lingeman, J. Kool, M. Wuhrer, G. Somsen

PL-13: MODULATION OF OXIDATIVE STRESS AS A PHARMACOLOGICAL STRATEGY
L. Saso, O. Firuzi

PL-14: STUDIES ON TURKISH ASTRAGALUS SPECIES: PAST, PRESENT AND FUTURE
E. Bedir

PL-15: PERSPECTIVES OF THE CREATION PREPARATIONS ON THE BASIS OF PLANT RAW MATERIALS
N. Abdullayev

PL-16: IMMUNOBIOPHARMACEUTICS: THE ROLE OF THE IMMUNE SYSTEM IN DRUG DELIVERY
G. Borchard

PL-17: ELECTROCHEMICAL CHARACTERIZATION AND REACTIVITY OF PLATINUM NANOPARTICLES
A. Ravalli, G. Marrazza

PL-18: BIOSENSORS: THE REVOLUTION IN THE CLINICAL ANALYSIS
J. M. Feliu, A. Boronat, R. Martinez

PL-19: NEW INSIGHTS IN THE TOXIC MODES OF ACTION OF NEUROTOXIC AND CARCINOGENIC METAL SPECIES
T. Schwerdtle

PL-20: POLYMERIC NANOCARRIERS FOR TARGETED DELIVERY OF SN-38
S. Dimchevska, N. Geshkovski, R. Koliqi, P. Petrov, K. Goracinova

PL-21: BACTERIOPHAGES AS THERAPEUTIC AND PROPHYLACTIC MEANS
N. Chanishvili

PL-22: METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS
B. Sancak

PL-23: UPDATE 2015 OF GUIDELINES FOR THERAPEUTIC DRUG MONITORING (TDM) IN PSYCHIATRY AND NEUROLOGY
C. Hiemke and the TDM task force of the Arbeitsgemeinschaft fur Neuropsychopharmkologie und Pharmakopsychiatrie (AGNP)
P-15: HPLC DETERMINATION OF LEVOFLOXACIN IN HUMAN BREAST MILK
G. Ergin, O. Sagirli, S. Erturk Toker

P-16: ELECTROCHEMICAL OXIDATION OF ANTIBACTERIAL DRUG CEFEPIME AND ITS QUANTITATIVE DETERMINATION FROM PHARMACEUTICALS AND SERUM WITH CARBON BASED ELECTRODES
G. Ozturk, D. Kul

P-17: ROOM-TEMPERATURE PHOSPHORESCENCE DETERMINATION OF MELAMINE IN DAIRY PRODUCTS USING L-CYSTEINE-CAPPED MN-DOPED ZINC SULFIDE (ZNS) QUANTUM DOTS
B. Er Demirhan, B. Demirhan, H. E. Satana Kara

P-18: CHEMICAL PROFILE OF THREE EDIBLE PLANTS BY USING LC-MS/MS AND GC-MS: SCOLYMUS HISPANICUS, CARDARIA DRABA SUBSP. DRABA AND CARDUUS PYCNOCEPHALUS SUBSP. ABIDUS
M. Boga, H. Temel, A. Ertas, M.A. Yilmaz, S. Ertekin, E. Aygun Tuncay

P-19: ELECTROCHEMICAL DETECTION AND DISCRIMINATION OF INFLUENZA A AND B VIRUSES BASED ON DNA HYBRIDIZATION BY USING NANOBIOSENSORS
H. Subak, D. Ozkan Ariksoysal

P-20: HPLC SEPARATION OF ENANTIOMERS OF SOME CHIRAL CARBOXYLIC ACID DERIVATIVES USING POLYSACCHARIDE-BASED CHIRAL COLUMNS AND POLAR ORGANIC MOBILE PHASES

P-21: METHOD VALIDATION FOR AFLATOXIN ANALYSIS IN COSMETIC CREAMS
I. Gazioglu, U. Kolak

P-22: THE DETERMINATION OF TRACE METAL CONTENT OF DIFFERENT PARTS OF EUPHORBIA MACROCLADA SPECIES FROM VARIOUS LOCALITIES
I. Yener, H.Temel, A. Ertas, K.Senturk, M. Firat

P-23: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF ELEVEN ALLIUM (SAVAGE GARLIC) SPECIES ETHANOL EXTRACT
H. Temel, E. Izol, A. Ertas, I. Yener, M.Firat

P-24: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF METHANOL EXTRACT OF NINE EUPHORBIA SPECIES
I. Yener, A. Ertas, Y. Yesil, K. Senturk, H. Temel

P-25: AN EFFICIENT LC-MS/MS METHOD VALIDATION FOR DETERMINATION OF WATER SOLUBLE VITAMINS IN THYMUS
M.A. Yilmaz, O. Cakir, A. Boukeloua, I. Yener, H. Temel,

P-26: EFFECT OF BASIC AND ACIDIC ADDITIVES ON THE SEPARATION OF SOME BASIC DRUG ENANTIOMERS ON POLYSACCHARIDE-BASED CHIRAL COLUMNS WITH ACETONITRILE AS MOBILE PHASE

P-27: DETERMINATION OF TARTRAZIN USING NOVEL ELECTROCHEMICAL SENSOR BASED ON NANOCERIA IN DRINK SAMPLES
S. Kart, O. Karasalli, D. Koyuncu Zeybek

P-28: A NEW 'TURN-ON' AND REVERSIBLE FLUORESCENT PROBE WITH HIGH AFFINITY TO IRON (II) IONS IN AQUEOUS SOLUTION
M. Ozdemir, S. Urus, M. Sonmez

P-29: A NOVEL MWCNT SUPPORTED 'OFF–ON' COLORIMETRIC FLUORESCENT PROBE FOR IRON (II) IONS DETECTION IN AQUEOUS MEDIUM
M. Ozdemir, S. Urus, M. Caylar, I. Karteri

P-30: DESIGN AND SYNTHESIS OF A NOVEL MAGNETIC CORE-SHELL Fe3O4@SiO2 SUPPORTED CHEMOSensor BASED ON RHODAMINE B: HIGHLY SELECTIVE AND SENSITIVE FLUORESCENT PROBE FOR CR3+ AND HG2+
M. Ozdemir, S. Urus, M. Caylar, I. Karteri

P-31: DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE DETERMINATION OF EMTRICITABINE, RILPIVIRINE AND TENOFOVIR FROM BIOLOGICAL SAMPLES
M. Gumustas, M.G. Caglayan, F. Onur, S.A. Ozkan

P-32: DETERMINATION OF ACID DISSOCIATION CONSTANT VALUES OF SOME NEW MANNICH BASES OF 5-METHYL and 5-NITRO-2-BENZOXYLATIONONES

P-33: DETERMINATION OF APIXABAN IN TABLETS USING ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
M. Kozanli, N. O. Can, G. Arli

P-34: A NEW ALTERNATIVE TO SOLID PHASE EXTRACTION OF CEFTRIAXONE IN HUMAN PLASMA SAMPLES: ULTRAFILTRATION-BASED EXTRACTION AND HPLC ANALYSIS
M. Celebier, I. Suslu, S. Boynuegri, S. Altinoz

P-35: EFFECT OF INTERNAL STANDARD ON HPLC ANALYSIS OF TABLET DOSAGE FORMS. AN EXPERIMENTAL STUDY WITH STATISTICAL COMPARISON
M. Celebier, M. Nenni, A. Dogan, I. Suslu, S. Altinoz

P-36: LIQUID CHROMATOGRAPHIC DETERMINATION OF TOFISOPAM IN TABLETS USING DIFFERENT TYPES OF STATIONARY PHASES
N. O. Can, S. Ozcan

P-37: OPTIMIZATION OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE DETERMINATION OF ANTAZOLINE AND TETRAHYDROXAZINE IN OPHTHALMIC SOLUTIONS
U. Alisha, N. G. Goger, N. Ertas

P-38: NITRATE, NITRITE AND BROMIDE DETERMINATION IN SWAP SAMPLES BY CAPILLARY ELECTROPHORESIS
O. Ozan Erol, B. Yavuz Erdogan, A.N. Onar

P-39: INVESTIGATION OF AN ANTICANCER DRUG; SORAFENIB REDOX BEHAVIOR AT DIFFERENT ELECTRODES AND ELECTROCHEMICAL IMPEDANCE BEHAVIOR OF THE DEVELOPED SENSOR
N. Karadas Bakirhan, S. Patris, S. A. Ozkan, J M Kauffmann
POLYMERIC NANOPARTICLES FOR INTRA-ARTICULAR APPLICATION OF DICLOFENAC SODIUM
Z.A. Cankaya, E. Yilmaz, B. Kucukturkmen, U.C. Oz, A. Bozkir

QUETIAPINE FUMARATE SUSTAINED RELEASE TABLET FORMULATION DESIGN BY USING INFORM V.4 ANN PROGRAM
E. Ozcelik, B. Mesut, B. Aksoy, Y. Ozsoy

PREPARATION AND EVALUATION OF RESPIRABLE MICROPARTICLES OF LEVOFLOXACIN
M. Alemdar, B. Devrim

METFORMIN HYDROCHLORIDE-LOADED CHITOSAN-ALGINATE (CS-AL) BEADS
B. Server, A. B. Ugur, M. Cetin

DEVELOPMENT AND EVALUATION OF A LIPID-POLYMER HYBRID NANOPARTICULATE SYSTEM
C.T. Sengel Turk, C. Hascicek

ANTITUMOR ACTIVITY OF GELS CONTAINING GEMSTABINE HCI-LOADED MICROSHERPES AND NANOPARTICLES ON BLADDER CANCER CELL LINES
G. Sevin, Y. Erzurumlu, S. Y. Karavana, D. Ilem Ozdemir, C. Caliskan, E. Baloglu

CYTOTOXIC EFFECTS OF GEMSTABINE HCI-LOADED MICROSHERPES AND NANOPARTICLES ON BLADDER CANCER CELL LINES
G. Sevin, Y. Erzurumlu, S. Y. Karavana, D. Ilem Ozdemir, C. Caliskan, E. Baloglu

USE OF THE RYSHKEWITCH-DUCKWORTH EQUATION TO PREDICT THE TENSILE STRENGTH OF TABLETS FROM BINARY MIXTURES OF VARIOUS EXCIPIENTS AND MODEL API'S
M. Lachmann, M. Weimer, Y. Ozalp, B. Tuncay, J. Khinast, M. Celik

IN VITRO INCORPORATION STUDIES OF RADIOLABELED MUCAOADESIVE MICROSHERPES AT BLADDER CARCINOMA CELL LINES
D. Ilem Ozdemir, S. Y. Karavana, Z. Ay Senyigit, M. Ekinci, Y. Erzurumlu, C. Caliskan, M. Asikoglu, E. Baloglu

RADIOLABELED BIOADHESIVE MICROSHERPES LOADED CHITOSAN GL DETRIMENTS INCORPORATION TO THE BLADDER PAPILLOMA AND CARCINOMA CELL LINES
D. Ilem Ozdemir, S. Y. Karavana, Z. Ay Senyigit, M. Ekinci, Y. Erzurumlu, C. Caliskan, M. Asikoglu, E. Baloglu

ABSORPTION ENHANCING EFFECT OF TOTAL SAPONINS DERIVED FROM ACANTHOPYLLUM SQUARRUSOM AND QUILLAJASAPONARIA ON NASAL PERMEATION OF GENTAMICIN SULFATE AND CARBOXYFLUORESCEIN
E. Moghimi, S. A. Sajadi Tabassi, M. Ramezani, R. Lobenberg, S. Handali
P-374: THE INVESTIGATION OF WOUND HEALING EFFECT OF THERANEKRON® IN RATS
I. Yilmaz, A. Cetin, S. Bindak, M. Badem

P-375: CHRYSIN PREVENTS BRAIN DAMAGE CAUSED BY GLOBAL CEREBRAL ISCHEMIA/REPERFUSION IN A C57BL/J6 MOUSE

P-376: EVALUATION OF ANTIOXIDANT EFFECTS IN RAT LIVER AND ESSENTIAL ELEMENT CONTENTS OF CHERRY LAUREL
LAUCERASUS OFFICINALIS ROEM.
A. Eken, A. Baldemir, B. Unlu Endirlik, E. Ozger, S. Ilgun

P-377: EFFECTS OF AKR1B1 SILENCING IN HCT-116 COLON CANCER CELLS
B. Taskoparan, M.S. Ceyhan, S. Tuncer, M. Stefek, S. Banerjee

P-378: EFFECT OF MELATONIN AND RESVERATROL ON CARBONTETRACHLORIDE-INDUCED ACUTE PANCREAS INJURY IN RATS
B. Yigitcan, M. Gul, S. Gul, C. C. Gul, N. Bayat

P-379: INVESTIGATION OF THE TiO2 SOLID NANOPARTICLES SAFETY FOR HUMAN HEALTH BY USING IN VITRO TESTS
C. O. Yalcin, A. Ustundag, O. Ulker Cemiloglu, Y. Duydu, A. Karakaya

P-380: CYTOTOXIC EFFECTS OF SODIUM ARSENITE AND CADMIUM CHLORIDE IN VERO CELLS: A PRELIMINARY STUDY
D. Pasli, A. Gurbay

P-381: MUTAGENICITY OF 4-ETHYL-6-SUBSTITUTED-2H-3,4-DIHYDRO-1,4-BENZOXAZIN-3-ONE DERIVATIVES AND THEIR METABOLITES THAT INHIBITED HUMAN TOPOISOMERASE I
E. Foto, F. Zilifdar, N. Diril, E. Aki Yalcin

P-382: EVALUATION OF IN VITRO GENOTOXIC POTENTIALS OF 4-METHYL-6-SUBSTITUTED-2H-3,4-DIHYDRO-1,4-BENZOXAZIN-3-ONE DERIVATIVES ON HELA CELLS
E. Foto, F. Zilifdar, S. Yilmaz, N. Diril, I. Yalcin, I. Yildiz, E. Aki Yalcin

P-383: COMPARISON OF ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF 5-METOXYINDOLE-3-ALDEHYDE HYDRAZONES AND INDOLE-3-ALDEHYDE HYDRAZONES
E. Ince, A. Tascioglu, C. Karasalan, S. Suzen, H. Gurer Orhan

P-384: PROTECTIVE EFFECTS OF DEXPANTHENOL ON THIACETAMIDE-INDUCED CARDIAC INJURY IN RATS
E. Tasliedere, E. Ozoler, A. Yildiz, N. Vardi

P-385: METHOTREXATE-INDUCED CARDIAC TOXICITY: ROLE OF DEXPANTHENOL IN TREATMENT
E. Tasliedere, E. Ozoler, A. Yildiz, N. Vardi

P-386: BIOLOGICAL ACTIVITY OF A SERIES OF 2-(4-SUBSTITUTED-BENZYL)-5-AMINO-BENZOXAZOLE DERIVATIVES
F. Zilifdar, E. Foto, N. Diril, T. Boletti, I. Yalcin

P-387: TWO NEW 4-SUBSTITUTED-N-(2´-HYDROXY-4´-NITROPHENYL)-BENZENE ACETAMIDE DERIVATIVES AS HUMAN TOPOISOMERASE I CATALYTIC INHIBITORS
F. Zilifdar, E. Foto, N. Diril, T. Boletti, A. Yalcin

P-388: PREVENTIVE EFFECTS OF RESVERATROL AGAINST AZOXYMETHANE INDUCED TESTIS INJURY IN RATS
M. Kurus, A. Bay Karabulut, E. Tasliedere, O. Otlu

P-389: THE COMPARISON OF THE EFFECTS OF ESTROGEN AND MELATONIN AGAINST CORNEAL DISORDERS IN OVARIECTOMIZED AND PINEALECTOMIZED RATS
E. Tasliedere, S. Tasdemir, M. Sagir, A. Yildiz, N. Vardi

P-390: COMPARING THE REGENERATIVE EFFECTS OF SILYMARIN (SILYBUM MARIANUM) AND APRICOT (PRUNUS ARMENIACA L.) ON LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN RATS
I. Yilmaz, E. Tasliedere, H. S. Hatipoglu, T. Arabaci, M. Karasalan

P-391: IN VITRO AND IN VIVO TOXICITY ANALYSES OF BARE AND CHITOSAN COATED MNPS

P-392: BIODISTRIBUTION OF MAGNETITE NANOPARTICLES CHEMICALLY MODIFIED WITH CHITOSAN IN MICE LIVER AND BRAIN TISSUE

P-393: CHANGES IN SEMEN PARAMETERS OVER A 16 YEARS PERIOD IN 24693 TURKISH MEN
I. Cok, G. Karababa, G. Goney, M.H. Satiroglu, A.C. Pehlivani, U.Ciftci

P-394: URINARY COTININE LEVELS OF ELECTRONIC CIGARETTE (E-CIGARETTE) USERS
G. Goney, I. Cok, U. Tamer, S. Burgaz, T. Sengezer, A.A. Ali

P-395: ANTIOXIDANT CAPACITIES OF CURCUMIN, RESVERATROL AND ROSMARINIC ACID
M. Bacanli, H.G. Goktas, Z. Sarigol, S. Aydin, A.A. Basaran, N. Basaran

P-396: CORRELATION BETWEEN SOME CYTOKINE GENE POLYMORPHISMS AND SUSCEPTIBILITY TO TYPE 2 DIABETES
I. Ates, D. Altuner, H. S. Suzen, A. Karakaya

P-397: THE ASSOCIATION OF SEROTONINE 2A RECEPTOR 102 T/C POLYMORPHISM AND NAUSEA IN CITALOPRAM TREATED PATIENTS
M. Demirbugen, B. Baskak, T. Kizil Ozel, H. Devrimci Ozguven, H. S. Suzen

P-398: PRELIMINARY INVESTIGATION OF SUBACUTE MELATONIN TREATMENT ON RATS
M.F. Abdallah, E. Karacaoglu, B. Kilicarslan, G. Girgin, G. Selmanoglu, T. Baydar

P-399: TOXICITY OF POLYHYDROXYBUTYRATE COATED MAGNETIC NANOPARTICLES IN MICE AND MDA-MB-231 CELL LINE

P-400: DOXORUBICIN LOADED DEXTRAN COATED MAGNETIC NANOPARTICLES ENHANCE CYTOTOXICITY IN DOXORUBICIN TREATED PATIENTS
S. Yalcin, G. Unsoy, R. Khodadust, U. Gunduz
<table>
<thead>
<tr>
<th>Conference Paper</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-402</td>
<td>TP53 (ARG72PRO) POLYMORPHISM AND CLINICAL OUTCOME IN LUNG CANCER</td>
<td>S. Bilgen, A. O. Ada, C. S. Kunak, M. Gulhan, M. Iscan</td>
</tr>
<tr>
<td>P-403</td>
<td>THE EVALUATION OF BIOMARKERS OF OXIDATIVE STRESS IN HASHIMOTO’S THYROIDITIS</td>
<td>U. Yaman, P. Erkekoglu, Derya Bulus, Nesibe Andiran, B. Kocer Gumusel</td>
</tr>
<tr>
<td>P-404</td>
<td>CHARACTERIZATION AND FUNCTIONAL EFFECTS OF CENTRIESTAT, A POTENT ALDO-KETO REDUCTASE INHIBITOR, IN DIABETES AND INFLAMMATION</td>
<td>M. Soltessova Pnovka, M. Majekova, J. Ballekova, M. S. Ceyhan, S. Enayat, S. Banerjee, M. Stafek</td>
</tr>
<tr>
<td>P-405</td>
<td>A COMPERATIVE STUDY ON ANTIOXIDANT POTENTIALS OF THE VARIOUS SOLVENT EXTRACTS FROM DIFFERENT PARTS OF MOMORDICA CHARANTIA</td>
<td>S. Yilmaz, G. Coksari, O. Bahadır Acikara, N. Artik, G. Saltan, T. Coban</td>
</tr>
<tr>
<td>P-406</td>
<td>SAFE HANDLING OF ANTINEOPLASTIC DRUGS: NURSES’ COMPLIANCE AND AWARENESS</td>
<td>V. Karacaoğlan, N. Tasdemir, S. Celik, M. Coplu, S. Akıgul</td>
</tr>
<tr>
<td>P-408</td>
<td>ISOLATION OF LACTIC ACID BACTERIA AND LACTIC ACID BACTERIA PHAGES FROM DIFFERENT SOURCES</td>
<td>A. Gumustes, E. Kukhadze, I. Janashia, A. Akin, N. Chanishvili</td>
</tr>
<tr>
<td>P-409</td>
<td>ANTIBACTERIAL EFFECTS OF CINNAMALDEHYDE AGAINST CARBAPENEM-RESISTANT NOSOCOMIAL ACINETOBACTER BAUMANII ISOLATES</td>
<td>B. Kaskatepe, M. E. Kiymaci, S. Suzuk, S. Aslan Erdem, S. Cesur, S. Yildiz</td>
</tr>
<tr>
<td>P-410</td>
<td>IN VITRO ANTIMICROBIAL EFFECTS OF MONTERPENE ALCOHOLS AGAINST PROPIONIBACTERIUM ACNES AND STAPHYLOCOCCUS EPIDERMIDIS</td>
<td>C. Elmeci, G. Iscan</td>
</tr>
<tr>
<td>P-411</td>
<td>IN VITRO ANTIMICROBIAL EFFICACY OF FOUR CONTACT LENS CARE SOLUTIONS</td>
<td>M. Eryilmaz, B. Kaskatepe, M. E. Kiymaci, D. Simsek, H. B. Erol, A. Gumustas</td>
</tr>
<tr>
<td>P-412</td>
<td>THE EFFECT OF BIO-ACTIVE COMPONENTS CONTAINING ADHESIVE SYSTEMS ON BACTERIAL MICROLEAKAGE: IN-VITRO STUDY</td>
<td>G. Demirel, G. Gur, M. Eryilmaz, N. Altanlar</td>
</tr>
<tr>
<td>P-413</td>
<td>EFFECT OF CAPSAICIN ON TRANSCRIPTION FACTORS IN 3T3-L1 CELL LINE</td>
<td>G. Bora, M. Berköz, M. Yıldırım, O. Türkmen, O. Allahverdiyev</td>
</tr>
<tr>
<td>P-414</td>
<td>APOPTOTIC EFFECTS OF TWO ETODOLAC DERIVATIVES, SGK-205 AND SGK-216, ON K562 LEUKEMIA CELL LINE</td>
<td>O. Orun, S. Averbek, P. Mega Tiber, P. Cikl Suzgun, S. G. Kucukguzel</td>
</tr>
<tr>
<td>P-415</td>
<td>SYNTHESIS OF SOME NOVEL THIAZIAZOLE DERIVATIVES AND EVALUATION OF THEIR ANTIDEPRESSANT-LIKE EFFECTS IN MICE</td>
<td>O. D. Can, Y. Ozkay, D. N. Yildiz</td>
</tr>
<tr>
<td>P-416</td>
<td>EVALUATION OF IN VITRO ANTIMICROBIAL EFFICACY OF SOME NEW GENERATION DISINFECTANTS</td>
<td>M. Eryilmaz, B. Kaskatepe, M. E. Kiymaci, H. B. Erol, D. Simsek, A. Gumustas</td>
</tr>
<tr>
<td>P-418</td>
<td>EFFECT OF CINNAMALDEHYDE ON BIOFILM FORMATION BY STAPHYLOCOCCUS EPIDERMIDIS</td>
<td>M.E. Kiymaci, N. Altanlar, A.Akin</td>
</tr>
<tr>
<td>P-419</td>
<td>ANTIMICROBIAL PROPERTIES OF THYME AND SAGE OILS AGAINST SOME PATHOGENS</td>
<td>B.Kaskatepe, M.E. Kiymaci, S.Suzuk, A.N.Yazgan, S. Aslan Erdem, S.Cesur</td>
</tr>
<tr>
<td>P-420</td>
<td>ANTIMICROBIAL ACTIVITIES OF ACTINOBACTERIA ISOLATED FROM ALGERIAN SAHARA SOILS</td>
<td>M. Harir, M. Bellahcene, Z Fortas</td>
</tr>
<tr>
<td>P-421</td>
<td>ANTIBACTERIAL EFFECT OF CORIANDER (CORIANDRUM SATIVUM L.) SEED EXTRACT AND SYNERGISTIC INTERACTION WITH CEFOXITIN</td>
<td>N. Ildiz, A. Baldeır, Y. Konca, H. Ozbilge</td>
</tr>
<tr>
<td>P-422</td>
<td>MICROBIOLOGICAL QUALITY CONTROL OF THE VETERINARY INJECTABLE VITAMIN PREPARATIONS MANUFACTURED IN TURKEY</td>
<td>S. Oztürk, S. Yıldız</td>
</tr>
<tr>
<td>P-423</td>
<td>PLANT EXTRACTS AS A CONTROL AGENT OF ASPERGILOSIS IN HUMAN AND AFLATOXIN FORMATION IN FOOD</td>
<td>Y. Alptekin</td>
</tr>
<tr>
<td>P-424</td>
<td>THE DEVELOPMENT OF BACTERIAL RESISTANCE AGAINST ANTIBIOTICS IN IMMUNOCOMPROMISED PATIENTS</td>
<td>G. Bora, Y. Bora, E. Kıyımacı, O. Türkmen, A. Sunnetçioğlu, Y. Bayram, M. Sunnetçioğlu, G. Acıkoğaz</td>
</tr>
</tbody>
</table>

AUTHOR INDEX 467

LIST OF PARTICIPANTS 477
PLENARY LECTURES
PL-1: IS BORIC ACID TOXIC TO REPRODUCTION IN HUMANS?
ASSESSMENT OF THE ANIMAL REPRODUCTIVE TOXICITY DATA AND EPIDEMIOLOGICAL STUDY RESULTS


Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, TURKEY

Boric acid and sodium borates are classified as toxic to reproduction in the CLP regulation under “Category 1B” with the hazard statement of “H360FD”. This classification is based on the reprotoxic effects of boric acid and sodium borates in animal experiments at high doses. However, boron mediated reprotoxic effects have not been proven in epidemiological studies so far. The epidemiological study performed in Bandırma boric acid production plant is the most comprehensive published study in this field with 204 voluntarily participated male workers. Sperm quality parameters (sperm morphology, concentration and motility parameters), FSH, LH and testosterone levels were determined in all participated employees as the reproductive toxicity biomarkers of males. However, boron mediated unfavorable effects on reproduction in male workers have not been determined even in the workers under very high daily boron exposure (0.21 mg B/kg-bw/day) conditions. The NOAEL for the reprotoxic effects of boron in rats is 17.5 mg/kg-bw/day. Accordingly the RfD of boron is 0.29 mg/kg-bw/day when using “60 (17.5/60)” as the chemical specific composite uncertainty factor for boric acid. The mean daily boron exposure level of the high exposure group (0.21 mg B/kg-bw/day) in boric acid production plant (Turkey) was lower than the RfD computed for boron. The NOAEL for rat reproductive toxicity is equivalent to a blood boron level of 2020 ng/g. This level is higher than the mean blood boron concentration (223.89 ± 69.49 ng/g) of the high exposure group workers in Bandırma boric acid production plant (Turkey) by a factor of 9.

The comparison of experimental and epidemiological studies clearly shows that human boron exposures, even in the highest exposure conditions, are too low to reach the blood boron concentrations that would be required to exert adverse effects on reproduction. Accordingly, classifying boric acid and sodium borates under “Category 1B” as “presumed reproductive human toxicant in the CLP regulation seems scientifically not reasonable. The results of the epidemiological studies (including the study performed in China) support for a down-classification of boric acid from the category 1B, H360FD to category 2, H360d, (suspected of damaging the unborn child).

Acknowledgement: This study was supported by Eti Mine Works General Management and BOREN.

PL-2: NANODIAGNOSTICS WITH SIMPLE PAPER/PLASTIC-BASED PLATFORMS

A. Merkoçi

ICREA & Catalan Institute of Nanoscience and Nanotechnology (ICN2), Bellaterra (Barcelona), Catalonia, Spain

Development of novel diagnostic tools with interest for point of care applications represents one of the main research fields for the nanobiotechnology. Either brand new devices or improvement of existing ones are being developed thanks to the use of nanomaterials and nanotechnologies. Between the various devices biosensing systems based on electrical and electrochemical transducing schemes are overall in the focus of interest given the simplicity and cost efficiency of detection. Among the various biosensing system performance requirements the high sensitivity and selectivity of the response are crucial for applications in clinical diagnostics. The fulfillment of such requirements means the detection of low levels of clinical biomarkers in human fluids. Due to the fact that the biomarkers are present in very low concentrations the need for biosensing systems that can detect these analytes with high sensitivity and selectivity that include very low detection limits along with high reproducibility is an important challenge. To overcome the difficulties in accomplishing all these requirements the main efforts are driven toward signal amplification and noise reduction of biosensing systems by the incorporation of nanomaterials. Nanomaterials (NMs) such as carbon nanotubes, graphene, metallic nanoparticles, nanowires and quantum dots are showing to be excellent materials to be used as electrochemical transducers or labels in DNA (or genosensors) sensors beside enzymatic sensors, immunosensors, or cell sensors. The amplification of the detection of biorecognition events (ex. DNA hybridization reactions etc) are the most important objectives of the current bioanalytical chemistry. In this context integration of the catalytic properties of some biomolecules with those of nanomaterials is appearing to be a promising way to enhance the sensitivity of the biossays. Examples related to various clinical biomarkers as well as contaminants detection will be shown. The developed devices and strategies are intended to be of low cost while offering high analytical performance in screening scenarios beside other applications. Special
emphasis will be given to lab-on-a-chip platforms with integrated electrochemical detection with interest for various applications. In addition simple paper-based platforms that operate in lateral flow formats with interest for various detections also will be shown.

**PL-3: USE OF NANO-ENTITIES AS SEPARATION MEDIA IN MODERN LIQUID PHASE SEPARATION TECHNIQUES**

Z. El Rassi, S. Alharthi, C. Aydogan, N. Ganewatta

Oklahoma State University, Department of Chemistry, Stillwater, Oklahoma, USA

**INTRODUCTION**

The aim of this investigation is to develop and characterize separation media based on nano-entities for modern liquid phase separation techniques, namely HPLC, capillary electrophromatography (CEC) and capillary electrophoresis (CE). In HPLC and CEC, monolithic columns with incorporated nanoparticles have been designed while in CE electrolytes containing the same nanoparticles have been investigated.

**MATERIALS AND METHODS**

*Materials:* Multiwalled carbon nanotubes (MWCNTs) as well as metal and silicon oxides (MOs) nanoparticles were obtained from various commercial sources. Test solutes including alkylbenzenes, phenoxy acid herbicides, benzene derivatives, benzonitrile, chlorophenols, DL-dansyl amino acids and aniline derivatives, reagents e.g., ethylene glycol, 1-dodecanol and cyclohexanol were purchased from Sigma Aldrich (Milwaukee, WI, USA). Glyceryl monomethacrylate (GMM) was from Dajac Labs (Trevose, PA, USA). Standard proteins were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile and isopropyl alcohol were purchased from Pharmco Aaper (Brookfield, CT, USA). Fused silica capillary columns were from Polymicro Technologies (Phoenix, AZ, USA).

*Methods:* Monoliths were prepared according to our established procedures (see typical refs. 1 and 2) with some slight modifications to fit the incorporated nano-entities and to adapt very well to HPLC and CEC running conditions.

**RESULTS AND DISCUSSION**

In both HPLC and CEC, the incorporation of MWCNTs and MOs led to significant changes in solute retention and selectivity. In one approach the nano-entities constituted entirely the stationary phase responsible for solute retention and separation. This second approach entailed the preparation and evaluation of an “ideal monolithic support” and coating it with nano-entities to yield real “nano-entities based stationary phases” for the HPLC and CEC separation of a wide range of solutes. The “ideal” monolith, which was an inert, relatively polar monolith based on the *in situ* polymerization of GMM and EDMA, proved to be the most suitable support for the preparation of “MWCNTs and MOs stationary phases”. The carbon nanotube “coated” monoliths proved useful in the HPLC and CEC separations of a wide range of small solutes including enantiomers (see Fig. 1). With MWCNTs, polar and π interactions were responsible for solute retention. With MOs based monoliths, polar and metal interactions were involved in solute retention and separations. Concerning CE with electrolytes containing nanoparticles, the presence of nano-entities allowed many samples to separate to a greater extent than in the absence of nano-entities thus proving the superiority of nano-entities interactive CE.

**CONCLUSIONS**

Because of their chemical and physical properties, nano-entities based separation media are very promising for the separation of a wide range of compounds providing unique selectivity and high resolving power.

**REFERENCES**


2. Mayadunne, E.; El Rassi, Z., Facile preparation of octadecyl monoliths with incorporated carbon nanotubes and neutral monoliths with coated carbon nanotubes stationary phases for HPLC of small and large molecules by

PL-4: NEWEST STRATEGIES IN THE SEARCH FOR BIOACTIVE SAPONINS FROM THE TROPICAL PLANT BIODIVERSITY

M.-A. Lacaille-Dubois

Laboratoire de Pharmacognosie, EA 4267 FDE/UFC, UFR des Sciences de Santé, Université de Bourgogne, 7, Bd Jeanne d’Arc, 21079 Dijon Cedex, France.

Pharmacognosy is probably the oldest pharmaceutical discipline. For thousands of years, man has used many plant extracts for medical treatments and nature is a huge reservoir of active molecules that are still being explored, for the search of new therapeutic strategies. Among these compounds, saponins known since antiquity for their detergent properties and toxicity to fish, have to date many uses in the food, therapeutic, and cosmetic areas..... Saponins, widely spread throughout the plant or marine organism kingdom are a group of steroid- or triterpene-glycosides offering a great molecular and biological diversity. Their activities are often related to their membrane interacting properties resulting in potential toxic or specific biological effects. Modern pharmacological studies have demonstrated a variety of activities for these compounds such as immunoadjuvant, immunomodulatory, cytotoxic, antitumor, antiinflammatory, hypcholesterolemic, antimicrobial properties, to mention just a few [1, 2].

This lecture will focus on our new optimization strategy leading to the discovery of new bioactive saponins by using a combination of successive advanced procedures in extraction, isolation, structure elucidation and bioassays. Among the most innovative extraction methods which are more environment friendly, time saving, using less energy and less solvent while producing higher yields, we used microwave-assisted extraction and ultrasonic extraction. These methods in agreement with the "green chemistry" replace now the conventional methods (maceration, soxhlet, refluxing). Then, the isolation of saponins which are sometimes highly complex molecules containing up to eight or nine sugars has been greatly improved through the optimization of the preparative solid/liquid chromatography conditions. The development of successive chromatographic steps involving flash chromatography, vacuum liquid chromatography, low, medium- and high-pressure liquid chromatography on silica gel and reversed-phase silica gel RP-18 (VLC, VLC, MPLC, HPLC), avoided the use of traditional partitions between butanol/water, dialysis procedures or precipitations in diethyl/ether. The structural elucidation is performed by extensive spectroscopic techniques which are basically used in routine analysis including 1D and 2D NMR (1H, 13C, DEPT, COSY, NOESY, TOCSY, HSQC, HMBC) and mass spectrometry (FAB-MS HRESIMS ) [3]. The bioassays include several in vitro tests such as cytotoxicity on human colon cancer cells (HT-29, HCT 116, SW 480), a cardiomyoblast cell line (H9c2), a human prostate carcinoma cell line (DU 145) and a murine mammary cancer cell line (mammary EMT6), immunoproliferation and apoptosis induction on Jurkat cells and splenocytes, antioxidant ORAC assay, antiinflammatory evaluation by determination of the modulation of the IL-1 production (major proinflammatory cytokine involved in tumor pathogenesis), and immunoadjuvant activity. The discussion will also focus on the understanding of their mechanism of action and structure/activity relationships.

This lecture will highlight examples of plant saponins from the African and Asian biodiversity, which have been studied in our laboratory (Polygalaceae, Mimosaceae, Caryophyllaceae, Apiaceae, Sapindaceae, Dioscoreaceae, Agavaceae...) [4-7]. We wish also to discuss new trends reported in the literature which have not yet widely introduced with saponins. They concern dereplication, and metabolomic approaches which are currently of considerable importance in the field of natural product discovery. The "rediscovery" of known structures in natural extracts is a major drawback in natural product chemistry. New NMR-based or MS-based dereplication approaches make the identification of known compounds early in the discovery line and enable the targeted isolation of unknown natural products. Ultra-performance liquid chromatography (UPLC) coupled with quadrupole time-of-flight mass spectrometry (QTOF MS) has become a powerful tool in the analysis of complex saponin metabolites with a short time of analysis and accurate mass measurements. This method has been successfully used to identify and differentiate complex saponins found in the stems, leaves and seeds of Polygala tenuifolia (Polygalaceae) [8].

REFERENCES

5. Pertuit et al. (2014) Phytochemistry 102, 182-188.
PL-5: CHEMISTRY AND SOME INTERESTING BIOLOGICAL ACTIVITIES OF SECONDARY METABOLITES FROM MARINE-DERIVED FUNGI

A. Kijjoa

Instituto de Ciências Biomédicas Abel Salazar & CIIMAR, Universidade do Porto, Rua de Jorge Viterbo, 228, 4050-313 Porto, Portugal

During the past 40 years, a myriad of novel compounds have been isolated from marine organisms and many of them have been reported as having interesting biological activities, some of which are of interest from the point of view of potential drug development. Lately, there has been an increasing interest on marine-derived fungi as a target source of bioactive marine natural products because many consider them among the world’s greatest untapped resources for new biodiversity as well as chemodiversity. Moreover, through established culture methods, they can produce quantity of compounds needed for medicinal chemistry development, clinical trials and even marketing. In our research program on drug discovery from natural sources, we have investigated the secondary metabolites produced by the cultures of the marine sponge and coral-associated fungi of the genera Aspergillus, Neosartorya and Talaromyces. Several structural types of the secondary metabolites, including the indole alkaloids of the tryptovaline, fiscalin and azsonalenin types, meroditerpenes, isocoumarin derivatives and cyclopeptide have been isolated. Since one of our objectives is to find new antibiotics to combat multi-drugs resistant bacteria, these compounds were evaluated for their antibacterial activity against Gram-positive and Gram-negative bacteria and multidrug-resistant isolates from the environment as well as for their synergistic effect with antibiotics and their antibiotic activity.

PL-6: CARDIOPROTECTIVE EFFECTS OF BETA-BLOCKERS MEDIATED BY SCAVENGING REACTIVE OXYGEN AND NITROGEN SPECIES IN DIABETES

B. Turan

Department of Biophysics, Ankara University Faculty of Medicine, Ankara, Turkey

Beta-adrenoceptors (β-AR), members of the G protein-coupled receptors play important roles in the regulation of heart function. A positive inotropic action of catecholamines is mediated through their interaction with β-AR, located on the sarcolemma, while they can also mediate some deleterious effects, such as cardiac arrhythmias or myocardial apoptosis. The well-known β-AR-associated signaling in heart is composed of a coupled mechanism among both β1- and β2-AR and stimulatory G protein (Gs). This coupled mechanism further leads to the activation of adenylyl cyclase, and thereby increases in intracellular cAMP level. However, recent studies have emphasized the contribution of constitutive β3-AR coupling to Gi proteins, thereby initiating additional signal transduction pathways, particularly under physiopathological conditions. It has been documented that abnormalities in β-AR signaling could be an important determinant for depressed heart function under pathological conditions including diabetes.

Diabetic cardiomyopathy, as a distinct entity, is recognized due to its diminished responsiveness to β1-AR agonist stimulation in the heart from diabetic rats with no important changes in the responses mediated with β2-AR. Furthermore, an upregulation of β3-AR has been shown in diabetic rat heart with a strong negative inotropic effect on left ventricular function. Experimental data provide evidences that the mechanisms for the negative inotropic effect with β3-AR activation appears to involve a pertussis toxin (PTX)-sensitive G protein and the activation of a nitric oxide synthase pathway. On the other hand, β-blockers demonstrate marked beneficial effects in heart dysfunction with scavenging free radicals and/or acting as an antioxidant with both sex- and dose-dependent manners.

Taken into consideration the preventive role of β-blockers on the structure and function of cardiac sarcoplasmic reticulum Ca2+ release channels, RyR2 macromolecular complex in various studies on both human and animal heart failure models, we aimed to investigate the mechanisms that underlie the benefits observed with β-blocker either timolol or propranolol treatment on diabetic cardiomyopathy. Our data showed that a long-term treatment of diabetic male rats with a β-AR blocker timolol or propranolol prevented diabetes-induced depressed basal activity of the left ventricle without any hypoglycemic effect while these two blockers attenuated the hypertrophy in cardiomyocytes. Furthermore, Western blot data indicated that the diabetes-induced alterations in cardiac ryanodine receptor Ca2+ release channel hyperphosphorylation, decreased FKBP12.6 protein level, and phosphorylated levels of PKA and CaMKII were prevented with propranolol or timolol treatment. Moreover, we report for the first time that this important preventive effect of timolol but not propranolol is associated with balanced intracellular Ca2+ and Zn2+ homeostasis, and a balanced level of oxidant to antioxidant ratio in both heart and circulation of diabetic rats. We also report that timolol-treatment significantly normalized depressed levels of some Ca2+ handling regulators, such as NCX and
phospho-PLN to PLN ratio. These observations may account for some of the beneficial effects of β-AR blockage, most importantly with timolol, in diabetic cardiomyopathy confirmed by severe heart dysfunction. (Acknowledgements: TUBITAK SBAG-113S466)

**PL-7: STEM CELL-BASED THERAPY: A DOUBLE-EDGED SWORD?**

A. B. Abdel-Mageed

Professor of Urology & Pharmacology, Zimmermann Endowed Professor of Cancer Research, Tulane Cancer Center, Tulane University School of Medicine, New Orleans, Louisiana, USA

Emerging evidence suggests that mesenchymal stem cells (MSCs) are often recruited to tumor sites, but their functional significance in tumor growth and disease progression remains elusive. Herein we report that prostate cancer (PC) cell microenvironment subverts PC patient-derived adipose derived stem cells (pASCs) to undergo neoplastic transformation. Unlike normal ASCs, the pASCs primed with PC cell conditioned media (CM) formed prostate-like neoplastic lesions in vivo and reproduced aggressive tumors in secondary recipients. The pASC tumors acquired cytogenetic aberrations and mesenchymal-to-epithelial transition and expressed epithelial, neoplastic, and vasculogenic markers reminiscent of molecular features of PC tumor xenografts. Our mechanistic studies revealed that PC cell-derived exosomes are sufficient to recapitulate formation of prostate tumorigenic mimicry generated by CM-primed pASCs in vivo. In addition to down-regulation of the large tumor suppressor homolog2 (Lats2) and the programmed cell death protein 4 (PDCD4), a neoplastic transformation inhibitor, the tumorigenic reprogramming of pASCs was associated with trafficking by PC cell-derived exosomes of oncogenic factors, including H-ras and K-ras transcripts, oncomiRNAs miR-125b, miR-130b, and miR-155 as well as the Ras superfamily of GTPases Rab1a, Rab1b, and Rab11a. Our findings implicate a new role for PC cell-derived exosomes in clonal expansion of tumors through neoplastic reprogramming of tumor tropic ASCs in cancer patients. Importantly, because of the potential risk of neoplastic transformation, our results implicate that allogeneic adult ASCs procured from donors with otherwise undetected malignancies may be non-amenable for therapeutic transplantations in clinical settings.

**PL-8: RECENT DEVELOPMENTS IN ENANTIOSEPARATION OF CHIRAL DRUGS**

B. Chankvetadze

Institute of Physical and Analytical Chemistry, School of Exact and Natural Science, Tbilisi State University, Chavchavadze Ave 1, 0179 Tbilisi, GEORGIA

This presentation summarizes the recent developments in analytical and preparative scale separation of enantiomers of chiral drugs. The techniques discussed include high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), nano-liquid chromatography (nano-LC) and capillary electrophochromatography (CEC). Chiral drugs and drug-like compounds discussed include arylpropionic acid derivatives, β-blockers, imidazole and triazole derivatives, dihydropyridines, chiral sulphoxides, amino acid derivatives and others. Various fundamental and applied aspects will be discussed in each technique. In HPLC part novel chiral selectors, mobile phases, mobile phase additives and inert carriers, as well as some unusual effects and approaches for a better understanding of the chiral recognition mechanisms will be summarized [1-3]. This part of the presentation will highlight the efforts of our group with regard to separations of enantiomers in the liquid phase with the highest possible coverage of analytes, separation selectivity, plate numbers and shortest analysis time. In order to achieve this goal, the systematic optimization of the composition of polysaccharide-based chiral selectors, the structures of the studied analytes (chiral sulfoxides), composition of the mobile phases, mobile phase additives and separation temperature have been performed. In a parallel project, the particle size of the silica, its morphology (porosity and pore size), the nature of the chiral selector and its content in the chiral stationary phase were optimized in order to reach the highest possible column performance. The nano-LC and CEC parts of the presentation will discuss the application of novel core-shell silica-based chiral stationary phases for separation of enantiomers of chiral drugs [4, 5]. The CE part will focus on chiral recognition mechanisms with cyclodextrin-type chiral selectors in aqueous and non-aqueous CE.

**ACKNOWLEDGMENTS**

This study was financially supported in part by the Rustaveli Georgian National Science Foundation (RGNSF) grant No 31/90 for fundamental research.

**REFERENCES**

1. Demetrashvili, M.; Chankvetadze, L.; Farkas, T.; Chankvetadze, B., Separation of enantiomers of selected chiral sulphoxides on polysaccharide-based chiral columns and polar organic mobile phases with the emphasis on the

PL-9: CAPILLARY / NANO-LIQUID CHROMATOGRAPHY: PRINCIPLES AND APPLICATIONS IN DRUG ANALYSIS

S. Fanali

Italian National Research Council, C.N.R., Institute of Chemical Methodologies, Monterotondo, ITALY

INTRODUCTION

Aim of this communication is to introduce the general principle of capillary/nano-liquid chromatography. These techniques have been recently developed and can offer several advantages over the conventional ones. Among their features: consumption of reduced volumes of sample and mobile phase, lower costs, high mass sensitivity, high resolution and perfect coupling with mass spectrometry due to a low flow rate (nL/min) are the most important. The separation can be carried out utilizing capillary columns of 10-300 Î¼m I.D. containing the selected stationary phase (packed particles, polymeric-monolithic or bonded on the capillary wall). In addition some selected applications dealing with drug analysis will be illustrated taking in mind how to select optimum experimental conditions (stationary phase – particles type and properties, mobile phase etc.). Attention will be paid to the method optimization and analysis of chiral compounds of pharmaceutical interest.

MATERIALS AND METHODS

Instrumentation: Experiments were performed utilizing a laboratory assembled apparatus composed of a conventional pump. The nL/min flow was obtained employing a split system (T union); a modified injection valve (60-100 nL) was used. The packed capillary was directly connected (on-column) with a UV detector or with a ESI-iontrap mass spectrometry.

Capillary packing: Column were packed in our lab applying the slurry packing method; the selected stationary phase was suspended in acetone or methanol, sonicated and packed. After flushing with water retaining frits were prepared at about 700 °C x 7 s.

RESULTS AND DISCUSSION

Fig. 1 shows a scheme of nano-LC instrumentation coupled with mass spectrometry (MS). The mobile phase coming from the pump is splitted reaching the injection valve and delivered to the packed capillary. The separated analytes are reaching the MS through the tip. High sensitivity and exact determination of mass compounds can be obtained. The capillary column contained different silica modified particles, e.g., vancomycin for chiral separations and C18 for smart drug analysis [1].

CONCLUSIONS

Nano-LC can be used for the analysis of a large number and type of compounds including enantiomers, drugs, nutraceuticals, herbicides, pesticides, peptides etc.

REFERENCE

PL-10: LIPOPHILIC VACCINE DELIVERY SYSTEMS

I. Toth

The School of Chemistry and Molecular Biosciences and School of Pharmacy, The University of Queensland, St. Lucia, Queensland, Australia

Subunit vaccines that contain the minimal microbial components necessary to stimulate appropriate immune responses have the potential to overcome allergic response or autoimmunity that can result from using classical vaccines. We developed new delivery systems by combining the adjuvant and antigenic peptide epitopes into one chemically bonded dendritic entity. The presentation of epitopes on the nanoparticles surface was optimized to elicit a strong immune response in mouse models.

Infection with group A streptococci (GAS), one of the most common and widespread human pathogens, can result in a broad range of diseases, with the potential to develop acute and post-infectious rheumatic fever and rheumatic heart disease. Immunity to GAS relies on the production of opsonic antibodies specific to the hypervariable N-terminal and conserved C-terminal regions of the coiled-coil α-helical M protein, a GAS major virulence factor. To improve vaccine delivery we developed a self-adjuvanting lipid core peptide (LCP) dendrimer system that included the antigen, a T helper epitope, a carrier, and the adjuvant within the same molecular entity. The system allowed the attachment of multiple copies of antigens. We investigated the structural requirements to elicit production of different antibodies (IgA, IgG) and assessed the influence of complex size on the level of antibody production1.

Recent developments in nanomedicine/vaccinology have identified that size and morphological characteristics of nanoparticle vaccines affect their efficacy. Preliminary investigations have demonstrated that polymer-based nanoparticles that displayed peptide epitopes on their surface induced very strong immune responses against those epitopes. We have also shown that this response was dependent on the size of the total construct. We explored the efficacy of nanoparticle vaccines using a human papillomavirus (HPV) model. HPV infection, most commonly HPV-16, is responsible for the vast majority of cases of cervical cancer, which is the second most common cancer in women worldwide.

The development of therapeutic vaccines that eliminate HPV infected cells and eradicate established HPV-associated tumors would therefore be beneficial and desirable. We established a synthetic pathway to conjugate human papillomavirus peptide antigens to the polymeric core to create macromolecular vaccine candidates to treat HPV-related cancers. These conjugates reduced tumor growth and eradicated established E7-expressing TC-1 tumors in mice after a single immunization, without the addition of an external adjuvant2.

We extended our vaccine delivery platform investigations by using luteinizing hormone-releasing hormone (LHRH) as antigen. An anti-LHRH vaccine aims to control the level of sex hormones FSH and LH by generating antibodies against LHRH in murine and ovine models.

We have observed significant IgG antibody response after primary immunization without the use of additional adjuvant. The antibody response was enhanced and longer lasting when we co-administered commercial adjuvant AdjuVac™ [Lab Anim (NY) 2007, 36 (9) 51-58] with our LCP-LHRH vaccine formulation3.


PL-11: INSIDE THE KINGDOM OF COMPLEMENT: THE TALE OF NANOPARTICLE AFFAIRS

S. Moien Moghimi

Centre for Pharmaceutical Nanotechnology and Nanotoxicology, Department of Pharmacy, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark.

The complement system is a key effector of both innate and cognate immunity and act in a wide variety of host defence, inflammatory, homeostatic and immune reactions. The function of complement in innate host defence is accomplished through highly orchestrated opsonisation, lytic and inflammatory processes. Polymers, synthetic nanoparticles and nanomedicines may trigger complement and a number of consequences ensue from complement activation. These comprise both beneficial and adverse reactions, depending on the extent and severity of complement activation and microenvironmental factors. Indeed, complement fixation is a central point for efficient clearance of nanoparticles and drug carriers by phagocytic cells. On the other hand, inadvertent
complement activation may initiate adverse reactions, which has been noted following infusion of therapeutic antibodies, contrast agents and nanomedicines into human subjects. Complement also plays a modulatory role in cancer, where recent studies have indicated that intratumoral accumulation of complement activating long-circulating nanoparticles/nanomedicines in immunocompetent mice can accelerate tumour growth through liberation of complement chemotactic molecules C5a, leading to the recruitment of regulatory T cells (resulting in deregulation or suppression of CD8+ cytotoxic T cell activity), immunosuppressive monocytes and alternatively activated macrophages into malignant tumours. C5a has also been suggested to promote tumour angiogenesis. Therefore, understanding of nanomaterial properties that incite complement is a prerequisite for design and engineering of immunologically safer nanomedicine and biomedical devices.

REFERENCES


PL-12: CLINICAL CHEMISTRY: IMMUNOLOGICAL VERSUS CHEMICAL ASSAYS

H. Lingeman, J. Kool, M. Wuhrer, G. Somsen

Division of BioAnalytical Chemistry, VU University Amsterdam, The Netherlands

Clinical enzymology and clinical immunology are the most frequently applied approaches in clinical chemistry. However, in a number of cases there are problems with the required selectivity and or specificity, resulting in false positive or false negative results. One of the possibilities to circumvent these problems are the use of hyphenated separation techniques combined with mass spectrometry (MS). In this presentation the potential of 2D liquid chromatography (LC), capillary electrophoresis (CE), advanced solid-phase extraction (SPE) approaches, and combinations of bio-assays and chemical approaches will be shown.

Based on a method that has been described in the literature [1] a method will be described for the quantitative analysis of thyroglobulin (Tg) in sample containing antithyroglobulin antoantibodies. Tg is a thyroid specific protein and is used as a biomarker in the follow-up of patients treated for differentiated thyroid cancer (DTC). About 10-30% of the patients have formed antithyroglobulin autoantibodies (Tg-Ab). These Tg-Ab can interfere with the technique which is used at the moment, immunoassays, and cause false negative results. The modified method [1] consists of a 2D-LC/MS method, a tryptic digestion and an affinity enrichment step with anti-peptide antibody. The way to increase the selectivity/specificity of the overall method is one by combining 2D-LC with a highly selective sample clean-up step based on solid-phase extraction using affinity coated magnetic beads and a triple quadrupole for MS detection.

Thyroglobulin is an example of a glycosylated protein. MS, LC/MS and CE-MS approaches nowadays are valuable additions to the whole range of available bioassays. Immunoglobulins (Ig) have shown to present pro- and anti-inflammatory functions according to the profile of carbohydrates attached to their Fc region. The potential of LC-MS, including MALDI, approaches to study the glycosylation features will be presented [2]. CE-based assessment of nanobody affinity and purity is another topic that will be shown [3]. The potential of combining bio-assays with LC-MS-based procedures will be shown by taking the identification of unknown snake venoms as an example [4].

REFERENCES


PL-13: MODULATION OF OXIDATIVE STRESS AS A PHARMACOLOGICAL STRATEGY

L. Saso1 and O. Firuzi2

1Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy (luciano.saso@uniroma1.it www.researchgate.net/profile/Luciano_Saso);
2Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Oxidative stress is linked with many pathologies ranging from cancer to neurodegenerative diseases and antioxidants are presumably of therapeutic value in such diseases. In the presentation, we will categorize different direct and indirect mechanisms by which natural antioxidants exert their action, including scavenging and metal chelating effects, mimicking the antioxidant enzymes or upregulation of their expression, activation of nuclear factor erythroid 2-
related factor 2 (Nrf2) and inhibition of pro-oxidant enzymes among others. Recent findings on natural antioxidants such as flavonoids and coumarins will be presented.

Since antioxidant therapy has failed in many instances, we have classified the reasons that may explain these shortcomings in different categories. Novel approaches to antioxidant therapy that include mitochondria-targeting drugs, antioxidant gene therapy and approaches for improvement of cell uptake and alteration of subcellular compartment localization will be described. In the end, “shadows” that are shortcomings of antioxidant therapy as well as “lights” that include positive outcomes will be addressed.

It is concluded that if we learn from failures, invest on agents with higher potential and take advantage of novel emerging approaches, antioxidants could be an asset for the management of some of the carefully chosen oxidative-stress-related diseases.

REFERENCES


PL-14: STUDIES ON TURKISH ASTRAGALUS SPECIES: PAST, PRESENT AND FUTURE

E. Bedir

Department of Bioengineering, Faculty of Engineering, Ege University, 35100, Bornova-Izmir, Turkey

Astragalus L., one of the largest genera of flowering plants with about 3000 different species belonging to the family Leguminosae, is represented by 445 species in the flora of Turkey, of which 224 are endemic [1,2]. Astragalus species, particularly A. membranaceus, are also well-known for their immunomodulator, antimicrobial, antiperspirant, anti-inflammatory, diuretic and tonic effects [3]. In Turkish folk medicine, the aqueous extracts of some Astragalus species are used to treat leukemia as well as healing wounds. Polysaccharides and saponins are major classes of metabolites in Astragalus species; however, the most investigated constituents are cycloartane-type saponins. Previous studies have shown that cycloartane- and oleane-type glycosides of the genus show interesting biological properties, including immunostimulating [4], anti-protozoal [5], antitrypanosomal [6], antiviral [7], cytotoxic [8] andcardiotonic [9] activities. Its diversity and unique chemistry together with its traditional use made Astragalus genera major subject of our continuing research program.

Until now, 31 out of 445 Turkish Astragalus species, from 17 different sections, have been investigated for their secondary metabolite contents. These studies resulted in the isolation of mainly cycloartane-type glycosides including five different aglycones, together with various group of secondary metabolites (oleane-type saponins, phytosterols, flavonoids, simple phenolic glycosides, amino acid conjugates and maltol glycosides). Our in vitro and in vivo bioactivity studies towards the immune system modulation and wound healing properties on the crude Astragalus extracts and their metabolites have provided significant results substantiating traditional claims. Besides, new compounds were prepared from Astragalus cycloartanes by microbial biotransformation and semi-synthesis studies to obtain more potent chemical entities.

This lecture will provide an overview about the chemistry and bioactivity studies performed on Turkish Astragalus species.

REFERENCES


PL-15: PERSPECTIVES OF THE CREATION PREPARATIONS ON THE BASIS OF PLANT RAW MATERIALS

N. Abdullaev
Biopharmaceutics examines the absorption, distribution, metabolism and elimination (ADME) of drugs, which largely depends on their physicochemical properties and physiological conditions. The resulting pharmacokinetic (PK) profile is used to describe the dosing regimen for the particular drug examined. Interaction with the immune system as a factor for the PK profile of low molecular weight drugs is generally considered to be of low importance, with two exceptions. First, allergies to small drugs is considered to result from hapten formation, i.e. the covalent binding of such drug to a peptide or protein and successive recognition by the immune system. Non-covalent binding to the major histocompatibility complex (MHC) and resulting T cell activation has been reported in this regard, as well [1]. Secondly, immune cells may be direct targets of low molecular weight drugs, e.g., in case of adjuvants or immune stimulatory molecules. In this case the affinity to target cell receptors and effects exerted determine to some extent the PK profile. In fact, activation of pathogen pattern recognition receptors (PRR), expressed at epithelial surfaces, by their low molecular weight ligands have been shown to modulate epithelial tight junctions. This mechanism is set to impact on the paracellular transport at affected epithelia [2].

With the advent of complex drugs - of biological as well as non-biological origin - the recognition by the immune system is in the focus when it comes to assuring the efficacy and safety of such drugs. Biological complex drugs, such as therapeutic proteins are mostly applied by injection, although the nasal and pulmonary route are still being considered. It was found that although therapeutic proteins such as antibodies can be completely "humanized", immune reactions do occur against almost all therapeutic proteins, especially during chronic therapy. These immune reactions are often due to aggregation, with the formation of particles recognized to be foreign by the immune system. An example are the injection side reactions (ISR) on subcutaneous injection of interferon-beta in multiple sclerosis therapy. However, other factors related to the treated disease itself, patient related factors, impurities, etc. play a role in the initiation of immune responses against therapeutic proteins.

The group of complex drugs of non-biological origin (NBCDs) includes so-called "nanomedicines" such as liposomes, nanoparticles, polymer conjugates, and glatirimoids [3]. This drug species is characterized by its complex structure, in which the active pharmaceutical ingredient is formed by both, the drug itself and formulation excipient(s). The composition of these complex drugs is essentially determining their efficacy and toxicity, as well as their recognition by the immune system. This was recently shown, e.g., in a comparison of the originator drug Doxil® and an FDA approved generic (liposomal doxorubicin produced by Sun). In conclusion, especially with the advent of more complex drugs the influence of the immune system on their efficacy and safety has to be integrated into the description of their biopharmaceutical profile.

REFERENCES
3. Duncan, R.; Gaspar R., Nanomedicine(s) under the microscope. Mol. Pharm. 2011, 8(6), 2101-2141.

PL-17: BIOSENSORS: THE REVOLUTION IN THE CLINICAL ANALYSIS
A. Ravalli, G. Marrazza
University of Florence, Department of Chemistry “Ugo Schiff” Florence, ITALY

Biosensors are analytical tools, which have gained much interest in detection and monitoring of various clinical manifestations. In order to improve patient care, molecular diagnostics laboratories have been challenged to develop new tests that are reliable, cost-effective and accurate and to optimize existing protocols by making them faster and more economical. Over the last decade, several procedures and applications of biosensors have been realized in order to obtain simple devices for cancer marker detection. The peculiar characteristics of the latest electrochemical biosensors development for cancer biomarker detection will be reviewed.

INTRODUCTION
Cancer still represents one of the leading causes of death worldwide. Cancer deaths are estimated to be nearly 1400 in the European Union in 2015 [1]. Due to the increasing efforts in technological advance for early detection and cancer treatments, 2015 prediction showed a falls in cancer incidence and mortality. A
useful tool, in order to perform an accurate early diagnosis, is represented by the analysis of cancer biomarkers found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process or of a condition or disease. Biosensor technology has the potential to provide fast and accurate detection, reliable imaging of cancer cells, and monitoring of angiogenesis and cancer metastasis, and the ability to determine the effectiveness of anticancer chemotherapy agents. Focusing on the most recent activity of our research group, the aim of the present review is to give a critical overview of novel micro- or nano-scale biosensors for the detection of cancer biomarkers, consisting in tumour associated antigens or gene mutations [2-4].

RESULTS AND DISCUSSION
In this section, biosensors design strategies and the electrochemical techniques involved in the signal detection will be briefly described. Furthermore, the main biological characteristics and the use in clinical analysis of cancer biomarkers will be discussed. Particular attention will be given to the FDA approved protein cancer biomarkers, followed by the analysis of new discovered cancer biomarkers with potential clinical applications. Finally, the development of electrochemical biosensors for cancer biomarkers detection will be reported. Due to the recent advance in nanotechnology (which leads to an improvement of analytical performance of the biosensors), the latest nanostructured electrochemical biosensors will be reviewed.

CONCLUSIONS
Electrochemical biosensors represent useful tools for the detection of cancer biomarkers combining high sensibility, low cost and rapid response. With further efforts, the realization of high sensitive point-of-care systems will probably make the analysis of cancer biomarkers available in few minutes directly on the bedside of the patients or in a close hospital ward.

REFERENCES

PL-18: ELECTROCHEMICAL CHARACTERIZATION AND REACTIVITY OF PLATINUM NANOPARTICLES

J. M. Feliu, A. Boronat, R. Martinez.
Alicante University, Faculty of Sciences Institute of Electrochemistry 03080 Alicante, SPAIN

INTRODUCTION
Most electrocatalytic reactions are structure sensitive, in such a way that particular atomic arrangements are more active than others. For practical purposes it is important to have tools to characterize the distribution of surface sites of platinum nanoparticles coming from different synthesis. Preferably, the characterization should be made in-situ and involve a statistical number of nanoparticles.

MATERIALS AND METHODS
Electrochemical experiments were made in two compartment cells, the main one contained the working and counter electrodes and the second one was connected through a Luggin capillary and contained the reversible hydrogen reference electrode. Platinum single crystals were oriented, cut and polished by standard procedures from small beads (2 mm diameter) obtained by fusion of 99.99% Pt wire (0.5 mm diameter, Goodfellow) [1]. Nanoparticles were prepared using different synthetic routes described in the literature, but particular emphasis will be given to a recent method, developed in our laboratory, which uses specifically adsorbed anions as inductors for cubic shaped nanoparticles[2]. Solutions were prepared with suprapure or p.a. quality grade reagents (Merck) in ultrapure water from Millipore. Single crystal working electrodes were flame annealed and cooled down in a reductive atmosphere (1:4 H2: Ar).

RESULTS AND DISCUSSION
The characterization of the polycrystalline platinum can be made by using platinum single crystals in contact with clean test solutions, usually 0.5 M sulphuric acid. In the latter hydrogen and anion adsorption states define particular pseudocapacitive features that can be used to understand the response of polycrystalline samples in a quantitative way. The procedure can be extended to platinum nanoparticles prepared by different synthetic routes [3]. The nanoparticles can be deposited in a suitable support from water suspensions, containing a statistically significant number of nanoparticles, which can be used as working electrodes. In this way, it is possible to rank nanoparticles from the point of view of a particular type of site. Results can be compared with those form ex-situ techniques, always limited to small numbers of nanoparticles.
Electrochemical ammonia oxidation can be carried out on platinum in alkaline solution. This reaction is almost uniquely sensitive to the presence of {100} symmetry sites and single crystal electrodes with other orientations are practically useless.

It has been found that high concentration HCl is convenient to produce shape-controlled cubic nanoparticles. Varying the HCl concentration the shapes change form spherical (0%) to almost cubic (25%) and then degenerate to bonelike shapes. The size of ne nanoparticles increases from 3-5 nm to 12-14 nm at the optimum cubic shape, and then remains constant [2].

**CONCLUSIONS**

Ammonia oxidation can be used to test the site distribution trends deduced in the characterization studies with excellent agreement.

**ACKNOWLEDGMENTS**

This contribution has been made in the framework of MINECO (Spain) Project CTQ2013-44083-P and GV (Feder) PROMETEOII/2014/013.

**REFERENCES**


**PL-19: NEW INSIGHTS IN THE TOXIC MODES OF ACTION OF NEUROTOXIC AND CARCINOGENIC METAL SPECIES**

T. Schwerdtle

Institute of Nutritional Science, Department of Food Chemistry, University of Potsdam, Potsdam, Germany

The toxic modes of action of numerous carcinogenic and neurotoxic metal species are to date not fully understood.

Concerning the strong human carcinogen inorganic arsenic, at exposure relevant concentrations neither arsenite nor its metabolites are mutagenic. Our *in vitro* data demonstrate a strong inhibition of nucleotide (NER) and base excision repair (BER), with arsenite and its metabolites affecting both common and different molecular key players. Thus, trivalent methylated arsenicals especially disturb NER damage recognition and sulphur-containing arsenicals especially diminish damage induced poly(ADP-ribosyl)ation. Additionally, after 21 days incubation, picomolar concentrations of two arsenicals caused DNA-hypomethylation. In summary, epigenetic and indirect genotoxic effects, among others a modulation of the cellular DNA damage response and DNA repair, are likely important molecular mechanisms contributing to inorganic arsenic induced carcinogenicity.

Strikingly, our very recent work indicates that some lipid soluble, organic arsenicals, so-called arsenolipids also might bear a risk to human health. Arsenic-containing hydrocarbons (AsHCs) belong to the group of arsenolipids and are frequently present in fatty fish and other seafood. Because of the total lack of toxicological data, so far no risk assessment has been carried out for these arsenolipids. In a first step, we characterized the cellular toxicity of three AsHCs in cultured human bladder (UROtsa) and liver (HepG2) cells. Cytotoxicity of the AsHCs was comparable to that for arsenite, which was applied as the toxic reference arsenical. Thereby, a large cellular accumulation of arsenic, as measured by ICP-MS/MS, was observed after incubation of both cell lines with the AsHCs. Moreover, the toxic mode of action shown by the three AsHCs seems to differ from that observed for arsenite. Evidence suggests that the high cytotoxic potential of the lipophilic arsenicals results from a decrease in the cellular energy level. Next, we confirmed the strong toxicity of AsHCs in a well-established *in vivo* model, in the entire organism *Drosophila melanogaster*. Here, AsHCs, in contrast to arsenite, massively disturb the development from the larval over the pupal stage to flies. These first *in vitro* and *in vivo* studies cannot exclude a risk to human health related to the presence of AsHCs in seafood. Furthermore, our recent bioimaging studies demonstrate that AsHCs are able to reach the brain of the flies. Thus, further toxicity studies in experimental animals are urgently needed, to fully assess a possible neurotoxicology related risk.

Concerning neurotoxic mercury and manganese species our data indicate a strong disturbance of DNA damage induced poly(ADP-ribosyl)ation in human astrocytes. Interestingly, the underlying molecular mechanisms seem to be different for the metal species. Current experiments in human neurons and first *in vivo* studies in *C. elegans* will hopefully help to clarify, whether the observed inhibition of poly(ADP-ribosyl)ation contributes to organic mercury species and manganese species induced neurotoxicity. Applying primary *in vitro* models of the blood-brain- and the blood-CSF-barrier, we could recently show that manganese species enter the brain in the first line via the blood-CSF barrier, whereas methyl mercury and thiomersal transfer especially via the blood-brain barrier. Most interestingly, in case of the organic mercurials a massive efflux of mercury out of the brain via the blood-CSF barrier was observed, whereas
for mercuric mercury an efflux was visible via the blood-brain-barrier.

In summary, our data clearly demonstrate that in case of both neurotoxic and carcinogenic metals, the metal species have to be taken into account when searching for the underlying toxic modes of action.

PL-20: POLYMERIC NANOCARRIERS FOR TARGETED DELIVERY OF SN-38

S. Dimchevska1, N. Geskovski1, R. Koliqi1, P. Petrov2 and K. Goracinova1

1 Faculty of Pharmacy, Ss Cyril and Methodius University, Skopje, Macedonia
2 Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria

7-Ethyl-10-hydroxy-camptotecin (SN-38) is an active metabolite of Irinotecan with high in vitro potency against different malignancies including colorectal, lung and ovarian cancer but its hydrophobicity substantially prevents its applications. Common approaches to improve its water solubility and increase the efficacy of the delivery to specific organ or target site are (i) chemical conjugation with hydrophilic polymers and (ii) encapsulation into carriers like polymer nanoparticles. Although, hydrophilic polymer conjugation is a very efficient way for solubility improvement, the efficacy and safety profile of SN-38 depends upon enzymatic or chemical cleavage of the bond and release of the active drug at the site of action. Therefore, noncovalent encapsulation of the hydrophobic drugs into carriers such as macromolecular nanoparticles is generally accepted, due to the uncomplicated manipulation for drug encapsulation and preservation of the intrinsic properties of the drug into the carrier. However, efficient encapsulation of high drug loadings of extremely hydrophobic substances is a very challenging objective mainly due to the fact that hydrophobic interactions responsible for the process of passive drug loading, are very poor driving force for efficient encapsulation and the risk for premature precipitation and crystallization of the drug causing formation of polymer particles with low drug loading. However, smart polymer selection will help us tailor the efficacy of encapsulation, drug loading as well as hydrophilicity/hydrophobicity, degradation, drug release rate, particle size, zeta potential and in vivo behavior of the targeted drug delivery systems. Also, in order to produce NP with high drug loading and adequate particle size using the process of nanoprecipitation, during the antisolvent addition, simultaneous occurrence of precipitation of the active substance and the polymer aggregation process is essential. Nevertheless, the polymer quantity is also important as it assembles on the surfaces of the drug particles and finally arrests the particle growth, influencing not only the drug loading but particle size as well. Accordingly, optimization of preparation conditions and formulation composition (selection of proper drug/polymer/surfactant ratio and appropriate solvent/non-solvent system) will be useful to define the design space for high encapsulation efficiency, controlled drug release rate and particle size suitable for passive targeting.

In order to evaluate the potential of several hydrophobic polymers as carriers for SN-38 and to investigate the factors influencing nanoparticle properties prepared by the process of nanoprecipitation method; D-optimal designs for response surface investigation were created and analyzed. Further, the optimized formulations were subjected to efficacy and toxicity studies using SW-480 human colon cancer cell lines.

D-optimal response surface designs for different formulation compositions (drug/polymer ratio and stabilizing agent (F 127) content) as factors of influence upon the efficacy of encapsulation, drug loading efficacy, zeta potential and particle size for PCL, PLA and PLGA NP pointed to low capacity of these polymers as carriers for SN-38. The particles were uniform in a size range from 80 to 100 nm, but the highest drug loading efficacy was limited to maximum of 1.5%. Supposing that, we will create physical and chemical properties gradient in the nanoparticle core that will result with increased capacity for SN-38 encapsulation, polycaprolactone (PCL) was combined with poly(lactic acid) (PDLLA) in a Poly(DL-Lactide-co-caprolactone)copolymer (10:90; LA:CL, Mw 77799 Da). D-optimal response surface design pointed that efficacy of encapsulation of up to 90% and drug content above 10% can be achieved for different formulation compositions (drug/polymer ratio and stabilizing agent (F 127) content) using this copolymer for NP preparation. We can hypothesize that along with the chemical properties gradient created in P(DL)LCL NP (Fig. 1, b), their structure and porosity will also be different.
comparing to Poly(DL-lactide)(PDLLA) and PCL NP alone, due to the gradient in the physical properties, and that will probably modulate not only the drug loading but the drug release rate as well. In order to evaluate the capacity of polymer micelles for solubilization of SN-38, and knowing the fact that low molecular amphiphilic self-assembly polymers pose low solubilization capacity for extremely hydrophobic substances, PAA– PCL–PAA (Mw 3300-9600-3300 Da) and PLGA-PEG-PLGA (Mw 70000- 8000-70000 Da) co-polymers with higher molecular mass and increased polymer hydrophobicity were selected. Also, due to the high crystallinity or large enough hydrophobicity of one of the blocks of the selected co-polymers, so called frozen or kinetically stable micelles will be produced during the process of solvent shifting or nanoprecipitation. The results from the D-optimal design pointed to very high encapsulation (above 95%) and drug loading of 12% and 18%, for the PLGA-PEG-PLGA efficacy of encapsulation (above 95%) and drug release rate as well. In general, the process of optimization for all polymers pointed that due to the very low solubility of SN-38 there is an upper limit (or small range around the limit) of the drug concentration into the medium for nanoprecipitation that will provide efficacious encapsulation. The limit concentration is highly dependent upon the stabilizing agent concentration, as F-127 actually helps the solubilization of the drug during the process of nanoprecipitation. In this small range of drug concentration the process of drug nucleation and the polymer aggregation on the surface of the drug nucleuses will start simultaneously, which will stop the drug substance crystal growth, produce small particles and improve drug loading. Concentrations above the limit concentration from the design resulted with premature drug precipitation and naked crystals formation, resulting with large waste of materials and low drug loadings.

Cell viability for non-loaded NP was assessed using MTT assay on SW-480 colon adenocarcinoma cell lines under different experimental settings. Broad concentration ranges of nanoparticles were tested, in several incubation periods (6, 24 and 48h) and in different cell growth media (with or without fetal bovine serum). The results indicated to statistically insignificant effect over the cell viability for NP with zeta potential close to neutral, like PLGA-PEG-PLGA and PDLLA NP, compared to the untreated controls. In contrast negatively charged NP (PAA-PCL-PAA) presented significant effect on growth inhibition and increased cell internalization. However, in the presence of protein corona (experiments with fetal bovine serum) the effect of the PAA-PCL-PAA NP on growth inhibition was significantly diminished. Loaded nanoparticles presented statistically significant increase in growth inhibition rate relative to similar concentrations of the drug solution, especially in the recovery period after 48h of drug or NP exposition.

REFERENCES

PL-21: SELF EMULSIFYING (SNEDDS-SMEDDS) DRUG DELIVERY SYSTEMS FOR POORLY SOLUBLE DRUGS

INTRODUCTION
Self nano emulsifying drug delivery system (SNEDDS) is an isotropic mixture of oil,surfactants and co-surfactants that form fine oil-in-water nanoemulsion, mild agitation, followed by administration into aqueous media, such as gastrointestinal fluids (1,2). Furthermore SNEDDS is a physically more stable formulation when compared to emulsions and easier to manufacture in a large scale (3) They can improve oral bioavailability and enhance permeation across the intestinal membrane through a wide distribution in the gastrointestinal tract (due to the small droplet size), and decrease food effect (4) Rosuvastatin (RS) is one of statin group drugs and approved by FDA in 2003. It is used for decreasing high cholesterol (5). Although current statin drugs have a great contribution to reduce low-density lipoprotein (LDL) and cardiovascular disease, the oral bioavailability of RS is only about 20% (6). Therefore, finding a new drug released system is important to
improve dissolution profile and drug permeability of RS.
The aim of this study is development of RS SNEDDS with the objective of increasing its solubility and permeability. The prepared SNEDDS which contain RS was evaluated for robustness to dilution, stability, in vitro drug release and permeability. In addition, RS SNEDDS were compared with commercial tablets of RS.

MATERIALS AND METHODS
RS was a gift from MSN Laboratories (Turkey). Tween 80, Corn oil, Soybean oil, Tween 80, Oleic acid, Span 80 were purchased from Sigma Aldrich (Germany). Labrasol, Transcutol, Capryol 90, Labrafil M 1944, Labrafil M 2125 were gifts from Gattefosse (France). All the other reagents and chemicals were used of HPLC or analytically grade samples.

Preparation of Rosuvastatin SNEDD
Once the SNEDD region was identified, the desired component ratios of SNEDDS were selected for drug incorporation and further optimization. 20 mg of drug and mixed surfactant, cosurfactant and oil were incorporated in their determined ratios. Finally homogeneous clear mixture was obtained by vortex mixing.

Characterization of SNEDD

HeatingCoolingCycle
Sixcyclesbetweenrefrigeratortemperature 5°C and 40°C withstorage at eachtemperature of not less than 24h wasstudied. Thoseformulations, whichwere stable at these temperatures, were subjected to centrifugation test (5 minutes at 3000 rev / min.). The phase separation and turbidity of SNEDDS were evaluated.

Robustness to Dilution
Robustness of RS SNEDDS to dilution was studied by diluting it 1:1, 1:100 and 1:500 ratios with various dissolution media i.e. water, buffer pH 1.2 and buffer pH 6.8. The diluted samples were stored for 0, 2 and 24 hours and observed for any signs of phase separation or precipitation. For each time, zeta potential and droplet size of formulations were measured.

Stability of Rosuvastatin SNEDDS
In order to evaluate the stability of SNEDDS, the SNEDDS were added into sealed glass vials which were stored at 25 ± 1°C and 40 ± 1°C in climate cabin for 12 months. The physical stability of formulations was determined the clarity, phase separation, droplet size studies. In the chemical stability tests, concentration of RS in the formulations was also measured.

Permeability of Rosuvastatin SNEDDS and Commercial Formulation
The in vitro permeability study was developed in Caco-2 cell monolayers. The cells were maintained at 37°C in an atmosphere. The medium was replaced every second day for 3 weeks. For the experiments with Caco-2 cell monolayers, the SNEDDS and marketed formulation in HBSS were used. The SNEDDS were diluted with HBSS (w/v) and the concentration of RS in these systems was found 100 μg/mL.

RESULTS AND DISCUSSION

Pseudo-Ternary Phase Diagram
The studied systems composed of safe constituents including oleic acid as oil phase, distilled water and the mixtures of Labrafil M 1944-Labrasol/Transcutol as surfactant/co-surfactant for SNEDDS (Figure 1). In this study, surfactant/cosurfactant ratio 1:1 was selected because this ratio is the most stable after formation of SNEDDS.

Characterization of SNEDDS
RS loaded SNEDDS were exposed to heating-cooling cycle and centrifugation study to check the stability of formulation. Phase separation signs were not observed for all formulations. Different fold dilutions of selected formulations were exposed to different media to mimic the in vivo conditions. According to this result, RS SNEDDS was stable in the same dilution ratio for each medium.

Stability of SNEDDS
After storage at 25 ± 1°C and 40 ± 1°C for 12 months, the RS SNEDDS were still clear and transparent without any phase separation. The concentration of RS and the droplet size of SNEDDS were not changed.

Permeability of Rosuvastatin from SNEDDS
The permeability value for the apical to basolateral direction (P_{app} (A→B)) was found above 1 × 10^{-5} cm/s for all formulations. However, the permeability value for the basolateral to apical direction (P_{app} (B→A)) was lower than 1 × 10^{-6} cm/s for all formulations. In addition, the permeated concentration of RS across Caco-2 cell monolayers in the apical to basolateral (A→B) direction is much higher than that in the basolateral to apical (B→A) direction for the RS commercial tablets and SNEDDS. Based on these
values, it can be concluded that RS is highly permeable through the Caco-2 cell monolayer.

CONCLUSIONS
In this study, the new SNEDDS for oral delivery of RS were developed through the construction of pseudo-ternary phase diagram and optimized with a simple method. According to the physicochemical characterization and permeability studies, it can be concluded that RS was incorporated into the SNEDDS formulations and increases permeability of RS across Caco-2 cell line when compared to commercial tablet.

ACKNOWLEDGMENTS
This project was supported by TUBITAK funding sources (112S637).

REFERENCES

PL-22: THE CONTRIBUTION OF ELECTROCHEMISTRY TO THE PHARMACEUTICAL SCIENCES
J.-M. Kauffmann

Department of Bioanalysis and Toxicology, Faculty of Pharmacy - Université libre de Bruxelles

Electrochemical techniques offer a broad selection of useful tools in drug research and development. Electrochemical synthesis and especially electroanalytical methods provide substantial impetus in drug compounds development. Analytical techniques are all facing major progress these last ten years and electroanalysis is also involved in this trend. The unique characteristics of the phenomenon occurring at the electrode-solution interface along with the variety of electrochemical methods allow for a broad spectrum of applications. Potentiometry, conductimetry, impedimetry, voltamperometry and amperometry are included in the tool box. New electrode material is available allowing for improved sensitivity and selectivity to be obtained. New biological material is being considered for surface modification and drug compounds investigation. Current electrochemical methods are briefly reviewed in the presentation with a critical view in terms of performances of the developed instrumentation and of application domains.

PL-23: A JOURNEY TO THE MYSTERIOUS WORLD OF THE BRAIN BY VOLTAMMETRY
Z. Şentürk

Yüzüncü Yıl University, Faculty of Science, Department of Analytical Chemistry, Van, TURKEY

Despite weighing only about 1.4 kg, the human brain has been called the most complex and magnificent biological structure in the known universe, and in many ways it’s the final frontier of science. Our brain gives us awareness of ourselves and of our environment, processing a constant stream of sensory data. It controls our muscle movements, the secretions of our glands, and even our breathing and internal temperature. Every creative thought, feeling, and plan is developed by our brain. The brain’s neurons (nerve cells) record the memory of every event in our lives. There is almost a quadrillion connections between a hundred billion neurons, and we don’t even fully understand a single cell. But even so, new techniques and technologies are rapidly advancing the state of the art in neurochemistry. Neurontransmitters which are chemical messengers that relay signals between neurons and other cells, and related compounds could be monitored by either in vivo sampling (primarily using microdialysis) coupled to analytical methods or microelectrode techniques such as voltammetry and electrophysiology. The former monitors the chemistry of the brain, whereas the latter measures its bioelectrical activity. Neurontransmitter content and release are also studied in vitro through analysis of cells in culture and ex vivo tissue preparations such as brain slices [1]. Voltammetry which is at the core of electroanalytical chemistry involves the application of a potential that varies with time and the measurement of the corresponding current that flows between the working and reference electrodes. Though birth of voltammetry dates back to Heyrovsky's experiments in 1922, the last quarter of the 20th century and the first decade of 21st century coincided with the rice and maturation of this methodology. Within a span of just over 90 years, different types of voltammetric techniques were
developed; instruments were designed and constructed by progressing in microelectronics and computer technologies. A few developments have revolutionized voltammetry, such as sample miniaturization, availability of modern spectroscopic techniques, developments of scanning probe microscopies, and advances in instrumentation that allowed nanosecond time gating and measurements of currents in the picoampere, and even femtoampere range. Due to the above mentioned developments and bearing in mind that redox processes are essential for the life of organisms, voltammetry has been used as a powerful analytical tool in neuroscience for solving the mysteries of the brain and its functions over the past four decades [2]. The early work was carried out by Ralph Adams and co-workers in the early 1970s, in which they demonstrated that electrochemistry could be used as a tool to measure fluctuations in levels of electroactive neurotransmitters and neuromodulators in whole animals. Second generation techniques utilized modified electrodes and more complex waveforms which allowed reliable measurements of neurotransmitter metabolites in ambulant animals with minute temporal resolution. More recent advances have resulted in the development of three in vivo voltammetric techniques for the real-time monitoring of neurotransmitters such as high-speed chronoamperometry, fast-scan cyclic voltammetry and amperometry. Carbon-fiber microelectrodes (~5 μm) with their excellent biocompatibility have been combined with these techniques to monitor in vitro or in vivo biological processes related to various physiological and pathological states with sub-second temporal resolution [3]. One of the strengths of voltammetric measurements in living brain tissue at wireless instrument is the ability to combine it with complementary methods including behavioral measurements, electrophysiological measurements and optical methods [4]. Although previously confined to animal research models, voltammetry has recently been successfully applied in humans to monitor real-time neurochemical changes during functional neurosurgery [5].

This presentation briefly surveys the use of voltammetric methods to probe brain chemistry.

REFERENCES


PL-24: DESIGNING CHEMICALS ACTIVE AGAINST MOSQUITOES

J. Devillers

CTIS, 3 Chemin de la Gravière, 69140 Rilléix la Pape, FRANCE

The goal of this review was to critically analyse all the existing structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) models on mosquitoes focusing on the discovery of new chemicals acting on larvae.

INTRODUCTION

Mosquito-borne human diseases have emerged or re-emerged in numerous countries worldwide due to a number of factors including the lack of progress in vaccine development, drug resistance in pathogens, insecticide resistance in mosquitoes, climate changes, societal behaviours, and economical constraints. Mosquito control relies heavily on the use of insecticides. However, because of increasing resistance to the different families of insecticides, reduction of mosquito populations is becoming increasingly difficult.

Despite the unquestionable utility of insecticides in fighting mosquito populations, there are very few new insecticides developed and commercialized for vector control. This is because the high cost of the discovery of an insecticide does not counterbalance the “low profitability” of the vector control market. Fortunately, the use of structure-activity modelling allows the reduction of time and cost in the discovery of new chemical structures potentially active against mosquitoes. The aim of this study was to review all the SAR and QSAR strategies that have been used to find new chemicals active on larvae of mosquitoes.

RESULTS AND DISCUSSION

Juvenile hormone III controls the development of larvae of mosquitoes. It has prototyped the design of structurally diverse insecticides mimicking its activity in larvae. Most of the SAR and QSAR models on mosquito larvae have been derived from these chemicals termed juvenoids [1]. Analysis of these chemicals shows that small changes in their structure can lead to a drastic change in their activity. Other chemicals are designed to act on the moulting process or the chitin synthesis of the mosquitoes.
In the SAR and QSAR models, the molecular descriptors encoding steric effects are very often the most important. The supervised nonlinear methods [2] outperform the classical linear methods in the design of powerful predictive SAR and QSAR models. Unfortunately, most of these new chemicals also present potential adverse effects on the development of non-target invertebrates. While various in silico approaches have shown their interest for estimating the activities and properties of nanomaterials and in the meantime, the nanomaterials are increasingly used in vector control, to our knowledge, no modelling methods still have been used to optimize the design of nanostructures active on mosquitoes.

CONCLUSIONS
Alternatives to insecticide-based vector control are the subject of intensive research worldwide. Nevertheless, the rational use of chemical substances remains the most operational strategy for controlling mosquito populations. Because the market of such substances is limited, it is obvious that in silico tools allow us to save time and money in the design of new insecticides.

ACKNOWLEDGMENTS
The financial support from the French Agency for Food, Environmental and Occupational Health & Safety (Anses) is gratefully acknowledged (contract #EST-2012/2/64).

REFERENCES

PL-25: PEPTIDE NANOSTRUCTURES AS MOLECULAR TRANSPORTERS OF THERAPEUTIC AGENTS
K. Parang

Associate Dean of Research, Graduate Studies and Global Affairs, Professor of Medicinal Chemistry, Chapman University School of Pharmacy, Irvine, CA, USA

Peptides and self-assembled peptide structures remain one of the most versatile systems as biomaterials for carrier-mediated drug delivery because they can carry a wide range of cargo molecules. The intracellular delivery of biological active cargos by employing linear cell-penetrating peptides (CPPs) has been previously reported. Conjugation to linear cationic CPPs, such as TAT, penetratin, antennapedia, or oligoarginine efficiently enhances the cellular uptake through different mechanisms. However, challenges to CPP delivery include achieving tissue or cellular selectivity, controlled release, and avoiding entrapment in endosomes. The cellular uptake of many CPPs along with the conjugated cargo occur predominantly via an endocytic pathway. Some compounds are trapped in endosomes and cannot reach the targets in the cytoplasm or nucleus. Thus, strategies that promote endosomal escape or avoid endosomal route are required for improving bioavailability.

We have discovered a new class of cyclic peptides containing alternative tryptophan and arginine residues [WR]n (n = 4, 5) that have diverse applications in noncovalent and covalent drug delivery, phosphopeptide, oligonucleotide and siRNA cellular delivery, generation of peptide capped gold nanoparticles (AuNPs), silver and selenium nanoparticles, surfactants, and protein stabilizers. Controlling and balancing electrostatic and hydrophobic interpeptide interactions in the cyclic peptides provided a range of unique structures with variable cellular and drug delivery efficiency. The mechanistic studies showed that the cellular uptake of [WR]n did not change in the presence endocytic inhibitors such as chloroquine, chlorpromazine, nystatin, methyl-β-cyclodextrin (Me β-CD), 5-(N-ethyl-N-isopropyl)amiloride (EIA), and sodium azide (NaN3) or at 4 °C, suggesting that the internalization pathway was independent of endocytic pathway. In a dose-dependent study comparing to current transfection reagents, [WR]n did not show any significant cytotoxicity against normal human kidney cells (HEK293) at 100 μM while polyArg CR7, TAT (YGRKKRQRRC), and oligofectamine 2000 (Invitrogen, a cationic lipid formulation) reduced the viability by 41-59%, thus making the peptide potentially superior to other CPPs.

To confirm the cellular uptake of cyclic peptides containing alternate Arg and Trp residues (e.g., [WR]n = 4, 5), fluorescence-labeled conjugates of the peptides, F-[W5R4K] and F-[W4R3K] (F = carboxyfluorescein), were synthesized, and their cellular internalization was studied. Incubation of F-[W5R4K] or F-[W4R3K] (10 μM) with human leukemia cells (CCRF-CEM) for 1 h showed significantly higher cellular uptake when compared with the corresponding fluorescence-labeled linear peptide, F-(KR)4, and FAM. Confocal microscopy also showed nuclear localization of the cyclic peptide (F-[W5R4K]) versus the corresponding linear peptide (F-(KR)4) confirming that the cyclic nature of the peptide is critical for the enhanced cellular permeability. The process used to determine whether the uptake is cell-specific included human ovarian adenocarcinoma (SKOV-3), colon adenocarcinoma (HT-29), and breast carcinoma (MDA-MB-468), which were incubated with F-[W5R4K] (10 μM) at 37 °C, and showed significantly high fluorescence mainly in the nucleus (as shown by FITC and DAPI), confirming the efficient cellular uptake of the representative cyclic peptide. The application of
these cyclic peptides in improving the cellular delivery of a number of compounds is described here.

**Cellular Delivery of Phosphopeptides, an Oligodeoxynucleotide, and a siRNA.** Negatively charged phosphopeptides are valuable probes for studying phosphoprotein-protein interactions, but their study in cellular systems is challenging because they do not readily cross cellular membranes. The cellular uptake of fluorescence-labeled PepYLGLD in CCRE-CEM was enhanced dramatically by 27-fold in the presence of [WR]₅ and was found to be time-dependent. [WR]₅ was also able to deliver a 15-mer oligodeoxynucleotide to SK-OV-3 cell nucleus while the oligonucleotide alone showed significantly low cellular uptake. The labeled oligodeoxynucleotide showed 7 times higher cellular uptake in SK-OV-3 cells, in the presence of [WR]₅ as shown by flow cytometry. Similarly, [WR]₅ (5 µM) enhanced the cellular uptake of fluorescence-labeled Glyceraldehyde 3-phosphate dehydrogenase (GADPH) siRNA (5 nM) by six-fold in SK-OV-3 cells. While siRNA alone did not have any effect on GAPDH expression, the expression was reduced by 2.2-fold in the presence of [WR]₅, suggesting that the CP enhanced the cellular delivery of cell-impermeable siRNA.

**Cellular Delivery of Doxorubicin.** After incubation of doxorubicin (Dox, 5 µM) with SK-OV-3 cells for 1 h, minimal cellular uptake and nuclear localization was observed. Conversely, in the presence of F-[W₅R₄K] (10 µM), significant uptake of both Dox and the fluorescence-labeled peptide was observed in the nucleus as shown by fluorescence microscopy, which suggests that the cyclic peptide acts as a molecular transporter of Dox.

**Cellular Uptake of Cyclic Peptide-Dox Conjugate.** A nontoxic concentration of 5 µM of cyclic peptide conjugate [W₅R₄K]-Dox was selected for cell-based studies. Confocal microscopy images of SK-OV-3 cells are shown after 1 h incubation. Free Dox and [W₅R₄K]-Dox were localized mainly in the nucleus. The results showed that the covalent conjugation of Dox with cyclic [W₅R₄K] did not prevent the nuclear accumulation of the drug. To compare the retention ability of cyclic [W₅R₄K]-Dox versus Dox alone against efflux effects, SK-OV-3 cells were incubated with both compounds for 1 h followed by incubation in drug-free media for 24 h at 37 °C. The fluorescence intensity of Dox in cells treated with cyclic [W₅R₄K]-Dox was found to be significantly higher than that in cells treated with Dox alone, indicating that cyclic [W₅R₄K]-Dox was retained in cells much longer than free Dox. Thus, this conjugate has a potential to be used as a tool for enhancing the nuclear retention of Dox.

**In Vivo Tumor-Targeting Studies.** We have conducted initial animal studies with HeLa cell xenografts nude mice model using a targeting cyclic peptide, Cy₅.₅-[W₅R₄K₅] with folate. Nude mice were injected with cancer cell line subcutaneously to form tumor xenografts. On one flank, mice were injected with folate receptor (FOLR1) underexpressing HEK293T cells and on the other flank mice were injected with FOLR1 overexpressing HeLa cells. Tumors were then allowed to grow for one to two weeks before mice were subjected to tail vein injections of the functionalized CP with folate and Cy₅.₅. Preferential systemic delivery of compounds to HeLa vs. HEK293T tumors were determined following mouse euthanasia, necropsy and isolation of tumors and organs. The animals survived, and since HeLa cells express nearly 1500-fold more FOLR1 than do HEK293T cells, folate functionalized compound dramatically preferentially accumulated in HeLa xenografts and not HEK293T. Other off-target accumulation of free Dox did not occur in liver, spleen, kidney, lung, and heart.

In conclusion, cyclic peptides containing alternative W and R residues have diverse applications in drug delivery, nucleic acid, and phosphopeptide cellular delivery. The diverse applications make them distinct from other known delivery systems. Peptides conjugated with a tumor-specific unit folate was localized in HeLa cells overexpressing folate receptor in animal studies. This system can be used for active targeting of anticancer drugs to tumors.

**PL-26: PUZZLING OUT THE CELLULAR EFFECTS OF ALLICIN, A NATURAL DEFENCE SUBSTANCE FROM GARLIC**

A.J. Slusarenko¹, F. Albrecht¹, J. Borlinghaus¹, H. Lühring², M.C.H. Gruhlke¹

¹Department of Plant Physiology RWTH Aachen University, Aachen, GERMANY, ²FZ Jülich, GERMANY

**INTRODUCTION**

Allicin (diallylthiosulfinate) is a biocide produced in garlic (Allium sativum L.) upon cellular damage and is a major deterrent to pest and pathogen attack [1]. A clove of garlic can produce ~5 mg allisin which is active in µg amounts. Allicin is a reactive sulfur species (RSS) and reacts with accessible thiol groups in proteins and glutathione in a thiol-disulfide exchange-like reaction [2]. It is thought that inactivation of essential cysteine-containing proteins is the basis for allicin’s biocidal activity (Fig. 1). As a natural product
from a common foodstuff allicin has potential for use in medicine and in plant protection in organic agriculture [3]. We are interested in the specific effects of allicin on the biochemistry and physiology of cells. We have worked mainly with baker's yeast (Saccharomyces cerevisiae) as a model fungus.

**MATERIALS AND METHODS**

The experiments were performed with synthesized allicin (NMR, HPLC authenticated, >98% pure) or allicin in crude garlic juice. The specific cellular protein targets of allicin have been investigated by characterizing the allicin redoxome using an isotope coded affinity tag procedure (OxICAT). In addition to effects on the proteome, allicin reacts with low $M_r$ thiols in the cell, e.g. glutathione, and we have investigated the effects of allicin on the cellular redox potential. Furthermore we have investigated the effects of allicin on cell membranes of various test organisms and artificial lipid bilayers using electrophysiological methods.

**RESULTS AND DISCUSSION**

We have shown that allicin is a cellular redox toxin that can shift the cell’s redox potential to a more oxidized state thereby inducing apoptosis or necrosis in a concentration-dependent manner [4].

The degree of oxidation of several proteins in yeast cells was shown to be specifically increased (Fig. 2). The biological significance of these results in relation to allicin’s antifungal activity is currently being investigated.

Allicin permeabilized membranes by forming fluctuating pores. Furthermore, beetroot cell membranes were approximately 200-fold more sensitive to allicin on a mol-for-mol basis than to dimethyl sulfoxide (DMSO) and approximately 400-fold more sensitive to allicin than to ethanol. In addition, allicin caused an immediate strong depolarization, and a decrease in membrane resistance at the plasmalemma of Chara cells, and caused pore formation in the tonoplast and in artificial lipid bilayers. These membrane effects are independent of allicin’s chemical reactivity as a thiol reagent [5].

**CONCLUSIONS**

The effects of allicin on cells are complex but we are slowly beginning to understand the workings of this physiologically active and fascinating molecule, produced in high amounts in garlic, which is a common foodstuff.

**ACKNOWLEDGMENTS**

Financial support from RFwN and RWTH Aachen University and from the [European Community’s] Seventh Framework Programme [FP7/2007-2013] under grant agreement No. [215009] are gratefully acknowledged.

**REFERENCES**

It is universally accepted that thyroid hormones (TH), tetraido-L-thyronine (T4) and 3,3′,5′ triido-L-thyronine (T3) modulates metabolism. Indeed TH affect both intermediate and energy metabolism (the well known “calorigenic effect”). These actions include, among others, effects on: basal metabolic rate (BMR); mitochondrial activities; lipids and cholesterol metabolism. Thyroid gland produces two main hormones, T4 and T3. All circulating level of T4 is released by the gland whereas about 80% of systemic T3 is formed by deiodination of T4 by peripheral tissues. T3 is considered to be the active form of TH while the T4 would be its precursor. However, today it seems pretty evident from the many papers in the literature that other iodothyronines or analogues/derivatives possess significant biological activities. Among the others the 3,5-diiodothyronine (T2) has attracted the interest of researchers because of its metabolic effects. Indeed, acute or chronic administration of T2 to rats (1) or humans (2) resulted in significant changes in energy metabolism. In these studies T2-treated rats/humans showed lower body weight and a significant reduction in the serum cholesterol and triglyceride levels. Several studies have confirmed these results on rodents and, seen the metabolic effects of T2, some scientist started to investigate on possible beneficial effects of its administration. Indeed: 1) Moreno et al. (3) and deLange et al.(4) reproted that T2 was able to prevents high-fat–diet-induced insulin resistance; 2) Markove et al (5) showed an antideoressant-like effect of T2 in rats; 3) Shang et al (6) showed that T2 was a protective agent against renal damage in diabetic-induced nephropathy in rats. These results generated the idea of a possible use of T2 as pharmacological agent to counteract some major diseases such as metabolic disorders and in particular the metabolic syndrome. However,so that it can be considered a real possibility of a pharmacological use of T2, some studies are still lacking leaving its potential long-term undesirable effects insufficiently explored. In particular: more comprehensive studies in humans and animal models, clinical trials, studies aimed to discriminate between beneficial and undesirable effects. Also long-term treatment with T2 to evaluate the effects on important organs such as brain and heart are to be investigated. Because of this, for the time being, the uncontrolled use of T2 as therapeutica agent should not be considered appropriate.


PL-28: BACTERIOPHAGES AS THERAPEUTIC AND PROPHYLACTIC MEANS

N. Chanishvili
Eliava Institute of Bacteriophage, Microbiology & Virology, Tbilisi, Georgia

Bacteriophage (from 'bacteria' and Greek φαγέω phagein "to devour") is any one of a number of viruses that infect bacteria. Bacteriophages (shortly “phages”) are among the most common and diverse entities in the biosphere. The estimated number of phages on earth is about 10^{31}. Sea waters, human intestines, etc. as natural sources of bacteriophages. Phages are not only the most abundant biological entities but also probably the most diverse ones. Bacteriophages are often isolated from environmental sources, such as water samples, etc. Before their clinical application bacteriophages are thoroughly characterized according to a number of properties. The selection criteria have been elaborated during the past 80 years. Therefore, bacteriophage mixtures (“cocktails”) have been traditionally used for therapy and prophylaxis of bacterial infections in Georgia and former Soviet Union. The phages nowadays are seen as a possible therapy against multi-drug-resistant strains of many bacteria. The therapeutic action of bacteriophages significantly differs from antibiotics.
which makes them still active against multi-drug-resistant bacteria. Bacteriophages have a number of other advantages in comparison with antibiotics. The presentation will focus on such important issues as efficacy of bacteriophages against multi-drug-resistant bacteria, past and present experiences in phage therapy and prophylaxis, safety of the phage preparations, regulatory issues, etc. The commercial phage preparations and their practical applications will be discussed as well.

PL-29: METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

B. Sancak

Hacettepe University Medical School, Department of Medical Microbiology, Ankara

Staphylococcus aureus is among the leading causes of nosocomial infections. In recent years antimicrobial resistance is a growing problem for these pathogens which complicates the treatment of important nosocomial and community-acquired infections. After the report of first case of methicillin-resistant Staphylococcus aureus (MRSA) in 1961, MRSA become a major problem in worldwide. Methicillin-resistant S. aureus infections were first detected in hospitals. However in recent years MRSA infections have emerged in the community. Therefore, both healthcare associated (HA-MRSA) and community associated MRSA (CA-MRSA) infections became an important problem worldwide. During recent years, the distinction between HA-MRSA and CA-MRSA has started to disappear and CA-MRSA now began to replace HA-MRSA in healthcare facilities of some countries such as USA and Taiwan. Glycopeptides (vancomycin and teicoplanin) are still the current mainstay of therapy for infections caused by MRSA. In the last decade dramatic changes have occurred in the epidemiology of MRSA infections. The high prevalence of MRSA infections has led to increased use of vancomycin and has resulted in the emergence of MRSA with reduced susceptibility to glycopeptides. Therefore, spreading of multiresistant strains between patients and between hospitals is of particular concern.

Recently, therapeutic alternatives introduced into clinical practice such as quinupristin/dalfopristin, linezolid, tigecycline and daptomycin for treating MRSA infections. Nevertheless, these drugs are only approved for certain indication and resistance has already been reported.

PL-30: UPDATE 2015 OF GUIDELINES FOR THERAPEUTIC DRUG MONITORING (TDM) IN PSYCHIATRY AND NEUROLOGY

Christoph Hiemke and the TDM task force of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP)

Department of Psychiatry and Psychotherapy, University Medical Center Mainz, D-55131 Mainz, Germany

In psychiatry and neurology, therapeutic drug monitoring (TDM) is established to improve the efficacy and safety of pharmacotherapies. TDM is a valuable tool for tailoring the dosage of the prescribed medication(s) to the individual pharmacokinetic characteristics of a patient. Proven evidence, however, that TDM should be used as a standard of care for treatment with the multiple neuropsychiatric medications is restricted to few compounds. Well designed clinical trials on medical and economic benefits of TDM are rare. Therefore, the use of TDM is limited in most countries to few antiepileptics, especially carbamazepine, phenobarbital and phenytoin, some mood stabilizers, especially lithium and valproic acid, some antidepressants, especially tricyclic antidepressants and some antipsychotics, primarily clozapine. On the other hand, many specific indications make TDM most useful for individualized pharmacotherapy with almost any neuropsychiatric drug.

Since potential benefits of TDM for optimization of pharmacotherapy can only be reaped if the method is adequately integrated into the clinical treatment process and current TDM use in psychiatric and neurologic care is far from optimal, the TDM expert group of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) issued consensus guidelines for best practice of TDM in psychiatry and neurology. A first version was published in 2004 (1). These guidelines were extended in 2011 (2) and an update 2015 is in preparation (3).

The aim of this presentation is to specify typical indications for TDM in psychiatry and neurology and explain the best practice of TDM in accordance with the actual consensus guidelines. A typical indication for TDM is suspected non-adherence or lack of clinical improvement under recommended doses: Loss of adherence is a major problem of long-term medications. In patients with schizophrenia and in patients with unipolar or bipolar disorders non-adherence ranges from 10 to 69 %. TDM is advantageous to control compliance compared with other methods, since it determines if the drug is in the body at a concentration that is potentially sufficient for the expected clinical response. When adverse effects are associated with clinical improvement under recommended doses,
TDM may clarify if side effects are related to excessively high drug levels in the blood and if the dose can be decreased without loss of efficacy. Another most important indication is polypharmacy. Combining of medications that are inhibitors or inducers of drug metabolizing enzymes in combination with victim drugs can lead to loss of action, poor tolerability or intoxication due to a pharmacokinetic drug-drug interaction. This may be controlled by TDM. To support relapse prevention is another indication for TDM. Relapse prevention is highly cost-effective when it reduces hospitalizations. For special groups of patients, such as children or adolescent patients, pregnant or breastfeeding patients, individuals with intellectual disabilities, forensic psychiatric patients, or elderly patients, TDM is also highly recommended. Using TDM in accordance with the actual AGNP consensus guidelines enables rational clinical decision making and thus improves efficacy and safety of pharmacotherapy in psychiatry and neurology.

REFERENCES

PL-31: ARYLATIN OF WEAKLY ACIDIC C–H’S

P.J. Walsh

University of Pennsylvania, Department of Chemistry 231 South 34th Street, Philadelphia, PA 19104-6323, USA

INTRODUCTION

Recently, sulfenic acids, and their conjugate bases, sulfenate anions (Figure 1), have gone from chemical curiosities to important intermediates in biological and synthetic chemistry. Small molecule sulfenic acids are highly reactive and difficult to isolate. Likewise, the conjugate bases of sulfenic acids, sulfenate anions, are highly reactive intermediates that have been generated and trapped in a number of organic and metal catalyzed processes. Despite their versatility, prior examples of small molecule sulfenate anions employed as catalysts are unknown. In this lecture, we describe our recent work on sulfenate anions as organocatalysts.

Fig. 1. Sulfenic acid and its conjugate base, the sulfenate anion.

MATERIALS AND METHODS

Procedure and Characterization for Formation of Stilbenes catalyzed by Sulfenate Anion Catalysis.

To an oven-dried microwave vial equipped with a stir bar was added benzyl phenyl sulfoxide (0.54 mg, 0.0025 mmol) and KOtBu (33.6 mg, 0.30 mmol, 3 equiv) in a nitrogen filled dry box followed by 1.0 mL dry CPME. The microwave vial was sealed with a vial cap with a rubber insert and the sealed vial removed from the dry box. Benzyl chloride (11.5 μL, 0.10 mmol) was then added by syringe under nitrogen. Note that if the benzylic halide is a solid, it was added to the reaction vial before sealed in the dry box. The reaction mixture was heated to 80 °C in an oil bath and stirred for 12 h. The sealed vial was cooled to room temperature, opened to air, and the reaction mixture was passed through a short pad of silica gel then rinsed with 10 mL ethyl acetate. The solvent was removed under reduced pressure. The resulting residue was purified by flash chromatography.

RESULTS AND DISCUSSION

We were inspired by our recent development of a palladium-catalyzed conversion of aryl benzyl sulfoxides to diaryl sulfoxides, wherein the palladium drives three distinct catalytic cycles (Figure 2) [1]. The key intermediate in this process was the sulfenate anion, ArSO, which was the leaving group in cycle B and underwent transmetallation in cycle C, ultimately forming a C–S bond. Based on the ability of sulfenate anions to behave as nucleophiles and leaving groups in this process, we hypothesized that sulfenate anions could be potential organocatalysts, and set out to test this hypothesis. As such, we proposed the sulfenate anion catalyzed coupling of benzyl halides to form symmetrical stilbenes in Figure 3.

Fig. 2. Palladium promotes the tri-catalytic cycle with sulfenate anion as the leaving group in cycle B and nucleophile in cycle C.
The starting point for proof of concept is benzylic sulfoxide A (Figure 3). Under basic conditions, benzylic sulfoxides can be α-deprotonated and the resulting sulfoxide anion (B) react with benzylic halide to generate sulfoxide D. Deprotonation of the β-C–H of D was envisioned to promote an E2 elimination generating trans-stilbene and expelling the sulfinyl anion catalyst. Sulfinyl anions are known to undergo nucleophilic substitution with benzylic halides, regenerating sulfoxide A and completing the catalytic cycle. The sulfinyl anion F is the smallest structural unit carried through this catalytic cycle.

We initiated a search for conditions for the sulfinyl anion-catalyzed synthesis of trans-stilbene from benzylic chloride by screening 6 bases: LiO’Bu, NaO’Bu, KO’Bu, LiN(SiMe3)2, NaN(SiMe3)2, and KN(SiMe3)2 in the presence of 10 mol % benzylic phenyl sulfoxide in cyclopentyl methyl ether (CPME) solvent at 80 °C (Table 1, entries 1–6). Assay yields of stilbene 2a ranged from 0% (LiO’Bu, entry 1) to >98% (KO’Bu, entry 3). Amide bases, MN(SiMe3)2, decomposed the starting materials (entries 4–6). Lowering the catalyst loading to 5 or 2.5 mol % benzylic phenyl sulfoxide led to quantitative assay yield of 2a (entries 7–8). Further reduction of the catalyst loading to 1 mol % also gave good yield (74%, entry 9). Screening 5 solvents under the conditions in entry 9 gave 2a in lower yields than with CPME (Table 1, entries 10–14). Using 2.5 mol % sulfoxide, lowering the equivalents of the base from 3.0, or the reaction temperature from 80 °C, resulted in a diminished assay yield of 2a (entries 15–16). An attempt to increase the reaction concentration from 0.1 M to 0.2 M resulted in slightly decreased yield (92%) due to formation of benzylic tert-butyl ether (entry 17). When the reaction was conducted under the conditions of entry 8 in the absence of the sulfoxide, no stilbene was formed (entry 19). Based on the results in Table 1, our optimized conditions for sulfinyl anion-catalyzed trans-stilbene formation from benzylic chloride were 2.5 mol % benzylic phenyl sulfoxide, 3.0 equiv KO’Bu in CPME at 80 °C for 12 h.

Table 1. Optimization of the formation of stilbene 2a from benzylic chloride 1a,[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Cat. (mol %)</th>
<th>2a NMR yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LiO’Bu</td>
<td>CPME</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>NaO’Bu</td>
<td>CPME</td>
<td>10</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>LiN(SiMe3)2</td>
<td>CPME</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>NaN(SiMe3)2</td>
<td>CPME</td>
<td>10</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>KN(SiMe3)2</td>
<td>CPME</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td>7</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>5</td>
<td>100 (95[b])</td>
</tr>
<tr>
<td>8</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>2.5</td>
<td>100 (95[b])</td>
</tr>
<tr>
<td>9</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>1</td>
<td>74</td>
</tr>
<tr>
<td>10</td>
<td>KO’Bu</td>
<td>Toluene</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>11</td>
<td>KO’Bu</td>
<td>THF</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>12</td>
<td>KO’Bu</td>
<td>Dioxane</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>13</td>
<td>KO’Bu</td>
<td>DME</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>14</td>
<td>KO’Bu</td>
<td>DCE</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>15[c]</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>2.5</td>
<td>88</td>
</tr>
<tr>
<td>16[d]</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>2.5</td>
<td>14</td>
</tr>
<tr>
<td>17[e]</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>2.5</td>
<td>92</td>
</tr>
<tr>
<td>18</td>
<td>KO’Bu</td>
<td>CPME</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

[a] Reactions performed using 1.0 equiv of 1a, 3.0 equiv of base on a 0.1 mmol scale at 80 °C. [b] Isolated yield. [c] 2.0 equiv of base used. [d] 50 °C. [e] 0.2 mmol of 1a used.

With the optimized reaction conditions in hand, the scope of benzylic halide substrates was examined. This will comprise the majority of the lecture, as well as new applications of sulfinate anions currently being developed in our research group [2,3].

CONCLUSIONS

In summary, we hypothesized that sulfinate anions could act as catalytic intermediates in organocatalytic reactions. This hypothesis was founded upon their ability to act as both leaving groups and as nucleophiles. As proof of concept, we developed the base-promoted conversion of benzylic halides to trans-stilbenes catalyzed by sulfinate anions. Furthermore, we have shown that a variety of sulfoxide precatalysts, including DMSO, can promote this reaction with loadings of 1–10 mol %. Reactivity studies provide support for the intermediacy of sulfinate anions and strong evidence that the catalyst resting state is the deprotonated sulfoxide. Based on these studies, we offer that sulfinate anions have significant potential in organocatalysis. Related reactions catalyzed by this novel class of catalysts are currently under investigation.

ACKNOWLEDGMENTS

We thank the National Science Foundation [CHE-0848460 (GOALI) and 1152488] for financial support.
connexin-43, a major ventricular gap junction protein as compared to Ad-LacZMI. Vascular density and decrease in collagen deposition in Ad-Peli1MI group for fibrosis with picrosirius red staining exhibited a compared to Ad-LacZ. Immunohistochemical analysis MAP Kinase 2 (1.7 fold) with Ad-Peli1 treatment phosphorylation of Akt (3.4 fold), eNOS (1.9 fold) and Western blot analysis 24 h after MI revealed increased remodeling in myocardial infarction (MI) model. increased ejection fraction and reduces ventricular of-function) significantly increased angiogenic effect, Peli1 by adeno-Peli1 gene therapy (Ad-Peli1) (gain-

**PL-32: KEY PLAYERS IN CARDIAC DISEASE: POTENTIAL AND CHALLENGES**

N. Maulik

University of Connecticut Health Center, Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, Farmington Avenue Farmington, Connecticut-06030

E-mail: nmaulik@uchc.edu

Therapeutic angiogenesis is a promising approach to the treatment of ischemic injury. Angiogenesis is a global highlight in the medical field. It offers enormous potentials for therapeutic intervention of many disorders in human. Many angiogenesis related factors are involved in the development of vessels during vasculogenesis, as well as the induction of new vessels in response to physiological or pathological stimuli. VEGF modulates the complex process of angiogenesis and other various aspects of endothelial cell function through either of its two tyrosine kinase receptors, VEGFR1/Flt-1 or VEGFR2/Flk1/KDR via its target protein MAPKinase 2. VEGF mediated angiogenesis signaling is widely accepted however relatively little is known regarding VEGF mediated downstream signaling through Flt-1 and/or Flk-1. The use of Affymetrix gene chip technology in Flk-1-/- knockout mice allowed us first time to identify several target genes downstream of VEGF/Flk-1 signaling in ischemic preconditioned myocardium. By Affymetrix gene chip analysis we demonstrated first time down regulation of Pellino-1(Peli1) after ischemic insult to the Flk-1-/-. Our study showed that overexpression of Peli1 by adeno-Peli1 gene therapy (Ad-Peli1) (gain-of-function) significantly increased angiogenic effect, increased ejection fraction and reduces ventricular remodeling in myocardial infarction (MI) model. Western blot analysis 24 h after MI revealed increased phosphorylation of Akt (3.4fold), eNOS (1.9 fold) and MAP Kinase 2 (1.7 fold) with Ad-Peli1 treatment compared to Ad-LacZ. Immunohistochemical analysis for fibrosis with picrosirius red staining exhibited a decrease in collagen deposition in Ad-Peli1MI group as compared to Ad-LacZMI. Vascular density and connexin-43, a major ventricular gap junction protein was found to be increased in Ad-Peli1MI group compared to Ad-LacZMI. Collectively, our study documents Peli1 as a promising molecule in the treatment of myocardial infarction, which could potentially lead to new therapeutic target.

**Disclosure:** Invited symposium speaker on the same topic in XI World Congress-ISAM, Japan, May 2015

**PL-33: MODULATION OF CALCIUM STORE STABILITY AS AN ANTIARRHYTHMIC INTERVENTION**

A. Zaza

Dipartimento di Biotecnologie e Bioscienze, Università degli Studi Milano-Bicocca (IT)

Sarclemmal ion channels are traditionally considered as the elective targets of antiarrhythmic therapy. The contribution of abnormal intracellular Ca\(^{2+}\) handling to arrhythmogenesis has been since long recognized for digitalis-induced arrhythmias, but has been for a long time circumscribed to this specific condition. An increasing amount of evidence points instead to its role as a common cause of arrhythmia, i.e. relevant to all arrhythmogenic mechanisms. Cytosolic Ca\(^{2+}\), acting as the signal to couple membrane excitation to sarcomere contraction, is largely released from the sarcoplasmic reticulum (SR). To serve its purpose, SR must be a “stable” store, i.e. it must release Ca\(^{2+}\) only upon membrane excitation (action potential). When stability of the intracellular Ca\(^{2+}\) store is compromised, Ca\(^{2+}\) may be released independently from excitation and lead to secondary perturbation of sarclemmal membrane potential. Indeed, the latter is coupled to cytosolic Ca\(^{2+}\) levels through an electrogenic Ca\(^{2+}\) transport mechanism (the Na\(^+/\)Ca\(^{2+}\) exchanger) and various Ca\(^{2+}\) -sensitive conductances. Ca\(^{2+}\) store stability is compromised, and contributes to the high incidence of arrhythmias, in common and more rare disease conditions (e.g. heart failure, genetic catecholamine induced arrhythmias). Therefore, SR stability may be considered an antiarrhythmic endpoint. Ca\(^{2+}\) store stability depends on the interplay between sarclemmal and SR “effectors” (ion channels and transports), which are mutually linked by Ca\(^{2+}\)-mediated feed-back control. While instrumental to cell homeostasis, such control makes any attempt to modulate SR stability dauntingly complex. In my presentation I will review current knowledge on the factors leading to SR instability, the mechanisms by which SR instability translates into arrhythmias and which interventions may be best suited to prevent SR instability. Although still at an initial stage of development, such interventions might represent the future of antiarrhythmic drug therapy.
ORAL PRESENTATIONS
INTRODUCTION

Temozolomide (TMZ) is an oral alkylating pro-drug and used to treat brain cancers such as glioblastoma multiform and malignant glioma with a good safety profile and proven efficacy. TMZ is a class of the imidotetrazine family and its active moiety is responsible for the physicochemical, anticancer and mutagenic properties of the molecule. The chemical structure of TMZ is showed in Figure 1.

Fig. 1 Chemical Structure of Temozolomide.

TMZ readily crosses the blood–brain barrier and delivers a methyl group to purine bases of DNA (O6-guanine; N7-guanine and N3-adenine) to exhibit its activity [1]. In the literature, TMZ–DNA interaction was detected generally by immunoassays [2, 3]. There are very few studies available for electrochemical investigation of TMZ with DNA [4]. In this study, electrochemical properties of TMZ and its interaction with probe (single stranded DNA) and hybrid (double stranded DNA) was studied extensively with electrochemical techniques as Differential Pulse Voltammetry (DPV) and Cyclic Voltammetry (CV). The oxidative damage caused to DNA bases by TMZ was detected electrochemically by monitoring the 8-oxoguanine oxidation peaks. The importance of the study, TMZ has oxidation signals at negative potentials and evaluation of these oxidation signals enabled us to determine hybridization independently from the guanine oxidation signals.

MATERIALS AND METHODS

DPV and CV was performed to monitor signals of guanine and TMZ using the AUTOLAB electrochemical analysis system. The 3 electrode system consisted of a pencil graphite electrode as the working electrode, a reference electrode and a platinum wire as the auxiliary electrode.

RESULTS AND DISCUSSION

The interaction of TMZ with probe and hybrid DNA was determined with both guanine and TMZ signals. In order to obtain best hybridization conditions, experimental parameters including probe, target and drug concentrations, hybridization, immobilization and interaction times, ionic strength and surface activation conditions were optimized.

The electrochemical oxidation signals of guanine and TMZ were detected at about +1.0 V and -0.4 V respectively vs. Ag/AgCl reference electrode with DPV. The drug signals were decreased after interaction with the DNA but higher decrease was observed with hybrid DNA when compared the probe DNA (Figure 2). These differences allowed hybridization detection without labeling or using redox probes.

Fig. 2 The oxidation signals of only drug and after interaction with probe (probe+drug) and hybrid (hybrid+drug).

CONCLUSIONS

The results are very promising in terms of using TMZ as a new DNA hybridization indicator. In the presentation, extensive electrochemical behaviors of TMZ and DNA interaction will be discussed and advantages of the study will be emphasized.

REFERENCES
OP-2: DIRECT ELECTROCHEMISTRY OF GLUCOSE OXIDASE ON GRAPHENE STANDALONE ELECTRODE FOR NON-INVASIVE DETECTION OF GLUCOSE IN SALIVA

A. Rabti, W. Argoubi, N. Raoufi

Tunis El-Manar University, Faculty of Sciences of Tunis, Department of Chemistry, Laboratory of Analytical Chemistry and Electrochemistry
Tunis, TUNISIA

INTRODUCTION

A lot of interest has been recently devoted to the development of 3rd generation of glucose biosensors based on the direct electron-transfer (ET) from immobilized glucose oxidase (GOx) on electrode modified with graphene and graphene-based hybrid materials, such as electrochemically reduced graphene (rGO) [1], rGO and silver NPs nanocomposite [2] and graphene/polyaniline/ AuNPs [3]. To the best of our knowledge, there are no reports for direct electrochemistry of GOx on graphene freestanding electrode. In this work, graphene electrode were prepared by drop casting rGO ink as the standalone-working electrode integrated within previously screen-printed reference and counter electrodes (SPEs). After immobilization of GOx, GOx/rGO/SPE has been examined as a sensitive amperometric biosensor. The accurate glucose determination achieved in artificial saliva proves that this biosensor could be used as a potential non-invasive probe for glucose detection in saliva.

MATERIALS AND METHODS

Glucose oxidase (Aspergillus niger ≥100,000 UI/g) and glucose were purchased from Sigma-Aldrich. Graphene oxide (5 mg/mL) solution was purchased from Angstron Materials.

A homemade SPEs printed on PET foils formed by a printed Ag/AgCl reference electrode and a carbon counter-electrode were used to perform the electrochemical experiments and they were printed on a DEK-248 screen printer (DEK International). The working electrode area was left blank to be filled with 5 µL of rGO/chitosan composite. For the fabrication of glucose biosensors, 5 µL of GOx (20 mg/mL) in PBS were casted onto the rGO layer on SPE and were dried at RT. The electrode were kept at 4 °C when not in use.

All CVs are recorded at scan rate: 0.1 Vs⁻¹ unless otherwise stated.

RESULTS AND DISCUSSION

To investigate electrochemistry of GOx, CVs of modified SPEs with (a) rGO and (b) GOx/rGO have been recorded in N₂-saturated PBS. In Fig. 1, a pair of well-defined redox peaks was observed at GOx/rGO (curve b). The formal peak potential was estimated as −756 mV and ∆Ep was 167 mV. These results are far different from those of GOx immobilized on graphene-modified GCE. This redox signal can be ascribed to GOx since bare rGO (curve a) did not show any redox peaks.

Fig. 1. CVs of of rGO (a) and GOx/rGO (b) in N₂-saturated PBS.

The influence of the scan rate on the performance of the GOx/rGO is investigated in Fig. 2. The peak current increased linearly vs. scan rates (Fig. 2 inset A). Moreover, ∆Ep also increased (Fig. 2 inset B) suggesting that ET on GOx/rGO electrode is a quasi-reversible surface-confined electro-chemical process.

According to Laviron’s model, the charge transfer coefficient (α) and the ET rate constant (kₜ) of the GOx were estimated to be 0.56 and 0.96s⁻¹, respectively. This kₜ value is lower than that reported for rGO-modified GCE (4.8 s⁻¹) [1]. Comparatively, these results suggest that rGO freestanding electrode has less active sites, which cause difficulties in the communication between GOx redox centre and electrode.

Fig. 3 displays CVs obtained at the GOx/rGO for different concentrations of glucose in air-saturated PBS solution. Upon increasing the glucose concentration, the reduction peak current (Iₚc)
decreased, while, the oxidation peak current (Ipa) increased, displaying a typical electrocatalysis of glucose at GOx-modified electrode.

Fig. 3. CVs of GOx/rGO in the air-saturated PBS (pH 7) solution containing various concentrations of glucose rates (from a to j: 0-1000 µM glucose); The inset shows the calibration curve of the linear dependence of Ipc on the glucose concentration.

The calibration curve corresponding to CV response was linear against the glucose concentration ranging (R²=0.9849) (Fig. 3 inset). The detection limit was estimated to be 5 µM (S/N=3). It was well-known that the normal range of glucose concentration in saliva was 20-240 µM [5] which indicated that this biosensor was suitable for its practical application for non-invasive detection of glucose in saliva. Good recovery results (~97%) in artificial saliva validate that the designed biosensor can be used for practical applications.

CONCLUSIONS
We have prepared rGO standalone electrode by simply drop casting rGO/CS solution on working electrode blank area integrated within previously screen-printed reference and counter electrodes. The rGO provided a good microenvironment for the immobilization of GOx, thus facilitating its direct electrochemistry and the electrocatalysis of glucose. The biosensor showed a good linear response toward glucose with good sensitivity. The selectivity study in artificial saliva revealed that this biosensor could be used as a potential candidate for real-time monitoring of glucose in saliva samples.

REFERENCES

OP-3: MODERN THIN LAYER CHROMATOGRAPHY (HPTLC) FOR QUALITY CONTROL OF BOTANICALS

A. Gökbunlüt
Ankara University Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara, TURKEY

INTRODUCTION
High performance thin layer chromatography is a sophisticated instrumental technique fundamentally based on the principles of conventional thin layer chromatography. For the analysis of the mixtures of organic, inorganic and biomolecules, HPTLC system presents and provides the advantages of automatic sample application and development, visualization, scanning, selective detection, quantification etc., therefore it is a powerful and multi-purpose device for chromatographic analysis of botanicals.

MATERIALS AND METHODS
HPTLC is one of the most commonly applied methods in phytochemical analysis due to its several benefits such as simplicity, low costs, parallel analysis of the samples, fastly gained results, more amount of application. Multicomponent samples can be analysed by spray-on technique using a wide choice of solvents as mobile phase. The environmental external factors supposed to effect the separation are minimized by controlling all the steps with the aid of a computer system.

RESULTS AND DISCUSSION
It is the only chromatographic technique offering the choice of obtaining and presenting results as an image. Also, both the qualitative and the quantitate analysis can be performed using HPTLC. Traditional Chinese medicines (TCM), ayurvedic medicines, nutraceuticals, herbal health products are commonly and frequently used worldwide. All pharmacopoeias set standards for the quality, purity, strength, and consistency of these products critical to the public health. For safety and efficiency of these natural products quality control studies had to be executed. HPTLC is one of the most convenient equipment to perform these analysis.
CONCLUSIONS
In this presentation, an overview on the fundamentals of HPTLC, the parameters effecting the separation, some methods applied to the analysis of phytochemical samples and qualitative and quantitative approaches in HPTLC will be presented.

REFERENCES

OP-4: PHYTO-ACTIVITY STUDIES ON TURKISH CENTAUREA SPECIES, FROM PAST TO FUTURE

INTRODUCTION
The objective of this study is to isolate the bioactive compounds from Centaurea species, evaluate them with NMR, HPLC-TOF/MS and other necessary techniques and present their anticancer activities against HeLa and C6 cell lines.

MATERIALS AND METHODS
Extraction and Isolation; Plant was extracted with methanol and boiled water, and chromatographic methods performed to isolate the active compounds. The extract was fractionated on a silica gel column, eluted with solvent systems of increasing polarity. The fractions were monitored by TLC and similar fractions were combined [1].

Cell culture and cell proliferation assay; HeLa and C6 cells were grown in DMEM, completed with fetal bovine serum and Pen Strep solution at 37 °C in a 5% CO2 humidified atmosphere. For proliferation assay, cells were plated in 96-well culture and incubated for overnight. The amount of cell proliferation was assessed by using a microplate reader or xCELLigence (RTCA).

RESULTS AND DISCUSSION
The structure isolated Centaurea species were elucidated by spectroscopic techniques, namely, 1D, 2D-NMR, HPLC-TOF/MS. Many compounds such as Solstitialin A were identified and displayed the anti-cancer activity.

The activities of samples were carried out at different concentrations. The compound 2 has higher antiproliferative activity against C6 compare to cisplatin and 5-florouracil. However, its activity was shown to increase depending to decreasing concentration. The Compound 1 has strong antiproliferative activity than standards.

The isolated compounds exhibited the significant anticancer activities against HeLa and C6 cell lines in different concentrations.

ACKNOWLEDGMENTS
The authors thank TUBITAK (TBAG-109T056).
OP-5: SYNTHETIC CAJANIN STILBENE ACID DERIVATIVES INHIBIT C-MYC IN BREAST CANCER CELLS

O. Kadioglu¹, T. Efferth²

¹Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Pharmaceutical Biology Department, Mainz, Germany
²Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Head of Pharmaceutical Biology Department, Mainz, Germany

INTRODUCTION
Breast cancer is the most common type of cancer in females with approximately 230,000 new cases of invasive cancer and 39,000 breast cancer deaths in the U.S.A. in 2011. In the present study, we investigated the activity and modes of action of synthetic derivative compounds in terms of cytotoxicity, gene expression profile and transcription factor activity.

MATERIALS AND METHODS
XTT assay on MCF-7 cells were performed and after the determination of IC₅₀ values, gene expression profiling was performed with Agilent microarray experiments. Microarray data was uploaded to GEO database with the ID GSE64430. Deregulated genes were determined with Chipster software, pathway and functional analyses were performed with Ingenuity pathway software. In order to identify the potential upstream regulators, MatInspector software was used to perform transcription factor binding motif search in the promoter regions of the deregulated genes. Molecular docking on MYC/MAX complex (PDB ID: 1NKP) and reporter cell line experiments were performed to validate the MYC inhibitory activity of the compounds. Two known MYC inhibitors; 10058-F4 and 10074-G5 were used as positive control.

RESULTS AND DISCUSSION
All compounds showed cytotoxicities in the micromolar range. Microarray analyses pointed to cell cycle, DNA damage, DNA repair as mainly affected cellular functions. Promoter motif analysis of the deregulated genes further supported the microarray gene expression analysis results emphasizing the relevance of transcription factors regulating cell cycle and proliferation, with MYC as being the most pronounced one. Luciferase-based reporter cell line experiments and molecular docking studies yielded supportive results emphasizing the inhibitory activity on MYC. The compounds are shown to be promising anti-cancer compounds with low toxicity. They inhibit the MYC activity better than the known inhibitors; 10058-F4 and 10074-G5 (Fig. 1).

CONCLUSIONS
Further studies are warranted to analyze the therapeutic applicability of these compounds in more detail.

ACKNOWLEDGMENTS
We are grateful to the Ph.D. position of the Johannes Gutenberg University (Mainz, Germany) for Onat Kadioglu.

REFERENCES

OP-6: EFFECT OF RECURRENT L-DOPA ADMINISTRATION ON HYDROGEN SULPHIDE-INDUCED RELAXATION RESPONSE ON CORPUS CAVERNOSUM TISSUE

S. S. Yıldırım³, F. İlişı², G. S. Öztürk Fincan², S. Erkan⁴, Y. Sarıoğlu²

¹Department of Medical Pharmacology, Kınıkkale University, Kınıkkale, TURKEY
²Department of Rational Drug Use and Drug Supply Management, Turkish Medicines and Medical Devices Agency, Ankara, TURKEY
³Department of Medical Pharmacology, Kınıkkale University, Kınıkkale, TURKEY
INTRODUCTION
Neuromodulatory action of hydrogen sulphide (H\textsubscript{2}S) was recently discovered. There has been exponential growth of scientific interest in H\textsubscript{2}S as a biological mediator in many systems, which varies from the brain to the gut [1]. Also H\textsubscript{2}S has a protective role in neurons against oxidative stress [2]. L-DOPA treatment has unwanted effects in the long term for example in Parkinson’s disease because of oxidation of L-DOPA [3, 4]. In recent years, hybrid compounds have been designed to release H\textsubscript{2}S from L-DOPA preparation in order to achieve beneficial neuroprotective effects of H\textsubscript{2}S donors. Nevertheless, the possible effects of L-DOPA treatment on H\textsubscript{2}S-induced relaxation response of corpus cavernosum are not known. The aim of this study is to investigate interaction between L-DOPA treatment and H\textsubscript{2}S-induced relaxation response of corpus cavernosum by using L-cysteine (substrate of endogenous H\textsubscript{2}S) and sodium hydrosulfide (NaSH, the standard H\textsubscript{2}S donor).

MATERIALS AND METHODS
Thirty-two adult albino male rabbits were divided into two groups as two- and four-week-treatment groups. And each groups were subdivided as control (saline-injected), 3 mg/kg/day (low dose) and 12 mg/kg/day (high dose) L-DOPA-treated groups. At the end of the treatment, rabbit corpus cavernosum tissues were placed in organ bath chambers containing Krebs solution. Isometric contractions of cavernosal smooth muscle were recorded via force displacement transducers. The EFS-mediated contraction responses were obtained in presence of atropine (10\textsuperscript{-5}M), L-NG-Nitroarginine methyl ester (L-NAME) (10\textsuperscript{-4}M) by 16 Hz, 70 V (duration 1ms; 10-s trains; 2-min intervals). L-cysteine (10\textsuperscript{-4}-10\textsuperscript{-2}M), NaHS (10\textsuperscript{-4}-10\textsuperscript{-2}M), propargylglycine (PAG) (10\textsuperscript{-4}-3x10\textsuperscript{-3}M) and aminooxyacetic acid (AOAA) (10\textsuperscript{-4}-3x10\textsuperscript{-3}M) were administered in separately on the EFS-mediated contraction responses. Also L-cysteine (10\textsuperscript{-4}-10\textsuperscript{-2}M) and NaHS (10\textsuperscript{-4}-10\textsuperscript{-2}M) were administered on PE-induced submaximal contraction.

RESULTS AND DISCUSSION
L-DOPA significantly increased NaHS relaxation responses on PE-induced submaximal contraction in high- and low-dose of two-week-treatment groups. There were no significant difference between control and high- and low-dose of four-week-treatment groups in NaHS responses on PE-induced submaximal contraction. L-DOPA significantly decreased NaHS responses on NO-independent EFS-mediated adrenergic contraction in high- and low-dose of four-week-treatment groups. L-DOPA significantly increased L-cysteine relaxation responses on PE-induced submaximal contraction in high-dose of two- and four-week-treatment groups. L-cysteine and AOAA responses on NO-independent EFS-mediated adrenergic contraction were similar in L-DOPA groups and controls. PAG administration had no effect on NO-independent EFS-mediated adrenergic contraction in control and L-DOPA groups.

<table>
<thead>
<tr>
<th>Emax</th>
<th>Control 2-week</th>
<th>L-DOPA 3mg/kg/day 2-week</th>
<th>L-DOPA 12mg/kg/day 2-week</th>
<th>Control 4-week</th>
<th>L-DOPA 3mg/kg/day 4-week</th>
<th>L-DOPA 12mg/kg/day 4-week</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>1.7±0.15</td>
<td>1.6±0.05</td>
<td>1.7±0.06</td>
<td>1.6±0.15</td>
<td>1.6±0.17</td>
<td>1.6±0.17</td>
</tr>
<tr>
<td>Carbachol</td>
<td>70.49±12.90</td>
<td>68.32±4.15</td>
<td>74.85±4.37</td>
<td>55.63±1.10</td>
<td>61.40±0.92</td>
<td>99.87±0.14*</td>
</tr>
<tr>
<td>SNP</td>
<td>97.34±1.01</td>
<td>93.00±1.13</td>
<td>100.25±1.15</td>
<td>39.03±1.17</td>
<td>94.42±2.72</td>
<td>102.10±1.18</td>
</tr>
<tr>
<td>Sodium thiocyanate</td>
<td>7.5±1.31</td>
<td>8.8±0.42.45*</td>
<td>8.8±0.42.33*</td>
<td>7.8±0.24.07</td>
<td>7.8±0.24.37</td>
<td>8.3±0.42.25*</td>
</tr>
<tr>
<td>Papaverine</td>
<td>88.74±4.33</td>
<td>93.02±2.70</td>
<td>83.25±0.72</td>
<td>88.84±0.71</td>
<td>83.07±0.39</td>
<td>92.34±0.76</td>
</tr>
<tr>
<td>pEC\textsubscript{50}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbachol</td>
<td>0.18±0.14</td>
<td>0.25±0.04</td>
<td>0.29±0.00</td>
<td>0.39±0.00</td>
<td>0.37±0.00</td>
<td>1.0±0.10</td>
</tr>
<tr>
<td>SNP</td>
<td>0.30±0.11</td>
<td>0.30±0.04*</td>
<td>0.30±0.05*</td>
<td>0.39±0.03</td>
<td>0.39±0.01*</td>
<td>0.38±0.01*</td>
</tr>
<tr>
<td>Sodium thiocyanate</td>
<td>3.50±0.02</td>
<td>4.4±0.01 (1)*</td>
<td>4.2±0.01 (1)*</td>
<td>4.3±0.01 (1)*</td>
<td>4.3±0.01 (1)*</td>
<td>3.5±0.01 (1)*</td>
</tr>
</tbody>
</table>

Maximum responses of 124mM KCl (E\textsubscript{max}= g/g contraction). Carbachol, SNP, sildenafil, papaverine (E\textsubscript{max}= % relaxing responses on PE-induced submaximal contractions). pEC\textsubscript{50} values of carbachol, sodium nitroprusside (SNP), sildenafil (-log ED\textsubscript{50}) (* P<0.05; vs control, controls n=4, L-DOPA administration groups n=6)

CONCLUSIONS
The correlation of NaHS responses on both NO-independent EFS-mediated adrenergic contraction and PE-induced submaximal contraction indicates that H\textsubscript{2}S response on corpus cavernosum smooth muscle may be due to its postsynaptic effect. L-DOPA-related changes in NaHS responses indicate sensitivity in relaxation mechanism of H\textsubscript{2}S. Lacking of sensitivity in the long-term treatment may be explained by the adaptive mechanisms.

REFERENCES
OP-7: DEVELOPMENT OF DRY POWDER INHALERS CONTAINING TRIAMCINOLONE ACETONIDE FOR SUSTAINED PULMONARY DELIVERY

A. Yurdasiper, M. Özyazıcı, M. Arıcı

Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Izmir, TURKEY

INTRODUCTION

Recently, new drug delivery systems for inhalation application of drugs have been developed on the basis of dry powder inhalers (DPI) [1]. Guidelines for asthma management recommend inhaled corticosteroids as first-line treatment for the inflammatory component of asthma [2]. In recent years, polymeric carriers have been shown to be effective in delivery of pharmaceuticals into target tissues.

The present study addressed the principles associated with the production of biodegradable microparticles intended for controlled pulmonary drug delivery by spray-drying technique.

MATERIALS AND METHODS

Materials

Triamcinolone acetonide (TA) from Deva Holding Inc. (Turkey). PLGA (50:50) from Boehringer Ingelheim (Germany). Mannitol (M) from Roquette (France). Leucine (L) from Sigma-Aldrich, Poole, (UK). Poly vinyl alcohol (PVA) from Acros (Belgium), sodium lauryl sulphate (SLS) were purchased from BDH Chemicals Ltd., (UK).

Preparation of PLGA dry powder inhalers containing TA

Mannitol and leucine were dissolved in PVA. TA and different ratio of PLGA was dissolved in acetone. This solution was added into PVA, mannitol and leucine solution and was homogenized using a high-speed homogenizer (Ultra-Turrax, IKA T-18, Germany) (Table 1). The resultant suspension was then spray-dried with constant stirring using a Büchi Nanospay dryer B-90.

Table 1. Formulation composition of the powders.

<table>
<thead>
<tr>
<th>Code</th>
<th>TA (mg)</th>
<th>M (%)</th>
<th>L (%)</th>
<th>PVA (%)</th>
<th>Acetone (ml)</th>
<th>TA:PLGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>250</td>
<td>50</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>10:1</td>
</tr>
<tr>
<td>F2</td>
<td>250</td>
<td>50</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>20:1</td>
</tr>
<tr>
<td>F3</td>
<td>250</td>
<td>50</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>30:1</td>
</tr>
</tbody>
</table>

Particle size and Zeta potential analysis

The size and polydispersities of the particles in suspension were determined by laser diffraction with a wet sampling system (Mastersizer, Hydro 3000, Malvern instruments, UK). Zeta potential of formulations were measured by photon correlation spectroscopy (Nano ZS, Malvern Instruments, UK).

Results and Discussion

SEM images of DPI formulations showed almost spherical structures with smooth surfaces (Fig. 1). Thermogravimetric (TGA) and Scanning electron microscopy (SEM) spectroscopy analysis

TGA was performed using a Perkin Elmer TGA-4000 instrument. A platinum sample pan was loaded with 5 ± 0.5 mg of sample and heated from 25 to 350°C at a rate of 10°C/min under dry nitrogen flowing at rate of 40 mL/min. The morphology of TA-DPI was evaluated using SEM, Emitech k-550X OL at 15 kV.

Powder flow characteristics

Bulk density was determined by filling the powder into a 10-ml measuring cylinder and tapped density was measured by tap density measurements following 1000 taps (Varian 2 Platform, USA).

Determination of process yield, drug content and entrapment efficiency of formulations

TA content in the dry powders was assessed by dispersing 3 mg of the powder in 5 mL of acetonitrile/water (50:50). The amount of TA in the supernatant was determined by HPLC.

Aerodynamic particle size analysis

The aerodynamic particle size distribution was determined using a Next Generation Impactor (NGI). The fine particle fraction of the emitted dose (ED), respirable fraction (RF), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined.

In vitro dissolution studies

Dissolution testing was performed on 10 mg spray dried powders using Paddle over Disc method USP apparatus 2, at 75 rpm. 300 ml PBS containing 1% (w/v) of SLS were used as dissolution media. Samples were analyzed by HPLC.

RESULTS AND DISCUSSION

SEM images of DPI formulations showed almost spherical structures with smooth surfaces (Fig. 1).
Table 2. The aerodynamic characteristics formulations measured by NGI.

<table>
<thead>
<tr>
<th>Code</th>
<th>FPF± SD (%)</th>
<th>ED± SD (%)</th>
<th>RF± SD (%)</th>
<th>MMAD± SD (µm)</th>
<th>GSD± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>54.17±1.3</td>
<td>98.31±3.9</td>
<td>78.51±2.7</td>
<td>3.77±0.8</td>
<td>1.73±0.2</td>
</tr>
<tr>
<td>F2</td>
<td>43.39±2.3</td>
<td>97.24±2.4</td>
<td>70.92±3.4</td>
<td>2.46±0.4</td>
<td>1.91±0.5</td>
</tr>
<tr>
<td>F3</td>
<td>39.42±1.4</td>
<td>93.68±2.8</td>
<td>78.06±2.9</td>
<td>4.9±0.6</td>
<td>1.83±0.4</td>
</tr>
</tbody>
</table>

Mass median aerodynamic diameter (MMAD) and fine particle fraction (FPF) ranged from 2.4±0.36 to 4.9±0.67 µm and 39±1.14 to 54±1.38 %, respectively. Emitted dose uniformity for all formulations was in the acceptable range (93.6±1.28–98±1.77%). The dissolution results indicate that the release of F1 formulation including large amount of PLGA was slower than other formulations [3]. The release profiles of TA from DPI formulations were presented in Fig. 2.

CONCLUSIONS

A dry powder prepared by spray-drying offers great promise as a formulation for the lung delivery of TA. The optimized formulation developed in this study exhibits good in vitro aerosolization properties. These results suggest that the pulmonary delivery of TA could be a new and safe administration route.

ACKNOWLEDGMENTS

This study was supported by a research grant from Ege University (13/ECZ017).

REFERENCES


OP-8: GC-MS BASED METABOLOMIC PROFILING OF HUMAN BONE MARROW

S. Ayhan¹, T. Reçber², E. Özkan², E. Nemutlu², R. Köksal Özgül³, D. Uçkan Çetinkaya², S. Kir²

¹Hacettepe University Stem Cell Research and Application Center, Ankara, TURKEY
² Hacettepe University, Faculty of Pharmacy Department of Analytical Chemistry, Ankara, TURKEY
³ Hacettepe University Faculty of Medicine Department of Child Health and Diseases Metabolism Unit, Ankara, TURKEY

In this study, comparative metabolomic analysis of bone marrow was carried out based on the GC-MS studies. The bone marrow plasma samples were derived from healthy human bone marrow transplant donors. The samples were isolated from two different regions of the bone in order to investigate microenvironments (niches) and their contents. GC-MS was used to scan wide range of metabolites.

INTRODUCTION

Today’s innovative technologies permit comprehensive screening of the genome, transcriptome, proteome, and metabolome. Detailed knowledge of genomic, proteomic and metabolomics processes converged in the integrated “omics” approach holds an immense potential for understanding mechanism of diseases, for their early diagnostics, choosing personalized therapeutic strategy and assessing its effectiveness. In metabolomics, the purpose is to identify and quantify as much as metabolites in a biological system. Analytes in a metabolomic sample comprise highly complex mixture. Combined gas chromatography with mass spectrometry (GC/MS) is one of the most powerful techniques and commonly used in metabolomics studies.

MATERIALS AND METHODS

The plasma sample was isolated from bone marrow aspirates by centrifugation for 20 min at 1500 rpm at +4°C temperature. 100 µL of plasma was extracted with 900 µL methanol:water (8:1, v/v). After extraction, 300 µL of the extract was evaporated to dryness in a vacuum dryer concentrator. Then, 20 µL of methoxymine hydrochloride (20 mg mL⁻¹) solution in pyridine was added to the sample for methoxymation. After 90 min at 30 °C, the sample were took out and derivatized with trimethylsilyl for 30 min at 37 °C by adding 80 µL of MSTFA with 1% TMCS. After derivatization, 50 µL of the samples were transferred into GC-MS vials. Metabolomic profiling of plasma samples were performed using GC-MS (Shimadzu). The derivatized sample was injected split (1:10) by an autosampler into
The GC-MS system is operative with a 30 m (+10 m dead) × 0.25 mm i.d. fused-silica capillary column having a chemically bonded 0.25-μm DB-5MS stationary phase. The gas flow rate through the column was 2.8 mL min⁻¹, and the purge was turned on after 60 s. The gas flow rate through the column was 2.8 mL min⁻¹, and the column temperature was held at 60°C for 1 min, then increased to 325°C, and held there for 10 min. The interface and the ion source temperatures were 230 and 290°C, respectively. Ions were generated by a 70-eV electron beam at an ionization current of 2.0 mA, and 30 spectra s⁻¹ were recorded in the mass range 50-600 m/z.

RESULTS AND DISCUSSION

Sixteen bone marrow samples were obtained from 8 volunteers at two different niche. The bone marrow samples were run on the GC-MS and 72 metabolites have been identified. These metabolites are belong to Krebs cycle, amino acid and fatty acid metabolism. From these metabolites, citric acid, alanine, theonine, lactic acid, glutamine and serine has shown an trend differention in Niche 1 and 2.

CONCLUSIONS

This is the first metabolomics study on the human bone marrow. We couldn’t find a significant differences between Nichel 1 and 2; however the trends showing changing in the Krebs cycle activity (citrate), oxidative stress (lactate and alanine) and amino acid metabolism (glutamine, serine and theonine).

ACKNOWLEDGMENTS

This work was supported by The Scientific and Technological Research Council of Turkey (Project no: 213S006).

REFERENCES


OP-9: SYNTHESIS OF NOVEL CHIRAL SULFOXIDES AND INVESTIGATION OF RELATIONSHIPS BETWEEN ENANTIOSELECTIVITY AND CHEMICAL STRUCTURE IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

R. Kakava1, A. Volonterio2, B. Chankvetadze1

1Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Chavchavadze Ave 3, 179 Tbilisi, Georgia
2Dipartimento di Chimica, Materiali ed Ingegneria Chimica "Giulio Natta", Politecnico di Milano, via Mancinelli 720131 Milano, Italy

INTRODUCTION

Separation of enantiomers is a hot topic for academic research, as well as in modern pharmaceutical industry. The reason for this is that more than 50 % of the drugs currently in use are chiral compounds and significant part of them are racemates consisting of an equimolar mixture of two enantiomers, stereoisomers with differences biological activity. In many cases, one enantiomer is the active pharmaceutical ingredient while the other inactive enantiomer shows unwanted side effects or even toxic effects. Removal of the unwanted enantiomer from the racemic mixture increases drug efficiency and safety [1].

MATERIALS AND METHODS

In order to investigate relationships between enantioselectivity and chemical structure of different chiral stationary phases (CSPs) and chiral compounds we have synthesized 17 chiral sulfoxides (some of them were not described in literature before) and 15 non-commercial cellulose trisphenylcarbamate-based chiral columns. We have carried out HPLC separation of enantiomers of synthesized chiral sulfoxides with polar organic (methanol, ethanol, 2-propanol, acetonitrile) and normal-phase mobile phases (n-hexane in combination with various alcohols) [2].

RESULTS AND DISCUSSION

At the time we found some structural features of chiral sulphoxides, chiral selectors and mobile phases, which use can lead to future optimization of enantioselectivity. From chiral sulfoxides the most promising seem to be – 2-(benzylsulfinyl) benzamide and its derivatives, from chiral selectors – cellulose tris(3-methyl, 4-chlorophenylcarbamate), tris(4-methyl, 3-chlorophenylcarbamate), tris(3,5-dichlorophenylcarbamate), tris(3-chlorophenylcarbamate) and tris(4-chlorophenylcarbamate) based adsorbents, from mobile phases – different combinations of 2-propanol/n-hexane mixture. In these conditions we
have already got unprecedented high value of selectivity in HPLC separation of enantiomers.

**CONCLUSIONS**

Changes in position of substituent from orto- to para-position decreased the enantioselectivity;
With increasing of concentration of non-polar eluent enantioselectivity increased significantly;
Nature of substituent (electron-donating or electron-withdrawing agent) affects on enantioselectivity.

**ACKNOWLEDGEMENTS**

Shota Rustaveli Georgian National Science Foundation (GNSF);
Georgian Research and development Foundation (GRDF).

**REFERENCES**


**OP-10: SYNTHESIS AND EVALUATION OF TRIAZOLOTHIADIAZINE DERIVATIVES AS NEW ANTICANCER AGENTS RELATED TO COX-2 INHIBITION**

B. Sever1, M. D. Altıntop2, G. Kuş2, M. Özkurt3, Z. Asım Kaplancıklı4

1Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 26470 Eskişehir, Turkey
2Anadolu University, Open Education Faculty, 26470 Eskişehir, Turkey
3Eskisehir Osmangazi University, Medical Faculty, Department of Physiology, Eskişehir, Turkey

**INTRODUCTION**

Cyclooxygenase-2 (COX-2) is an inducible form of cyclooxygenases that catalyses the rate-limiting step in arachidonate metabolism. High levels of COX-2 have been observed in several cancer forms including gliomas [1].

Gliomas are considered the most malignant form of brain tumors and one of the most aggressive human cancers. Although there are advanced therapies for gliomas, prognosis of patients remains weak. Another attempt for the treatment of gliomas that has gained interest is cyclooxygenase, especially COX-2 [2]. Indomethacin, a COX inhibitor, has a well-documented anticancer activity [3]. New analogs of triazolothiadiazines were found to exhibit notable anticancer activity [4].

In the current work, new indomethacin-based triazolothiadiazine derivatives were synthesized and evaluated for their anticancer activity related to COX-2 inhibition.

**MATERIALS AND METHODS**

Initially, 4-amino-5-[(5-methoxy-2-methyl-1-(4-chlorobenzoyl)-1H-indol-3-il)methyl]-4H-1,2,4-triazol-3-thione was synthesized via the solvent-free reaction of indomethacin with thiocarbohydrazide. Then the ring closure reaction of this compound with 2-bromoacetophenone derivatives afforded new triazolothiadizaines.

MTT was carried out to determine the cytotoxic effects of the compounds against T98 human glioblastoma cells [5]. Apoptosis (Flow Cytometric Method) and PCR experiments were carried out only for compound 8 because it was the only compound that showed a reasonable cytotoxic activity according to MTT results. COX-2; caspase 3, 8 and 9; cytochrome c mRNA relative quantity values of the most effective compound were evaluated with PCR method.

**RESULTS AND DISCUSSION**

3-[5-Methoxy-2-methyl-1-(4-chlorobenzoyl)-1H-indol-3-yl)methyl]-6-(4-methylphenyl)-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine (8) (Fig 1), the active compound, reduced cells' survival rates at 50 and 100 μM doses with 25% and 40% reasonable values, respectively. The apoptosis stimulating percentages of compound 8 in comparison with the control (only medium) group (5%) at 50 and 100 μM doses were calculated as 11% and 12%, respectively. PCR results showed that compound 8 reduced only COX-2 in cells, whereas it did not change other parameters. The increased activity of compound 8 can be attributed to the presence of p-methyl group due to its −σ and +π effects.

**CONCLUSIONS**

In the present paper, new indomethacin-based triazolothiadiazine derivatives were synthesized and evaluated for their anticancer activity related to COX-2 inhibition. Among these compounds, compound 8 can be considered as a promising agent due to its notable anticancer and apoptotic effects on T98 cells via the inhibition of COX-2.
OP-11: PHENOLIC COMPOUNDS OF CINNAMOMUM ZEYLANICUM AND INHIBITION EFFECT ON THE ENZYMES OF BACTERIAL REPLICATIVE DNA POLYMERASE

E.A. Turumtay1, E.K. Selvi1, H. Er2, A. Demir1, A. Midilli2, E.E. Budak2, H. Baykal1, A.O. Belduz4, C. Sandalli2

Recep Tayyip Erdogan University, Faculty of Arts & Sciences, 1Department of Chemistry, 2Department of Biology, Pazar Vocational School, 3Department of Plant and Animal Production, 4Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey

INTRODUCTION
Cinnamon is a common spice obtained from the inner bark of trees from the genus Cinnamomum known as tarçın in Turkish food culture. C. zeylanicum and C. aromaticum are two main varieties used for this spice in Lauraceae family. Cinnamon is considered as a remedy for respiratory, digestive and gynaecological ailments besides as an aromatic additive for foods. The replication of chromosomal DNA is essential for the growth of pathogen microorganisms and the inhibition of proteins involved in replication mechanism which are considered as targets for developing new drugs rapidly causes the death of microorganisms. We determined an inhibition effect of the methanol extract of C. zeylanicum on the DNA polymerase enzyme and started to elucidation of active compound which has this medical effect.

MATERIALS AND METHODS
5 gr of fresh plant material was used to prepare the extracts in a final volume of 5 ml [1]. Inhibition effect of each extract was investigated in 10 µl reaction volume as using below components; (1) 100 nm of 5'-prime CY5 labeled fluorogenic substrate (45/20-mer double-stranded DNA), (2) 300 µM from each dNTPs, (3) 10 mM of MgCl2 and (4) 50 nM from each polymerase at 30 °C for 10 minutes [2]. Replicative DNA polymerase III and plant extracts was kept for 10 minutes at room conditions before the polymerization experiments. The reaction products were run in 8 M urea/16% PAGE and imaged by Typhoon FLA9500. The phenolic components of the extracts were determined by HPLC-DAD method. Twelve phenolic standards were used for method development and comparison. A Luna C18 column (15cmx3.0mmi.d., 5µm particles) (phenomenex, Torrance, CA, USA) was used in HPLC.

RESULTS AND DISCUSSION
We investigated the inhibition of Gram - (E. coli) and Gram + (B. subtilis) bacterial replicative DNA polymerase III enzyme in methanol extract of the C. zeylanicum. Inhibition of primer extension in the presence of plant extracts was evaluated as having inhibitory activity. The plant extract have inhibition effect on B. subtilis DNA polymerase III (PolC protein) and E. coli DNA polymerase III (DnaE). It was concluded that the plant is the potential carrier of inhibitor molecules for bacterial replicative DNA polymerases and we try to purify these molecules from plant extracts. According to HPLC-DAD analysis, we quantified Protocatechuic acid (53.7mg/L extract), p-OH benzoic acid (1.6mg/L extract), p-coumaric acid (20.5mg/L extract) and ferulic acid (1.1mg/L extract).

CONCLUSIONS
Although p-coumaric acid is not a major compound of this plant according to the HPLC-DAD chromatograms, it is well known for its DNA polymerase inhibition. This study is going to find the compound(s) which cause this inhibition effect.

ACKNOWLEDGMENTS
This work was supported by TUBITAK-113Z054 number project.

REFERENCES

OP-12: NEUROPROTECTIVE EFFECTS OF NICOTINAMIDE ON APOPTOTIC MECHANISMS IN AB(1-42) INDUCED IN VIVO NEURODEGENERATION

E. Turunc Bayrakdar1, Y. Uyanikgil2, L. Kanit3, A. Yalcin1
INTRODUCTION
Alzheimer’s Disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline and associated with widespread senile plaques (SP) and neurofibrillary tangles (NFT) that ultimately lead to progressive synaptic dysfunction and neuronal death. In some animal models intracerebral administration of Aβ(1-42) induces neurodegenerative disorders that mimic AD. Mitochondrial dysfunction, oxidative stress and cell death mechanisms have been proposed as important contributing factors in AD [1]. This study aimed to test the neuroprotective effects of nicotinamide (NA), NAD+ precursor, on mitochondrial function and mRNA expressions of proapoptotic Bax, Bim, Puma and antiapoptotic Bcl-2 in experimental AD model induced by intrahippocampal injection of Aβ(1-42).

MATERIALS AND METHODS
Adult male Sprague-Dawley rats were used for in vivo experiments and divided into four groups as control, Aβ(1-42), Aβ+NA-100 mg and Aβ+NA-500 mg. All groups were stereotaxically injected bilaterally into the hippocampus with either aggregated Aβ(1–42) or saline (Fig. 1a). After surgery NA treatments were administrated i.p. to the rats for seven days. Then rats were decapitated and the hippocampus samples dissected on ice. Total RNA was extracted and was used for cDNA synthesis. Real-time PCR amplifications were carried out using SYBR Green I reagent. The relative gene expressions were quantified with 2^(-ΔΔCT) method. Mitochondrial function was assessed by MTT assay. One-way analysis of variance (ANOVA) was used to identify significant differences between the experimental groups.

RESULTS AND DISCUSSION
Aβ(1-42) treatment significantly increased the proapoptotic Bax, Bim and Puma mRNA expressions while decreased the levels of antiapoptotic Bcl-2 mRNA and mitochondrial function (Figs. 1b-2). Treatments with NA against Aβ significantly reduced the levels of Bax, Bim and Puma mRNAs. Also the improved levels of mitochondrial function and Bcl-2 mRNA expression were found in Aβ+NA groups (Figs. 1b-2).

CONCLUSIONS
According to our results, treatments with NA provide a protection against Aβ(1-42) induced in vivo neurodegeneration due to the improvement of mitochondrial function, elevated Bcl-2 expression and the repressed levels of proapoptotic Bax, Bim and Puma. Therefore it may possible to suggest that NA has a potential for neurodegenerative conditions where apoptosis is triggered in.

ACKNOWLEDGMENTS
This study was supported by Ege University Scientific Research Foundation (10/ECZ/011).

REFERENCES

OP-13: NEWLY SYNTHESIZED INDOLE-3-IMINE-2-ON DERIVATIVES INHIBITED MMP-9 mRNA EXPRESSION OF HUMAN BREAST CANCER CELLS

F. Bakar 1, M.G. Çağlayan2, I.M. Palabıyık2, Z. Kurt1, S. Öğen1

1Ankara University, Faculty of Pharmacy Department of Biochemistry, Ankara, TURKEY
INTRODUCTION
Cancer is one of the leading causes of morbidity and mortality worldwide [1]. The results of long-term studies indicate that Matrix metalloproteinases (MMPs), a group of extracellular matrix remodeling proteinases, are the principle mediators for the alterations of microenvironment during cancer development [2]. MMPs have been considered as potential diagnostic and prognostic biomarkers in many types and stages of cancer [3]. The studies reported that MMP-9 (Gelatinase B), a 92 kDa member of the family, has been overexpressed in breast cancer resulting the invasion of tumor and plays critical role on extracellular matrix degradation and cell migration [4]. On the basis of the pivotal roles that MMPs play in several steps of cancer progression, the pharmaceutical industry has been investigating potent MMP inhibitors for several years. Recent studies have shown that some indole derivatives have MMP inhibitory effects [5].

The aim of this study is to evaluate the effects of newly synthesized indole-3-imine-2-on derivatives on proliferation, apoptosis and MMP-9 mRNA expression of human MCF-7 breast adenocarcinoma cells.

MATERIALS AND METHODS
The indole-3-imine-2-on derivatives [compounds 1-3] were synthesized by aniline reaction. Human MCF-7 breast carcinoma cells were cultured in DMEM medium supplemented with 10% FBS and the assays were performed at a concentration range of compounds between 1 nM to 0.1 μM. The cytotoxicity assays were performed via MTT assay and cell proliferation was determined by measurement of bromodeoxyuridine (BrdU) incorporation into newly synthesized DNA of actively proliferating cells. The quantification of apoptosis and necrosis in MCF-7 cells were determined by cell death detection elisa method. Quantitative real-time reverse transcription PCR was performed to quantify the mRNA expression of human MCF-7 breast adenocarcinoma cells.

RESULTS AND DISCUSSION
Compounds [1-3] have significantly inhibited cell growth with IC values of 0.52 μM, 0.59 μM and 0.18 μM, respectively. Although the BrdU-treated cells demonstrated a statistically significant dose-responsive decrease in cell numbers for all compounds, compound [1] was the most potent anti-proliferative agent that the proliferating cell amount was measured as 35.99% when compared with control. The results also showed that compound [1] inhibited the expression of MMP-9 protein even at 10 μM concentration.

CONCLUSIONS
In conclusion, the newly synthesized indole derivatives inhibited cell growth and proliferation of MCF-7 cells significantly. The results also showed that these compounds display their cytotoxic effects through inhibition of MMP-9 mRNA expression.

ACKNOWLEDGMENTS
The study was supported by Scientific and Technological Research Council of Turkey Grant, Tubitak-SBAG-113S254.

REFERENCES

OP-14: EFFECTS OF INDOLE α-LIPOIC ACID DERIVATIVES ON CELL VIABILITY PARAMETERS IN K562 LEUKEMIA CELL LINE

A.Z. Karabay1, A. Koc2, A.S. Gurkan-Alp3, Z. Buyukbingol1, E. Buyukbingol3
Ankara University, Faculty of Pharmacy2
1Department of Biochemistry, 3Department of Pharmaceutical Chemistry. Ankara, TURKEY
INTRODUCTION
Alpha lipoic acid is a naturally occurring sulfurous compound which exhibits direct and indirect antioxidant functions. A number of experimental and clinical studies investigated the potential usefulness of lipoic acid as a therapeutic agent for various pathologies such as hypertension, ischemia-reperfusion injury, diabetes, cancer, immune and neurodegenerative diseases. In this study, we aimed to investigate the effects of different indole α-lipoic acid derivatives on viability parameters of K562 cell line. These derivatives have been synthesised from α-lipoic acid and suitable indole compounds and we have shown that they served anti-oxidant [1] and anti-inflammatory functions [2] in different experimental and cell culture models.

MATERIALS AND METHODS
K562 cell line was cultured in 25 cm² flasks, at 37°C and 5% CO₂ saturated air, in RPMI-1640 medium containing 1% L-glutamine, 1% penicilline-streptomycine antibiotics and 10% fetal bovine serum. Cells were collected from flasks and seeded to 24 well plates before incubation with different concentrations (8.75-140 μM) of indole α-lipoic acid derivatives for 48 hours. After incubation time, cells were collected for further analysis for markers related with cell viability.

RESULTS AND DISCUSSION
Lipoic acid has been shown to inhibit cancer cell growth by different biochemical mechanisms in different cell lines and models [3,4]. Our results showed that cell viability of K562 leukemia cell line was significantly (p<0.05) inhibited by some of the indole lipoic acid derivatives at concentrations ≥17.5 μM. We also determined the mode of cell death and found out that different proteins associated with apoptosis were differently expressed in treated leukemia cells compared to untreated controls.

CONCLUSIONS
In conclusion, our results showed that various indole lipoic acid compounds inhibited K562 cell viability by modulating different proteins related to cell death. Further research is needed to analyse the effects of these derivatives on different biochemical pathways related to cell viability.

REFERENCES

OP-15: ANTIOXIDANT AND CYTOTOXIC MICROALGAE SPECIES FROM FOUR LOCAL GEOTHERMAL SOURCES
M. Sensoy1, Z. Dogan1, M. Conk Dalay2, Z. Demirel1, I. Saracoglu1
1Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY
2Ege University, Engineering Faculty, Department of Bioengineering, Izmir, TURKEY

INTRODUCTION
Microalgae have become an important commercial source of many valuable compounds, such as carotenoids, proteins, polysaccharides, etc., used in pharmaceutical and industrial purposes. As a result of this rich composition, microalgae-derived products are used for health improvement, as cosmetics and as food supplements. Spirulina platensis, Dunaliella salina, Haematococcus pluvialis and Chlorella sp. are very well-known species which are used in pharmacologic field. In general, microalgae have hepatoprotective, antimicrobial, antioxidant, immune stimulant activities; and used as natural dyes for medicines, foods, cosmetics and analytical applications [1]. In the present study, microalgae samples were collected from four different geothermal water supplies in Afyonkarahisar, Turkey to determine antioxidant and cytotoxic activities of isolated single strains. Results showed that these microalgae species possessed different antioxidant and cytotoxic activities depending on the extracted chemicals, in concentration dependent manner. Analysis of the extracts by HPLC-DAD system showed that lutein is major carotenoid in all species.

MATERIALS AND METHODS
Four microalgae strains (M1-M4) were isolated as a single strain. DNA analysis were made in Refgen Gene Research and Biotech. Co. They were determined as Getlerinema sp. (M1), Oscillatoria sp. (M2) and two Leptolyngbya sp. (M3 and M4) according to DNA analysis. The species were cultured in their own geothermal water, at 30±2°C, 24 h light period of 20 μmol m⁻² s⁻¹. Biomass concentration was determined and simultaneously controlled by chlorophyll-α and dry mass analysis. For carotenoid
extraction, freeze-dried microalgal powder was mixed with hexane and shaken for 1 h, then 40% methanolic KOH was added. The solution was saponified in the dark for 16 h under nitrogen, followed by adding hexane. Then 10% Na2SO4 solution was added and the mixture was allowed to stand in the dark for 1 h. The supernatant layer was collected and evaporated to dryness for further analysis [2]. DPPH and SO radical scavenging activity methods were used to determine antioxidant potential of extracts [3]. Cytotoxic activity experiments were also conducted on the extracts against HEp-2 cell line by MTT assay [4].

An HPLC method was developed to discern the variety and content of microalgae carotenoids. Comparison of the carotenoid extracts of four microalgae species to the *Spinacia oleracea*, *Spirulina platensis*, *Chlorella sp.*, N2 deprived and light stressed *Chlorella sp.* and commercial canthaxantin as standards using HPLC-DAD system indicated the presence of lutein in all tested microalgae.

RESULTS AND DISCUSSION
All concentrations (25-800 µg/ml) of tested carotenoid extracts were found to possess SO radical scavenging activity, but none of them showed over than 40% inhibition. Comparing all extracts, M1 showed the highest activity. Inhibition degrees of the highest concentrations of samples M1, M2 and M4 were higher than the standart, butylated hydroxyanisole. Extracts showed no significant DPPH radical scavenging activity. Carotenoid extract of M3 showed highest cytotoxic activity with the IC50 value of 66 µg/ml.

Comparison of the carotenoid extracts to the *Spinacia oleracea*, *Spirulina platensis*, *Chlorella sp.*, N2 deprived and light stressed *Chlorella sp.* and commercial canthaxantin as standards using HPLC-DAD system showed that the presence of lutein as a major carotenoid in all species.

CONCLUSIONS
In conclusion, all of the samples showed significant concentration dependent antioxidant and cytotoxic activities. Microalgae can be cultured in geothermal water as a source of bioactive compounds for medicinal and nutritional use.

REFERENCES

OP-16: DEVELOPMENT OF A SUITABLE SIMULTANEOUS DISSOLUTION METHOD FOR ATORVASTATIN AND EZETIMIBE IN THEIR PHARMACEUTICAL DOSAGE FORM BY RP-LC

C. Kose Ozkan, O. Esim, S. Kurbanoglu, A. Savaser, Y. Ozkan, S.A. Ozkan

1Gulhane Military Medical Academy, Department of Pharmaceutical Technology, Etlik, 06018 Ankara, Turkey
2Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Tandogan, 06100 Ankara, Turkey

INTRODUCTION
Dissolution tests are one of the most used tests in the characterization of drugs and in the quality control of dosage forms [1]. Dissolution testing is also used to identify bioavailability problems and to assess the need for further bioequivalence studies relative to scale-up and post-approval changes, where it can function as a signal of bioinequivalence (2). Ezetimibe is a cholesterol absorption inhibitor that reduces low-density lipoprotein cholesterol levels. Atorvastatin is a commonly used statin with greater LDL-C lowering efficacy than most other statins. Adding ezetimibe to atorvastatin lowers LDL-C significantly more than doubling the atorvastatin dose. There is no USP method available then the dissolution testing should be conducted using a method recommended by the FDA.

In our study, we aimed to develop a new, suitablesimultaneous dissolution method which FDA-recommended method is inadequate for the combined oral product.

MATERIALS AND METHODS
Ezetimibe and Atorvastatin was obtained from Neutec Pharmaceutical Company (Istanbul, Turkey). All other reagents were either of analytical grade or chromatographic grade. The commercial brand of ezetimibe atorvastatin tablet tested was Ezator® 10/10 mg tablet (batch no. 00456, Mentis, Turkey) as the test product.

The dissolution test was performed in a six-station rotating paddle (Apparatus 2) (Caleva 7, UK). LC system consisting of the following components: a Hewlett-Packard Model 1100 series with a Model Agilent series G-13159 UV detector and Model Agilent 1100 series G-1329 ALS auto sampler and HP chemstation. The Zorbax (150 mm, 4.6 mm, 5 µm particle size) column was used for chromatographic separation using an isocratic elution. The mobile phase components were phosphate buffer (pH 3.0,
acetonitrile:methanol (35:32.5:32.5, v/v/v) at a flow rate of 1.5 mL min⁻¹. Detector was set at 242 nm. An injection volume of 10μL was used for studies. Amlodipine was used as an internal standard. The following parameters were evaluated: dissolution medium (distilled water, 0.10 M hydrochloric acid, 0.01-0.02 M sodium phosphate solution, potassium phosphate buffer (pH 6.8) and sodium acetate buffer (pH 4.50) and Tween 80 and SLS (0.2-0.7 %) were also added. Apparatus 2 (paddle) at a rotation speed 50, 75 rpm, where these rotation speeds were preset for each apparatus according to U.S. Pharmacopeia (USP) and the Food and Drug Administration (FDA) Guide. The dissolution medium was heated and sonicated, the volume used in the tanks was 500-900 mL, and stabilization of solution temperature at 37 ± 0.50 °C and the aliquots were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min.

RESULTS AND DISCUSSION
The high selectivity and efficiency of the chromatographic column employed is proven through the system suitability tests such as theoretical plate results, which is approximately 5297 for AML, 7309 for EZE, 6379 for ATOR; the tailing factor 1.12 for AML, 1.07 for EZE, 1.02 for ATOR, selectivity to previous peak 2.66 for EZE, 1.64 for ATOR, selectivity to next peak 2.66 for AML, 1.64 for EZE, resolution to previous peak 12.10 for AML, 9.97 for EZE, 6.96 for ATOR, resolution to next peak 9.97 for AML, 6.96 for EZE. It was chosen to work with the first concentration level of 10.00 µg/mL of EZE and ATOR for being representative of ± 5% of the drug dissolved in the dissolution medium, presenting the method to be capable of detecting, EZE and ATOR for being representative of ± 5% of the drug dissolved in the dissolution medium, presenting the method to be capable of detecting, EZE and ATOR within the linearity range, from 6.11x10⁻⁷ M to 6.11x10⁻³ M for EZE and 4.48x10⁻⁸ M to 4.48 x10⁻³ M for ATOR with LOD values of 2.13 x10⁻⁸ M for EZE and 2.52x10⁻⁸ M for ATOR.

In-vitro drug dissolution testing has been a critical component of pharmaceutical product development and is increasingly used to evaluate the drug release characteristics of the pharmaceutical products(3). As the drug release with a poorly soluble drug like EZE, as used in the study, is usually driven more by the medium than the formulation ingredients. The current methods do not seem adequate in light of this information. The paddles setup was selected due to its inherent advantages over the basket system. The dissolution characteristics of oral formulations evaluated over the physiologic pH range of 1.2–6.8. For this point of view, we determined the test conditions at three pH points (1.2, 4.5 and 6.8). For very poorly soluble compounds, aqueous solutions may contain a percentage of a surfactant (sodium lauryl sulphate, Tween 80) that is why we used to enhance drug solubility.

CONCLUSIONS
The initiative in the development of a fresh dissolution is the evaluation of physical and chemical information of drug content. Knowledge of this information will help the selection of dissolution medium and its volume. Solubility plays a prime role in the dissolution of a drug substance from a solid dosage form.

REFERENCES
2. United States Pharmacopeia (USP).

OP-17: NOVEL NANO FORMULATION FOR SYNTHETIC and PLANT BASED METAL CHELATORS with BLOOD-BRAIN BARRIER TARGETING PROPERTY for TREATMENT of ALZHEIMER’S DISEASE
F. Bahadori1, F. Kazdal2, B. Celik3, A.S. Kozanoglu4, B. Filiz4, A. Ertas5
Bezmialem Vakif University, Faculty of Pharmacy, 1Department of Pharmaceutical Biotechnology, 2 Institute of Health Sciences, Biotechnology Master’s Program Student, 3Department of Pharmaceutical Technology, 4 Undergraduate Student, 34093, Istanbul, TURKEY

INTRODUCTION
Lots of pathological factors are contributed in the progress of Alzheimer’s Disease (AD), the most important one being aggregation of Amyloid β (Aβ) in brain. It has been very well defined Aβ chains pose histidin units which have affinity to bind Cu²⁺ ve Zn²⁺ metal ions and this is the way that Aβ makes aggregates.

Although there are number of metal chelators (MC) approved by FDA, all of them are neurotoxic. More importantly, all are hydrophilic and are not able to cross Blood Brain Barrier (BBB).

Based on studies made on synthetically obtained MCs or on the natural ones, it has been approved that the poly-phenolic compounds bearing hydroxyl or keto groups in ortho position are the best MCs. Also Pyrrolidine Dithiocarbamate (PDTC) is an active and non-toxic synthetic material known for beneficial effect in transgenic AD mice through Cu⁺²-activated Akt pathway [1].

Metal chelating is not the only property which is expected from the new formulation. The MC shall enter the BBB and take out the excess metal. For this reason the plant extracts rich in phenolic content and PDTC was uploaded to nano-micelles (NMs) made of
Poly(lactic-co-glycolic acid) (PLGA) with modified surface by Apo-lipoprotein E (Apo-E). Apo-E4 is known for mimicking LDL (low density lipoprotein) transport mechanism in getting in and out of BBB [2]. It is not enough for a MC to scavenge the metal ions, but it needs to show more affinity to metal than Aβ (metal protein attenuating activity (MPAA)).

In this study we scanned nearly 25 plant extracts which previously has been reported for their rich polyphenols for their metal chelating activity (ChA). The active extracts were uploaded to micelles made of PLGA and the surface of micelle was decorated with Apo-E4. The same process was followed for PDTC. Metal protein attenuating activity of these formulations were also determined.

MATERIALS AND METHODS
Collected plants consisted of Astragalus leporinus, Astragalus schizopterus, Astragalus distinctissimus, Centaurea lycopifolia, Centaurea balsamita, Centaurea Iberica, Centaurea diffusa, Centaurea urvillei subsp. urvillei, Verbascum flavidum, Stachys thirkei, Melissa officinalis subsp. officinalis, Calystegia silvatica, Hypericum capitatum, Hypericum triquetrifolium, Sedum sediforme, Cardaria draba subsp. draba, Carlina corymbosa, Pulicaria dysenterica, Onopordum polycephalum, Onopordum carduchorum, Gundelia tournfortii L. var. tournfortii, Scolymus hispanicus, and Rosmarinus officinalis were air dried in shadow, extracted with methanol and consequently with deionized water. The extracts were evaporated under vacuum.

Fe²⁺ ChA was measured using Decker and Welch’s method with basic modifications to correct the deviations raised from extract’s colour[3]. The methods for Cu²⁺ and Zn²⁺ [4] result in obtaining wrong results. In this study we measured the deviations occur in max. absorbance of murexide upon chelating the metal ions.

MC-loaded PLGA NMs were synthesized via the modified-SESD technique [5] and Apo-E4 was incorporated by shaking (R.T., 1h) [2]. The MPAA of MCs were measured using commercially available Aβ (1-42), where the resolubilized Aβ concentration was measured against a concentration curve obtained using albumin by coomassie plus method.

RESULTS AND DISCUSSION
The most active Fe²⁺, Cu²⁺ and Zn²⁺ chelator plant extracts were found to be Hypericum capitatum, Melissa officinalis, Pulicaria dysenterica and Rosmarinus officinalis (RO) (Figure 1).

The size of PLGA micelles were obtained as 65 (Figure 2) nm. Uploading plant extracts or PDTC to nano particles didn’t change their size while Apo-E4 decorating of micelles enlarged the particles to 350 nm (Data not shown).

PDTC was the highest metal protein attenuating activity compared to all plant extracts, while methanolic extract of RO was the most active plant extract in this frame.

CONCLUSIONS
Currently used methods for evaluating the Fe²⁺, Cu²⁺ and Zn²⁺ ChA of pure chemical compounds are not appropriate for coloured materials such as plant extracts. We introduce new methods for investigation of this activity on plant extracts. PDTC and methanolic extract of RO both are very active materials for chelating bivalent metals while RO is the best candidate for further investigations because of its known safe profile in terms of toxicity for centuries.

REFERENCES
2. Kreuter, J.; D. Shamenko; V. Petrov; P. Ramge; K. Cychutek; C. Koch-Brandt, and R. Alyautdin, Apolipoprotein-mediated transport of nanoparticle-bound
OP-18 : ELECTROSYNTHESIS, CHARACTERISATION, AND SENSOR APPLICATION OF POLY(METHYL RED) ELECTROACTIVE POLYMER FILMS

D. Kul

Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, 61080, Trabzon, TURKEY

INTRODUCTION
Methyl red (MR) is a nitrogen-containing aromatic dye used as indicator in microbiology to identify enteric bacteria performing mixed-acid fermentation of glucose [1]. MR can be electrochemically polymerised and used for electrochemical determination of the molecules such as dopamine [2] and acetaminophen [3].

MATERIALS AND METHODS
All chemicals were used as received. Phosphate buffer (PB, 50 mM) solutions at different pH values from 5.0 to 8.0 and Britton Robinson buffer (BRB) solutions at pH values between 5.0 and 10.0 were prepared. All experiments were achieved using a three-electrode electrochemical cell containing a GC working electrode, a platinum wire as counter electrode, and an Ag/AgCl electrode as reference. All measurements were performed using an Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm, The Netherlands).

RESULTS AND DISCUSSION
Electropolymerisation of MR was achieved on glassy carbon (GC) electrodes by potential cycling in PB and BRB solutions at pHs 7.0 – 11.0. The catalytic effect of Cl⁻, NO₃⁻, and SO₄²⁻ anions onto electropolymerisation of MR were also investigated. Electrochemical characterization of poly-MR modified GC electrodes were carried out by CV in BRB and found that the process of the film was adsorption controlled. The best polymer film formation was obtained in BRB at pH 9.0+0.1 M KCl containing 0.5 mM MR monomer (Fig. 1). Quantitative determination of uric acid was achieved with poly-MR modified GC electrode by cyclic voltammetry (CV), differential pulse voltammetry (DPV), and fixed potential amperometry (AMP) in BRB at pH 5.0. The anodic peak current increased linearly with UA concentration ranging from 0.4 to 60 μM for CV, from 0.08 to 100 μM for DPV, and from 0.04 to 2.9 μM for AMP at 0.6 V. Detection limits were 3.80x10⁻², 9.17x10⁻³, and 1.78x10⁻³ μM for CV, DPV, and AMP, respectively. Interference study of UA was also carried out in the presence of ascorbic acid and dopamine in BRB at pH 5.0.

Fig. 1. Cyclic voltammogram of 0.5 mM methyl red in BRB at pH 9.0 + 0.1 M KCl. Scan rate 100 mV s⁻¹.

CONCLUSIONS
Electropolymerisation of methyl red has been achieved on GC electrodes by potential cycling. The electrochemical properties of the polymer have been investigated. The poly-MR modified electrodes exhibited good sensitivity, wide linear range, and good stability, showing that they can be used as sensors for uric acid as compared literature assay. Thus, poly-MR films can be used in electrochemical sensors and biosensors, to be addressed in future work.

REFERENCES
OP-19: THE CYTOTOXICITY PROFILE OF RESVERATROL IN HUMAN CERVICAL CANCER (HELA) AND CHINESE HAMSTER LUNG FIBROBLAST (V79) CELLS

H. Gül Göktaṣ¹-², M. Baçanlı¹, A.A. Başaran³, N. Başaran³

¹ Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100, Ankara, Turkey
² Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Adana, Turkey
³ Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100, Ankara, Turkey

INTRODUCTION

Plant derived phenolic compounds in human diet can be found in significant amounts in many fruits and vegetables. According to the data obtained from previous studies, it is known that vegetables and fruits rich in phenolic compounds and other plant derived foods (like tea and vine) could be protective against various diseases. It is suggested due to their cytotoxic effects on cancer cells, phenolic compounds have been found to have protective effects against cancer. Resveratrol is a polyphenolic compound, naturally found as an ingredient in many plant species including grapes, peanuts, mulberries. It has become an interesting and attracting subject for the research because of its role as a cancer preventive, cardioprotective and neuroprotective features[1].

Fig. 1. Chemical structure of trans-resveratrol

MATERIALS AND METHODS

In this study, the cytotoxic effects of resveratrol (0-400 μM) were evaluated in human cervical cancer (HeLa) and Chinese hamster lung fibroblast (V79) cells by neutral red uptake (NRU) and (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide) (MTT) assays after 24 h exposure. NRU assay was performed following the protocols described by Saquib et al. (2012) and MTT assay was performed by the method of Kuzma et al (2012)[2][3].

RESULTS AND DISCUSSION

In our study, resveratrol has been found to exert less cytotoxic effects both in HeLa and in V79 cells. The IC50 values cannot be calculated in studied concentrations (Fig. 2 and 3).

Figure 2. Effects of resveratrol on cell viability (%) to HeLa cells.

Figure 3. Effects of resveratrol on cell viability (%) to V79 cells.

Although resveratrol was shown to have cytotoxic effects on prostate carcinoma and melanoma cell lines, in our study both in cancer and in healthy cell lines at the studied concentration no cytotoxicity was observed by NRU and MTT assays[4][5].

CONCLUSIONS

Resveratrol was not found to be cytotoxic to V79 and HeLa cells. When comparing the cytotoxicity assays, MTT assay appears to be more sensitive in detecting loss of viability than NRU assay.

REFERENCES

2. Saquib Q.; Al-Khedhairy AA.; Siddiqui MA.; Abou-Tarboush FM.; Azam A., Musarrat, J., Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnionepithelial (WISH) cells. Toxicology in vitro, 2012, 26, 351-361.
INTRODUCTION

Lung cancer is an increasing worldwide public health problem particularly in men. It is the main cause of deaths arising from cancers in most countries. Majority of lung carcinomas have non-small cell lung carcinoma (NSCLC) histology and its prognosis is poor. Smoking and environmental carcinogens are well known risk factors in the development of lung cancer. However, 10% of smokers eventually suffer from lung cancer that addresses individual susceptibility. Possible cancer susceptibility genes have been sought among tumor suppressor genes such as TP53 and genes encoding phase I and phase II metabolizing enzymes.

Therefore, the aim of this study was to investigate the role of TP53, CYP2E1*5B, GSTM1, GSTT1, and GSTP1 polymorphisms as a genetic modifier of risk for NSCLC in a Turkish population.

MATERIALS AND METHODS

The study population was consisted of 172 NSCLC patients and 172 healthy controls. The TP53 (Arg72Pro) (rs1042522) gene polymorphism was determined by Real-Time PCR method modified from Talseth et al., (2006). CYP2E1*5B (c1/c2) (rs2031920) polymorphism was also determined by Real-Time PCR technique modified from Choi et al. (2003). The genetic polymorphism analyses for the GSTM1 and the GSTT1 genes were determined by multiplex PCR method described by Abdel-Rahman et al. (1996). GSTP1 exon 5 (Ile105Val) (rs1695) and GSTP1 exon 6 (Ala114Val) (rs138272) genetic polymorphism analyses were determined by PCR method described by Park et al. (1999). Multivariate logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals adjusted for age, gender, and smoking status between genotypes and NSCLC.

RESULTS AND DISCUSSION

Multivariate analyses showed a significant association between TP53 and GSTP1 exon 6 variant genotypes and NSCLC risk (OR 2.21, 95% CI 1.39-3.51; P= 0.001 and OR 1.92, 95% CI 1.01-3.65; P= 0.047 respectively). The combination of TP53 variant with GSTM1 null, GSTT1 null and GSTP1 exon 6 variant genotypes significantly increased the risk of developing NSCLC (OR 2.22, 95% CI 1.23-4.04; P= 0.009; OR 2.98, 95% CI 1.49-5.94; P= 0.002 and OR 3.38, 95% CI 1.54-7.41; P= 0.002 respectively). Combinations of GSTT1 null with GSTM1 null and GSTP1 exon 6 variant genotypes also increased the risk of developing NSCLC significantly (OR 2.04, 95% CI 1.01-4.14; P= 0.048 and OR 3.00, 95% CI 1.04-8.71; P= 0.043 respectively).

CONCLUSIONS

This study revealed that TP53 and GSTP1 exon 6 mutations alone, in combination with each other and GSTM1 and GSTT1 deletions; also GSTM1 and GSTT1 deletions together might be important in determining NSCLC susceptibility.

ACKNOWLEDGMENTS

This study was supported by the grants from Research Fund of Ankara University Nos: 2006-08-03-002HPD, 2008-08-03-006HPD and 10A3336002.

REFERENCES

1. Talseth, B.A.; Meldrum, C.; Suchy, J.; Kurzawski, G.; Lubinski, J.; Scott, R.J., Age of diagnosis of colorectal cancer in HNPCC patients is more complex than that predicted by R72P polymorphism in TP53. Int J Cancer 2006, 118(10), 2479-84
INTRODUCTION
Platelet response to clopidogrel, an antiplatelet agent used for the treatment of thromboembolic cardiovascular disorders shows high interindividual variability. The purpose of this study was to investigate the role of P-glycoprotein (P-gp) in modulating the intestinal permeability of clopidogrel.

MATERIALS AND METHODS

MATERIALS
Clopidogrel bisulfate, metoprolol tartrate and verapamil hydrochloride were kindly supplied from Deva Holding (Istanbul, Turkey), Novartis Pharma AG (Basel, Switzerland) and Abbott Laboratories (Istanbul, Turkey), respectively. Phenol red, CaCl₂, MgCl₂, NaH₂PO₄, D-glucose, MES, HEPES, KCl, NaCl and HPLC grade acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany).

METHODS

Single-Pass Intestinal Perfusion (SPIP) Studies
Effective permeability (Peff) of clopidogrel was investigated in the proximal jejunum and distal ileum of rats. The study protocol of animal experiments was approved by Gazi University Ethics Committee (G.U.ET-13.053). In situ SPIP was performed on male Wistar albino rats (250-300 g, Kobay Laboratories, Ankara, Turkey) according to previously published report [1]. Phenol red (0.5 mM) was added to the perfusion buffer as a nonabsorbable marker for water flux measurements. Metoprolol was used as a reference standard for high permeability. After 1 h steady state period, perfusion samples were taken in 10 min intervals for 1 h. The length of each perfused intestinal segment was measured at the end of the experiment. Samples were quantified by a validated UPLC method.

Data Analysis
The measured \( \frac{C_{\text{out}}}{C_{\text{in}}} \) ratio was corrected for water transport according to Eq. 1:

\[
\frac{C_{\text{out}}'}{C_{\text{in}}'} = \frac{C_{\text{out}}}{C_{\text{in}}} \times \frac{C_{\text{in phenol red}}}{C_{\text{out phenol red}}} \quad \text{Eq. 1}
\]

where \( C_{\text{in phenol red}} \) and \( C_{\text{out phenol red}} \) are equal to the concentrations of phenol red in the inlet and outlet samples, respectively.

Peff (cm/s) through the gut wall was determined using Eq. 2:

\[
P_{\text{eff}} = -\frac{Q}{2\pi RL} \ln \left( \frac{C_{\text{out}}'}{C_{\text{in}}'} \right) \quad \text{Eq. 2}
\]

where Q is the perfusion buffer flow rate (0.2 mL/min), \( C_{\text{out}}'/C_{\text{in}}' \) is the ratio of the outlet and inlet concentrations of drug that has been adjusted for water transport, R and L is the radius (0.2 cm) and length of the intestinal segment, respectively.

RESULTS AND DISCUSSION
Clopidogrel displayed a concentration-dependent but not site-dependent permeability in jejunum and ileum (Fig.1). Following the perfusion of 30 μM clopidogrel, Peff values were 3.0 and 1.4 times higher than those of metoprol in jejunum and ileum, respectively. Peff values of clopidogrel in the presence of a known P-gp inhibitor, verapamil is presented in Fig.2. Coperfusion with verapamil significantly enhanced intestinal Peff of clopidogrel.

CONCLUSIONS
This SPIP study shows that clopidogrel is a P-gp substrate. However, P-gp inhibition had no significant effect on the Peff of clopidogrel at high intestinal concentration of the drug, indicating that the inhibition appears not to be of clinical relevance in terms of intestinal absorption.

ACKNOWLEDGEMENTS
This study was supported by the research grant (113S514) from TUBITAK. The authors would like to thank Deva Holding (Istanbul, Turkey), Novartis Pharma AG (Basel, Switzerland) and Abbott.
Laboratories (Istanbul, Turkey) for kindly providing drug substances.

REFERENCE

OP-22: EFFECT OF RESIDUAL URINE IN BLADDER TO INTRAVESICALLY APPLIED CHITOSAN AND POLOXAMER GELS

1Z. Ay Şenyiğit, 2D.l. Özdemir, 3S.Y. Karavana, 2Ç. Çalışkan, 3C. Waldner, 3A. Bernkop-Schnürch, 4E. Baloğlu

Ege University, Faculty of Pharmacy, 1Department of Pharmaceutical Technology, 2Department of Radiopharmacy, 3ThioMatrix, 6020, Innsbruck, Austria

INTRODUCTION
Local administration of chemotherapeutic agents is commonly used for treatment of superficial bladder cancer. To achieve an effective treatment, sustained-retention delivery platforms such as bioadhesive particles or hydrogel systems could be used to extend the drug exposure in bladder beyond the voiding of urine [1]. Thence; in our previous studies, bioadhesive chitosan-thioglycolic acid (chitosan-TGA) nanoparticles of Gemcitabine HCl was prepared and suspended in bioadhesive chitosan gel or in situ Poloxamer (Plx) gel to increase the remaining time in bladder.

The maximum urine volume in bladder is generally 250 – 350 mL and due to incomplete emptying, a urine volume of nearly 50 mL generally residues even after voiding. Chemotherapeutic drugs are applied intravesically in catheterized patients by installation of 20-60 mL of formulations [1]. In this study, the properties of Plx and chitosan gel formulations were evaluated after the dilution with urine in a ratio of 1:1 to mimic the in vivo conditions in bladder.

MATERIALS AND METHODS

Preparation of Nanoparticles (NPs): The NPs were prepared by ionotropic gelation of chitosan-TGA with tripolyphosphate [2].

Preparation of Gels: For the preparation of chitosan gel, chitosan was dissolved in lactic acid solution. In situ Plx gel was prepared according to cold method. After the preparation of gels, Gemcitabine HCl loaded NPs were suspended in gels.

Dilution of Gels with Artificial Urine: NP loaded chitosan and Plx gels were diluted with Tyrode solution used as artificial urine in a ratio of 1:1 [3]. After the dilution of formulations; the gelation temperature and time, viscosity, mechanical properties, rheological properties, mucoadhesive properties of the formulations were determined [4].

RESULTS AND DISCUSSION
The gelation time and temperature of in situ Plx gel are important parameters for the in vivo behavior and they were determined firstly. The results showed that, the gelation time of NP + Plx gel and NP + Plx gel:Tyrode solution were 288±0.004 and 483.1±1.849 sec, respectively. The gelation temperature of NP + Plx gel and NP + Plx gel:Tyrode solution were 37.858±0.004 and 53.658±1.849°C, respectively. The results showed that both gelation time and temperature of Plx gels increased significantly with dilution of Tyrode solution. The results of rheological properties of the formulations were given in Table 1. The results of rheological evaluation were represented in Fig. 1 and 2.

Table 1. Viscosity and mechanical properties of the formulations at 37°C (±SD)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Viscosity (cP)</th>
<th>Hardness (N)</th>
<th>Adhesiveness (N.sec)</th>
<th>Compressibility (N.sec)</th>
<th>Cohesiveness (N)</th>
<th>Elasticity (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP + chitosan gel</td>
<td>736.33</td>
<td>2.51</td>
<td>0.01</td>
<td>0.05</td>
<td>1.06</td>
<td>0.94</td>
</tr>
<tr>
<td>NP + chitosan gel:Tyrode solution</td>
<td>131.33</td>
<td>1.52</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>1.03</td>
</tr>
<tr>
<td>NP + Plx gel</td>
<td>268.0</td>
<td>2.64</td>
<td>0.11</td>
<td>0.10</td>
<td>0.19</td>
<td>1.09</td>
</tr>
<tr>
<td>NP + Plx gel:Tyrode solution</td>
<td>35.0</td>
<td>0.26</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Table 2. The mucoadhesive properties of the formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mucoadhesion (mN.mm) ±SD</th>
<th>Detachment Force (mN) ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP + chitosan gel</td>
<td>1795.9 ± 42.7</td>
<td>1684.1 ± 123.6</td>
</tr>
<tr>
<td>NP + chitosan gel:Tyrode solution</td>
<td>1003.6 ± 48.1</td>
<td>462.1 ± 33.6</td>
</tr>
<tr>
<td>NP + Plx gel</td>
<td>1103.6 ± 81.0</td>
<td>975.5 ± 3.315</td>
</tr>
<tr>
<td>NP + Plx gel:Tyrode solution</td>
<td>378.2 ± 22.4</td>
<td>423.7 ± 48.509</td>
</tr>
</tbody>
</table>
**CONCLUSION**

Intravesically applied gels were diluted with Tyrode solution to mimic the in vivo conditions. The in vitro evaluation results of gels showed that all of the parameters are significantly affected in the presence of urine. Also, this alteration is more pronounced in Plx gels than chitosan gels. In addition; it should be suggested that before the intravesical administration of these formulations, no liquids should be taken by the patients at least 4 h before the treatment and bladder needs to be completely emptied.

**ACKNOWLEDGMENTS**

This study was supported by TUBITAK (112/S/293). The authors would like to thank to the T.R. Prime Ministry State Planning Organization Foundation (Project Number: 09/DPT/001).

**REFERENCES**


**OP-23: ELECTROCHEMICAL BEHAVIOR OF 1,4-DIHYDRO PYRIDINE DERIVATIVES**

H. Celik², Z. Ozçicek², S. Isık², M. S. Baymak³, E.T. Turkoz-Acar³, M. G. Gündüz³, E. Albayrak³, R. Şimşek³, C. Şafak³

¹Yeditepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, TURKEY
²Marmara University, Faculty of Arts and Sciences, Department of Chemistry, Istanbul, TURKEY
³Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

**INTRODUCTION**

Calcium ions play a versatile role in variety of biological functions and also in pathological processes such as muscle contraction, neuronal transmitter release, calcium dependent gene transcription, neuronal excitability regulation and even cell death [1]. Calcium entry blockers are the class of drugs that inhibit selectively the calcium influx through cell membranes. 1,4-Dihydro pyridines (DHPs) represent the most important group of calcium-channel modulating agents and are widely prescribed as treatments for cardiovascular diseases, particularly hypertension and angina pectoris [2]. Therefore, sixteen DHP derivatives have been newly synthesized. To evaluate their myorelaxant activities, the Eₘₐₓ and pD₂ values of the compounds and nifedipine were determined on isolated rabbit gastric fundus smooth muscle strips. The obtained results indicated that introduction of long chain alkyl groups such as 2-methoxyethyl or 2-(methacryloyloxy) ethyl moiety to the ester group led to the most active compounds. However, there is no information in the literature about the electrochemical reduction and oxidation mechanisms for these compounds. The purpose of this study is to elucidate the oxidation and reduction mechanisms at glassy carbon electrode (GCE) by cyclic voltammetry (CV) and the dropping mercury electrode (DME) by polarography for DHP derivatives in aqueous buffered solutions pH between 1 and 12. Based on experimental evidences, electrochemical behavior of DHPs has been investigated and both oxidation and reduction mechanisms have been proposed.

**MATERIALS AND METHODS**

Electrochemical current-voltage recordings were obtained by using a BIOLOGIC SP50
potentiostat/galvanostat controlled via EC-Lab V10.21. The working electrode was a glassy carbon electrode (GCE) with a surface area of 0.196 ± 0.005 cm². The auxiliary and reference electrodes were Pt wire and Ag/AgCl, respectively. Polarographic measurements were followed according to literature [3]. Buffer solutions within the pH 1.0 to 12.0 range were prepared from simple buffer systems.

RESULTS AND DISCUSSION

For every analytical method it is of importance to understand the nature of chemical and physical processes involved in the procedure used. Only when such processes and their sequence are understood it is namely possible to understand and perhaps predict the influence of other components in the studied matrix on analytical results. Investigation of electrochemical oxidation mechanism of sixteen DHP derivatives has been achieved by carrying out a pH dependence of the compound by cyclic voltammetry (CV) in common buffer solutions with pH values in the range of 1 to 12. DHP derivatives have shown well developed oxidation peaks in the studied pH values (See the figure). The peak current was controlled by diffusion. Investigation of reduction mechanism on DME for DHP derivatives has been achieved. The reduction mechanism proposed.

Figure. Cyclic voltamogram of 0.1 mM Compound 1 in pH 3.75 acetate buffer.

CONCLUSIONS

The number of electrons transferred in the oxidation and reduction processes were determined and redox mechanisms have been proposed based on the comparison with sixteen structurally related DHPs derivatives.

REFERENCES


OP-24: LEADERSHIP BEHAVIORS IN THE PHARMACEUTICAL SECTOR

B. Sözen Şahne1, S. Şar2

1Hacettepe University Faculty of Pharmacy Department of Management, Ankara, TURKEY
2Ankara University Faculty of Pharmacy Department of Management, Ankara, TURKEY

INTRODUCTION

Competition in the health sector is increased as a result of the raising awareness of public about maintaining a healthy life. It’s known that this competition is very high in the pharmaceutical sector. Companies in the pharmaceutical sector need managers who can provide a competitive advantage (1). In this study, it’s aimed to present the Leadership behaviors of managers in the Turkish Pharmaceutical Sector with the Leadership Behavior Description Questionnaire (LBDQ).

MATERIALS AND METHODS

A survey was made with the participation of 112 managers for determining the leadership characteristic of the managers in the Turkish Pharmaceutical Sector. After the factor analysis for assessing the scale, t-test was applied for two-level variables in order to understand whether the resultant factors differ according to some demographic information. ANOVA were utilized for more than two levels of variables. Pairwise comparisons of the results obtained by the ANOVA test were performed by the Tukey test.

RESULTS AND DISCUSSION

In accordance with the findings of the LBDQ, four factors “Behaviors related with supporting the subordinates”, “Behaviors related with communication with subordinates”, “Behaviors related with recognition” and “Behaviors related with establishing the authority” put forward the consideration and initiations dimensions. The majority of the participants were pharmacists. However there is no statistically difference between the professions in terms of the LBDQ. It is known that leadership and training are intertwined concepts (2). So the responses that study participants give to the LBDQ were compared according to whether they have received leadership training. However, it was determined that whether they received training didn’t cause statistically difference in terms of the resultant factors. It is thought that the contents of the training won’t be the cause of differences in leadership behaviors.
CONCLUSIONS

Adopting effective leadership forms has great importance in health-related organizations. It is thought that pharmacist in pharmaceutical sector can be effective about the accurate and effective health policies. Therefore, it is evaluated that the addition of lecturers about leadership on the curriculum and expansion of the existing lecturers could be useful in terms of development of the leadership behaviors. Currently, it is known that it is possible to take consulting services from firms operating professionally in this area for people working in these positions for the development of leadership behavior. It is thought that leadership behavior develops by effective training it will be possible to improve the health legislation and practices.

REFERENCES


OP-25: PROVIDING PATIENT CONSULTANCY IN COMMUNITY PHARMACY: A LITERATURE REVIEW AND QUALITATIVE STUDY IN ANKARA

Z. Çalgan, S. Yeşenoglu

Hacettepe University, Faculty of Pharmacy, Department of Pharmacy Management

INTRODUCTION

Community pharmacists have an important role in patients’ access to medicines and pharmaceutical services (1). However, pharmacists have obstacles in providing professional services. The aim of this study is to examine current situation of pharmacy services in Ankara and compare it to the community pharmacy services in other countries.

MATERIALS AND METHODS

In this study, 20 in-depth interviews were performed with community pharmacists in Ankara and literature on pharmacy services in different countries is reviewed. In addition, an interview with the international award-winner pharmacist in Ankara was conducted to learn more about the ways to provide pharmacy services in Turkey.

RESULTS AND DISCUSSION

Pharmacists interviewed stated that they spend most of their time for explaining reimbursement rules and medical examination fee, medication distribution and business management instead of providing patient consultancy. Pharmacists in other countries also stated that the main driver of pharmacy systems is economic considerations, and time needed for counseling on medicine use is taken by advice giving on reimbursement (2).

Similarly, provision of pharmaceutical care is limited across Europe. Pharmacists conduct routine activities like patient record screening; however, they perform patient-centered professional activities such as implementation of therapeutic objectives and monitoring plans infrequently (3).

Although workload is an important barrier for providing health care services in pharmacy, inadequacy in professional knowledge and self-confidence of pharmacists are important handicaps to pharmacy care as well. Turkish pharmacists who attended the clinical pharmacy and pharmaceutical care continuing education programs point out the lack of clinical knowledge of disease states and technical knowledge on how to provide pharmaceutical care as the primary barriers to providing pharmaceutical care in Turkey (4).

On the other hand, good examples show that it is possible to overcome both lack of knowledge on pharmaceutical care and market pressure and workload in pharmacy. The award-winner community pharmacist in Ankara shares her experience in building human resource capacity of pharmacy personnel and creating a management system to delegate pharmacist’s overload (5).

CONCLUSIONS

Pharmacy workload is seen as a main barrier for providing consultancy activities both in Turkey and in other countries. However, insufficiency of pharmacists’ professional knowledge and self-confidence are also important barriers to providing health care. Pharmacists could overcome challenging conditions in their work life by creating innovative solutions and improve their professional capacity in order to provide health care services in their pharmacy.

ACKNOWLEDGMENTS

Authors thank to community pharmacists who participated to the in-depth interviews.

REFERENCES


OP-26: ASSESSMENT OF THE EPILEPSY KNOWLEDGE OF EPILEPTIC PATIENTS, NON-EPILEPTIC PATIENTS AND COMMUNITY PHARMACISTS

C. Macit 1, M. Macit2, C. Aykut Bingol2, P. M. Clark1
1 Yeditepe University, Faculty of Pharmacy, Department of Clinical Pharmacy, Istanbul, TURKEY
2 Yeditepe University, Faculty of Medicine, Department of Neurology, Istanbul, TURKEY
3 Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Istanbul, TURKEY

INTRODUCTION
Epilepsy is a chronic condition characterized by recurrent unprovoked epileptic seizures. Epilepsy has the greatest incidence in young children and the elderly [1]. In treatment, variety of antiepileptic drugs are used according to the seizure type [2, 3]. Epileptics meet lots of difficulties like misunderstanding, exclusion and limitation of social life due to having insufficient information about epilepsy [4]. The objective of study is to measure epilepsy knowledge of the epileptic patients, community pharmacists and other patients.

MATERIALS AND METHODS
This study was performed on 13 pharmacies in Atasehir, Istanbul. Besides owner of the pharmacies, epileptic and non-epileptic patients who came to these pharmacies were included into the study. The data of the study was collected via 23 questioned survey that performed during 6 months. The questions asked in survey are related with social life, nutrition, sleep patterns, drugs that are used and fertility of the epileptic patients. Answers of the many questions were accepted as valid. 47 of all participants were epileptics and remaining 172 were non-epileptics. All collected information was recorded as excel file and then transferred to SPSS program version 18 in order to assess.

RESULTS AND DISCUSSION
According to the results, community pharmacists, non-epileptic patients and epileptic patients do not have enough knowledge about epilepsy. However, compared with non-epileptics, epileptics have more knowledge, especially about their social problems as shown in Table 1. Additionally, it is observed that female participants have more knowledge about questions related with their selves.

Table 1. Knowledge comparison between epileptics and non-epileptics on social life and psychological problems

<table>
<thead>
<tr>
<th></th>
<th>PSYCHO-SOCIAL PROBLEMS</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES</td>
<td>NO</td>
<td>NO IDEA</td>
<td>Total</td>
</tr>
<tr>
<td>EPILEPTIC</td>
<td>Count</td>
<td>35</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Expected Count</td>
<td>25.3</td>
<td>13.9</td>
<td>7.7</td>
<td>47.0</td>
</tr>
<tr>
<td>% within Q1</td>
<td>74.5%</td>
<td>19.1%</td>
<td>6.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Q9</td>
<td>29.7%</td>
<td>13.8%</td>
<td>8.3%</td>
<td>21.5%</td>
</tr>
<tr>
<td>NON EPILEPTIC</td>
<td>Count</td>
<td>83</td>
<td>56</td>
<td>33</td>
</tr>
<tr>
<td>Expected Count</td>
<td>92.7</td>
<td>51.1</td>
<td>28.3</td>
<td>172.0</td>
</tr>
<tr>
<td>% within Q1</td>
<td>48.3%</td>
<td>32.6%</td>
<td>19.2%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Q9</td>
<td>70.3%</td>
<td>86.2%</td>
<td>91.7%</td>
<td>78.5%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>Count</td>
<td>118</td>
<td>65</td>
<td>36</td>
</tr>
<tr>
<td>Expected Count</td>
<td>118.0</td>
<td>65.0</td>
<td>36.0</td>
<td>219.0</td>
</tr>
<tr>
<td>% within Q1</td>
<td>53.9%</td>
<td>29.7%</td>
<td>16.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Q9</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

χ²: 10.624, p: 0.005

CONCLUSIONS
As a conclusion; it is revealed that non-epileptic patients and pharmacists who possess an important mission that providing first degree health counselling to the public, must be educated about epilepsy disease, especially about the social problems of the epileptic subjects.

ACKNOWLEDGMENTS
I thank to all 13 pharmacists in Atasehir for their support to this research.

REFERENCES
OP-27: CYTOTOXICITY OF THE DIETARY FLAVONOID, APIGENIN, AS DETERMINED BY PHARMACOGENOMICS AND MOLECULAR DOCKING

M. Saeed1, O. Kadioglu1, H. Khalid2, Y. Sugimoto3, T. Efferth1

1Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Pharmaceutical Biology Department, Mainz, Germany
2The Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research, Khartoum, Sudan
3Division of Chemotherapy, Faculty of Pharmacy, Keio University, Tokyo, Japan

INTRODUCTION

Apigenin is a natural flavonoid found in many fruits or vegetables, including French peas, snake gourd, garlic, Chinese cabbage, bell pepper, bilimbi fruit, guava, wolfberry leaves, local celery, kamok and swiss chard [1, 2]. Apigenin also occurs in medicinal plants, such as Salvia officinalis (Lamiaceae) [3], Lawsonia inermis (Lythraceae) [4], Turnera aphrodisiaca (Turneraceae) [5], Ocimum basilicum (Lamiaceae) and Tamarindus indica (Fabaceae) [6]. Interestingly, apigenin also occurs in post-harvested chamomile flowers (Matricaria chamomilla) as a result of enzymatic degradation of apigenin-7-glycoside [7].

Apigenin has a considerable cytotoxic activity in vitro and in vivo. Despite many mechanistic studies on its mode of action, less is known about factors that may hamper apigenin’s activity towards cancer cells.

MATERIALS AND METHODS

Cytotoxicity assays. The resazurin assay was performed to determine the cytotoxicity of apigenin towards drug-sensitive and -resistant cell lines. Flow cytometry. The uptake of doxorubicin on cell lines overexpress both ABC transporters (P-gp and BCRP) with and without addition of apigenin was measured by flow cytometry. The assay was carried using BD FACSCalibur™ and CellQuest Pro software (Beckton Dickinson, GmbH, Heidelberg, Germany).

Synergism and antagonism assay. The assay is based on Bliss independence model, predicted fractional inhibition (YP) was calculated for drug combinations according to equation (YP = YDrug_1 + YDrug_2 - YDrug_1 * YDrug_2).

Molecular docking and modelling. Both structure of P-glycoprotein/ABCB1 and ABCB5 structure were predicted by Protein Homology analogY Recognition Engine V 2.0 (PHYRE2). The affinity of apigenin to homology models of P-glycoprotein/ABCB1 and ABCB5 was studied by molecular docking using Autodock4. The whole protein and domains sequences of mouse P-glycoprotein, human P-glycoprotein/ABCB1 and human ABCB5 were obtained from UniProt, ClustalW2 is multiple sequence alignment program for proteins, was used to align proteins sequences and to determine the identity of predicted proteins (human P-glycoprotein/ABCB1 and human ABCB5) with known structure protein (mouse P-glycoprotein).

Bioinformatical methods. COMPARE analyses were performed for a transcriptome-wide search for correlations between gene expressions and apigenin response (IC50 values). Agglomerative hierarchical cluster analysis was done to cluster cell lines according to their mRNA expression of genes identified by COMPARE analysis.

RESULTS AND DISCUSSION

Apigenin inhibited not only P-gp but BCRP as well by increasing cellular uptake of doxorubicin and synergistically work with established anticancer drugs towards multidrug-resistance tumors. This indicates that apigenin might return sensitivity to refractory and multidrug-resistant tumors potentially leading to improved treatment outcome in patients.

Apigenin binds to P-glycoprotein and ABCB5 at ATP binding domain without being transported. This binding disturbs the outward transport of substrates leading to increased intracellular accumulation of anticancer drugs and improved tumor cell killing. The highest degrees of protein sequence homology were found in the nucleotide binding domains (ATP binding site) of murine ABCB1, human P-glycoprotein/ABCB1 and human ABCB5 (89-100%). This indicates that the binding and cleavage of ATP is of crucial importance for the function of ABC transporters.

classical multidrug resistance phenotypes conferred by ABC transporters did not hamper apigenin’s cytotoxicity towards tumor cells, whereas a number of genes with diverse functions point to a multifactorial determination of responsiveness of tumor cells to apigenin.

CONCLUSIONS

In conclusion, apigenin’s activity is not hampered by classical mechanisms of multidrug resistance and the inhibition of ABC transporters by apigenin indicates that apigenin may overcome multidrug resistance in otherwise refractory tumors.

REFERENCES

**OP-28: IN VITRO ANTIOXIDANT EFFECT OF RIBES L. SPECIES IN TURKEY**

G. Kendir, A. Köroğlu

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoğan, Ankara-TURKEY

**INTRODUCTION**

Ribes genus (Grossulariaceae) including about 200 species in the world which is shrubs, usually evergreen, sometimes deciduous. It is represented by eight species in Turkey. Seven of the species growing wild in Turkey [R. biebersteinii, R. nigrum L., R. uva-cripa L., R. alpinum L., R. orientale Desf., R. multiflorum Kit. ex Romer &Schultes (rare), R. anatolica Behçet (endemic)] and one of the species commonly cultured (R. rubrum L.). These species usually are known as “Frenk üzümü”, widespread in northeastern Anatolia [1-4]. Leaves of R. nigrum, R. rubrum and R. orientale species have been used as diuretic and diaphoretic in folk medicine of Turkey [5].

**MATERIALS AND METHODS**

The phenolic contents and antioxidant activities of different extracts prepared with different polarities of solvents from the leaf and branch of Ribes species. The phenolic contents of the samples were determined using Folin-Ciocalteu's phenol reagent. Antioxidant activities of the extracts were studied by qualitative using DPPH* (1,1-diphenyl-2-picrylhydrazyl radical) to detect the free radical scavenging activity and thiobarbituric acid (TBA) assays for determining the amount of liposome lipid peroxidation.

**RESULTS AND DISCUSSION**

The highest total phenolic content were determined in ethyl acetate extracts of the leaves in the ranging from 1067.19 to 345.31 mg/g of dry weight expressed as gallic acid equivalents. All extracts showed a strong antioxidant activity with both DPPH* test. The highest activity was observed in the water extracts of leaf and branch (0.30 and 0.44 µg/mL, respectively) of R. orientale when compared to the other plant extracts in the TBA test.

**CONCLUSIONS**

Ribes orientale leaf and branch of water extracts showed good antioxidant activity on both in vitro assays.

**ACKNOWLEDGMENTS**

The financial support of the research fund of Ankara University (Project Number 09B3336001) is gratefully acknowledged.

**REFERENCES**


**OP-29: WHEATGRASS PHYTOTHERAPY OF PB INDUCED TOXICITY IN MALE WISTAR RAT**

C. Abdennour1 and O. Mansouri2

1Faculty of Medicine, Laboratory of Animal Ecophysiology, Department of Biology, University of Annaba, Annaba 23000, Algeria

The aim of this study is to search for an effective treatment to reduce Pb toxicity by using wheatgrass Triticum durum. Rats were divided into 3 groups; the control, the group exposed to a diet containing 600 mg Pb acetate/Kg diet (Pb), and the group received a combination of Pb and 7g wheatgrass /100g diet (Pb-WG). After a period of 6 weeks, some biological markers were evaluated. Results showed that Pb group has decreased the concentration of serum hormone T3 compared to the control. The supplementation of wheatgrass caused a considerable increase in the levels of this hormone. The TSH level has been affected deeply by the two treatments. The activity of AST and ALT were significantly increased in rats treated with Pb compared to the control. In the Pb-WG group only the AST activity was higher compared to the control. The Pb treated animals has enhanced the level of urea, but it decreased the concentration of calcium. On the
other hand, the presence of Pb-WG has kept the concentration of urea, calcium almost as that of the control. Cholesterol level has not been affected by Pb, but it was lowered by wheatgrass. In the Pb group, the levels of sperm concentration and motility were lower compared to the control, while no noticeable difference was recorded between the pb-WG group and the control. The histological architecture confirms such variations. Thyroid histological profile showed shrinking of follicles exposed to Pb, but that of Pb-WG was much better and close to the control. To conclude, the presence of wheatgrass in the daily diet of rat has reduced the risk of pb intoxication.

OP-30: IRON SUPPLEMENTATION INCREASES OXIDATIVE STRESS IN NON-ANAEMIC PREGNANT WOMEN

A. F. Ceylan-Isik1,2*, O. B. Tulmac3, Y. Y. Yucel4, A. Yesilada5

Departments of Medical Pharmacology1 and Gynecology3, School of Medicine, Kirikkale University, Kirikkale, TURKEY
Departments of Pharmacology3, Biochemistry4 and Basic Pharmaceutical Sciences5, School of Pharmacy, Istanbul Kemerburgaz University, Istanbul, TURKEY

INTRODUCTION

Universal prenatal daily supplementation with iron plus folic acid prevents anaemia where iron deficiency is prevalent [1]. On the other hand, excessive iron consumption during pregnancy can lead to increased oxidative stress in maternal body which may result in negative consequences [2,3]. Excessive oxidative stress initiates NADPH oxidase-mediated harmful effects which endothelial dysfunction is the most abundant one of them all. Although relationship between excessive iron consumption and oxidative stress was documented, role of NADPH in this process has not been clarified yet. The aim of the present study is to determine oxidative stress and potential role of NADPH in this process in anaemic and non-anaemic pregnant women.

MATERIALS AND METHODS

The study participants were 60 pregnant women (20-30 years) who were taking iron plus folic acid supplementation. Diagnosis of anaemia was set according to haemoglobin (Hb) levels. While Hb≤11.5 g/dl was accepted as anaemic, Hb>11.5 g/dl was accepted as non-anaemic pregnant. The treatment was started in the first trimester of the pregnancies and continued for 12 weeks. Some of the pregnant women, who were non-anaemic, did not take the supplementation (control group). Plasma samples from pregnant women were used in order to determine concentration of malondialdehyde (MDA) which is the end product of lipid peroxidation and activities of key antioxidant scavenging enzymes (catalase, CAT and superoxide dismutase, SOD). NADPH p47 and gp91phox, indicator of increased oxidative stress, was also assessed by Western Blot analysis.

RESULTS AND DISCUSSION

We found that, the daily supplementation of iron plus folic acid, prevented anaemia in non-anaemic pregnant women. On the other hand, plasma MDA concentration and CAT activity increased by the treatment in this group compared to the control group. The treatment also prevented anaemic pregnant women from severe anaemia which has also beneficial effect on oxidant/antioxidant status of plasma. MDA concentration and CAT activity were comparable in anaemic pregnant and control pregnant women. SOD activities were comparable among the groups. Western blot analysis showed that NADPH subunits,p47 and gp91phox, increased in non-anaemic treated group compared to control group. p47 and gp91phox were comparable in anaemic pregnant and control pregnant women.

CONCLUSIONS

This study demonstrates that without anaemia, iron consumption during pregnancy, may induce oxidative stress while it prevents from anaemia. Iron supplementation not only had beneficial effect on anaemic state but also ameliorated oxidant/antioxidant status of plasma in anaemic pregnant women.

ACKNOWLEDGEMENTS

This work was supported by Istanbul Kemerburgaz University Research Project Fund.

REFERENCES


OP-31: ANTIMICROBIAL ACTIVITY OF SOME ALNUS SPECIES

Ç. Altnay1, M. Eryılmaz2, A.N. Yazgan3, B. Sever Yılmaz2, M.L. Altun3

1The Ministry of Health of Turkey, Turkish Medicines and Medical Devices Agency, 2Ankara University, Faculty of Pharmacy Department of Pharmaceutical Microbiology, 3Department of Pharmacognosy, Ankara, TURKEY
INTRODUCTION
The genus *Alnus* Miller, belongs to Betulaceae family, consists of about 35 species and is distributed in temperate regions of the northern hemisphere. There are six taxones of *Alnus* species growing wild in Turkey. The plants of this genus contain various types of plant secondary metabolites predominantly diarylheptanoids, flavonoids, terpenoids, phenols, steroids and tannins. In folk medicine they are used for the treatment of rheumatism, uterus cancer, hemorrhoids, dental abscesses, nail inflammation, various skin diseases and also for wound healing [1, 2]. The objective of this study was to evaluate the in vitro antimicrobial activities of leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens* against some bacteria and yeast.

MATERIALS AND METHODS

In vitro antibacterial and antifungal activities of *Alnus* Species

Aqueous and ethanolic leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens* were screened for their potential in vitro antibacterial activities against *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (MRSA), *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and antifungal activities against *Candida albicans* ATCC 10231. Prior to testing all extracts were filter-sterilized through a 0.45 µm membrane filter. Broth dilution assay was used for determination of the minimum inhibitory concentrations (MIC) [3, 4].

RESULTS AND DISCUSSION

Ethanolic extracts of tested species exhibited better antimicrobial activity than aqueous extracts. Ethanolic extracts of tested species possessed activity having MIC values of 0.125-0.250 mg/ml against the tested microorganisms. No antibacterial activity was observed against *B. subtilis*, *E. coli*, *P. aeruginosa* for all the aqueous extracts. The MIC values of aqueous extracts were 1.000 mg/ml against both *S. aureus* strains and *C. albicans*.

CONCLUSIONS

In conclusion, this study provides significant information about antimicrobial activities of leaf extracts of *A. glutinosa* subsp. *glutinosa*, *A. orientalis* var. *orientalis*, *A. orientalis* var. *pubescens*. It is conceivable that one of the reasons for the usage of *A. glutinosa* in treatment of wound healing in folk medicine is because of its antimicrobial activity. To our knowledge, this is the first investigation on the evaluation of antimicrobial activities on aqueous and ethanolic leaf extracts of these species.

REFERENCES


OP-32: RHAMNOLIPID PRODUCTION BY PSEUDOMONAS SPP. IN PULPS

B. Kaskatepe1, S. Yildiz1, M. Gumustas2, S.A. Ozkan2

Ankara University, Faculty of Pharmacy, 1Department of Pharmaceutical Microbiology, 2Department of Analytical Chemistry, Ankara, TURKEY

INTRODUCTION

Biosurfactants are microbial origin surfactants produced by bacteria, yeast and fungi. In recent years, the studies directed to improve the production has increased to meet growing needs of raw material in many areas where biosurfactants can be used. Rhamnolipid is a biosurfactant produced by *Pseudomonas spp.* and has a strong potential to be used in industries and bioremediation. Although interest for biosurfactants is increasing, they are not economically competitive with their synthetic counterparts. Obtaining maximum yield with a low cost substrate is significantly important in biotechnological processes. The aim of this study was to isolate highly productive rhamnolipid producer *Pseudomonas spp.* strains and also try to increase the rhamnolipid production of these strains by formulating media using pulps of barley, hazelnut and sunflower.

MATERIALS AND METHODS

Isolation and identification of bacteria

*Pseudomonas* strains used in this study were isolated from water and soil samples taken from different regions in Turkey. Isolated bacteria were identified with conventional methods as *Pseudomonas spp.* (morphologically and biochemically), two of these strains determined as rhamnolipid producer identified using a 16S rRNA gene sequencing analysis according to standard protocol. The 16S rRNA gene was amplified from the genomic DNA by a PCR using the forward primer 5'- AGAGTTTGATCCTGACTCA- 3’ and reverse primer 5'-TACGGTTACCTTGTACACTT- 3’. The 16S rRNA gene sequence for first isolate showed
the highest similarity (99%) to that of *Pseudomonas putida* and also the 16S rRNA gene sequence for second isolate was nearly identical (99%) to that of the type *Pseudomonas pachastrellae*. *P.putida* and *P.pachastrellae* isolated from environmental samples and *P. aeruginosa* ATCC 9027 strain known rhamnolipid producer were used for rhamnolipid production in this study.

**Rhamnolipid production, purification and quantification:** The production of rhamnolipid by the strains was determined by using Mineral Salt Medium (MSM), recommended by Zhang et al. [1]. For this purpose media formulated by using pulp of barley, hazelnut and sunflower were prepared by adding water in a different ratio depend on water absorption capacity and kept at 4 °C for overnight. Next day, The mixture was filtered, and filtrate was enhanced by adding 0.5 g NaCl and 1 mL trace element solution and then autoclaved at 121 °C for 15 min. Bacterial suspensions were adjusted to McFarland 2. And were inoculated into media at the ratio of 1/20. Bacterial cultivation was performed in 500 mL flask containing 200 mL medium at 35 °C, stirred in an orbital rotary shaker (STIR) at 150 rpm for 7 days. The obtained rhamnolipids were purified and quantified according to Dubois et al. [2].

**Statistical Analyses**
Statistical significance was assessed by one way ANOVA using SPSS for Windows (Version 17.0) packet program. The level of significance was defined at p<0.05.

**RESULTS AND DISCUSSION**
The quantities of rhamnolipid obtained in the study are given in table 1 and 2.

**Table 1.** The rhamnolipid concentration in barley pulp (g/l)

<table>
<thead>
<tr>
<th>Strains</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4±0.4</td>
<td>9.3±0.05</td>
<td>6.3±1</td>
<td>5±0.9</td>
</tr>
<tr>
<td>2</td>
<td>2.6±0.2</td>
<td>9.2±0.2</td>
<td>3.4±0.01</td>
<td>4.3±0.3</td>
</tr>
<tr>
<td>3</td>
<td>2.4±0.2</td>
<td>2.5±2.1</td>
<td>3.7±0.2</td>
<td>3.7±0.2</td>
</tr>
</tbody>
</table>

1.*P. aeruginosa* ATCC 9027, 2:*P. pachastrellae*, 3:*P. putida* A: basic medium with barley pulp, B: A medium +glycerol, C: A medium+brews’ yeast, D: A medium+NaNO₃

When the results were statistically evaluated, rhamnolipid amounts obtained in B (p=0.000) and C (p=0.036) media were found statistically different compare to MSM media. The results in B medium (p = 0.000) were statistically significant higher than in other media (A, C and D).

The increase in rhamnolipid obtained in medium prepared with using hazelnut pulp compared with MSM is statistically significant for all strains (*P.aeruginosa* ATCC 9027 p=0.003, *P. pachastrellae* p = 0.035, *P. putida* p = 0.003). There was no statistically significant difference between the amounts of rhamnolipids in MSM and sunflower pulp for *P. aeruginosa* ATCC 9027 (p = 0.121). The increase for *P. pachastrellae* and *P. putida* was found statistically significant and p values were p = 0.049 and p = 0.010 respectively.

**Table 2.** The rhamnolipid concentration in hazelnut and sunflower pulp (g/l)

<table>
<thead>
<tr>
<th>Strains</th>
<th>Hazelnut pulp (g/l)</th>
<th>Sunflower pulp (g/l)</th>
<th>MSM (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em> ATCC 9027</td>
<td>11.1±1</td>
<td>5.3±0.3</td>
<td>1.5±0.04</td>
</tr>
<tr>
<td><em>P. pachastrellae</em></td>
<td>5.4±0.2</td>
<td>5±0.6</td>
<td>3.6±0.03</td>
</tr>
<tr>
<td><em>P. putida</em></td>
<td>8.5±1.2</td>
<td>6.7±1</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**
When the rhamnolipid quantities obtained in this work compare with the studies in the literature, especially rhamnolipid achieved in the medium formulated with sunflower and hazelnut pulps were higher than the rhamnolipid obtained in the medium prepared by adding oil of the same substances in other studies. Therefore, these pulps for being produced from waste and being low cost, can be a more suitable source for rhamnolipid production.

**REFERENCES**
1. Zhang G.; Wu Y.; Qian X.; Meng Q., Biodegradation of crude oil by *Pseudomonas aeruginosa* in the presence of rhamnolipids. J. Zhejiang Univ. 2005,8,725-730

**OP-33: CHNQ EXERTS ANTITUMOR ACTIVITY AGAINST COLORECTAL CANCER THROUGH THE INDUCTION OF APOPTOSIS AND OXIDATIVE STRESS**

S. Enayat*1, M.Ş. Ceyhan*1, Ç.D. Son1, M. Ştefek2, S. Banerjee1

1METU Department of Biological Sciences, Ankara, TURKEY2Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia. * Equal contribution, § Corresponding author banerjee@metu.edu.tr

1METU Department of Biological Sciences, Ankara, TURKEY2Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia
Phytochemicals such as quercetin (Qc) have poor bioavailability; synthetic derivatives with better solubility have therefore been designed. Here we describe the effects of CHNQ, a novel synthetic Qc derivative, on the induction of oxidative stress, autophagy and the related signal transduction pathways in colorectal cancer cell lines.

INTRODUCTION
Clinical application of well known phytochemical reagents such as quercetin (Qc) is limited due to poor water solubility and bioavailability. In a screening of novel semi-synthetic derivatives of Qc, 3,7-dihydroxy-2-[4-(2-chloro-1,4-naphthoquinone-3-yloxy)-3-hydroxyphenyl]-5-hydroxychromen-4-one (CHNQ) was the most promising in terms of biological efficacy[1]. Since chronic inflammation very significantly contributes towards neoplastic transformation, we have hypothesized that CHNQ may also have potential as an anti-inflammatory, anti-cancer agent.

MATERIALS AND METHODS
Using colon cancer cell lines HCT-116 and HT-29, we compared the effect of CHNQ and Qc on cellular proliferation, cytotoxicity, and apoptosis. Cell proliferation and cytotoxicity was assayed by BrdU incorporation and Muse cell viability assays. Apoptosis was examined by Annexin V staining and flow cytometry, multi caspase activity assay and the expression of pro- and anti-apoptotic proteins using immunoblotting. Cell cycle was analyzed using PI staining and flow cytometry. Detailed functional analyses were carried out to determine the effect of CHNQ treatment on cellular oxidative stress, cell cycle, apoptosis, autophagy and the associated signal transduction pathways. The pro- or antioxidant effect of CHNQ was examined by dihydroethidium staining analyzed by flow cytometry and NBT colorimetric assay. The expression of stress related proteins was determined by western blotting. The induction of autophagy was examined by acridine orange staining and Cyto-ID staining and flow cytometry and confocal microscopy; autophagy related proteins were determined by western blot.

RESULTS AND DISCUSSION
CHNQ treatment strongly inhibited cell proliferation along with a more efficient and robust induction of apoptosis compared to Qc. In addition, treatment of cells with CHNQ resulted in the increased superoxide production and ROS formation followed by oxidative stress leading to cell cycle arrest at G2/M. This was accompanied by the increased phosphorylation of MAP Kinases including; ERK1/2, p38 and JNK and decreased phosphorylation of Akt/PKB. Interestingly, HCT-116 cells treated with CHNQ resulted in a dramatic increase in oxidative stress related autophagy as shown by increased expression and conversion of LC-3-I to LC-3II, acidic vesicle accumulation and microscopy imaging of LC-3 puncta formation. However, CHNQ treatment induced only accumulation and conversion of LC-3-I to LC-3II in HT-29 cells; acidic vesicle formation was inhibited. Moreover, simultaneous treatment of cells with CHNQ and N-acetyl cysteine, a well-known antioxidant, led to inhibition of ROS formation and autophagy induction suggesting that oxidative stress is upstream and a leading cause of autophagy.

CONCLUSIONS
Overall, we propose that CHNQ, a semi-synthetic derivative of Qc, induces cancer cell death through the induction of oxidative stress and apoptosis.

ACKNOWLEDGMENTS
This project is supported by TUBITAK-SAS Bilateral Collaboration Program (113S006).

REFERENCES

OP-34: CYTOKINE PROFILES OF LYMPH NODE CELL CULTURES OF IRRITANT AND ALLERGEN EXPOSED MICE GROUPS
O. Cemiloglu Ulker1, S. Arancioglu2, A. Karakaya1
1Ankara University Faculty of Pharmacy, Department of Toxicology,Ankara, Turkey
2Kulu State Hospital, Kulu, Konya, Turkey

INTRODUCTION
In recent years, in the industrialized countries, the incidence of skin toxicity has a tendency to increase in parallel to the increase in the number of potential chemicals. The two most frequent manifestations of skin toxicity are irritant contact dermatitis and allergic contact dermatitis1. In our study we aimed to distinguish the In this study, we aimed to compare the differences between irritation and sensitization potency of some chemicals in terms of the cytokine profiles.

MATERIALS AND METHODS
Mice
Female Balb/c mice (8-12 weeks of age) were used.

Chemicals
Sodium lauryl sulfate (Prolab, Belgium), croton oil (Sigma-Aldrich, MO, USA), DNCB (Sigma-Aldrich,
MO, USA), isoeugenol (Acros Organics, NJ, USA),
eugenol (Acros Organics, NJ, USA), and
paraaminobenzoic acid (PABA) (Sigma-Aldrich,
MO, USA) in acetone:olive oil (AOO; 4:1 v/v;
Sigma-Aldrich, MO, USA) were applied to the animals in these studies.

Culture of Lymph Node Cells and the Measurement of Cytokine Levels

Harvested lymph node cells from the ex vivo protocols outlined previously were seeded in a 24-well culture plate in 1 mL of RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin. Following the seeding steps, the wells were supplemented with 5 mg/mL of phytohemagglutinin-L (PHA-L; Biochrom) immediately. After 72 hours of culture in a 37°C incubator containing 5% CO2, supernatants were collected and stored at -80°C until analyzed for levels of IL-2, IFN-g, IL-4, IL-5, and TNF-a. The levels of each cytokine in the culture supernatants were measured using commercially available ELISA kits (BenderMed Systems), according to the manufacturer’s instructions. The SI was calculated as the ratio of the

RESULTS AND DISCUSSION

Table 1. Cytokine Stimulation Index (SI) Values for Lymph Node Cells after Treatment of Mice With Different Concentrations of Chemicals in Acetone:Olive Oil (AOO; 4:1 v/v).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration, w/v%</th>
<th>IL-2</th>
<th>IFN-g</th>
<th>IL-4</th>
<th>IL-5</th>
<th>TNF-a</th>
<th>IL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNCB</td>
<td>0.025</td>
<td>1.6</td>
<td>1.98</td>
<td>2.44</td>
<td>1.19</td>
<td>2.54</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>3.12</td>
<td>4.53</td>
<td>3.31</td>
<td>1.47</td>
<td>1.83</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4.96</td>
<td>5.22</td>
<td>3.90</td>
<td>1.62</td>
<td>3.94</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>5.23</td>
<td>6.09b</td>
<td>7.61</td>
<td>2.99</td>
<td>4.60</td>
<td>1.46</td>
</tr>
<tr>
<td>Eugenol</td>
<td>2.6</td>
<td>1.6</td>
<td>2.14</td>
<td>2.30</td>
<td>2.06</td>
<td>4.19b</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.75</td>
<td>2.24</td>
<td>2.32</td>
<td>2.25</td>
<td>2.3</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.78</td>
<td>3.12</td>
<td>2.76</td>
<td>3.53b</td>
<td>4.43</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.32</td>
<td>3.24</td>
<td>10.10</td>
<td>4.51b</td>
<td>1.56</td>
<td>1.23</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>0.5</td>
<td>1.5</td>
<td>1.63</td>
<td>1.89</td>
<td>1.97</td>
<td>6.9b</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.65</td>
<td>4.10</td>
<td>2.71</td>
<td>3.58</td>
<td>1.15</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.12</td>
<td>2.06</td>
<td>5.55</td>
<td>2.99</td>
<td>1.96</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.73</td>
<td>5.58b</td>
<td>6.15</td>
<td>3.74b</td>
<td>2.22</td>
<td>1.19</td>
</tr>
<tr>
<td>PABA</td>
<td>1</td>
<td>1.09</td>
<td>1.06</td>
<td>1.12</td>
<td>1.08</td>
<td>1.47</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.53</td>
<td>1.20</td>
<td>1.45</td>
<td>1.71</td>
<td>1.11</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.42</td>
<td>1.29</td>
<td>2.01</td>
<td>1.72</td>
<td>1.36</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.74</td>
<td>1.42</td>
<td>2.22</td>
<td>2.02</td>
<td>1.61</td>
<td>1.25</td>
</tr>
<tr>
<td>Croton oil</td>
<td>0.05</td>
<td>1.78</td>
<td>1.02</td>
<td>1.25</td>
<td>1.55</td>
<td>2.71</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>2.57</td>
<td>4.65</td>
<td>3.33</td>
<td>2.50</td>
<td>3.30</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>1.5</td>
<td>6.11</td>
<td>2.25</td>
<td>1.72</td>
<td>2.95</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.45</td>
<td>5.42</td>
<td>4.41</td>
<td>2.43</td>
<td>3.45</td>
<td>1.93</td>
</tr>
<tr>
<td>SLS</td>
<td>0.05</td>
<td>4.09</td>
<td>5.34</td>
<td>6.18</td>
<td>2.78</td>
<td>3.40b</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4.91</td>
<td>6.74</td>
<td>3.42</td>
<td>2.5</td>
<td>3.42</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>4.74</td>
<td>3.70</td>
<td>8.28</td>
<td>2.44</td>
<td>3.40</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.72</td>
<td>1.76</td>
<td>2.87</td>
<td>1.97</td>
<td>1.91</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Abbreviations: DNCB, dinitrochlorobenzene; IL, interleukin; IFN-g, interferong; PABA, paraaminobenzoic acid; SLS, sodium lauryl sulfate; TNF-a, tumor necrosis factor a. aThe differences in cytokine SI values between the vehicle group and each treatment group were statistically analyzed with Mann-Whitney U test, bP < 0.05 was taken as the level of significance.

CONCLUSIONS

Our results demonstrated that 4 cytokines including IL-2, IFN-g, IL-4, and IL-5 were released in lymph node cell cultures with a clear dose trend for sensitzers whereas only TNF-a was released in response to irritants. In conclusion, according to cytokine analyses results we can suggest that this method can be useful for discriminating irritants and allergens.

REFERENCES


OP-35: A MOLECULAR MODELLING APPROACH TO RATIONALE THE INHIBITOR ACTIVITY OF DERIVATIVES OF COUMARIN AGAINST CARBONIC ANHYDRASE ISOFORMS II

Z. E. Boylu1, H. Unan2, M. Tekpinar2, E. Eröğlu3

1Aksaray University, Faculty of Sciences and Arts, Department of Physics, Aksaray, TURKEY
2Yüzüncü Yıl University, Faculty of Sciences, Department of Physics, Van, TURKEY
3Akdeniz University, Faculty of Education, Department of Science Education, Antalya, TURKEY

Here, we present DFT-based QSAR models that demonstrate the correlations between the inhibition constant (Ki) data of the 52 derivatives of coumarin against carbonic Anhydrase Isozyme II (CA II) and the structural features of these molecules. Stability, dynamics and interaction of different coumarin derivatives with their surrounding amino acids in CA II active pocket are also studied by using Molecular Dynamics Simulation (MDS) method.

INTRODUCTION

Carbonic anhydrases (EC 4.2.1.1) that belong to the lyase family are ubiquitous zinc enzymes present in prokaryotes and eukaryotes, all over the phylogenetic tree. Many of the CA isozymes involved in some important physiological processes, are important therapeutic targets with the potential to be inhibited to treat a range of disorders including edema, glaucoma,
obesity, cancer, epilepsy and osteoporosis [1]. In the inhibition process of CAs, inhibitors are usually coordinated to Zn^{2+} ions with a tetrahedral geometry. In recent years [2], it has been shown that CAs can be inhibited by coumarin derivatives with a different mode of action. This study aims to understand at atomic level the inhibition mechanism of coumarin derivatives with CA II by using the molecular modelling approaches.

MATERIALS AND METHODS
For all the coumarin derivatives investigated, 3D modelling and calculations were performed using the Gaussian 03 (G03) Quantum Chemistry package. For QSAR modal development, CodessaPro (Comprehensive Descriptors for Structural and Statistical Analysis), Version 2.7.2 has been used for descriptors calculation, feature selection and statistical analysis. Molecular Dynamics Simulations were performed by using NAMD package. VMD package was used for the ligand parameterization, preparation and analysis of trajectory files. The X-ray structure of CA II-coumarin adduct which is deposited in Protein Data Bank with the PDB accession code 3F8E was used for MDS as a template.

RESULTS AND DISCUSSION
Among the obtained QSAR models statistically the most significant one which comprises physically interpretable molecular descriptors is a three-parameter linear equation with the statistical values as below;

\[ N_{\text{training}} = 39, \quad R^2 = 0.81, \quad Q^2 = 0.75, \quad F = 56 \]
\[ s^2 = 867.2, \quad N_{\text{test}} = 13, \quad R^2_{\text{test}} = 0.83, \quad \text{RMSE} = 39.43 \]

Results presented in this study shows that the frontier orbital energies, polarizability tensors and atomic partial charges are dominant factors which determine the inhibitory activity of the coumarin derivatives. MDS results support the experimental finding that is given in the literature [2] about inhibitory mechanism of coumarin derivatives.

CONCLUSIONS
Results presented in this study shows that quantum chemical based molecular descriptors are very useful to construct a QSAR model which may be used to design new coumarin derivatives with desired inhibitory activities. MDS results enable us to further understand the inhibitory mechanism of the coumarin derivatives.

ACKNOWLEDGMENTS
This project has been financially supported by TÜBİTAK with the contract number 114Z045.

REFERENCES
2. Maresca A.; Temperini C.; Vu H.; Pham N.B.; Poulsen S.A., Scozzafava A.; Quinn R.J.; Supuran C.T., Non-Zinc Mediated Inhibition of Carbonic Anhydrases: Coumarins Are a New Class of Suicide Inhibitors, JACS 2009 131, 3057-3062.
POSTER SESSION I
(Poster 1 - 131)

June 9, 2015
P-1: RAPID DETERMINATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE BY CE-DAD

A. G. Dal, S. Koyutürk
Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, TURKEY

A rapid, sensitive and specific method is developed for the determination of telmisartan (TEL) and hydrochlorothiazide (HCT) by CE-DAD. The method was validated and LOQ values were for 3.80x10⁻⁷ M and 3.14x10⁻⁷ M TEL and HCT, respectively. The method was applied both TEL and TEL/HCT combined tablets and is proposed for routine analysis for TEL and HCT.

INTRODUCTION
TEL is an angiotensin II receptor antagonist and HCT is a diuretic which are both used in hypertension treatment in combined forms. For the determination of both TEL and HCT, there are only a few studies which used spectrophotometry [1], LC [2,3] and a CE method [4] which proposes a total run time of 18 min. The aim of the study is to develop a rapid and sensitive method for the determination of TEL and HCT.

MATERIALS AND METHODS
All chemicals used were analytical grade. An Agilent 7100 model CE with DAD (230 nm) was used for CE experiments. Separation was achieved by a fused silica capillary with 40 cm effective (48.5 cm total, 50 µm i.d.) length. The run buffer was composed of 15 mM borate containing 10% methanol (pH 9.0). The applied potential was 25 kV and the samples were injected at 50 mbar pressure for 10 s. Candesartan was used as IS.

RESULTS AND DISCUSSION
All parameters effecting separation were investigated. The optimum conditions resulted a total run time of 7 min for HCT, TEL and IS. The method was validated for linearity, precision, accuracy, specificity, sensitivity and stability. The method was linear over the range of 2.51x10⁻⁷-1.19x10⁻⁴ M for HCT and 3.04x10⁻⁷-1.44x10⁻⁴ M for TEL. The LOQ values were found to be 3.80x10⁻⁷ M and 3.14x10⁻⁷ M for TEL and HCT, respectively. The validated method was applied to both TEL (80 mg) and TEL/HCT (80mg/12.5 mg) combined tablets. The recoveries were between 100.97-101.63% for TEL tablets. For the combined tablets the recoveries were 99.75-103.17% for HCT and 99.03-100.65% for TEL.

CONCLUSIONS
The method developed and validated here is rapid, specific and sensitive. It is proposed for routine analysis of these drugs and because of the low LOQ values, the method can be also used for the analysis of the drugs in biological samples.

ACKNOWLEDGMENTS
This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1207S122.

REFERENCES

P-2: A FULLY VALIDATED CHROMATOGRAPHIC DETERMINATION OF VORICONAZOLE FROM INFUSION SOLUTION AND TABLET

A. Doğan, N. E. Başıç
Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, TURKEY

In this study, an ultra flow liquid chromatographic (UFLC) method for determination of VOR in intravenous infusion and tablet preparations was developed and fully validated according to the ICH guidelines. The analysis of VOR together with internal
standard Phenazopyridine (IS) was performed using an analytical column of ACE Excel 2 C18 (50 mm x 3 mm i.d., 2 µm). Substances were eluted by a mobile phase consisted of methanol and water (50: 50, v/v) at a flow rate of 0.40 mL/min. Diode array detector was set to 255 nm. Under these chromatographic conditions retention times of VOR and IS were 6.09 and 7.35 min, respectively. LOD and LOQ values for VOR were found to be 50 ng/mL and 100 ng/mL. Developed method was linear over the range from 0.5 to 100 µg/mL VOR. The highest RSD value was calculated as 1.81% in inter-day and intra-day studies. The mean recovery for VOR was 100.64 ± 0.48% (RSD: 1.18%, n = 6). Developed method for the analysis of VOR in intravenous infusion and tablet preparations was found to be rugged and robust in terms of different analyst and minor changes in chromatographic conditions. System suitability values of developed method on the basis of criteria stated in pharmacopeias were over the limit values suggesting that was suitable in order to determine VOR in its intravenous infusion and tablet preparations. The long term reliability of the results from developed and fully validated UFLC method presented here was demonstrated via Shewhard’s quality control chart of VOR.

INTRODUCTION
VOR is a major drug used in the treatment of invasive aspergillosis which may occur in immunocompromised patients [1, 2]. UFLC method for analysis of VOR from tablet and intravenous infusion preparations was developed, and fully validated according to ICH Guidelines [3].

MATERIALS AND METHODS
Standards and stock solutions:
VOR (1000 µg/mL): in methanol
IS (1000 µg/mL): in water

Sample Preparations:
Infusion: Content of one preparation (200 mg) was dissolved in 250 mL methanol.
Tablet: The average weight of one tablet was transferred into a 250 mL volumetric flask and dissolved with methanol.

Equipment: UFLC-DAD (255 nm)
Column: ACE Excel2C18(50mmx3 mm i.d.,2 µm)
Mobile phase: Methanol and water (50:50, v/v)
Flow rate: 0.40 mL/min

RESULTS AND DISCUSSION
The mobile phase consisted of methanol and water (50:50, v/v) at a flow rate of 0.40 mL/min resulted in best separation for VOR, at which \( k' = 7.08, N = 2952.54, R_s = 2.74 \) and \( A_S = 1.16 \), and all chromatographic parameters were acceptable on the basis of these scientific criteria.

<table>
<thead>
<tr>
<th></th>
<th>VFEND* Tablet</th>
<th>VFEND* IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \bar{x} )</td>
<td>200.50 ± 0.47</td>
<td>199.53 ± 0.40</td>
</tr>
<tr>
<td>SD</td>
<td>1.15</td>
<td>0.97</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.57</td>
<td>0.49</td>
</tr>
</tbody>
</table>

CONCLUSIONS
Developed method for the analysis of VOR in intravenous infusion and tablet preparations was simple, sensitive, precise, accurate and cost reduced with low solvent consumption because of its low flow rate for routine quality controls.

REFERENCES

P-3: DETERMINATION OF SOME ANIONS IN DAIRY PRODUCTS BY CAPILLARY ELECTROPHORESIS

B. Yavuz Erdoğan¹, A.N. Onar²

Ondokuz Mayıs University, ¹Department of Food Technology Programmes, Technical Vocational School of Higher Education, 55600 Terme, ²Faculty of Art and Sciences, Department of Chemistry, 55139 Kurupelit, Samsun,TURKEY

In this study, a capillary electrophoretic method has been developed for determination of nitrate and nitrite ions in dairy products (yogurt, fresh kasar cheese, halloumi cheese, cottage cheese and Turkish white cheese). Large volume sample stacking was used to improve the limit of detection of ions. The electroosmotic flow is reversed by using low pH running buffer.

INTRODUCTION
Nitrate and nitrite occur widely in human and animal foodstuffs, both as intentional additives and as undesirable contaminants [1]. Nitrite and nitrate after being metabolized or reduced to nitrite; can react with secondary and tertiary amines to form N-nitroso compounds [2]. N-nitroso compounds are known to have mutagenic, teratogenic and carcinogenic activity [3].
MATERIALS AND METHODS
The analysis was carried out in Agilent HP3D capillary electrophoresis with diode array detector that was set at 200 and 214 nm. Separations were conducted using an untreated fused silica capillary (Polymicro Technologies) of 50μm inner diameter and with an effective length of 35 cm (total length 42 cm). The background electrolyte system consisted of 0.050 mol L⁻¹ phosphate buffer solution. In this research we investigated different applied potentials (-20 and -22) kV; capillary column temperatures (23 and 30 °C) and phosphate buffer pHs (2.5 and 3.5).

RESULTS AND DISCUSSION
The optimum capillary electrophoretic experimental conditions were decided to be: pH 3.5; temperature 30°C; applied potential (-20 kV). In order to improve the limit of detection sample stacking was employed using with hydrodynamic injection into the capillary by applying 50 mBar pressure. Injection times were manipulated for signal enhancement and injection time of 50 sec was decided. Reproducibility (intraday and day-to-day) was acceptable with relative standard deviations (less than RSDs 3.0%) for relative migration times in both standard solutions and dairy product samples.

CONCLUSIONS
Capillary electrophoretic (CE) method presented in this work is a valid technique for fast and economic analysis of nitrate and nitrite content of food samples. The proposed method was successfully applied to different kinds of dairy food samples.

ACKNOWLEDGMENTS
This work was supported by Ondokuz Mayı̇s University (Project No: PYO.TMY.1901.11.001).

REFERENCES
2. Garcia Roche, M.O.; Del Pozo E Izquierdo, L.; Fontaine, M., Nitrate and nitrite contents in cuban cheese of the gouda type, Die Nahrung 1993, 27 (2) 125-128.
3. Atawodi, S.E.; Mende, P.; Preussmann, R.; Spiegelhalder, B., Detection of methylating activity due to nitrosamide in some nitrosated nigerian foodstuffs. Food and Chemical Toxicology 1996, 34 (2) 147-151.

P-4: SIMULTANEOUS ELECTROCHEMICAL DETERMINATION OF ASCORBIC ACID AND URIC ACID USING POLY(GLYOXAL-BIS(2-HYDROXYANIL)) MODIFIED GLASSY CARBON ELECTRODE
E. Ergün¹, Ş. Kart², B. Zeybek³
¹Dumlupınar University, Faculty of Science and Arts, Department of Chemistry, Kütahya, Turkey; ²Dumlupınar University, Faculty of Science and Arts, Department of Biochemistry, Kütahya, Turkey

A novel polymer film of poly(glyoxal-bis(2-hydroxyanil), P(GBHA), was prepared by electropolymerization of glyoxal-bis(2-hydroxyanil) (GBHA) on the surface of a glassy carbon electrode (GCE) and this electrode was used to the simultaneous determination of the ascorbic acid (AA) and uric acid (UA).

INTRODUCTION
AA (vitamin C) is widely required for biological metabolism and expended in large quantities as an antioxidant agent food, beverages, cosmetic and pharmaceutical formulations applications [1, 2]. UA is the primary end product of purine metabolism [3,4]. Abnormal concentration level of UA in the urine and serum are symptoms of several diseases such as gout, hyperuricemia, and Lesch–Nyhan syndrome [4].

MATERIALS AND METHODS
Electrochemical analyzer: Ivium CompactStat
Working electrode: GCE (BAS MF-2012)
Reference electrode: Ag/AgCl (BAS MF-2052)
Counter electrode: Pt wire (BAS MW-1032).

RESULTS AND DISCUSSION
Electrochemical synthesis of P(GBHA) was performed on the surface of a GCE by using cyclic voltammetry method in the 0.1 mol L⁻¹ PBS (pH 7) containing 1 mmol L⁻¹ GBHA.

Fig. 1 exhibits the cyclic voltammograms (CVs) of the bare (BGC) and P(GBHA) modified GC electrodes in the solution of 0.1 mol L⁻¹ KCl containing 5.0 mmol L⁻¹ Fe(CN)⁶³⁻⁻⁻⁻ ions. A well characterized reversible redox peaks are observed for both electrodes. At the P(GBHA) modified GCE, compared to BGCE, the peak current intensity and the ΔEₚ of (Fe(CN)⁶³⁻⁻⁻⁻)
significantly increases and decreases, respectively. It is suggested that the P(GBHA) film increases the electroactive surface area of GCE.

The cyclic voltammetric response of AA and UA at the bare and modified GCEs are shown in Fig. 2. Compared to bare GCE, the peak currents remarkably increase at GC/P(GBHA) electrode for the both species.

CONCLUSIONS
In the study, P(GBHA) film on the GCE has been deposited for the first time. The P(GBHA) film modified GCE was successfully employed to determine AA and UA simultaneously.

REFERENCES

P-5: DIFFERENT SHAPE AND SIZE OF GOLD NANOPARTICLES FOR IMMUNOASSAY DETECTION

E. Kasap1, G. Çağlayan2, D. Cetin3, Z. Suludere4, U. Tamer1

1Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, 2Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, 3Science Teaching Programme, Faculty of Education, Gazi University, Ankara; 4Department of Biology, Faculty of Science, Gazi University, Ankara

INTRODUCTION
The analysis of biological samples requires complex equipment or time-consuming sample preparation procedures. One of the goal is to develop simple, reliable and rapid analytical techniques that can be applied by untrained persons on-site. Immunoassay techniques such as lateral flow immunoassay (LFIA) can be a powerful one to reach this goal [1]. In this study, we synthesized different shape of gold nanoparticles, especially anisotropic gold nanoparticles, to increase the surface area of the nanoparticles for the efficient immobilization of biological samples. Chemical and physical properties of nanoparticles are size and shape dependent and different sizes and shapes of nanoparticles affect the analytical performance of immunoassays.

MATERIALS AND METHODS
LFIA test strip was composed of four elements including sample pad, conjugate pad, nitrocellulose membrane and absorbent pad. Briefly, conjugation pad was prepared from glass fibers 3x8 mm in size. These pads were dipped into the modified gold nanoparticle solutions and dried under vacuum. Sample pad and absorbent pads were prepared from cellulose fiber 3x10 mm in size. Here, 3x40 mm nitrocellulose membranes were used as detection pads. Transmission electron microscopic analyses were performed with JEOL JEM 1400 instrument at 80 kV and 90 kV.

RESULTS AND DISCUSSION
In the present study, gold nanosphere, nanostars and nanorods particles were used as label. The performance on the detection of biological samples was evaluated. Different shape of the nanoparticles alters the surface area. Higher surface area means more sensing molecule on the surface and higher sensitivity of the assay. However changing the shape and sizes of nanoparticles also affect the mobility of
the particles on the membranes. Optical properties of gold nanoparticles are also size and shape dependent. Therefore sensitivity of the immunoassay is subject to these properties. TEM image of anisotropic gold nanoparticles used in this study can be seen in Fig. 1.

Fig. 1. TEM image of anisotropic gold nanoparticles

CONCLUSIONS

Paper based immunoassays are easy-to-use and on-site techniques. The analytical performance of the technique can be improved by modification of the materials. In this study we evaluated the effects of modifications of the gold nanoparticles for the detection of biological samples.

ACKNOWLEDGMENTS

The work is supported by The Scientific and Technological Council of Turkey (TÜBİTAK - Grant No: 114Z783)

REFERENCE


P-6: DETERMINATION OF ANTINEOPLASTIC DRUG 5-FLUOROURASIL USING ELECTROCHEMICAL DNA BIOSENSOR BASED ON GLASSY CARBON ELECTRODE WITH MODIFIED POLY(BROMOCRESOL PURPLE)

D. Koyuncu Zeybek¹, B. Demir², B. Zeybek³, S. Pekyardımcı³

Dumlupınar University, Faculty of Science and Arts
¹Department of Biochemistry, ²Department of Chemistry-Kutahya, TURKEY
³Ankara University, Faculty of Science Department of Chemistry, Ankara, TURKEY

This work demonstrates a genosensor based on poly(bromocresol purple) (P(BCP)) for observing of interaction between anticancer drug 5-fluorouracil (5-FU) and dsDNA. The dsDNA was immobilized on P(BCP) modified glassy carbon electrode. The proposed electrodes were characterized via CV and EIS techniques. The interaction mechanism of DNA-drug was investigated by differential pulse voltammetry. A decrease in the guanine oxidation peak current was observed after the interaction dsDNA and 5-FU. Under optimum conditions, the linear response was observed in the 5-FU concentration range of 5–50 mg L⁻¹. The obtained data exhibit the applicability of the developed electrochemical biosensor based on GC/P(BCP)/dsDNA electrode for the determination of 5-FU in pharmaceutical samples. To understanding of the interaction mechanism between dsDNA and 5-FU, UV-Vis spectroscopy and viscometer were also used.

INTRODUCTION

5-FU, uracil derivative, is one of the most effective antimetabolite agents and is used in treatment of many types of cancer such as colorectal, breast, stomach, pancreatic, and cervical cancer [1]. 5-FU in overdoses causes neurotoxicity, inducing mortality and morbidity due to accumulation of toxic metabolites. The determination of 5-FU is of vital importance; therefore many analytical methods have been developed [2]. Electrochemical approach, providing a new perspective on drug design in particular provide a better understanding of the interaction mechanisms between DNA with antineoplastic drugs [3].

MATERIALS AND METHODS

Polymerization: − 0.4–1.8 V /100 Vs⁻¹/50 cycles
Accumulation time and potential (dsDNA): 240 s.
+0.5V dsDNA conc.: 30 mg L⁻¹
Interaction time (5-FU): 120 s.

RESULTS AND DISCUSSION

Table 1. Analytical parameters and results

<table>
<thead>
<tr>
<th>Linear range (mg L⁻¹)</th>
<th>Slope (μA mg⁻¹ L⁻¹)</th>
<th>R²</th>
<th>Intercept (μA)</th>
<th>SE of slope (μA mg⁻¹ L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 – 50.0</td>
<td>−0.0037</td>
<td>0.9969</td>
<td>0.4432</td>
<td>8.482×10⁻⁵</td>
</tr>
<tr>
<td>SE of intercept (μA)</td>
<td>Reproducibility (%)</td>
<td>Repeatability (%)</td>
<td>Recovery (real samples)</td>
<td></td>
</tr>
<tr>
<td>0.0032</td>
<td>3.6</td>
<td>4.3</td>
<td>3.9</td>
<td>%93.2–%102.7</td>
</tr>
</tbody>
</table>

CONCLUSIONS

In this paper, we have reported an electrochemical DNA biosensor based on P(BCP) to the detection of 5-FU for the first time. The work was summarized in Fig 1.
INTRODUCTION

Biosensors are devices that combine a biological component with a detector component. Electrochemical mechanisms are important to all redox chemistry including biological systems related to electron transport chains. Electrodes could be miniaturized for mass production and arranged in different shapes. The electrode can convert the biological recognition event into a useful electrical signal: electron transfer, potential or impedance change at the electrode-solution interface.

Electrochemical biosensors consist of three parts: i) the biological component (DNA/protein), ii) the electrochemical transducer that transforms the detected signal in a readable and quantified output and iii) the signal processor that display the transformed signal in a user-friendly way.

In this study, various electrochemical biosensors were used for drug-DNA interactions and direct protein detection. These studies have attracted much attention because of the requirement of understanding the fundamental reactions of biomolecules.

MATERIALS AND METHOD

Voltammetric studies were carried out on AUTOLAB-PGSTAT 30 electrochemical analyzer with a conventional three electrode system including glassy carbon (GC) (φ = 1.5 mm) and pencil graphite (PG) electrodes as working, an Ag/AgCl electrode as a reference electrode and a platinum wire as an auxiliary electrode. The thin layer biological component modified electrodes were prepared by depositing two/three drops of 5 μL biomolecules solutions on the electrode surfaces. After placing each drop on the electrode surface, the modified electrodes were allowed to dry at 35°C. Then the electrode was immersed into blank supporting electrolyte and DP voltammograms were recorded.

RESULTS AND DISCUSSION

The interaction of leuprolide (LPR) with double-stranded DNA (dsDNA) immobilized onto pencil graphite electrode (PGE) have been studied by electrochemical methods. The linear response was obtained in the range of 0.20 ± 6.00 ppm LPR concentration with a detection limit of 0.06 ppm on DNA modified PGE. The guanine signal was lower with dsDNA-treated PGE than the untreated electrode after the interaction with Efavirenz (EFV) was occurred. The linearity was obtained between 2 and 24 ppm EFV concentration using guanine signal decreasing.

The electrochemical biosensor was designed in order to show the interaction between ct-dsDNA and lapatinib (LPT). The fresh prepared ct-dsDNA-electrochemical biosensor was incubated for 60 s, 180 s, 240 s and 480 s LQ—JP—1 LPT. The peak potential shifting in a positive direction with the addition of ct-dsDNA was confirmed that the planar aromatic ring structure of LPT is expected to facilitate its intercalation into the DNA helix. Electrochemical methods were advanced for the detection of changes in protein structures based on the anodic response of tyrosine or tryptophan and histidine using different solid electrodes. The largest difference between peaks of native and denatured alpha 2 macroglobulin (α2M) was observed at GCE.

CONCLUSION

Electrochemical biosensors are experimentally convenient and sensitive so that they require only a small amount of materials. Future progress in sensor development would require extensive multidisciplinary efforts for meeting emerging needs.

ACKNOWLEDGMENTS

The authors acknowledge the Dumlupınar University, Scientific Research Fund for financial support.

REFERENCES


P-7: APPLICATION OF VARIOUS ELECTROCHEMICAL BIOSENSORS

B. Dogan-Topal, B. Bozal-Palabiyk, B. Uslu, S. A. Özkan

Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry
Ankara, TURKEY

INTRODUCTION

Biosensors are devices that combine a biological component with a detector component. Electrochemical mechanisms are important to all redox chemistry including biological systems related to electron transport chains. Electrodes could be miniaturized for mass production and arranged in different shapes. The electrode can convert the biological recognition event into a useful electrical signal: electron transfer, potential or impedance change at the electrode-solution interface.

Electrochemical biosensors consist of three parts: i) the biological component (DNA/protein), ii) the electrochemical transducer that transforms the detected signal in a readable and quantified output and iii) the signal processor that display the transformed signal in a user-friendly way.

In this study, various electrochemical biosensors were used for drug-DNA interactions and direct protein detection. These studies have attracted much attention because of the requirement of understanding the fundamental reactions of biomolecules.

MATERIALS AND METHOD

Voltammetric studies were carried out on AUTOLAB-PGSTAT 30 electrochemical analyzer with a conventional three electrode system including glassy carbon (GC) (φ = 1.5 mm) and pencil graphite (PG) electrodes as working, an Ag/AgCl electrode as a reference electrode and a platinum wire as an auxiliary electrode. The thin layer biological component modified electrodes were prepared by depositing two/three drops of 5 μL biomolecules solutions on the electrode surfaces. After placing each drop on the electrode surface, the modified electrodes were allowed to dry at 35°C. Then the electrode was immersed into blank supporting electrolyte and DP voltammograms were recorded.

RESULTS AND DISCUSSION

The interaction of leuprolide (LPR) with double-stranded DNA (dsDNA) immobilized onto pencil graphite electrode (PGE) have been studied by electrochemical methods. The linear response was obtained in the range of 0.20 – 6.00 ppm LPR concentration with a detection limit of 0.06 ppm on DNA modified PGE. The guanine signal was lower with dsDNA-treated PGE than the untreated electrode after the interaction with Efavirenz (EFV) was occurred. The linearity was obtained between 2 and 24 ppm EFV concentration using guanine signal decreasing.

The electrochemical biosensor was designed in order to show the interaction between ct-dsDNA and lapatinib (LPT). The fresh prepared ct-dsDNA-electrochemical biosensor was incubated for 60 s, 180 s, 240 s and 480 s LQ—JP—1 LPT. The peak potential shifting in a positive direction with the addition of ct-dsDNA was confirmed that the planar aromatic ring structure of LPT is expected to facilitate its intercalation into the DNA helix. Electrochemical methods were advanced for the detection of changes in protein structures based on the anodic response of tyrosine or tryptophan and histidine using different solid electrodes. The largest difference between peaks of native and denatured alpha 2 macroglobulin (α2M) was observed at GCE.

CONCLUSION

Electrochemical biosensors are experimentally convenient and sensitive so that they require only a small amount of materials. Future progress in sensor development would require extensive multidisciplinary efforts for meeting emerging needs.
ranging from early detection of disease biomarkers or minimally invasive continuous monitoring of glucose and lactate, to early detection of biological warfare agents.

P-8: LC-MS/MS METHOD FOR THE DETERMINATION OF LEVETIRACETAM IN RAT BRAIN MICRODIALYSATE

D. Dogrukol-Aka, E. Senera, O. T. Korkmazb, S. Torunc, L. Gencd and N. Tuncelb

aAnadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, Türkiye; bOsmangazi University, Faculty of Medicine, Department of Physiology, Eskişehir, Türkiye; cAnadolu University, Faculty of Health Science, Eskişehir, Türkiye; dAnadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, Türkiye

This is the first study to develop and describe a simple and rapid method of liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS) for the determination of levetiracetam in rat brain microdialysate samples.

INTRODUCTION

Levetiracetam (LVT) is a new antiepileptic drug used in the treatment of partial onset seizure in patient. For the determination of LVT in human plasma a few methods based on high performance liquid chromatography (HPLC) with UV detection, micro-emulsion electrokinetic chromatography with UV detection, gas chromatography (GC) or in combination with mass spectrometry (GC/MS) have been described in recent years. Less sensitive LC-MS/MS methods were also reported for the determination of LVT in plasma. The objective of this study is to develop and validate a high throughput LC-MS/MS method for accurate and more sensitive measurement of LVT in biological fluids especially rat brain microdialysate samples.

MATERIALS AND METHODS

Chemicals and reagents: LVT was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Water was purified using Milli-Q water device (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade and solvents were of HPLC grade.

Equipment: Agilent 1290 series LC system consisted of a solvent delivery system with binary pump, an autosampler, and column oven (Agilent Technologies, USA). The separation of LVT was successfully performed on Supelco Ascentis Express (100 mm x 2.1 mm, 2.7 μm) with programmed gradient elution consisting of 10 mM ammonium formate in water (A) and in 10 ammonium formate in 90 % (v/v) acetonitrile (B) as mobile phase components and delivered at a flow rate of 0.3 ml/min to a triple-quadruple mass spectrometer equipped with a jet stream electrospray ionization source (6460 Trip-Quad, Agilent Technologies, USA). Injection volume was 2 μl. Data was analysed by using Mass Hunter programme.

Microdialysis samples: Wistar rats were used to obtain brain microdialysate samples in the region of hippocampus by using a specific prob (PAES 3mm membrane length with cut of 20000 D, CMA). LVT was administrated to rats at a LVT dose of 50 mg/kg by i.p.

RESULTS AND DISCUSSION

LVT was investigated generated the prominent protonated molecular ion [M+H]+ in positive ion mode. It was successfully separated at 3.4 min and monitored MRM transition of m/z 126>69.2. The method was linear over the range of 0.139-1390 ng/mL with the correlation coefficients greater than 0.998 (r²) for three separate batches in Ringer’s solution. Precision and accuracy were determined by measuring the concentration of LVT in Ringer’s solution in six replicates of quality control standards at three different concentrations (1.39, 139 and 1390 ng/mL) for three separate batches. Intra-day precision was <2%, and calculated recovery was >93% in Ringer’s solution. For the inter-day studies precision was <4%, and calculated recovery was >96% in the Ringer’s solution. To demonstrate the applicability of the method, rat brain microdialysate samples were directly analyzed after administration an i.p. dose of LVT and collection of samples was performed at the different time points over 12 hours (Fig. 1-2).
CONCLUSIONS
Rapid LC-MS/MS method for the quantitation of LVT in rat brain microdialysate samples was developed and validated. It was successfully applied to the analysis of rat brain microdialysate samples obtained from brain hippocampal region after i.p. LVT application and it was showed the concentration time profile of LVT in rat brain.

ACKNOWLEDGMENTS
This work was supported by the Scientific Research Projects Commission of Anadolu University (Project No 1301S009). The authors acknowledge the instrumental support of Anadolu University Medicinal Plants and Medicine Research Center (AUBIBAM). All animal experiments were performed in accordance with the principles of animal use and care approved by the ethical committee of the Medical Faculty of Osmangazi University (Approval File No. 04/2007).

REFERENCES

INTRODUCTION
Quetiapine fumarate (QF) is an atypical type antipsychotic drug used primarily for the treatment of schizophrenia, bipolar disorder, and also episodes of mania or depression associated with bipolar disorder [1].

MATERIALS AND METHODS
All experiments were achieved using a three-electrode electrochemical cell containing a GC working electrode, a platinum wire as counter electrode, and an Ag/AgCl electrode as reference. Modification of GC electrode was done by dropping 0.2% and 0.5% (w/w) of multiwalled carbon nanotube (MWCNT)/dimethyl formamide (DMF) suspension on the surface of GC electrode. All measurements were performed using an Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm, The Netherlands). All chemicals were used as received. Stock solution of QF (1x10^-3 M) was prepared in deionised water. Phosphate buffer (PB), Britton Robinson buffer (BRB), and acetate buffer (AC) solutions at different pH values between 2.0 and 9.0 were prepared.

RESULTS AND DISCUSSION
Electrochemical properties of QF were investigated on the anodic direction using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV), after MWCNT concentration was optimized to obtain the best electrode modification for QF. The effect of pH on the redox reaction of QF was studied by CV in all buffer solutions. It was seen that QF had an irreversible redox process in all pHs. The highest anodic peak current with a sharp and better peak shape was obtained in PB at pH 3.0, thus, this supporting electrolyte was selected for the further studies (Fig. 1).

Scan rate study was also carried out with CV at different scan rates between 5 and 200 mV s^-1 and found that QF had an adsorption controlled process. Therefore, differential pulse stripping voltammetry (DPSV) and square wave stripping voltammetry (SWSV) methods were used for the quantitative analysis of QF. The resulting linearity ranges of the calibration graphs were determined as 4x10^-9 – 2x10^-7 M (correlation coefficient 0.999) for DPSV and 2x10^-9 – 2x10^-7 M (correlation coefficient 0.996) for SWSV with detection limits of 5.39x10^-11 and 3.11x10^-10 M, respectively. The results of calibration graphs were compared with the results in the literature. Finally, quantitative analysis of QF from its pharmaceutical dosage forms was performed without any separation and filtration and it was seen that the excipients did not affect the analysis results.

P-9: ELECTROCHEMICAL ANALYSIS OF ANTIPSYCHOTIC DRUG QUETIAPINE FUMARATE AT CARBON NANOTUBES MODIFIED GLASSY CARBON ELECTRODE

B. Kaya, D. Kul
Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical, Chemistry, 61080, Trabzon, TURKEY
Fig. 1. Cyclic voltammograms of QF in (a) PB at pH 2.0, b) PB at pH 3.0, c) AC at pH 4.5, d) PB at pH 6.0, and e) PB at pH 8.0. QF concentration: 8x10^{-5} M. Scan rate: 100 mV s^{-1}.

CONCLUSIONS
Glassy carbon electrode was modified with MWCNT and used for the determination of quetiapine fumarate. Consequently, effective and economical modified electrode with high sensitivity and selectivity was obtained for the electrochemical analysis of quetiapine fumarate compared to the methods in the literature.

REFERENCES

P-10: LC-MS/MS METHOD FOR THE DETERMINATION OF GABAPENTIN IN RAT BRAIN MICRODIALYSATE

E. Sener, D. Dogrukol-Aka, O. T. Korkmaz, S. Torunc, L. Genc and N. Tuncel

Microdialysis samples: Wistar rats were used to obtain brain microdialysate samples in the region of hippocampus by using a specific prob (PAES 3 mm membrane length with cut of 20000 D, CMA). GBP was administrated to rats at a GBP dose of 50 mg/kg by i.p. 2.

RESULTS AND DISCUSSION
GBP was investigated generated the prominent protonated molecular ion [M+H]^+ in positive ion mode. It was successfully separated at 2.14 min and quantified using at m/z of 172>154 MRM transition. The method was linear over the range of 0.116-116 ng/mL with the correlation coefficients greater than 0.93 (r^2) for three separate batches in Ringer’s solution. Precision and accuracy were determined by measuring the concentration of GBP in Ringer’s solution in six replicates of quality control standards at three different concentrations (1.16, 116 and 1160 ng/mL) for three separate batches. Intra-day precision was <2%, and calculated recovery was >94% in Ringer’s solution. For the inter-day studies precision was <4%, and calculated recovery was >95% in the Ringer’s solution. To demonstrate the applicability of GBP in human plasma a few methods based on high performance liquid chromatography (HPLC) with fluorescence detection and capillary electrophoresis after derivatization, gas chromatography in combination with mass spectrometry (GC/MS) have been described in recent years. Less sensitive LC-MS and LC-MS/MS methods were also reported for the determination of GBP in plasma 1. The objective of this study is to develop and validate a high throughput LC-MS/MS method for accurate and more sensitive measurement of GBP in biological fluids especially rat brain microdialysate samples.
the method, rat brain microdialysate samples were directly analyzed after administration an i.p. dose of GBP and collection of samples was performed at the different time points over 12 hours (Fig. 1-2).

![Fig. 1. MRM chromatograms of Ringer’s solution (top), QC samples (100 ng/mL GBP) (middle) and GBP in microdialysate sample at 2h time point (bottom)](image)

![Fig. 2. The concentration time profiles in rat brain tissue after administration of 50 mg/kg of GBP to individual rats (n=5)](image)

**CONCLUSIONS**
Rapid LC-MS/MS method for the quantitation of GBP in rat brain microdialysate samples was developed and validated. It was successfully applied to the analysis of rat brain microdialysate samples obtained from brain hippocampal region after i.p. GBP application and it was showed the concentration time profile of GBP in rat brain.

**ACKNOWLEDGMENTS**
This work was supported by the Scientific Research Projects Commission of Anadolu University (Project No 1301S009). The authors acknowledge the instrumental support of Anadolu University Medicinal Plants and Medicine Research Center (AUBIBAM). All animal experiments were performed in accordance with the principles of animal use and care approved by the ethical committee of the Medical Faculty of Osmangazi University (Approval File N°. 04/2007)

**REFERENCES**

**P-11: SIMULTANEOUS DETERMINATION OF AMLODIPINE AND ROSUVASTATIN IN HUMAN URINE BY LC-MS/MS METHOD**

E. Can¹², F.Onur², I.M.Palabiyik², M.G.Caglayan², H.Koyuncu¹

¹ The Council of Forensic Medicine, Ankara, TURKEY
² Ankara University, Faculty of Pharmacy, Analytical Chemistry Department, Ankara, TURKEY

The aim of this study is to develop a LC-MS/MS method for the simultaneous determination of amlodipine (AML) and rosuvastatin (ROS) in human urine. Multiple reacting monitoring (MRM) transitions of m/z [M+H]+ 409.01→238.01 and, m/z [M+H]+ 482.3→258.3 were used to quantify AML and ROS respectively. The analytes were isolated from urine samples by solid phase extraction (SPE) method. Limit of detections (LOD) for AML and ROS are 0.61 and 0.91 ng/mL, respectively in the method. The described method was linear over a range of 2.5 - 100 ng/mL both for AML and ROS.

**INTRODUCTION**
Amlodipine (AML), is a potent calcium channel blocker and Rosuvastatin (ROS), is a member of a class of cholesterol-lowering drugs, are used as their combination for the treatment of hypertension and dyslipidemia. In urine an LC-MS/MS method (1) was used for the determination of AML and a spectrofluorometric method (2) was used for the determination of ROS previously. But there is no method for their simultaneous determination in human urine. Therefore we aimed to develop a simple, fast, precise, sensitive a LC-MS/MS method for simultaneous determination of these two drugs in spiked human urine.

**MATERIALS AND METHODS**
Amlodipine besylate (AML) and rosuvastatin calcium (ROS) kindly donated by Salutis (Istanbul, Turkey). All the reagents and solvents are of LC-MS grade (Merck). Stock solutions of AML and ROS were prepared by dissolving in methanol (1 µg/mL). The working standards for urine obtained by diluting the stock solution in drug-free urine, The chromatographic study was carried out using WATERS ACQUITY
TQD triple quadrupole mass spectrometer equipped with an electro-spray ionization source LC-MS/MS system. Chromatographic separation was achieved on ACQUITY HSS-C18 (2.7 μm, 2.1 x 150 mm) column using formic acid- 15 mM ammonium formate buffer (pH 5) – % 1 formic acid in acetonitrile (8:92, v/v) as mobile phase. The ESI-MS spectrometer was operated in the positive ion mode. The electro-spray capillary voltage was set to 3000 V. Protonated analyte molecules were subjected to collision induced dissociation using Nitrogen as the collision gas to yield product ions for each analyte. The collision energy was 12 and 34 eV for AML and ROS, respectively. The scan time was 1s. Multiple reaction monitoring (MRM) of the precursor–product ion transitions of m/z [M+H]+ 409.0₁→238.0₁ and m/z [M+H]+ 482.3→258.3 were used for quantitation of AML vs ROS. The corresponding LC-MS/MS chromatograms for each analyte are depicted in following figures:

RESULTS

Retention times for AML and ROS were found as 0.85 and 0.88 minutes respectively. The analytes were isolated from urine samples by SPE. The recovery of the extraction method (SPE) was found as 99.68% for AML and 100.10% for ROS. Calibration curve were constructed by plotting the peak areas of urine standards versus nominal concentrations. The regression equations of calibration curves were:

\[ y = 222.51 x - 24.31 \ (r^2 = 0.9999) \] for AML and \[ y = 225.11 x - 12.74 \ (r^2 = 0.9999) \] for ROS where \( y \) represents the peak area of analytes, and \( x \) represent the urine concentration of analytes in ng/mL. LOD for AML and ROS are 0.61 and 0.91 ng/mL, respectively in the method. The described method was linear over a range of 2.5 - 100 ng/mL both for AML and ROS. In intra- and inter-day assays, RSD values in the method, were < 1% for all concentrations studied.

CONCLUSIONS

The proposed LC-MS/MS assay method is rapid, simple, precise and sensitive for quantification of AML and ROS in human urine, and is fully validated as per the FDA guidelines. This is the first report on simultaneous assay of AML and ROS in human urine and it has been successfully applied to the simultaneous determination of AML and ROS in spiked urine samples.

REFERENCES


P-12: ELECTROCHEMICAL INVESTIGATION AND DETERMINATION OF AMOXICILLIN ON POLY(ACRIDINE ORANGE) MODIFIED GLASSY CARBON ELECTRODE

F. Ağın

Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, Trabzon, Turkey

The electrochemical behavior of amoxicillin (AMX) has been investigated at poly(acridine orange) modified glassy carbon electrode (GCE) was prepared by acridine orange potential cycling electropolymerization. Quantitative determination of AMX was achieved by differential pulse and square wave voltammetry in 0.04 M BRT, pH 5.0.

INTRODUCTION

Amoxicillin (AMX), is one of the most frequently used b-lactam antibiotics in the world. B-lactam antibiotics is a structure based on a b-lactam ring responsible for the antibacterial activity and AMX has variable side chains that account for the major differences in their chemical and pharmacological properties. AMX is one of the more important antibiotics used in the treatment of bacterial infections and its determination [1, 2].

Acridine orange (AO), is an aromatic dye containing nitrogen which is generally used in cell biology [3]. Due to the structure and molecular recognition capability of AO, it is can be easily polymerized electrochemically on the surface of solid electrodes as a redox mediator for the determination of electroactive molecules, such as dobutamin [4].

MATERIALS AND METHODS

A three-electrode electrochemical cell was used for the experiments. It contained a GC electrode (BAS, φ: 3 mm diameter) as working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference. All measurements by CV, DPV and SWV were performed using a computer-controlled Autolab
potentiostat/galvanostat with Nova 10.0 software (Metrohm-Autolab, The Netherlands).

RESULTS AND DISCUSSION
Modification of glassy carbon electrode was carried out by AO polymerized electrochemically on the surface GCE using potential cycling in 0.025 M phosphate buffer solution (PBS), pH 5.5. The electrochemical behavior of AMX was investigated using cyclic, differential pulse and square wave voltammetry poly(AO) modified electrode at different pH values between 5.0-9.0. A diffusion-controlled irreversible oxidation peak was observed in cyclic voltammetry for AMX. Quantitative determination of AMX was carried out at poly(AO) modified electrode using differential pulse and square wave voltammetry in 0.04 M Britton Robinson buffer at pH 5.0. The peak current showed a linear dependence with concentration in the range of 0.4-20 µM for DPV and 0.8-80 µM for SWV.

CONCLUSIONS
Simple, selective, sensitive, fully validated, rapid and reliable, DPV and SWV were applied for the analysis of AMX pharmaceutical dosage forms. No electroactive interferences from the pharmaceutical dosage forms excipients were found.

REFERENCES

P-13: POLY-3-AMINO-1,2,4-TRIAZOLE-5-THIOL MODIFIED GLASSY CARBON ELECTRODES FOR DNA AND DNA – ANTIBACTERIAL AGENT INTERACTION

G. Aydoğdu¹, G. Güneşli¹, S. Pekyardimci¹, T. Ertaş Bolelli², İ. Yalçın²

¹Ankara University, Faculty of Science, Department of Chemistry; ² Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

INTRODUCTION
Studies on the binding mechanism of some small molecules with DNA have been identified as one of the important aspect during the past few decades [1]. Electrochemical DNA biosensors involve a nucleic acid recognition layer which is immobilized over an electrochemical transducer. The recognition layer detects the changes that occurred in the DNA structure during interaction with DNA-drug molecules [2]. Substituted benzimidazoles are found to be associated with various chemotherapeutical activities such as antibacterial, antifungal, antitumor, antiviral activities [3]. 2-(2-phenyl ethyl)-5-methyl benzimidazole (G24) is an antibacterial agent which could be used against HepG2 liver cancer cells [4].

MATERIALS AND METHODS
All electrochemical measurements were carried out by using an AUTOLAB-PGSTAT 302N electrochemical analyzer connected with a three-electrode cell stand (Bioanalytical Systems, BAS, Inc., USA). A conventional three-electrode system consist of the unmodified or modified GCE as working electrode, a platinum wire as counter electrode, and Ag/AgCl (3 mol L⁻¹ NaCl,) as reference electrode. The CVs and DPVs were analyzed by using NOVA 11.0 software (ECO Chemie). The electropolymerization of 3-amino-1,2,4-triazole-5-thiol (AT) on GCE was carried out by using CV technique. 15 successive potential sweeps were applied between −0.20 V and +1.70 V at a scan rate of 50 mV s⁻¹ in 0.1 mol L⁻¹ H₂SO₄. 2-(2-phenyl ethyl)-5-methyl benzimidazole (G24) was synthesized by Ankara University Faculty of Pharmacy Department of Pharmaceutical Chemistry. For adsorption of dsDNA, poly-AT modified GCEs (GCE/p-AT) were immersed into vials containing 40 mg L⁻¹ dsDNA in 0.5 mol L⁻¹ NaCl) for 15 min. Electrochemical detection of the interaction between G24 and dsDNA on the GCE/p-AT is based on the decreases of guanine signal. After the adsorption of dsDNA, the electrode was immersed into G24 solution in 0.5 mol L⁻¹ ABS (pH 4.8) for selected times. After the interaction, the electrode was rinsed and replaced in G24-free ABS, where DPVs were recorded.

RESULTS AND DISCUSSION
In this study, GCE/p-AT was constructed and this electrode was used for the electrochemical monitoring of interaction between the dsDNA and G24 for the first time. The p-AT film was characterized by scanning electron microscopy (SEM). Electrochemical behaviour of GCE/p-AT was investigated by using CV and EIS and compared with those of the bare GCE. The GCE/p-AT/dsDNA electrode was prepared by adsorption of dsDNA upon the GCE/p-AT and the binding of G24 with dsDNA was investigated via DPV method. The decrease in the guanine oxidation peak current at +0.8 V was used as an indicator for the
interaction between dsDNA and G24 in ABS. The experimental parameters such as dsDNA concentration, G24 concentration, adsorption time, interaction time were optimized. Obtained results showed that amount of dsDNA was found to be 40 mg/L and dsDNA adsorption time was chosen as 15 min. Moreover, the reproducibility, repeatability, stability and applicability of the analysis in human serum and urine samples were also investigated. These results showed that this DNA biosensor could be used for the sensitive, accurate and precise determination of G24–dsDNA interaction.

CONCLUSIONS
An electrochemical DNA biosensor based on GCE/p-AT was proposed for the determination of chemotherapeutical active benzimidazole derivative, G24. The GCE/p-AT improved the rate of electron transfer at the solution/electrode interface according to the results obtained with CV and EIS methods in Fe(CN)$_6^{3-/4-}$ as redox probe. In order to determination the interaction of the G24 with dsDNA, the DPV and UV–vis spectroscopy techniques were used. The fabricated DNA biosensor will be used for recognition of biomolecules as drugs and nucleic acids and drug-DNA interactions in the future.

ACKNOWLEDGMENTS
This work was supported by the Ankara University Scientific Research Fund (Project No: 14L0430004) for their financial support.

REFERENCES

**P-14: ELECTROCHEMICAL BEHAVIOUR OF ANTVIRAL DRUG TENOFOVIR ON GLASSY CARBON ELECTRODE**

G. Ozcelikay, B. Dogan, S. Ozkan

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Analytical Chemistry, Ankara, TURKEY

**INTRODUCTION**
The use of electrochemical techniques to expand information about drug molecules and their mechanism of action is getting one of the important events in drug discovery. So it is easy to find relations between electrochemical and biological reactions concerning electron transfer pathways (1). Electrochemical mechanisms are important to all redox chemistry including biological systems relating to electron transport chains. Tenofovir is a type of anti-HIV medicine called a nucleoside reverse transcriptase inhibitor (NRTI). NRTIs work by blocking HIV reverse transcriptase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood. The electro-oxidation behaviour of tenofovir (TEN) at glassy carbon electrode (GCE) was studied using various voltammetric techniques. It was investigated by cyclic, differential pulse (DPV) and square wave (SWV) adsorptive stripping voltammetric techniques. Different parameters were tested to optimize the conditions for the determination of TEN (2). The dependence of intensities of currents and potential on pH, concentration, scan rate, nature of the buffer was investigated. The developed method was applied to the pharmaceutical dosage forms.

**MATERIALS AND METHODS**
The cyclic, DPV, SWV and experiments at a stationary electrode were performed using a BAS 100W Electrochemical analyzer. A three-electrode cell system incorporating the glassy carbon electrode as working electrode: a Ag/AgCl (3 M KCl) reference electrode and platinum-wire auxiliary electrode were also used. Before each experiments the glassy carbon electrode was polished manually with alumina (D=0.01 mm) in the presence of distilled water on a smooth polishing cloth. DPV conditions were: pulse amplitude, 50 mV; pulse width 50 ms; scan rate, 20 mV s$^{-1}$ and SWV conditions were: pulse amplitude, 35 mV; frequency, 30 Hz; potential step 8 mV. The parameters of stripping methods were also optimized.

**RESULTS AND DISCUSSION**
The dependence of the peak currents and potentials on pH were investigated within the range of pH 0.6–12.0 by various voltammetric techniques. The one-defined peak was observed at about 1.30V in the optimum pH 4.70 acetate buffer by differential pulse voltammetry. The effects of scan rate on TEN signal were determined in detail by cyclic voltammetry. The oxidation behavior of TEN was found as irreversible and diffusion-adsorption mix controlled on the glassy carbon electrode. In the selected electrolyte solution (pH 4.70 acetate buffer solution), accumulation potential and accumulation time were optimized for differential pulse stripping (DPSV) and square wave stripping voltammetric (SWSV) techniques and found as 1000 mV, 60 s for both techniques. Step potential, pulse amplitude and frequency were also optimized.
CONCLUSIONS
The electrochemical behavior of TEN was examined for the first time with this study. The electro-oxidation of TEN at bare electrodes was investigated in great detail so that the behavior of TEN at carbon-based electrodes might be used for analytical purposes, particularly as a sensor. These methods were successfully applied for the analysis of TEN in the pharmaceutical dosage forms. The repeatability and reproducibility precision and accuracy of the methods for all media were determined.

REFERENCES
1. Antiretroviral Therapy and the Kidney, Volume 22 Issue 3 June/July 2014

P-15: HPLC DETERMINATION OF LEVOFLOXACIN IN HUMAN BREAST MILK

G. Ergin, O. Sağırlı, S. Ertürk Toker

Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, 34116, Beyazit, Istanbul, TURKEY

INTRODUCTION
Levofoxacin is one of the third generation synthetic fluoroquinolone antimicrobial agent with a broad-spectrum antibacterial activity and is used to treat severe bacterial infections which failed to respond to other antibiotic classes [1-3]. In this work, a simple, rapid and sensitive HPLC method was developed and validated for the determination of levofloxacin (LEV) in human breast milk.

MATERIALS AND METHODS
Levofoxacin, its pharmaceutical preparation (Tavanic®) (containing 500 mg of Levofoxacin per tablet) were kindly supplied by Sanofi (Istanbul, Turkey). Breast milk was obtained from healthy human volunteers and were stored at approximately -20 °C until they were analyzed. Moxifloxacin was used as internal standard (IS). Sample preparation was assured by one step protein precipitation with acetonitrile.

The stock solution of LEV and IS (1 mg mL⁻¹, calculated as free base) was prepared and diluted with acetonitrile to give standard solutions of 0.25 µg mL⁻¹. Standard calibration samples were prepared daily by spiking 0.1 mL of drug-free human breast milk with 0.5-100 µL of LEV and 50 µL IS standard solutions to achieve final concentrations of 2.5-500 ng mL⁻¹ of LEV.

A Shimadzu (Kyoto, Japan) LC 20 A liquid chromatograph consisted of a LC-20 AT solvent delivery system, SIL-20AC autosampler, DGU-20A5 vacuum degasser, CTO-10ASVP column oven and SPD-M20A fluorescence detector. The data were collected and analyzed via the automation system software.

RESULTS AND DISCUSSION
The chromatographic separation was achieved on a C18 column (5 µm, 250 x 4.6 mm, ID) using a mobile phase of acetonitrile-10 mM o-phosphoric acid (25:75, v/v) at a flow rate of 1 mL min⁻¹. The retention time of LEV and IS 6.0 and 8.2 min under these conditions. Representative chromatogram of the milk sample spiked with LEV (200 ng mL⁻¹) and IS (250 ng mL⁻¹) was given in Figure 1. The peaks were detected by fluorescence detection at 292 nm for the excitation and 494 nm for the emission.

Calibration graph was rectilinear over the range of 2.5-500 ng mL⁻¹ of LEV. The mean regression equation was as follows: A = 0.0128 C + 0.01657 (r=0.9985). Limit of detection and limit of quantification were found 0.63 and 2.11 ng mL⁻¹, respectively. Assay precision and recovery of LEV from breast milk at three different concentrations were assessed. Intra-day and inter-day relative standard deviation (RSD %) values were found less than 4.9 %. Mean absolute recovery was also found 94.5%.

CONCLUSIONS
The proposed HPLC method was applied to the determination of LEV in human breast milk samples. The LEV concentrations obtained from collected milk samples versus time graph was plotted. As shown at this graph, LEV mostly excreted to human breast milk and this result accord with literature [4]. Drug excretion into breast milk should be evaluated in view of a possible risk to neonates and infants. Complex nature of milk demands application of special cleanup procedures for drug determination. For this purpose we developed a simple protein precipitation technique followed by the reversed phase
chromatography. Data obtained by this approach show that the method is reliable and suitable for LEV determination in human breast milk.

REFERENCES

P-16: ELECTROCHEMICAL OXIDATION OF ANTIBACTERIAL DRUG CEFEPIME AND ITS QUANTITATIVE DETERMINATION FROM PHARMACEUTICALS AND SERUM WITH CARBON BASED ELECTRODES

G. Öztürk, D. Kul
Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, Trabzon, TURKEY

INTRODUCTION
Cefepime (CEF) is a new parenteral cephalosporin described as a fourth-generation, broad spectrum cephalosporin. This drug is a semisynthetic antibacterial drug and mainly used for the treatment of febrile neutropenic events, sepsis, and also lower respiratory, urinary tract, skin soft tissue, complicated intra-abdominal, and gynecological infections[1].

MATERIALS AND METHODS
All electrochemical experiments were performed on an Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm, The Netherlands) with a three-electrode system including BDD electrode or CP electrode as a working electrode, an Ag/AgCl as reference electrode and a platinum wire as counter electrode.

All chemicals and reagents were analytical grade and used without any purification. Stock solutions of CEF (1x10^-3 M) were prepared by dissolution of the solid substance in deionized water. Phosphate buffer (PB), Britton Robinson buffer (BRB), and acetate buffer (AC) solutions at different pH values were prepared.

RESULTS AND DISCUSSION
The electrochemical study of CEF at BDD and CP electrodes was investigated on the anodic direction using cyclic (CV), differential pulse (DPV), and square wave voltammetry (SWV). After the effect of pH on the redox reaction of CEF was studied by CV in all buffer solutions, supporting electrolytes were selected as Britton–Robinson buffer at pH 2.0 for DPV and SWV with BDD electrode; phosphate buffer at pH 5.0 for DPV and Britton–Robinson buffer at pH 10.0 for SWV with CP electrode. CV results showed that CEF had an irreversible redox reaction. Scan rate study was carried out and found that the process of CEF was diffusion and adsorption controlled for BDD and CP electrodes, respectively. Linear ranges of the calibration graphs were between 0.04 and 10 μM for DPV and between 0.4 and 60 μM for SWV for BDD electrode. The detection limits were calculated as 1.58x10^-3 μM for DPV and 1.10x10^-2 μM for SWV at BDD electrode. For CP electrode, linearity was in the range of 0.08 to 10 μM for DPV and 0.4 to 10 μM for SWV. The detection limits were 3.74x10^-3 and 2.21x10^-2 μM for DPV and SWV, respectively. The repeatability of the methods (RSD%) was calculated from the peak currents and found to be 0.40 for BDD electrode and 0.73 for CP electrode using DPV method. In the same way, the values of RSD% were 0.73 for BDD electrode and 0.76 for CP electrode using SWV method. The practical analytical values of the methods are demonstrated by quantitative determination of CEF in pharmaceutical dosage forms and human blood serum, without need of the separation procedure, since there was no interference from the excipients.

CONCLUSIONS
The electrochemical oxidation of CEF at BDD and CP electrodes was studied with a good limit of detection. The analysis exhibited satisfactory results with good sensitivity and wide linear range for electrochemical determination of CEF compared to the methods in the literature.

REFERENCES

P-17: ROOM-TEMPERATURE PHOSPHORESCENCE DETERMINATION OF MELAMINE IN DAIRY PRODUCTS USING L-CYSTEINE-CAPPED MN-DOPED ZINC SULFIDE (ZNS) QUANTUM DOTS

B. Er Demirhan1, B. Demirhan1, H. E. Satana Kara2
Gazi University, Faculty of Pharmacy, 1Department of Food Analysis, 2Department of Analytical Chemistry, Ankara, TURKEY
INTRODUCTION
Melamine (MEL; 1,3,5-tiazine-2,4,6-triamine) is a trimer of cyanamide and is a nitrogen-rich chemical. Although MEL may be used the manufacturing of packing for food products, it is not approved for direct addition to human food or animal feeds. Because of its high nitrogen level, MEL increases the protein level, but it cannot be detected by the Kjeldahl method. MEL is inexpensive and easily accessible and thus is illegally added to protein-rich foods such as milk products to enhance the protein content.
In this study, a room temperature phosphorescence (RTP) method was developed for the determination of MEL in dairy products using L-cysteine capped Mn-doped ZnS quantum dots (QDs) as a probe. This method based on the quenching of the phosphorescence signal of quantum dots by the interaction with MEL.

MATERIALS AND METHODS
Synthesis of Quantum Dots: Fifty mL of 0.02 M L-cysteine, 5 mL of 0.1 M ZnSO₄, and 1.5 mL of 0.01 M MnCl₂ were added to flask and mixed. The pH of the mixture was adjusted to 11 with 1M NaOH. After stirring, argon gas was passed through the flask contents at room temperature for 30 min to remove air, and 5 mL of 0.1 M Na₂S was added quickly to the solution to allow nucleation of the nanoparticles. The mixture was stirred for 20 min, and then the solution was aged at 50°C in the open air for 2 h to form L-cysteine-capped Mn-doped ZnS quantum dots. Phosphorescence Experiments: Phosphorescence measurements were carried out at an excitation wavelength of 290 nm in the absence and presence of MEL. One hundred microliters of QD was diluted with 10 mM phosphate buffer (pH 7.4), and different volumes of MEL solution were added to detect the phosphorescence quenching effect. All measurements were done 2 min after the mixing of MEL and QD.

RESULTS AND DISCUSSION

Table 1. Analytical merits of RTP method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng mL⁻¹)</td>
<td>50-500</td>
</tr>
<tr>
<td>Slope/ SE of slope</td>
<td>0.71 / 0.11</td>
</tr>
<tr>
<td>Intercept/ SE of intercept</td>
<td>44.06 / 3.20</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.995</td>
</tr>
<tr>
<td>LOD/LOQ</td>
<td>5.95 / 19.83</td>
</tr>
<tr>
<td>Inter-/intra-day precision</td>
<td>0.15 / 0.49</td>
</tr>
</tbody>
</table>

Table 2. Results of samples and recovery analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>MEL (ng mL⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee creamer</td>
<td>866</td>
<td>101.8</td>
</tr>
<tr>
<td>White cheese</td>
<td>&lt;DL</td>
<td>100.5</td>
</tr>
<tr>
<td>Yogurt</td>
<td>1275</td>
<td>101.8</td>
</tr>
<tr>
<td>Cream</td>
<td>1531</td>
<td>101.4</td>
</tr>
<tr>
<td>Follow-on-milk</td>
<td>&lt;DL</td>
<td>100.6</td>
</tr>
</tbody>
</table>

CONCLUSIONS
The proposed method was used to quantify MEL in dairy products. Concentration of MEL could be directly monitored according to the changing of RTP intensity of QD. Proposed method is sensitive, selective, fast, and inexpensive. Also, it avoids interference from autofluorescence or cations such as Na⁺, K⁺, Mg²⁺, and Ca²⁺, and does not need removing oxygen, adding modifier.

P-18: CHEMICAL PROFILE of THREE EDIBLE PLANTS by USING LC-MS/MS AND GC-MS: SCOLYMUS HISPANICUS, CARDARIA DRABA SUBSP. DRABA and CARDUUS PYCNOCEPHALUS SUBSP. ABIDUS

M. Boğa¹, H. Temel², A. Ertaş³, M.A. Yılmaz⁴, S. Ertekin⁵, E. Aygün-Tuncay⁶

Dicle University, Faculty of Pharmacy, ¹Department of Pharmaceutical Technology, ²Department of Pharmaceutical Chemistry, ³Department of Pharmacognosy, ⁴Dicle University Research and Application of Science and Technology Center (DUBTAM), Diyarbakır, TURKEY; Dicle University, Faculty of Science ⁵Department of Biology, ⁶Department of Chemistry

This study aimed determination of chemical profiles of the three edible plants including Scolymus hispanicus, Cardaria draba subsp. draba and Carduus pycnocephalus subsp. abidus. Phenolic and flavonoid profiles have been investigated with LC-MS/MS and
essential oils and fatty acids have been determined with GC-MS analysis.

INTRODUCTION

*S. hispanicus* known as “şevketi bostan,altın diken, sardıken, akkız, sarıçakız, akıdkız” in Turkish [1]. Although *S. hispanicus* is widespread in Turkey, it is consumed as vegetable only in İzmir and around area [2]. *Cardaria draba* subsp. *draba* and *Carduus pycnocephalus* subsp. *abidus* are used as vegetable in southeastern part of Turkey. Root and aerial parts of *S. hispanicus* and infusion of *Cardaria* species used as diuretic and for kidney and gall stone. Decoction of *Carduus* species is used as appetizer and for antidiabetic [1].

MATERIALS AND METHODS

**Plant materials:** *S. hispanicus* was collected from İstanbul-Belgrad in 2012 and was identified by Dr. Yeter Yeşil (ISTE 98060). *Cardaria draba* subsp. *draba* was collected from Mardin-Kızıltepe in 2012 and was identified by Prof. Dr. Selçuk Ertekin (ISTE 97148). *Carduus pycnocephalus* subsp. *abidus* was collected from Diyarbakır-Çınar in 2012 and was identified by Prof. Dr. Selçuk Ertekin (ISTE 97145).

**Instruments:** A Shimadzu UHPLC coupled to a tandem MS instrument was used to perform LC-ESI-MS/MS analysis of the methanol extract. The equipments, used for liquid chromatograph, were LC-30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. Clevenger type apparatus was used for obtaining essential oil of the three plants. GC-FID and GC-MS instruments were used for determination of essential oil composition of the three plants.

RESULTS AND DISCUSSION

Twenty-seven compounds including (L)-malic acid, quercetin, protocatechuic acid, chrysin, rutin, hesperetin, naringenin, rosmarinic acid, vanillin, p-coumaric acid, caffeic acid, chlorogenic acid, hyperoside, myricetin, coumarin, kaempferol, quinic acid, tr-acetic acid, 4-hydroxybenzoic acid, fisetin, gallic acid, tannic acid, salicylic acid, hesperidin, luteolin, apigenin, rhamnetin have been investigated with LC-MS/MS analysis.

CONCLUSIONS

Chemical profile of the three edible plants was investigated with LC-MS/MS for the first time. Essential oil and fatty acid contents of the three edible plants were determined with GC-MS analysis.

ACKNOWLEDGMENTS

The authors are thankful to Dicle University Science and Technology Research and Application Center (DUBTAM) for its support.

REFERENCES


P-19: ELECTROCHEMICAL DETECTION AND DISCRIMINATION OF INFLUENZA A AND B VIRUSES BASED ON DNA HYBRIDIZATION BY USING NANOBIOSENSORS

H. Subak1,2, D. Ozkan-Ariksoysal2*

1 Department of Analytical Chemistry, Faculty of Pharmacy, Yuzuncu Yıl University, Van, Turkey; 2 Ege University, Faculty of Pharmacy, Analytical Chemistry Department, İzmir, Turkey

In this work, a label-free electrochemical genosensor was designed for the detection and discrimination of DNA sequences related to the Influenza A and B viruses based on the guanine oxidation signal at about 1.0V. The probe-modified sensor was able to clearly distinguish Influenza A and B virus types in only 45 min. detection time by differential pulse voltammetry (DPV). The obtained data were also confirmed and compared with inosine-modified DNA sequences. Various factors effect on the hybridization were studied to maximize sensitivity and selectivity. These parameters were applied to nanomaterial-enhanced surfaces in the second part of the project. Thus, these methodologies were evaluated for use in biological sensor systems and the use of a hand-held portable genosensors.

INTRODUCTION

The primary cause of respiratory infections in both adults and children has been known to be an influenza virus for a long time. These viruses cause epidemics
and are responsible for existing morbidity and mortality in humans. Biosensors are cost effective analytical devices, which do not demand sophisticated procedures and offer fast and simple analysis.

**MATERIALS AND METHODS**

Hybridization of Inf-A and Inf-B determined by using electrochemical techniques as DPV (AUTOLAB PGSTAT-30 potentiostat) based on DNA-modified and/or nanomaterial immobilized carbon electrodes. The three-electrode system was comprised of a disposable PGE, an Ag/AgCl reference electrode, and a platinum wire as the auxiliary electrode. Acetate buffer (ACB) and phosphate buffer solutions (PBS) are buffer used for analysis.

**RESULTS AND DISCUSSION**

For the effective electrochemical monitoring of hybridisation and discrimination of full-match and non-complementary sequences, rapid tests were performed to detect hybridisation conditions of probe and target sequences. Hybridization is determined to compare peak potentials of probe, non complementary (NC) and hybrid as shown in the Fig 1.

![Fig. 1. Shematic illustration of the electrochemical biosensor for detection of hybridization.](image)

**CONCLUSIONS**

The decrease or appearance in the intrinsic guanine signal simplified the detection procedure and shortened the assay time because protocol eliminates the label binding step. Besides this, the primary advantage of the new protocol is its nanomaterial-based detection scheme providing an enhanced response with high sensitivity.

**ACKNOWLEDGMENTS**

The authors acknowledge financial support from Ege University Project Coordination Center (Projects 12/ECZ/033) and the Pharmaceutical Sciences Research Centre (FABAL) of Ege University, Faculty of Pharmacy.

**REFERENCES**


**P-20: HPLC SEPARATION OF ENANTIOMERS OF SOME CHIRAL CARBOXYLIC ACID DERIVATIVES USING POLYSACCHARIDE-BASED CHIRAL COLUMNS AND POLAR ORGANIC MOBILE PHASES**

I.Matarashvili, L. Chankvetadze, B. Chankvetadze

Department of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Tbilisi, Georgia

**INTRODUCTION**

In the present study the separation of enantiomers of chiral carboxylic acid derivatives was attempted on different polysaccharide-based chiral HPLC columns with polar organic eluents. Along with the successful separation of analyte enantiomers the emphasis of this study was on differences in enantiomer elution order between various columns and mobile phases. In addition, the effect of column temperature on the retention and separation of enantiomers was studied [1].

**MATERIALS AND METHODS**

The separation of enantiomers of 14 chiral carboxylic acid derivatives was studied on 6 different polysaccharide-based chiral columns in high-performance liquid chromatography with methanol, ethanol and acetonitrile as mobile phases with emphasis on the elution order of enantiomers.

**RESULTS AND DISCUSSION**

Some interesting examples of enantiomer elution order reversal were observed function of the nature and composition of chiral selector and mobile phase. For instance, the enantiomer elution order for carprofen, ketorolac, naproxen, proglumide and suprofen reversed with changing the chemical structure of the chiral selector. Also, the enantiomer elution order for carprofen, ketorolac and naproxen was changed by varying the composition of the mobile phase. In addition, the interesting effect of column temperature on the retention and separation of some analytes was observed. For instance, the enantiomers of suprofen were only partially resolved at lower
temperatures but baseline resolved at higher temperature.

CONCLUSIONS

As this study illustrates, the affinity of enantiomers of chiral carboxylic acid derivatives towards polysaccharide-based chiral stationary phases is dependent on the nature of the chiral selector and on the mobile phase composition. Sometimes only subtle changes in the structure of a chiral selector or mobile phase composition may lead to a reversal in the affinity for a particular pair of enantiomers. Temperature can be considered as a very useful parameter for improving the separation. Further studies of these phenomena may provide useful information for understanding the chiral recognition mechanisms with polysaccharide-based chiral stationary phases.

ACKNOWLEDGMENTS

This study was financially supported in part by the Rustaveli Georgian National Science Foundation (RGNSF) grant No 31/90 for fundamental research.

REFERENCES


P-21: METHOD VALIDATION FOR AFLATOXIN ANALYSIS IN COSMETIC CREAMS

I. Gazioğlu¹, U. Kolak²

¹Bezmialem Vakif University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, TURKEY; ²Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, TURKEY

Current tolerance levels set by the European Community for most food products are 2 ppb aflatoxin B1 and 4 ppb total aflatoxins, although cosmetic creams including plant extracts are not specifically addressed. Many studies indicated that their aflatoxin levels were found to be high. Cosmetics which include contaminated plant materials possess high risk for direct consumption on human health. In this study, a high performance liquid chromatography method coupled with fluorescence detector (HPLC-FLD) after post-column derivatization by KOBRA-cell was used. The method was developed for determination of aflatoxins in 10 cosmetic products including plant extract. Hand and body creams from Turkey were selected for analysis and analyzed for AFs detection.

INTRODUCTION

Aflatoxins are secondary toxic metabolites of Aspergillus flavus and A. parasiticus. Aflatoxins can be absorbed through the skin. Potent carcinogens like AFs should be completely absent from cosmetics, this can be achieved by careful quality control of the raw plant materials.

MATERIALS AND METHODS

Samples: 15 random samples were taken from cosmetic markets in Istanbul, Turkey. Cosmetics containing the raw materials under consideration were usually peeling (scrub) creams or powders to be mixed with water, or creams, powders and emulsions for intensive skin cleaning.

Methods: Method validation was done by AOAC 999.07 [1] method with some modifications. Samples were analysed according to this improving method.

RESULTS AND DISCUSSION

The results showed that most of the samples were contaminated with aflatoxins above LOD level. Aflatoxins are known to be highly potent tumour initiating compounds. For such compounds there is no toxicologically harmless level and therefore they should never be ingested. Even if absorption of aflatoxins through the skin is apparently slow from less than 0.05 to 3.41% in 46 hours. The concentration of aflatoxins in cosmetics should be minimized by careful quality control of those raw materials that might contain these compounds [2]. It is possible to produce aflatoxin-free cosmetics.

CONCLUSIONS

In conclusion, the modified methods showed acceptable results at the same laboratory with different analysts for cosmetic products at all levels.

REFERENCES


P-22: THE DETERMINATION OF TRACE METAL CONTENT OF DIFFERENT PARTS OF EUPHORBIA MACROCLADA SPECIES FROM VARIOUS LOCALITIES

I. Yener1, H. Temel2, A. Ertas3, K. Şentürk4, M. Fırat5

Dicle University, Faculty of Pharmacy, 1Department of Analytical Chemistry, 2Department of Pharmaceutical Chemistry, 3Department of Pharmacognosy, 4Department of Pharmaceutical Toxicology, Diyarbakır, TURKEY; 5Yüzüncü Yıl University, Faculty of Education Department of Biology, Van, TURKEY

In this study Euphorbia macroclada plant from different localities (root, stem, leaves, flower, seed and also mixed parts separately) were dried and then homogenized. Homogenized plant samples are digested by microwave oven. Heavy metal contents of prepared samples are observed by ICP-MS device. Depending on our results, our samples that collected from different localities and separated to different parts are including different rates of trace metals, were observed.

INTRODUCTION

Euphorbia is one of the largest genus which belongs to the Euphorbiaceae family, and represented by 105 species in Turkey [1]. Euphorbia species are named as 'Sütleğen' and 'Xaşılı' [2]. Euphorbia species are commonly used in Turkish folk medicine for the treatment of rheumatism, swelling as well as a wart remover. However, inflammation and diarrhoea are the two potential side effects that might occur during the treatment [2]. It’s known that plant’s root parts are accumulate trace metal elements more than plant’s other parts. Additionally some Euphorbia species show accumulating few metals effect was known in literature [3].

MATERIALS AND METHODS

Plant Material

Root and aerial parts (stem, leave, flower and seed) of E. Macroclada were collected from Diyarbakır, Trabzon, Malatya and Van in flowering period. Plants were identified at Istanbul University Faculty of Pharmacy Department of Pharmaceutical Botany and Van Yüzüncü Yıl University Faculty of Education Department of Biology.

Method

Identified E.macrocладa plant samples homogenized by grinding in sterile laboratory conditions. Average 0.2 g of this plant samples is taken. Then 6 mL HNO₃ and 2 mL H₂O₂ added on samples and the samples are digested by closed microwave system. Digested samples are kept at +4°C to analyse day. Mixed metal standart package was bought. And then this standart isuesed for determining heavy metal contents of digested samples. Depending on prepared standart serial, trace metal concentrations of these plant samples are determined by drawing calibration lines in ICP-MS device.

RESULTS AND DISCUSSION

In analyzed E. Macroclada species in terms of metal content Cu and Zn elements possess the highest concentration that is determined. Collected samples from Trabzon Pb element concentration is more than the plants from other cities is determined additionally. Especially it was observed that in working samples, roots of plants have more metal content than the other parts of plants.

CONCLUSIONS

E. macroclada species which was collected from different areas, were separated different parts as seed, root, stem, leave and mixed parts. It was determined that heavy metal contents of the various parts of E. Macroclada found different in each other. As the result, it was observed that E. macroclada plant accumulates Cu, Pb, Cr, Ni, Co and Se elements.

REFERENCES


P-23: TOTAL PHENOLIC AND FLAVONOIDS CONTENTS OF ELEVEN ALLIUM (SAVAGE GARLIC) SPECIES ETHANOL EXTRACT

H. Temel1, E. İzol2, A. Ertas3, İ. Yener4, M. Fırat5

Dicle University, Faculty of Pharmacy, 1Department of Pharmaceutical Chemistry, 2Department of Pharmacognosy, 4Department of Analytical Chemistry, Diyarbakır, TURKEY; 5Yüzüncü Yıl University, Faculty Of Education Department of Biology, Van, TURKEY

In this study underground and aerial parts of eleven different Allium species (A. kharputense, A. rhetoreanum, A. shatakiense, A. scorodoprasum, A. khorasanicum, A. sativum, A. carinatum, A. tuberosum, A.
vinedale, A. tripedale, A. atrovioileaceum, A. akaka, A. scabriscapum, A. chrysantherum, A. shirnakiense) were separated. Ethanol extracts of these parts were prepared and then total phenolic and flavonoid contents of prepared extracts were determined. Total phenolic and flavonoid contents are calculated as respectively pyrocatechol and quercetin equivalents.

INTRODUCTION
In Turkey Allium genus is represented by 179 species [1]. On the earth there are more over 800 species depending on Allium genus. Species that featured in Allium genus are splits some parts between each other. Allium genus includes known species like onion, garlic and leek. Some species is joining in the group whose head smells like onion, generally in appeared on Northern hemisphere (specially on area between Europe’s east and Asia’s west) [1]. In Turkey, traditionally consumed Allium genus like kormen, rock garlic, savage onion, savage garlic, dog onion and shepherd garlic are also used as food or used in treating aim [2].

MATERIALS AND METHODS
Plant material
A. kharputenense, A. rhetoreanum, A. shatakiense, A. scorodoprasum, A. vineale, A. tripedale, A. atrovioileaceum, A. akaka, A. scabriscapum, A. chrysantherum, A. shirnakiense, which were collected by Dr. A. Ertaş, I. Yener, Y. Yeşil and M.Fırat from Turkey (Diyarbakir, Van, Malatya, Kayseri) in 2013-2014 and characterized by M. Fırat and Dr. Y. Yeşil. Voucher specimens have been strowned in the Herbarium of Yüzüncü Yıl University and Istanbul University.

Determination of total phenolic and flavonoid contents
The concentrations of phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs) [3]. The solution (100 µL) of the samples in methanol was added to 4.6 mL of distilled water and 100 µL of Folin-Ciocalteu’s Reagent, and mixed thoroughly. After 3 min, 300 µL sodium carbonate (2 %) was added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of flavonoid compounds was calculated according to the following equation:

\[
\text{Absorbance} = 0.1917 \text{ quercetin (µg)} - 0.1644 (R^2 = 0.9979)
\]

RESULTS AND DISCUSSION
Underground and aerial parts of Allium species which collected from different locals of Turkey was separated. Then their ethanol extracts was prepared and total phenolic and flavonoid contents of prepared 25 extracts was observed. When the results was regarded in species terms it was determined that underground extract of A. atrovioileaceum was richer than other species in terms of Phenolic content, and also aerial parts extract of A. scorodoprasum was found richer than other species in terms of flavonoid content. Generally, worked Allium species was determined to be rich at average level in terms of Phenolic content. And also it can be said that these Allium species were found poor about flavonoid content.

CONCLUSIONS
As the result; total phenolic and flavonoid contents of eleven Allium species are determined which are used as food. In the light of these data, activities and chemical contents of these species will be able to determine in future.

REFERENCES

P-24: TOTAL PHENOLIC AND FLAVONOID CONTENTS OF METHANOL EXTRACT OF NINE EUPHORBIA SPECIES

[Yener1, A.Ertas2, Y.Yeşil3, K.Şentürk4, H.Temel5

Dicle University, Faculty of Pharmacy, 1Department of Analytical Chemistry, 2Department of Pharmacognosy, 4Department of Pharmaceutical Toxicology, 3Department of Pharmaceutical Chemistry, Diyarbakir, TURKEY; 5Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, TURKEY

In this study various parts of nine different Euphorbia species (E. craspeda, E. macrolacta, E. denticulata, E. aleppica, E. eriophora, E. grisophylla, E.
**INTRODUCTION**

The Euphorbiaceae is a large family of the flowering plants, that includes 300 genera and over 5,000 species ranging from annuals to trees. *Euphorbia* is one of the largest genus which belongs to Euphorbiaceae family, and represented by 105 species in Turkey. *Euphorbia* species are named as “Sütlügen” and “Xasil”. *Euphorbia macroclada* and *Euphoria gaillardotii* are commonly used in Turkish folk medicine for the treatment of rheumatism, swelling, as well as a wart remover [1]. However inflammation and diarrhea are the two potential side effects that might occur during the treatment. A literature survey of the genus showed that many of its constituents are highly bioactive in phytochemical analyses. Many different parts of the *Euphorbia* species like roots, seeds, latex, stem, stem barks, leaves and whole plants have been studied. Moreover, it is found that the plants in the Euphorbiaceae family are well known for the chemical diversity of their isoprenoid constituents. The major constituents of the genus are diterpenoids Many biological activities of the constituents of the *Euphorbia* species have been reported for a decade [2].

**MATERIALS AND METHODS**

**Plant material**

*E. craspedia*, *E. macroclada*, *E. denticulata*, *E. aleppica*, *E. eriophora*, *E. grisophylla*, *E. seguiriana*, *E. fistulosa*, *E. falcata*, which were collected by Dr. A. Ertas, İ. Yener, Y. Yeşil and M.Furat from Turkey (Mardin, , Malatya, Trabzon, Kayseri, Diyarbakır, Van) in 2013-2014 and characterized by Dr. Y. Yeşil (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University). Voucher specimens have been strored in the Herbarium of Istanbul University, Faculty of Pharmacy.

**Determination of total phenolic and flavonoid contents**

The concentrations of phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs) [2]. The solution (100 µL) of the samples in methanol was added to 4.6 mL of distilled water and 10 µL of Folin-Ciocalteu’s Reagent, and mixed thoroughly. After 3 min, 300 µL sodium carbonate (2%) was added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds were calculated according to the following equation that were obtained from standard pyrocatechol graphics:

\[ \text{Absorbance} = 0.021 \text{pyrocatechol(µg)} + 0.0229 \quad (R^2 = 0.9913) \]

\[ \text{Absorbance} = 0.0248 \text{pyrocatechol(µg)} + 0.0158 \quad (R^2 = 0.9929) \]

Measurement of flavonoid content of the crude extracts were based on the method described by Moreno et al. with a slight modification and results were expressed as quercetin equivalents [2]. An aliquot of 1 mL of the solution (contains 1 mg of crude extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was determined at 415 nm. The concentration of flavonoid compounds were calculated according to the following equations:

\[ \text{Absorbance} = 0.1797 \text{ quercetin (µg)} - 0.1256 (R^2 = 0.9980) \]

\[ \text{Absorbance} = 0.1568 \text{ quercetin (µg)} - 0.0880 \quad (R^2 = 0.9953) \]

**RESULTS AND DISCUSSION**

*Euphorbia* species are dismembered to gain various parts of the plant which are collected from different locals in Turkey. And these parts of the plant are converted to their methanol extract. Then total phenolic and flavonoid contents of prepared 61 extracts are observed. When the results regarded in species terms, it is determined that *E. macroclada* is richer about both phenolic and flavonoid content levels than the other species. When this species is regarded in its own, leave and flower extracts of the plant include higher percentage of phenolic and flavonoid contents than the other parts of the plant is determined.

**CONCLUSIONS**

As the result; total phenolic and flavonoid contents of nine *Euphorbia* species was determined. These data will able to benefit on science world by using in activity and isolation studies of *Euphorbia* species.

**REFERENCES**


2. Ertas, A.; Yilmaz, M. A.; Firat, M., Chemical profile by LC-MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two Euphorbia species. Natural Product Research 2015, 29(6), 529–534
P-25: AN EFFICIENT LC-MS/MS METHOD VALIDATION FOR DETERMINATION OF WATER SOLUBLE VITAMINS IN THYMUS

M.A. Yilmaz1, O. Cakir1, A. Boukeloua2, I. Yener1,3, H. Temel1,3

1Dicle University Science and Technology Research and Application Center, Diyarbakır, TURKEY
2University of Constantine 1, Laboratory of Toxicology and Pharmacology, Constantine, ALGERIA
3Dicle University, Faculty of Pharmacy, Diyarbakır, TURKEY

A comprehensive and effective UHPLC-MS/MS method for the simultaneous determination of 8 water-soluble vitamins including vitamin B1 (thiamine), B2 (riboflavin), B3 (both nicotinic acid and nicotinamide), B5 (pantothenic acid), B6 (pyridoxine), B9 (folic acid) and B12 (cyanocobalamine) in plants was developed and validated. Edible thymus was used as sample. The extraction protocol and method validation parameters such as LOD, LOQ, linearity, repeatability, recovery and uncertainty were determined.

INTRODUCTION

Being biologically active organic compounds, vitamins are that are essential compounds involved in metabolic and physiological functions in the human body. Additionally, vitamins are present in almost all foods we consume and are essential for our health [1]. There are 13 vitamins identified that are classified according to their solubility into fat-soluble vitamins (FSV) (A, E, D, and K) and water-soluble vitamins (WSV) (B-group vitamins and vitamin C) [2]. These compounds greatly differ in their chemical composition, physiological action and nutritional importance in the human diet, even within the same group [3]. For the determination of water-soluble vitamins UHPLC tandem mass spectrometry (LC-MS/MS) is a promising method for the simultaneous determination of multiple components in plants because of its high sensitivity and specificity. In this study we screened the water soluble vitamins in Thymus that is widely consumed as tea and in meals.

MATERIALS AND METHODS

Thymus samples were purchased from a local market in Diyarbakır, Turkey. Dried thymus samples were grounded and extracted by 0.1 M acetic acid and 2 M ammonium acetate solutions. After that the extract was centrifuged and filtered through 0.2 µm filter prior to LC-MS/MS analysis. A Shimadzu LCMS 8040 model LC tandem MS instrument were used for this method.

RESULTS AND DISCUSSION

In this simple and efficient LC-MS/MS method was validated according to EUROCHEM. Calibration points of the vitamin standards (vitamin B1 (thiamine), B2 (riboflavin), B3 (both nicotinic acid and nicotinamide), B5 (pantothenic acid), B6 (pyridoxine), B9 (folic acid) and B12 (cyanocobalamine)) were between 25 ppb and 1000 ppb. The method validation parameters were limit of detection (LOD), limit of quantification (LOQ), linearity, repeatability, recovery and uncertainty. The regression coefficients of the calibration curves were over 0.99.

CONCLUSIONS

A UHPLC-MS/MS method was developed and validated for the simultaneous analysis of vitamin B1, B2, B3 (nicotinamide and nicotinic acid), B5, B6, B9, B12 in plants. The sample plant was chosen to be thymus. It is a simple, low-cost and time-efficient method and can be used for the analysis of plants, vegetables, fruits and mushrooms.

ACKNOWLEDGMENTS

Authors involved in this study are thankful to Dicle University and DÜBTAM for their great efforts and helps.

REFERENCES


P-26: EFFECT OF BASIC AND ACIDIC ADDITIVES ON THE SEPARATION OF SOME BASIC DRUG ENANTIOMERS ON POLYSACCHARIDE-BASED CHIRAL COLUMNS WITH ACETONITRILE AS MOBILE PHASE

K. Gogaladze, B. Chankvetadze

Institute of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Tbilisi, Georgia

INTRODUCTION

Polysaccharide-based chiral selectors are established as the most useful materials for analytical and preparative-scale separation of enantiomers in liquid chromatography and several related techniques, such as super-/sub-critical fluid chromatography, nano-liquid chromatography and capillary electrochromatography. In spite of wide application of polysaccharide phenylcarbamates and ethers in liquid-phase separation of enantiomers, the chiral recognition mechanism of these materials is still poorly understood. Although many efforts involving various experimental and computation techniques have been
made in the past, at present we are still far from the status allowing us to develop a tailor-made chiral selector for separation of enantiomers of a given chiral analyte, to predict the most useful separation mode, mobile phase and mobile phase additives not to mention at all the enantiomer elution order (EEO).

MATERIALS AND METHODS
Diethylamine, formic acid and commercially available chiral beta-blockers, acebutolol, alprenolol, atenolol, betaxolol, bisoprolol, bupranolol hydrochloride, bumetanol hydrochloride, celiprolol, isoproterenol, mabuterol, metoprolol, metipranolol, nifenalol, oxprenolol, sotalol and tolprolol were purchased from Sigma Aldrich (Taufkirchen, Germany). Acetonitrile of HPLC quality was acquired from Carl Roth (Karlsruhe, Germany). The amylose tris(3,5-dimethylphenylcarbamate) (ADMPC)-based column was an experimental column provided by Enantiosep GmbH (Münster, Germany) while the other chiral columns, Lux Amylose-2, Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3 and Lux Cellulose-4, were kindly provided by Phenomenex (Torrance, CA, USA). All columns (Fig.1).

All HPLC experiments were performed with an Agilent 1200 HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a G1367C HiP ALS-SL autosampler, a G1316B TCC-SL temperature controller, a G1311A quaternary pump and a G1314D VWD variable wavelength detector. Chemstation software (version B.03.02-SR2) was used for instrument control, data acquisition and data handling. If not stated otherwise, the samples were dissolved in the mobile phase used for the respective separation at a concentration of 0.2 mg/ml. HPLC separations were performed at 20°C with 1.00 ml/min mobile phase flow rate and detection was performed at 220 nm.

RESULTS AND DISCUSSION
Interesting tendency was observed when screening 6 polysaccharide-based chiral columns on their separation ability towards 16 chiral β-blocker drugs. Thus, both amylose-based columns, Lux Amylose-2 and ADMPC were more universal than cellulose-based columns. Based on their applicability the tested columns could be ranked as Lux Amylose-2>ADMPC>Lux Cellulose-4>Lux Cellulose-1>Lux Cellulose-2. No separation of enantiomers was observed on cellulose ester type column, Lux Cellulose-3. Significant difference in the applicability of 2 cellulose-based chiral columns with similar chemistry of chiral selectors, namely Lux Cellulose-2 and Lux Cellulose-4 was quite unexpected. This might be caused by statistically small number of analytes together with low overall success rate with ACN as the mobile phase. From the mobile phases used ACN with 0.1% DEA was most universal in the case of both Lux Amylose-2 and ADMPC. The success rate with ACN+FA and ACN+DEA+FA was very similar in the case of both chiral selectors. The mobile phase with DEA was more successful than the mobile phase with FA. No complementarity was observed between these additives, i.e. no new separation was observed with FA as additive. However, there was a complementarity observed from the viewpoint of the EEO that is discussed in the next subsection.

Thus, for instance, the enantioseparation of several β-blockers (atenolol, sotalol, tolprolol) improved not only by the addition of a more conventional basic additive to the mobile phase, but also by the addition of an acidic additive. Moreover, an opposite elution order of enantiomers was observed depending on the nature of the additive (basic or acidic) in the mobile phase (Fig.2).

![Fig. 2. Separation of enantiomers of atenolol (1:2 ratio of S- and R-enantiomers, respectively) on Lux Amylose-2 column with the following mobile phases: ACN (a), ACN+0.1% DEA (b), ACN+ eq. FA (c), and ACN+0.1% DEA+ eq. FA (d).](image-url)
CONCLUSIONS

Based on the study of HPLC enantioseparation of 16 β-blocker drugs on 4 cellulose- and 2 amylose-based chiral columns and acetonitrile as a bulk mobile phase more universal enantiomer resolving ability of amylose-based columns was observed. In addition, interesting examples of the reversal of enantiomer elution order based on the chemistry of the chiral selector and the nature (basic or acidic) of minor additives to the mobile phase was established. Of special interest seems to be the possibility of enantiomer elution order adjustment for atenolol, sotalol and toliprolol by alternative use of diethylamine or formic acid additives.

REFERENCES


P-27: DETERMINATION OF TARTRAZIN USING NOVEL ELECTROCHEMICAL SENSOR BASED ON NANOCERIA IN DRINK SAMPLES

S. Kart, O.Karasalli, D. Koyuncu Zeybek
Dumlupınar University, Faculty of Science and Arts, Department of Biochemistry, Kutahya, TURKEY

This paper described a novel electrochemical sensor based on nanoceria (CeO_2NP) modified carbon paste electrode to determination of tartrazine. The electrochemical characterization of this sensor was actualized via CV and EIS methods. The proposed sensor was successfully used to the electrochemical determination of tartrazine in drink samples and the results were in good agreement with those obtained using HPLC.

INTRODUCTION

Synthetic dyes have been widely used as food additives to optimize food color. Generally, synthetic dyes contain azo functional groups and aromatic ring structures, so they are harmful to human health [1]. Tartrazine (TA), one of the food colorants, can cause allergies, eczema and even cancer if they were excessively consumed [2, 3]. Until now, different methods such as spectrophotometry and HPLC have been reported for the determination of TA. Electrochemical sensors have also been proposed for their advantages of high sensitivity, simplicity and low cost to the TA analyses.

MATERIALS AND METHODS

The BCPE was prepared by mechanically mixing graphite powder and mineral oil. CeO_2NP/MCPE was composed of nanoceria and graphite powder in various ratios of CeO_2NP/graphite powder (w/w) by keeping constant amount of mineral oil. The electrocatalytic properties of nanoceria were studied via CV and EIS techniques in the redox probe solution. Electrochemical behaviour of TA at the CeO_2NP/MCPE was studied by DPV technique in the potential range of −0.4 to 1.3 V vs. Ag/AgCl scan rate of 20 mV s^−1.

The effects of scan rate and pH on the electrochemical response of TA were investigated. CeO_2NP/MCPE sensor was used to determination of TA in drinks. HPLC method was also employed for comparison.

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>Working ranges (molL^−1)</th>
<th>R^2</th>
<th>LOD (molL^−1)</th>
<th>Repeatability (%)</th>
<th>Reproducibility (%RSD)</th>
<th>Working pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1×10^−3 - 1×10^−4</td>
<td>0.9950</td>
<td>0.9902</td>
<td>6.7×10^−10</td>
<td>1.77 (n=5)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytic application</th>
<th>Drink samples</th>
<th>CeO_2NP/MCPE (mg/L)</th>
<th>HPLC (mg/L)</th>
<th>Recovery (%)</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23.61±0.35</td>
<td>23.64</td>
<td>99.86</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8.57±0.35</td>
<td>8.49</td>
<td>100.97</td>
<td>1.22</td>
<td></td>
</tr>
</tbody>
</table>

Fig.1. Electrooxidation mechanism of TA and calibration plot

CONCLUSIONS

In this paper, we have reported an electrochemical sensor based on nanoceria for determination of TA. Nanoceria exhibits high electrocatalytic activity and...
CeO2NPs/MCPE has been shown to be efficient for oxidation of TA.

REFERENCES

P-28: A NEW ‘TURN-ON’ AND REVERSIBLE FLUORESCENT PROBE WITH HIGH AFFINITY TO IRON (II) IONS IN AQUEOUS SOLUTION

M. Ozdemir¹*, S. Uruş², M. Sonmez³

¹Kilis 7 Aralık University, Faculty of Arts and Sciences, Department of Chemistry, 79000-Kilis, TURKEY
²University of K. Maras Sutcu Imam, Faculty of Arts and Sciences, Department of Chemistry, 46100-K. Maras, TURKEY
³Gaziantep University, Arts and Sciences Faculty, Department of Chemistry, 27310-Gaziantep, TURKEY

INTRODUCTION
Fluorescent chemosensors have recently received attraction in the fields of biology and environmental science due to their capability to sense and identify special chemical species [1]. Furthermore, coumarine based organic molecules have been increasingly studied by researchers as fluorescent probes on account of their high photo stability, large Stokes shift, and high fluorescence quantum yield [2].

MATERIALS AND METHODS
Reagents: N-amino-pyrimidine and 7-N, N-dietylamino-coumarine-3-aldehyde were synthe-sized according to the methods [2,3]. Metal salts and other chemical reagents were purchased from Sigma-Aldrich, Merck and Fluka.

Spectroscopic measurements were recorded using Varian Carry Eclipse Fluorescence and UV-VIS spectrophotometers. Fluorometric metal ion titration solutions were prepared and diluted to the respective concentration (concentration: 1 mM, metal ions: Na⁺, K⁺, Mg²⁺, Ca²⁺, Pb²⁺, Cu²⁺, Mn²⁺, Ni²⁺, Ag⁺, Fe³⁺, Cr³⁺, Zn²⁺, Al³⁺, Hg²⁺, Fe³⁺, Cd²⁺, Co²⁺). The solution of sensor ‘CNAM’ (1 mM) was prepared in dimethyl sulfoxide-aqueous solution (DMSO/H₂O, 8:2, v/v). Each time, a 100 μL of CNAM solution and the mixture was completed to the 2 ml with DMSO/PBS buffer (2:1).

RESULTS AND DISCUSSION
Selectivity and sensitivity of CNAM as the fluorescent sensor for Fe²⁺ has been studied in presence of various competing metal ions. A job’s plot obtained from absorption data showed 1:1 stoichiometric ratio between sensor and Fe²⁺ during the complexation. CNAM in acetonitrile solution displayed photophysical properties that colour changes have been significantly different from those of other analytes.

CONCLUSIONS
Herein, a coumarine based chemosensor was synthesized and this compound exhibited enhanced fluorescence with high selectivity in presence of Fe²⁺ towards most competing metal ions. Ongoing coumarine-based fluorescent ligand design studies in our laboratory to further develop the selectivity and sensitivity of receptor for specific labeling and detection of iron (II) ions are presently under investigation.

REFERENCES
1. Ma, J., Sheng, R., Wu, J., Liu, W., Zhang, H., 2014, A new coumarine-derived fluorescent sensor with red-


**P-29: A NOVEL MWCNT SUPPORTED “OFF – ON” COLORIMETRIC FLUORESCENT PROBE FOR IRON (II) IONS DETECTION IN AQUEOUS MEDIUM**

M. Ozdemir1*, S. Urus2, M. Caylar3, İ. Karteri4

1Kilis 7 Aralik University, Faculty of Arts and Sciences, Department of Chemistry, 79000-Kilis, TURKEY
2University of K.Maras Sutcu Imam, Faculty of Arts and Sciences, Department of Chemistry, 46100-K.Maras, TURKEY
3Research and Development Centre for University–Industry–Public Relations (USTKIM), KSU, K.Maras, TURKEY
4University of K.Maras Sutcu Imam, Faculty of Arts and Sciences, Department of Physics, 46100-K.Mar resolution.

**INTRODUCTION**

The detection and sensing of biologically and environmentally important metals have gained interest in the field of chemical sensors in recent years [1]. Therefore, the design and development of fluorescent chemosensors for various analytes have received considerable attention due to their biological and environment important roles [2]. Colorimetric and fluorescent chemosensor based on the interaction of ligands with metal ion, leading to the change of signals visible to the naked-eyes can be successfully implemented in many fields of research.

**MATERIALS AND METHODS**

Multi-walled carbon nanotube (MWCNT), Rhodamine B, Hydrazine monohydrate, 2,6-diacetylpyridine, 3-aminopropytriethoxysilane (APTES) and other chemical reagents were purchased Sigma & Aldrich, Merck and Fluka. Spectroscopic measurements were conducted using Varian Carry Eclipse Fluorescence and UV-VIS spectrophotometers. Fluorometric metal ion titration solutions were prepared and diluted to the respective concentration(concentration:1 mM, metal ions: Na$^{+}$, K$^{+}$, Mg$^{2+}$, Ca$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Ag$^{+}$, Fe$^{2+}$, Cr$^{3+}$, Zn$^{2+}$, Al$^{3+}$, Hg$^{2+}$, Fe$^{3+}$, Cd$^{2+}$, Co$^{2+}$).

**Synthesis of fluorescent probe MWCNT-AP-RhDAP:** Firstly, rhodamine hydrazide was synthesized according to method [3]. Then, rhodamine hydrazide and 2,6-diacetylpyridine were refluxed in the ethanolic solution at the equal amount. Finally, MWCNT-APTES prepared from MWCNT, TEOS and APTES was taken into ethanolic solution of Rh-

DAP and heated to reflux at 85 $^\circ$C. After the purification process, it was characterised by FT-IR, NMR ($^1$H, $^13$C), XRD, EDX, SEM and TEM.

**RESULTS AND DISCUSSION**

* In order to investigate the fluorescence selectivity and sensitivity of synthesized compound toward some special metal ions, fluorescence and UV-VIS measurements were successfully performed in CAN/Tris-HCl buffer (2:1).

* Herein, it was found that the fluorescent chemo-sensor ‘MWCNT-AP-RhDAP’ showed a highly selectivity and sensitivity exhibiting “turn-on” response to Fe$^{2+}$ with a colour change from colourless to purple.

**CONCLUSIONS**

In conclusion, a novel naked-eye fluorescent probe has been designed and synthesized by covalently attaching to the surface of MWCNT modified using APTES. It has also been found that this fluorescent nano-sensor shows a high sensitivity and selectivity for Fe$^{3+}$ ions towards other competing metal ions with no significant signal changes. Consequently, this nano-sensor design may provide a significant strategy for synthesis of the novel fluorescent chemosensors for the analytes in environmental and biological applications.
REFERENCES


P-30: DESIGN AND SYNTHESIS OF A NOVEL MAGNETIC CORE-SHELL Fe₃O₄@SiO₂ SUPPORTED CHEMOSENSOR BASED ON RHODAMINE B: HIGHLY SELECTIVE AND SENSITIVE FLUORESCENT PROBE FOR CR³⁺ AND HG²⁺

M. Ozdemir¹*, S. Urus², M. Caylar³, İ. Karteri⁴

¹Kilis 7 Aralık University, Faculty of Arts and Sciences, Department of Chemistry, 79000-Kilis, TURKEY
²University of K.Marş Sütçü İmam, Faculty of Arts and Sciences, Department of Chemistry, 46100-K.Marş, TURKEY
³Research and Development Centre for University–Industry–Public Relations (USTKIM), KSU, K.Marş, TURKEY
⁴University of K.Marş Sütçü İmam, Faculty of Arts and Sciences, Department of Physics, 46100-K.Marş, TURKEY

INTRODUCTION

Heavy metal pollution has currently become a significant issue for the human health, living resources, and ecological systems. Also, on account of their not biodegradability and accumulating properties in living organisms, they have been reported to be associated with many diseases and disorders concerned with nervous, immune, and gastrointestinal systems [1]. For human and environmental health, those pollutants must be detected and removed from media. For this reason, new solutions must be developed to overcome and to deal with this problem. In this context, magnetic core-shell Fe₃O₄@SiO₂ nanoparticles have been attracted by many researchers on the detection of the various heavy and transition metal ions as a fluorescent probe due to the their biocompatibility, renewability and stability against the degradation. Rhodamine based fluorescent chemosensors have increasingly been gained an attention on account of their high fluorescence quantum yield, broad absorption values, emission wavelengths, and large absorption coefficients [2].

MATERIALS AND METHODS

Rhodamine B, Hydrazine monohydrate, 2,6-Diacetylpyridine, 3-Aminopropyltriethoxy silane (APTES), Tetraethoxysilane (TEOS), FeCl₂, FeCl₃.6H₂O and other chemical reagents have been purchased from Sigma-Aldrich, Merck and Fluka.

Spectroscopic measurements were conducted using Varian Carry Eclipse Fluorometer and UV-VIS spectrophotometers. Fluorometric metal ion titration solutions were prepared and diluted to the respective concentration (concentration:1mM, metal ions: Na⁺, K⁺, Mg²⁺, Ca²⁺, Pb²⁺, Cu²⁺, Mn²⁺, Ni²⁺, Ag²⁺, Fe²⁺, Cr³⁺, Zn²⁺, Al³⁺, Hg²⁺, Fe³⁺, Cd²⁺, Co²⁺).

Synthesis of fluorescent probe Fe₃O₄@SiO₂-APTES-RhDAP: Firstly, rhodamine hydrazide was synthesized according to method [3]. Next step, rhodamine hydrazide and 2,6-diacetylpiridine were refluxed in the ethanolic solution at the equal amount. After purification processes, Fe₃O₄@SiO₂-APTES was prepared using two steps illustrated on Scheme 1, and RhDAP was taken into ethanolic solution and heated to reflux at 85°C. Then, it was characterised by FT-IR, NMR (¹H, ¹³C), XRD, EDX, SEM and TEM.

RESULTS AND DISCUSSION

Figure 1. Fluorescence spectra changes of probe Fe₃O₄@SiO₂-APTES-RhDAP (0.02 g/L) with Hg²⁺, Cr³⁺ at 0.25 µM and other various metal ions (0.25 mM) (λₑₓ=530 nm, λₑᵐ=587 nm)

CONCLUSIONS

In summary, herein, a novel magnetic core-shell, Fe₃O₄@SiO₂, nanoparticles as special immobilizing carrier of fluorescent chemo-sensor has been synthesized and characterized. This organic-inorganic hybrid material showed as a highly selective and sensitive FRET-based ratio- metric sensor for Cr³⁺ and Hg²⁺ ions in acetonitrile/water solutions.
P-31: DEVELOPMENT OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE DETERMINATION OF EMTRICITABINE, RILPIVIRINE AND TENOFOVIR FROM BIOLOGICAL SAMPLES

M. Gumustas, M.G. Caglayan, F. Onur, S.A. Ozkan
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, TURKEY

INTRODUCTION
Combination of emtricitabine, tenofovir and rilpivirine are used in treatment of virologically suppressed patients who have HIV-1 RNA plasma viral load (pVL) of ≤100,000 copies/mL [1]. This combination has been used worldwide by patients suffered from human immunodeficiency virus (HIV). The goal of present study to develop and validate rapid, accurate, selective and reproducible capillary electrophoresis method for determination of emtricitabine (EMT), rilpivirine (RIL) and tenofovir (TEN) (Fig 1.) from biological samples.

MATERIALS AND METHODS
Stock solutions of EMT, TEN and RIL were prepared in methanol as final concentration of 1000 µg.mL⁻¹. Phosphate buffer was used as background electrolyte. Capillary electrophoretic measurements were conducted with an Agilent 7100 capillary electrophoresis system coupled with diode array detector (Agilent Technologies, Germany). Samples were separated in a bare fused silica capillary with an inner diameter of 50 µm and effective length of 31.5 cm (total length of 40 cm). Hydrodynamic injection was used as 50 mbar pressure for 1 second and detection wavelength was selected as 210 nm. Temperature of capillary cassette was hold at 15 °C.

RESULTS AND DISCUSSION
The method was optimized and validated according to the ICH Guidelines [2]. Optimization were carried out for minimizing the analysis time without detriment to peak symmetry and resolutions, pH 2.5, voltage 30 kV and concentration 53.1 mM in order to keep the analysis time minimum. Calculated charges are 0.0, +1.0 and +0.1 for EMT, RIL and TEN respectively at pH 2.5. They were separated in order of RIL, TEN and EMT. Ranges for the samples were 5-1000 µg mL⁻¹ for emtricitabine and rilpivirine and 10-400 µg mL⁻¹ for tenofovir.

CONCLUSIONS
Proposed capillary electrophoretic method offers rapid, low-cost and reliable analysis for the determination of EMT, TEN and RIL with small amount of samples and reagents consumption. The samples are well separated within 5 minutes. All method validation parameters investigated according to ICH guideline. After optimization, present method was applied to spiked urine samples with an acceptable accuracy.

REFERENCES
INTRODUCTION

Between the two main groups of analgesics, the non-narcotic ones (nonsteroidal anti-inflammatory drugs (NSAIDs)) have more therapeutic importance as they have anti-inflammatory effects and do not cause drug dependence. NSAIDs are among the most widely used therapeutics, primarily for the treatment of pain and inflammation. Most pharmaceutical compounds are either protonated or deprotonated in aqueous solution. The ionization ability is measured by a parameter called the acid ionization constant (K_a). The ionized form is usually more water soluble, while the neutral form is more lipophilic and has higher membrane permeability. Hence, knowledge of the possible ionization states of a pharmaceutical substance by determining their K_a values is vital for drug development since it can allow a reduction of attrition rates and shorter development times in drug discovery programs [1]. Spectrophotometric methods are generally used for measuring pK_a values. On the other hand, potentiometric titration is the standard method for pK_a measurements. However, this method is subject to some limitations such as the sample amounts and solution volumes used. The determination of pK_a values by capillary zone electrophoresis (CZE) is based on the observation of the effective mobility of an ionizable compound in a series of electrolyte solutions with constant ionic strength and at different pHs. The first purpose of this study is to determine the pK_a values of 5-(Methyl/Nitro)-2-(3H)-benzoxazolinone and its 21 derivatives by using a spectrophotometry, potentiometry and CZE methods. This research is of great importance since knowing K_a of the compound is essential for understanding the chemical interactions and its pharmacological effects. Furthermore, the relationship between the acidity constant and analgesic/anti-inflammatory activities of the drug candidates were discussed.
an official liquid chromatographic method on its determination in pharmaceutical preparations. The aim of this study was to develop a validated liquid chromatography method for determination of Apixaban in tablets.

![Chemical Structure of Apixaban](image)

**Figure 1. Chemical Structure of Apixaban.**

**MATERIALS AND METHODS**

**Liquid chromatograph:** Analyses were performed using a Nexera series of ultra high performance liquid chromatography system from Shimadzu, which was composed of two binary pumps equipped with separate degassers and a low-pressure gradient unit, an auto-sampler and an column oven with cooler functions, a communications bus module, and a diode array detector; the system was controlled and chromatograms were integrated by using LabSolutions software (All Japan).

**Sample preparation:** Thirty tablets were used for the assay. Each tablet was transferred to a separate 100-mL volumetric flask and 50 mL of acetonitrile/water solution was added. The content of the flask was stirred using a magnetic stirrer for 20 min until dissolution. Subsequently, the flask was filled up to volume with the same solvent and content was sonicated for 10 min. Ten milliliter portion of the resulting mixture was transferred into a polypropylene centrifuge tube and centrifuged to precipitate insoluble excipients. About two milliliters of the supernatant was taken and filtered through a 0.20-ȝm syringe filter. After subsequent dilution, the resulting solution was injected on to the column.

**RESULTS AND DISCUSSION**

Analysis of Apixaban was achieved with very high efficiency in term of theoretical plates, using ultra high performance liquid chromatography. After performing initial method development applications, the proposed method was applied on different columns to verify its validity. Isocratic elution of Apixaban was successful, observing good system suitability values. Effects of mobile phase composition and detection wavelength on the retention time and signal intensity were evaluated. Similar retention properties were observed when using C18 type stationary phases. As an important point, the effects of particle size and its distribution on the analyses was clearly observed once more, as a crucial factor in separation sciences.

**CONCLUSIONS**

The proposed method in this study was successfully applied on the real samples, possessing very high chromatographic efficiency.

**ACKNOWLEDGMENTS**

The authors would like to thank Anadolu University Scientific Research Projects Commission for valuable support on this research (Project No: 1404S124.)

**REFERENCES**


**P-34: A NEW ALTERNATIVE TO SOLID PHASE EXTRACTION OF CEFTRIAXONE IN HUMAN PLASMA SAMPLES: ULTRAFILTRATION-BASED EXTRACTION AND HPLC ANALYSIS**

M. Çelebier1, İ. Süslü2, S. Boynueğri2, S. Altınöz2

1Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey
2Ankara Numune Education and Research Hospital, 3rd Serves Ear Nose and Throat Clinic, Ankara, Turkey

In this study, an ultrafiltration-based extraction technique was developed as an alternative to solid phase extraction of ceftriaxone prior to HPLC analysis. The developed extraction technique and HPLC method was validated according to FDA guidelines and it was successfully applied for analysis of ceftriaxone in human plasma samples. The newly developed extraction technique presents simplicity in comparison to solid phase extraction of ceftriaxone and could be applied on pharmacokinetic studies.

**INTRODUCTION**

Ceftriaxone (CEF), a semisynthetic third-generation cephalosporin, is effective against a wide variety of Gram - positive and Gram -negative bacteria [1]. In
this study an ultrafiltration-based extraction was developed for determination of CEF in human plasma samples. The extracted samples were analysed by HPLC.

MATERIALS AND METHODS
Chemicals and Apparatus: Millipore Microcon® 3K 0.5 mL Centrifugal Filters and methanol (MeOH) were supplied from Merck (Darmstadt, Germany). Citric acid and sodium hydroxide were from Sigma-Aldrich (St Louis, USA). All the solutions were prepared by using Milli-Q water obtained from BarnsteadNanopure™ system from Thermo Scientific.

Extraction procedure: 500 µL of plasma sample was taken into an eppendorf tube. 500 µL of MeOH added and centrifuged at 14,000 rpm for 10 min. The supernatant (500 µL) was transferred into the Microcon® tube and centrifuged at 14,000 rpm for 15 min. 250 µL of filtered part was taken and the dried by using SpeedVac Vacuum Concentrator (1.5 hours, 35 °C, 1700 rpm). The dried part was dissolved in 100 µL of citrate buffer at pH 3.0 and centrifuged. 20 µL of the centrifuged part was diluted by mobile phase to 1000 µL in a vial and injected into the HPLC system.

HPLC analysis: Separations were carried on a Nucleosil C18 100-3 (125 mm x 4.6 mm, 5µm i.d.) HPLC column. The flow rate was 1.0 mL min⁻¹ while using isocratic elution with pH 3.0 citrate buffer: MeOH (75:25 v/v) mixture. Injection volume was 20 µL and UV detection was performed at 270 nm.

RESULTS AND DISCUSSION
HPLC chromatograms of standard CEF (1.0 µg mL⁻¹) and one of the extracted sample are given in Figure 1.

Fig. 1. Chromatograms of plasma sample and standard CEF (1.0 µg mL⁻¹) sample

The developed method was precise, accurate, sensitive and specific.

CONCLUSIONS
CEF is a well-known and widely used active pharmaceutical ingredients, its analysis individually and simultaneously with other compounds in biological samples have been reported by HPLC for a long time. By the ultrafiltration-based extraction technique presented in this study, it would be easy to analyse CEF in human plasma in comparison to the solid phase extraction techniques.
MeOH:Water (50:50 (v/v)) mixture. Injection volume was 20 µL. The peak area of PA and the peak area ratios of PA to IS were evaluated in the λ max for PA.

Preparation of Calibration Standards: 1, 5, 10, 20, 30, 40 and 50 µg mL⁻¹ of PA solutions were prepared by using 5, 20 and 40 µg mL⁻¹ of IS of IS in HPLC vials. Each IS concentration indicates one calibration set. There are three calibrations sets in this study.

Preparation of Sample Solutions: Parol® Tablets containing 500 mg PA were used. Tablet solutions contain 5 and 20 µg mL⁻¹ PA where 5, 20 and 40 µg mL⁻¹ of IS were being used. There are six sample sets in this study.

RESULTS AND DISCUSSION
All the calibration sets and sample sets were prepared by same analyst and same instrument. The results on calibration curve techniques should be identical with internal standard technique theoretically if there was no errors. The relative standard deviations were from 0.385 to 2.90 and 1.39 to 3.17 whereas the bias values were from -0.21 to 4.51 and -5.97 to 1.75 for calibration curve and internal standard techniques, respectively. These results indicate that the effect of internal standard on the precision and accuracy was not significant.

CONCLUSIONS
According to the results on our experimental conditions, the internal standard technique statistically effect the analysis results but did not improve the precision and accuracy.

REFERENCES

P-36: LIQUID CHROMATOGRAPHIC DETERMINATION OF TOFISOPAM IN TABLETS USING DIFFERENT TYPES OF STATIONARY PHASES
N. Ö. Can, S. Özcan
Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry

INTRODUCTION
Tofisopam (TOF) is a 2,3-benzodiazepine type anxiolytic compound, which is generally prescribed for the treatment of alcohol withdrawal, anxiety and irritable bowel syndrome. Although there are some previously published protocols [1-3], there is still not an official LC method focused on determination of TOF in pharmaceutical preparations. Thus, the aim of this study was to develop a validated liquid chromatography method for determination of TOF in tablets. Assay of TOF was performed applying the same instrumental conditions on different types of stationary phases, which were C₁₈-bonded conventional particle-based, core-shell, and monolithic silica columns.

MATERIALS AND METHODS
Liquid chromatograph: Analyses were performed using a Nexera series of liquid chromatography system from Shimadzu (Japan), which was composed of two DGU-20A5R online degassers, two LC-30AD parallel plunger pumps equipped with a low-pressure gradient unit, SIL-30AC auto-sampler, CTO-20AC column oven, FCV-32AH high-pressure flow-line selection valve, CBM-20A communications bus module, and SPD-M20A diode array detector. The system was controlled and chromatograms were integrated by using LabSolutions software (Japan).

Sample preparation: Ten tablets were used for the assay. Each tablet was transferred to a separate 100-mL volumetric flask and 50 mL of acetonitrile/water solution was added. The content of the flask was stirred using a magnetic stirrer for 20 min until dissolution. Subsequently, the flask was filled up to volume with the same solvent and content was sonicated for 10 min. Ten milliliter portion of the resulting mixture was transferred into a polypropylene centrifuge tube and centrifuged to precipitate insoluble excipients. About two milliliters of the supernatant was taken and filtered through a 0.20-µm syringe filter. After subsequent dilution, the resulting solution was injected on to the column.

RESULTS AND DISCUSSION
Isocratic elution of TOF was successfully achieved with adequate capacity factor, utilizing a mobile consisted of phosphate buffer, acetonitrile and water. Effects of the detection wavelength, column temperature and mobile phase composition on the retention time, system backpressure and signal intensity were evaluated, and comparison of system suitability parameters and chromatographic performances between the columns were realized. The results of the study reveal that a single liquid chromatographic method can be applicable on different type of stationary phases, observing similar retention properties. As a remarkable point, novel stationary phases possessed better performance in terms of peak capacity and theoretical plate counts, due to recent advancements in silica-chemistry.

CONCLUSIONS
The method described herein was developed to fill a gap in the field, regarding the assay of TOF in
pharmaceutical preparations. As a conclusion, although some minor enhancements are required, the proposed method was found to be rapid and applicable for the determination of TOF in tablets.

REFERENCES

P-37: OPTIMIZATION OF A CAPILLARY ELECTROPHORESIS METHOD FOR THE DETERMINATION OF ANTAZOLINE AND TETRAHYDROZOLINE IN OPHTHALMIC SOLUTIONS

U. Alshana, N. G. Göker, N. Ertaş
Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, Ankara, TURKEY

Capillary electrophoresis was applied for the determination of antazoline (AN) and tetrahydrozoline (TET) in ophthalmic formulations. Optimum conditions were achieved with a background electrolyte of 20 mM phosphate buffer at pH 7.0, capillary temperature of 25°C, separation voltage of 22 kV and pressure injection of the sample at 50 mbar for 13 s. Calibration graphs showed good linearity with coefficients of determination (R²) of 0.9979 and 0.9994 for AN and TET, respectively. Intraday and interday precision (expressed as %RSD) were lower than 2.8%. The developed method was demonstrated to be simple and rapid for the determination of AN and TET in ophthalmic solutions providing recoveries of 98.2 and 97.9%, respectively.

INTRODUCTION

Antazoline is a histamine H1 receptor antagonist. It can prevent histamine from acting on target cells through a reversible competition effect on histamine receptor sites of these cells. [1]. Tetrahydrozoline (TET) is a sympathomimetic agent with α-adrenergic activity. It acts as a local vasoconstrictor. Solutions and suspensions of TET are used as a conjunctival decongestant [2]. A combination of these two drugs is now available in the pharmaceutical market for ophthalmic use.

High-performance liquid chromatography (HPLC) is commonly reported for the determination of AN and TET [3]. Capillary electrophoresis (CE) offers several unique characteristics that make it particularly attractive, such as high resolving power, minimal reagent consumption, rapidness and low cost analyses. To the best of our knowledge, this is the first report on applying CE for the simultaneous determination of AN and TET in ophthalmic formulations.

MATERIALS AND METHODS

AN-phosphate, TET-hydrochloride and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium dihydrogen phosphate (KH2PO4), dipotassium hydrogen phosphate (K2HPO4) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). Deionized (DI) water (18.2 MΩ cm) treated with Millipore (Simplicity, 185 water purification system) was used. Individual stock solutions of AN and TET at a concentration of 2000 μg mL⁻¹ were prepared in methanol and stored at -15°C. Mixed standard solutions were freshly prepared from the stock solutions by proper dilutions with DI water.

HPCE (Agilent Technologies, Waldbronn, Germany) equipped with an online diode-array UV detector (DAD) that was operated at a wavelength of 192 nm was used. Separations were achieved using an uncoated fused-silica capillary (Agilent Technologies, USA) of 75 μm i.d. and 64.5 cm total length with effective length to the detector of 56 cm.

RESULTS AND DISCUSSION

Optimization of CE conditions was mainly based on corrected peak area (CPA). Among other buffers, the highest CPA was achieved using 20 mM phosphate buffer at pH 7.0. Separation voltage was investigated in the range of 18-27 kV; CPA increased gradually up to 22 kV where it started to decrease. Thus 22 kV was applied. Capillary temperature was tested over the range of 12-30°C. CPA increased gradually up to 25°C and then started to decrease. Figures of merit under optimum conditions are summarized in Table 1. A typical electropherogram of unspiked eye drop after being diluted ten times with DI water is shown in Figure 1.
Table 1. Analytical performance parameters

<table>
<thead>
<tr>
<th>Analytical parameter</th>
<th>AN</th>
<th>TET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>y=4.83, (±0.08)x-7.44 (±2.42)</td>
<td>y=6.06, (±0.05)x-1.13 (±1.13)</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9979</td>
<td>0.9994</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>Intraday: 1.9</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Interday: 1.8</td>
<td>2.1</td>
</tr>
<tr>
<td>LOD (µg mL$^{-1}$)</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>LOQ (µg mL$^{-1}$)</td>
<td>5.3</td>
<td>2.3</td>
</tr>
<tr>
<td>LDR (µg mL$^{-1}$)</td>
<td>5.3–100</td>
<td>2.3–100</td>
</tr>
<tr>
<td>Recovery</td>
<td>Reported concentration (µg mL$^{-1}$)</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Found concentration (µg mL$^{-1}$)</td>
<td>147.3</td>
</tr>
<tr>
<td></td>
<td>%R</td>
<td>98.2</td>
</tr>
</tbody>
</table>

*Peak area = slope (±SD) × [concentration (µg mL$^{-1}$)] + intercept (±SD);

b Limit of detection
c Limit of quantitation
d Linear dynamic range

---

Fig. 1. A representative electropherogram of unspiked eye drop after being diluted ten times with DI water. Peaks: 1, TET; 2, AN

CONCLUSIONS

In this study, a CE method is presented for the determination of antazoline and tetrahydrozoline in ophthalmic formulations which offers numerous advantages, such as rapidity, use of minimum amounts of organic solvents, simplicity, low cost, ease of operation, and high selectivity. Good recoveries, high reproducibility and interference-free electropherograms were also achieved.

ACKNOWLEDGMENTS

The authors are thankful to Gazi University for the financial support of this work “Project No: BAP-02/2010-02”.

REFERENCES


P-38: NITRATE, NITRITE AND BROMIDE DETERMINATION IN SWAP SAMPLES BY CAPILLARY ELECTROPHORESIS

Ö. Özen Erol, B. Yavuz Erdoğan and A.N. Onar

Here a capillary electrophoretic method has been proposed for the determination of nitrate, nitrite and bromide in gunshot residue (GSR). In order to improve the detection sensitivity, a large volume sample stacking was employed which was necessary especially for nitrite ion. The electroosmotic flow is reversed by using low pH running buffer without using any additives.

INTRODUCTION

Gunshot residues are produced when a firearm is discharged and can be deposited on the hands of the perpetrator. Nitrite and nitrate determinations can be used as screening tools for investigating residues of firearm discharge due to the fact that these ions are major inorganic components of gunshot residues[1].

MATERIALS AND METHODS

The analysis was carried out in Agilent HP3D capillary electrophoresis with diode array detector. The detection was by UV absorption at 200 nm. A bare fused-silica capillary (Polymer Micro Technologies, 50 µm inner diameter) was used. Phosphate solution was selected as running buffer at pH 3.5. The applied potentials were 20 and 22 kV, with reversed polarity, to obtain the migration of anions with high mobility towards the detector. Capillary column temperatures (25 and 27 °C), concentrations of phosphate buffer (50, 75, 100 mM) and hydrodynamic injection periods (1-50 s, 50 mbar) were investigated.

RESULTS AND DISCUSSION

Optimum experimental conditions were found as: 50 mM phosphate buffer solution (pH 3.5), 20 kV applied potential; 25°C capillary and the background electrolyte temperature and hydrodynamic sample injection (50s, 50mbar). LOD values were 6.7 µM, 4.3 µM and 1.67 µM for nitrate, nitrite and bromide respectively.
Reproducibility (intraday and day-to-day) was acceptable with relative standard deviations (less than RSDs 3.0%) for relative migration times in both standard solutions and real swap samples.

CONCLUSIONS
Capillary electrophoresis (CE) technique offered promising, effective and economic approach for the separation of anions in GSR. The proposed method was successfully applied to real swap samples. In traditional swap procedure mechanical agitation is employed for extraction, here in this work ultrasound mixing for 30 minutes was found sufficient.

ACKNOWLEDGMENTS
This work was supported by Ondokuz Mayıs University (Project No:1904.12.026).

REFERENCES

P-39: INVESTIGATION OF AN ANTICANCER DRUG; SORAFENIB REDOX BEHAVIOR AT DIFFERENT ELECTRODES AND ELECTROCHEMICAL IMPEDANCE BEHAVIOR OF THE DEVELOPED SENSOR

N. Karadas-Bakirhan1, S. Patris2, S. A. Ozkan1, J-M Kauffmann2

1Ankara University, Faculty of Pharmacy,Department of Analytical Chemistry, Ankara, TURKEY
2 Université Libre de Bruxelles, Faculty of Pharmacy, Campus Plaine, CP 205/6, 1050 Bruxelles, Belgium

INTRODUCTION
Sorafenib (SOR) (Figure 1), (4-[4-[[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl amino] phenoxy]-N-methyl-pyridine-2-carboxamide), is a drug approved for the treatment of primary kidney cancer (advanced renal cell carcinoma), advanced primary liver cancer (hepatocellular carcinoma), and radioactive iodine resistant advanced thyroid carcinoma [1]. Our aim is to investigate redox behaviour of sorafenib at a carbon based screen printed electrode (SPE) and at a chitosan/carboxylic acid functionalized multiwalled carbon nanotube modified glassy carbon electrode (Chitosan/-COOH MWCNT/GCE).

MATERIALS AND METHODS
SOR pure form (99%) was supplied by Bayer (Istanbul, Turkey). Carbon nanotubes were purchased from DropSens. Chitosan (low molecular weight) was purchased from Sigma-Aldrich. All chemicals for preparation of buffers and supporting electrolytes were reagent grade (Merck or Sigma). Buffer solution pH was adjusted with NaOH solution. For electrode modification, a 0.1 % (m:v) chitosan with 1 mg/mL – COOH MWCNT suspension was prepared in 0.1 M acetate buffer solution (pH 5.0).

CV studies at the SPE were realized in 0.1 M PB + 0.1 M KCl in the presence of 10 % methanol.

Electrochemical impedance spectroscopy was performed using the Autolab potentiostat/galvanostat PGSTAT100 with NOVA software. The impedance diagrams were recorded at the peak potential by applying a 10 mV sinusoidal potential through a frequency domain from 100 kHz down to 0.1 Hz.

RESULTS AND DISCUSSION
The pH of the supporting electrolyte has a major impact for oxidation potential of SOR at the Chitosan/-COOH MWCNT/GCE. The pH dependency was studied in 0.1 M PB (2.0, 3.0, 6.0, 7.0, 8.0) and 1.0 M acetate buffers (3.7, 4.7, 5.7) by CV. EIS results showed that the higher electric conductivity, improved electron transfer and larger surface area were gained.

CONCLUSIONS
The voltammetric behavior of sorafenib was investigated by CV method. At both electrodes (GCE and SPE), cyclic voltammetry indicated one irreversible oxidation peak followed by a second one embedded in the solvent oxidation current. The impedance behavior of Chitosan/-COOH MWCNT/GCE sensor was investigated.
ACKNOWLEDGMENTS
The authors would like to gratefully acknowledge the Ankara University Scientific Research Project Foundation (Project No: 12L 3336002), Turkey. Thanks are expressed to TUBITAK for grant support to Nurgul Karadas-Bakirhan for her scientific stay in the Université Libre de Bruxelles.

REFERENCES

P-40: SIMULTANEOUS QUANTITATIVE ESTIMATION OF ACTIVE COMPONENTS IN A BINARY MIXTURE BY USING RATIO SPECTRA-DERIVATIVE SPECTROPHOTOMETRY

Ö. Üstündağ and E. Dinç
Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Tandoğan/Ankara-Turkey

INTRODUCTION
In this study, a ratio spectra-derivative spectrophotometric (RD) method was developed for the simultaneous quantitative estimation of candesartan (CAN) and hydrochlorothiazide (HCT) in a tablet dosage form.

MATERIALS AND METHODS
The RD method was applied to the UV spectra of the CAN and HCT. The calibration equations were obtained by measuring the amplitudes at 243.1 nm for the CAN determination and at 262.7 nm for the HCT determination, respectively.

RESULTS AND DISCUSSION
The validity and applicability of the proposed ratio spectra-derivative spectrophotometric method was carried out by analysing an independent set of the synthetic binary mixtures consisting of CAN and HCT. Also, standard addition technique and intra-day and inter-day tests were performed.

CONCLUSIONS
The proposed method was successfully applied to the analysis of the CAN-HCT in pharmaceutical tablets.

REFERENCES

P-41: PHENOLICS OF ATRIPLEX HORTENSIS L. BY SPE/HPLC-UV

P. Köseoğlu Yılmaz, U. Kolak
Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, TURKEY

In this study, a new SPE/HPLC-UV method was developed and validated for the quantitative analysis of phenolics in the aerial parts of Atriplex hortensis L. Hydrophilic-lipophilic balance solid phase extraction cartridges were used for sample preparation. The separation was achieved by a C18 column and a gradient elution was performed. Analytes were detected by a UV detector. The developed method was validated in terms of linearity, LOD, LOQ, repeatability, accuracy and recovery. Calibration equations and correlation coefficients were calculated by the weighted least squares method.

INTRODUCTION
The family Chenopodiaceae (Goosefoot) includes approximately 100 genus and 1500 species. There are 31 genera and 99 species belonging to this family in Turkey. In this family, Atriplex L. is a medium-size
cosmopolitan genus comprising about 270 species, and is represented by 16 species in Turkey, one of them is endemic [1, 2]. *Atriplex hortensis* L. (garden orach), also known as mountain spinach, sea purslane, or salt bush, is considered to be one of the oldest cultivated plants, valued primarily for its leaves. *A. hortensis* has been used both as a potherb for traditional medicinal purposes and as a soil erosion control [3].

In this study, a new SPE/HPLC-UV method was developed and validated for the quantitative analysis of phenolics in the aerial parts of *A. hortensis*.

**MATERIALS AND METHODS**

The aerial parts of *A. hortensis* were collected from Istanbul, Hadimköy (September, 2012), dried and grinded. Extraction of the analytes was performed by maceration (water/MeOH/acetone, 40:40:20; water/acetonitrile, 50:50) followed by solid phase extraction (SPE) with hydrophilic-lipophilic balance (HLB) cartridges. HLB cartridges were consisted of hydrophilic modified styrene polymer. Separation of the analytes were carried out using a C18 column (250 x 4.6mm, 5.0 μm I.D.) and analytes were detected by a UV detector.

**RESULTS AND DISCUSSION**

A new SPE/HPLC-UV method was developed for the quantitative analysis of phenolics in the aerial parts of *A. hortensis*. HLB cartridges were used after maceration to clean and concentrate the *A. hortensis* sample. The developed method was validated in terms of linearity, LOD, LOQ, repeatability, accuracy and recovery according to the validation parameters of ICH (2005) [4].

**CONCLUSIONS**

The developed and validated SPE/HPLC-UV method was found to be appropriate for the quantitative determination of the phenolics of *A. hortensis* considering the validation parameters.

**ACKNOWLEDGMENT**

This study was supported by the Research Fund of Istanbul University (Project number: 28183).

**REFERENCES**


**P-42: VOLTAMMETRIC DETERMINATION OF GALLIC ACID IN RED WINE USING A POLY(CAFFEIC ACID) MODIFIED GLASSY CARBON ELECTRODE**

P. Talay Pınar1, Y. Yardım2, Z. Şentürk3

Yüzüncü Yıl University, 1Faculty of Science and 2Faculty of Pharmacy, Department of Analytical Chemistry, Van, TURKEY

**INTRODUCTION**

Gallic acid (GA), 3,4,5-trihydroxybenzoic acid, is one of the main natural phenolic components encountered in wine, gallnuts, sumac, tea leaves, and several other plants. The rapid and accurate determination of GA is of great interest because it has not only strong antioxidant, anti-inflammatory, antimicrobial, and anticancer activities but also potential health effects which have been found recently. The electrochemical methods using chemically modified electrodes have been widely used for the determination of individual polyphenols, in particular GA [1-3]. The goal of the current work was to demonstrate the practical applicability of a novel chemically modified electrode for the determination of GA. For this purpose, a poly(caffeic acid) modified glassy carbon electrode (PCA/GCE) was fabricated and used to investigate the electrochemical behavior of GA. This sensor was based on an electrochemically polymerized caffeic acid (CA) layer on a GCE.

**MATERIALS AND METHODS**

Stock standard solutions of GA were prepared with deionized water. Three different supporting electrolytes, namely sulfuric acid (H2SO4, 0.2 M), Britton-Robinson buffer (0.1 M, pH 2.0-7.0), and phosphate buffer (0.1 M, pH 2.0 and 10.0) solutions were used. All experiments were performed using a Autolab type III electrochemical system driven by the GPES 4.9 software. A three electrode system in a 10-mL one-compartment voltammetric cell was employed consisting a bare or modified GCE, a platinum wire counter electrode and Ag/AgCl reference electrode. The polished bare GCE was continuous cyclic scanned in phosphate buffer solution, pH 7.4 containing 8×10-4 M CA between -0.6 and +1.6 V at 100 mV s-1 for 8 scans to form PCA/GCE.

**RESULTS AND DISCUSSION**

A detailed study of the electrochemical properties of GA on GCE and PCA/GCE was carried out by the use of cyclic (CV) and square-wave (SWV) voltammetry. The experimental conditions that affect the electrode reaction process were studied in terms of pH, concentration of CA, electro-polymerization cycle and
scan rate. Using SW mode, GA \((1 \times 10^{-4} \text{ M})\) exhibited an electrochemical response with a peak current of 105 \(\mu\text{A}\) at \(+0.46\text{ V}\) on bare GCE, while a peak current of 446 \(\mu\text{A}\) at \(+0.47\text{ V}\) was observed on PCA/GCE (Fig. 1). In phosphate buffer \((\text{pH} 3.0)\), the SW peak current correlates linearly with GA concentration from \(2.0 \times 10^{-9}\) to \(1.0 \times 10^{-6}\) \(\text{mol L}^{-1}\) with a detection limit of \(6.0 \times 10^{-9}\) \(\text{mol L}^{-1}\). GA content in red wine samples was successfully determined on applying the prepared sensor.

![Fig. 1. SW voltammograms of GA \((1 \times 10^{-4} \text{ M})\) in sensor.](image)

In this study, formation constants of the complexes of Cu(II) with L-Arginine (Arg) and L-Arginine Methyl Ester (ArgE) and pK\(_{\text{a}}\)s of them were determined potentiometrically at \(5.0, 20.0\) and \(35.0\text{ oC}\) and \(l = 0.10\) mol L\(^{-1}\) (NaClO\(_4\)). \(\Delta G^o\), \(\Delta H^o\) and \(\Delta S^o\) were determined for protonation and for Cu(II) complexations of these ligands.

**INTRODUCTION**

Determination of the formation constants and the other thermodynamic quantities of metal complexes of biologically important substances (bioligands) would provide useful information about live metabolism. Our aim is to study the complex formations between transition metal ions and amino acids or drugs \([1, 2]\).

**MATERIALS AND METHODS**

Potentiometric pH titration measurements were performed on an automatic titrator (Radiometer Analytical, TIM 860 Titration Manager) with a pH combination electrode equipped with a temperature probe. The temperature of the solution to be titrated was kept constant by a thermostat (ThermoHaake DC10).

The experimental procedure involved the potentiometric titration of the solutions prepared according to Irving and Rossoitti’s method (Fig. 1) \([3]\). Using potentiometric pH titration data, the values of average proton–ligand formation number, \(n_\Lambda\), at various pH levels were determined by the equation given earlier. Then values of the protonation constants, pK\(_{\text{a}}\), were calculated by a PC (using an electronic spreadsheet software, e.g., MS Excel) from the \(n_\Lambda = f\) (pH) relationship at \(n_\Lambda = 0.5, 1.5\) \([2, 4]\).

For the first and second formation constant (logK\(_{1}\) and logK\(_{2}\)) of binary complexes, the values of average metal–ligand formation number, \(n_L\), at various pH values and the values of pL were calculated using the equations given elsewhere \([2, 4]\). For the corresponding values of \(n_L\) and pL, the formation constants were calculated by a PC at \(n_L = 0.5\) and 1.5 \([2, 4]\).

The \(\Delta H^o\), \(\Delta G^o\) and \(\Delta S^o\), values for protonation and complexation reactions were calculated from the formation constants determined at \(5.0, 20.0\), and \(35.0\text{ oC}\) by equations given previously \([4]\).

**RESULTS AND DISCUSSION**

The pK\(_{\text{a}}\)s of both ligands decreases with increasing temperature. So protonation of –NH\(_2\) groups were found to be exothermic for H–Arg and H–ArgE systems. pK\(_{\text{a}}\)s are: 9.16 and 7.31 at \(20\text{ oC}\). pK\(_{\text{a}}\)s of R-group of Arg and ArgE cannot be determined under the experimental condition of this work. Driven force is both of \(\Delta H^o\) and \(\Delta S^o\) in these reactions. Contributions of \(\Delta H^o\) to \(\Delta G^o\) are both 70\% \([1, 2, 4, 5]\). pK\(_{\text{a}}\) of Arg also cannot be determined under the experimental condition of this work. \(\Delta H^o\) of both 1:1 and 1:4 Cu–Arg complexations are found to be negative. \((\log\text{K}_1 = 7.95, \log\text{K}_2 = 6.65\text{ at }20\text{ oC and and})\)

**P-43: COMPLEXATIONS OF L-ARGININE AND ITS METHYL ESTER WITH Cu(II): TEMPERATURE DEPENDENCE OF THE FORMATION CONSTANTS**

R. Y. Cantürk Talman, A. S. Baştug

Marmara University, Faculty of Pharmacy, Division of General Chemistry, Istanbul, TURKEY

**REFERENCES**

conditional coefficients are the same). Contribution of $\Delta H^o$ and $\Delta S^o$ to $\Delta G^o$ are 43 and 70% for Cu-Arg and Cu-Arg$_2$ [4-5]. $\Delta H^o$ of both 1:1 and 1:4 Cu-ArgE complexations are found to be positive (driven force is 100% $\Delta S^o$). $\log K_1 = 3.12$, $\log K_2 = 2.94$ and conditional coefficients are 2.34 and 2.16 respectively at 20 °C.

Fig. 1. Potentiometric titration curves of (A) HClO$_4$ (AL) HClO$_4$+Ligand (ALM) HClO$_4$+Ligand+Cu solutions (Left: for Arg, Right: for ArgE (20.0 °C)

CONCLUSIONS
Occurring both enthalpic and entropic stabilization together for Cu-Arg and Cu-Arg$_2$ and furthermore entropic stabilization for Cu-ArgE and Cu-ArgE$_2$ complexations implies that O atom of $\pm COO\pm$ group is participated more effectively in formation of these complexes [2, 4, 5]. They may be included Cu-N bond or bonds also, but their strength should be weaker than that of Cu-O bonds.

REFERENCES

P-44: DETERMINATION OF THE IONIZATION CONSTANTS OF SOME 1,8-DIOXOACRIDINE CARBOXYLIC ACID DERIVATIVES IN ETHANOL–WATER MIXTURES

R. Saygılı1, R. Ulus1, I. Yesildag1, E. K. Inal2, M. Kaya1, O. M. Kalfa1, B. Zeybek1

1Dumlupınar University, Faculty of Arts and Sciences, Department of Chemistry, Kütahya, TURKEY; 2Ankara University, Faculty of Science, Department of Chemistry, Ankara, TURKEY

Four novel compounds of 1,8-dioxoaacidine carboxylic acid derivatives were prepared. The stoichiometric ionization constants of these compounds were determined in ethanol–water mixtures by potentiometric titration method and the ionization constants were calculated with three different ways. The effects of solvent composition and substituent groups on ionization constants of 1,8-dioxoaacidine carboxylic acids were also discussed.

INTRODUCTION
The acridinedione derivatives have high fluorescence efficiency and are members of a class of laser dyes [1,2]. Acrdines are toxic towards bacteria and especially malaria parasite because of their ability to inhibit DNA and RNA synthesis [3]. These dyes are also important because of their structural similarity to those of 1,4-dihydropyridines (DHP). The DHPs are analogous to the biologically important NADH, which are coenzymes in biological systems [1,4]. The DHP derivatives are well known as the group of calcium channel blockers and have also potassium channel opener activities. They are used in the clinic as vasodilator and antihypertensive [5].

MATERIALS AND METHODS
Titrator: Mettler Toledo DL50 (Mettler-Toledo AG, Schweizerland) automatic titrator. Electrode: Combined glass pH electrode (9104SC, Thermo Scientific, USA)
RESULTS AND DISCUSSION

![Chemical Structure](image)

Fig. 1. The ionization equilibrium of 1,8-dioxoacridine carboxylic acid derivatives.

Table 1. The $pK_a$ values of some 1,8-dioxoacridine carboxylic acids calculated with BEST computer software program at $25.0 \pm 0.1$ °C for various ethanol–water mixtures ($I = 0.10$ mol L$^{-1}$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>50% ethanol-50% water</th>
<th>60% ethanol-40% water</th>
<th>70% ethanol-30% water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.57 ± 0.04</td>
<td>4.75 ± 0.08</td>
<td>5.20 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>4.34 ± 0.04</td>
<td>4.64 ± 0.03</td>
<td>4.99 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>4.64 ± 0.09</td>
<td>4.78 ± 0.02</td>
<td>5.00 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>4.31 ± 0.02</td>
<td>4.58 ± 0.03</td>
<td>4.98 ± 0.02</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The $pK_a$ values of synthesized four carboxylic acid derivatives increases with increasing the percentage of ethanol in the solvent mixture. This study also indicated that the changes in the $pK_a$ values of a compound are affected by solvent effects and structural effects.

ACKNOWLEDGEMENTS

We thank the Dumlupınar University Scientific Research Fund (Project No: 2012-4) for their support.

REFERENCES


P-45: SQUARE-WAVE VOLTAMMETRIC DETERMINATION OF CEFUROXIME AXETIL IN PHARMACEUTICAL PREPARATIONS

S. Erdoğan Kablan, N. Özaltın

Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Sihhiye, 06100, Ankara, TURKEY

A simple, sensitive, and selective square-wave voltammetric (SWV) method was developed for the determination of Cefuroxime axetil (CEFA). The best results were obtained in 0.1M KH$_2$PO$_4$ and Na$_2$B$_4$O$_7$ buffer of pH 7.0. The peak currents were measured with hanging mercury drop electrode (HMDE) at $-1050$ mV vs. Ag/AgCl. The electrochemical behaviour was studied by using different voltammetric techniques. The calibration curve for CEFA was found as linear at a concentration range from 0.263 to 14.773 µg/mL. The limit of detection and the limit of quantification were found 0.0868 and 0.263 µg/mL respectively. Validation parameters, such as sensitivity, accuracy, precision, ruggedness, robustness and recovery were evaluated. The developed method was applied for the determination of CEFA in pharmaceutical dosage forms.

INTRODUCTION

Cefuroxime axetil is an ester pro-drug which permits the oral administration of cefuroxime. This ester product of cefuroxime increases the lipophilicity of the parent compound and its oral bioavailability [1]. The aim of the present study is to develop and validate simple, sensitive, accurate, precise and reproducible SWV method for determination of CEFA in pharmaceutical dosage forms and to investigate the reduction behavior of CEFA at HMDE, using cyclic voltammetric (CV) and square-wave voltammetric (SWV) techniques.

MATERIALS AND METHODS

All experiments were carried out using a BAS 100 B/W electrochemical analyzer (Bioanalytical System, USA) in combination with a BAS controlled growth mercury electrode (CGME). A three-electrode system consisted of an HMDE as the working electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode.
Standard stock solutions of CEFA were prepared by dissolving in methanol, the subsequent dilutions were made with water.

RESULTS AND DISCUSSION
The influence of the species and concentration of the supporting electrolyte, pH, initial potential was examined. The best results were obtained in 0.1 M KH₂PO₄ and Na₂B₄O₇ buffer (FB) of pH 7.0. The peak currents were measured with HMDE at -1050 mV vs. Ag/AgCl. Under the optimum conditions, the peak current was linearly increased with CEFA concentration (Fig.1). A linear relationship was observed between 0.263 - 14.773 μg/mL. Validation parameters were evaluated according to ICH Guidelines [2]. The validated method was applied to pharmaceutical formulation and amount of drug estimated was found in good agreement with the label claim.

CONCLUSIONS
Developed SWV method can be used for determination of CEFA in pharmaceutical dosage forms. It is simple, fast, low cost, and has sufficient precision, accuracy and sensitivity. This method can be a good alternative to expensive methods for the determination of CEFA.

REFERENCES

P-46: DESIGN OF AN ELECTROCHEMICAL NANOBIOSENSOR FOR THE DETERMINATION OF CATECHOL

S. Kurbanoglu1, C.C. Mayorga-Martinez2, M. Medina-Sánchez2, L. Rivas2, S. A. Ozkan1, A. Merkoçi2

1Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, TURKEY
2Nanobioelectronics & Biosensors Group, ICN2- Institut Catala de Nanociencia i Nanotecnologia, Campus UAB, 08193 Bellaterra, Barcelona, Spain

INTRODUCTION
Nanostructured materials possess unique optical, electronic and magnetic properties depending on their core materials and they have large surface-to-volume ratio that favors miniaturization. In the last few years, nanostructured materials such as magnetic nanoparticles (MNPs) have been reported in a wide range of applications that include the immobilization of cells, enzyme, proteins and nucleic acids. Among various nanostructured materials, iridium oxide nanoparticles (IrOx NPs) attract attention in the design of novel electrochemical sensors for their stability and catalytic properties. Tyrosinase (Tyr, EC 1.14.18.1) is a bifunctional copper-containing enzyme that has both cresolase and catecholase activities. Tyr catalyzes the o-hydroxylation of monophenols to the corresponding catechols (called as cresolase activity), and the oxidation of catechols to the corresponding oquinone (called as catecholase activity). The electrocatalytic activity of the MNP/Tyr/IrOx NPs platform for catechol detection can be observed through electrochemically reduced o-quinone to catechol at low applied negative potentials [1-4].

MATERIALS AND METHODS
Tyrosinase from mushroom (Z1000 unit/mg), catechol was purchased from Sigma-Aldrich (St. Louis, MO). As the electrochemical detector, screen printed carbon electrodes (SPEs) consisted of a set of three electrodes: carbon working electrode with a diameter of 3 mm, Ag/AgCl pseudo reference electrode (with a potential of 10 mV with respect to a commercial Ag/AgCl electrode) and carbon counter electrode with an approximate thickness of 4 μm were used. IrOx NPs suspension (250 μL) was shaken with 20 μL of Tyr solution of 19.61 U during 12 h at 4 °C. After that, 50μL of NH₃ functionalized MNPs were added to the mixture, and shaken 4 °C for 12 h. 8 μL of the resulting nanocomposite was dropped onto the working electrode of SPE and it was pre-concentrated using magnet under the working electrode of SPE.

RESULTS AND DISCUSSION
Morphological studies: The Scanning Electron Microscope (SEM) images were captured to observe the morphology of each component of the nanocomposite.
Fig. 1A, B and C show the SEM images of MNPs and Tyr, the bioconjugate formed between the IrOx NPs and tyrosinase (Tyr), and the product of the IrOx NPs–Tyr bioconjugate interaction with MNPs, respectively. Optimization of the catechol detection

The best biosensing response toward catechol detection was optimized using different strategies during the preparation of IrOx NPs/Tyr/MNPs biocomposite. Different parameters such as shaking (incubation) time (6 h and 12 h) of the mixture of Tyr with IrOx NPs, IrOx NPs amount (250, 125, 62.5 ȝ/L) and Tyr amount (19.61 U, 4.9 U, 9.81 U) were evaluated. The best response was found when 250 ȝ/L IrOx NPs and 19.61 U Tyr were mixed for 12 h.

The chronoamperometric response of the MNPs/Tyr/IrOxNPs biosensor to successive additions of catechol at different concentrations was further evaluated. A linear biosensor response for catechol was observed. The biosensor shows LOD and LOQ values of 0.043 ȝM and 0.13 ȝM, respectively for catechol.

CONCLUSIONS

A novel biosensor based on the use of a biocomposite made of MNPs, IrOx NPs and tyrosinase for catechol detection is developed.

ACKNOWLEDGMENTS

We acknowledge MINECO (Spain) through Project MAT201125870. S. Kurbanoglu acknowledges the support given by Ankara University BAP14L0237002 for her Ph.D. thesis project.

REFERENCES


P-47: ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF OMEPRAZOL IN A CAPSULE DOSAGE FORM

S. Dermis, C. Ertekin, E. Beker and E. Dinc

Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, TURKEY

INTRODUCTION

Omeprazole (OMP) has been widely used as a drug substance in pharmaceutical formulations. OMP is used as a proton pump inhibitor in the treatment of dyspepsia, peptic ulcer disease, gastro esophageal reflux disease, laryngo pharyngeal reflux and Zollinger-Ellison syndrome. In addition OMP suppresses gastric acid secretion and blocks the final step in acid production, thus reducing gastric acidity [1, 2]. In this study, a new ultra-performance liquid chromatographic (UPLC) method was developed and validated for the quantitative analysis of OMP in a capsule dosage form.

MATERIALS AND METHODS

The separation and analysis of the related drug in the presence of ondansetron (OND) as internal standard (IS) were performed on Waters UPLC BEH C18 column (50mm x 2.1mm i.d., 1.7µm) using a mobile phase consisting of acetonitrile and 0.05 M H3PO4 (28:72, v/v). Flow rate was 0.28 mL/min with the column temperature of 40°C.

RESULTS AND DISCUSSION

Chromatograms of the samples containing OMP with IS were recorded using the detection at 302.0 nm. As can be seen in Figure 1, the retention time for OMP and IS was found to be 0.787 and 1.060 min, respectively. A calibration graph for OMP in the concentration range of 4.0–46.0 ȝg/mL was calculated by using linear regression analysis based on concentration and peak area ratio of OMP and IS in their chromatograms. In the method validation process, percent mean recovery and relative standard deviation was found as 101.6 % and 1.20 %, respectively.
CONCLUSIONS
In this study, a new, fast, precise, accurate and reliable UPLC method was developed for the quantitative analysis of OMP in a commercial formulation. It was observed that the application of the newly developed UPLC method gave us successful results for the quantitative estimation and routine analysis of OMP in capsules.

REFERENCES
1. http://www.drugbank.ca/drugs/DB00338#pharmacology

P-48: INTERACTION OF THE Pd(II) COMPLEX OF 5,10,15,20-TETRAKIS(1-METHYL-4-PYRIDIYL)-PORPHYRINE WITH NATURAL DNA

S. Aydinoglu¹, T. Biver²

¹Pharmacy Faculty, Department of Analytical Chemistry, University of Cukurova (Turkey), ²Department of Chemistry and Industrial Chemistry – University of Pisa (Italy)

A mechanistic, thermodynamic and kinetic analysis of the binding of the Pd(II)/5,10,15,20-tetrakis(1-methyl-4-pyridiyl)-porphyrine metal complex is done. Fluorescence and absorbance titrations under different conditions of temperature and salt content concur in indicating that the binding is strong. Kinetic and equilibrium parameters for the complex interaction with the nucleic acid are obtained and the binding mechanism is discussed. An intercalative binding is found to be active.

INTRODUCTION
In 1979 Fiel and co-workers demonstrated the intercalative binding mode of porphyrins to DNA and this lead to intense investigations on interaction of porphyrins with synthetic and natural nucleic acids [1]. Porphyrins offer advantages for studies on drug binding to nucleic acids such as planar aromatic structure and large molar absorption coefficient [2]. The study on porphyrin chemistry has received high interest in recent years, mainly in connection with the use of these molecules in biology [3]. DNA/porphyrin systems have been analysed using very different methods but some details still need to be elucidated. We present here a combined thermodynamic and kinetic analysis of the binding of the Pd(II) complex of 5,10,15,20-tetrakis(1-methyl-4-pyridiyl)-porphyrine (Figure 1) to DNA.

P-48: INTERACTION OF THE Pd(II) COMPLEX OF 5,10,15,20-TETRAKIS(1-METHYL-4-PYRIDIYL)-PORPHYRINE WITH NATURAL DNA

S. Aydinoglu¹, T. Biver²

¹Pharmacy Faculty, Department of Analytical Chemistry, University of Cukurova (Turkey), ²Department of Chemistry and Industrial Chemistry – University of Pisa (Italy)

A mechanistic, thermodynamic and kinetic analysis of the binding of the Pd(II)/5,10,15,20-tetrakis(1-methyl-4-pyridiyl)-porphyrine metal complex is done. Fluorescence and absorbance titrations under different conditions of temperature and salt content concur in indicating that the binding is strong. Kinetic and equilibrium parameters for the complex interaction with the nucleic acid are obtained and the binding mechanism is discussed. An intercalative binding is found to be active.

INTRODUCTION
In 1979 Fiel and co-workers demonstrated the intercalative binding mode of porphyrins to DNA and this lead to intense investigations on interaction of porphyrins with synthetic and natural nucleic acids [1]. Porphyrins offer advantages for studies on drug binding to nucleic acids such as planar aromatic structure and large molar absorption coefficient [2]. The study on porphyrin chemistry has received high interest in recent years, mainly in connection with the use of these molecules in biology [3]. DNA/porphyrin systems have been analysed using very different methods but some details still need to be elucidated. We present here a combined thermodynamic and kinetic analysis of the binding of the Pd(II) complex of 5,10,15,20-tetrakis(1-methyl-4-pyridiyl)-porphyrine (Figure 1) to DNA.

MATERIALS AND METHODS
The porphyrine ligand used is the tetra-p-tosylate salt by Sigma-Aldrich, its molar concentration is indicated by C_D. Pd(Cl)_4 was a kind gift from Chimet. Calf thymus DNA (lyophilised sodium salt, highly polymerised) was purchased from Sigma and sonicated as previously described [4]. The concentrations of DNA are expressed in molarity of base pairs and indicated as C_P.

Experiments were performed at pH = 7. The solutions were buffered using 0.01M NaCac ((CH3)2AsO2Na = Sodium calcodylate). A Perkin-Elmer Lambda 35 spectrophotometer was used to record absorption spectra and to perform spectrophotometric titrations, whereas a Perkin Elmer LS55 fluorometer is used for fluorescence measurements. Both apparatuses are equipped with jacketed cell holders, and allows to control the temperature within ±0.1°C. Equilibrium studies on the interaction of palladium(II)-porphyrin complex with DNA were carried out by addition of increasing amounts of DNA to the spectrophotometric cell containing the solution of Pd(II)-porphyrin by means of a syringe connected to a micrometric screw (Mitutoyo).

The kinetics were performed using a T-jump apparatus that enables the measure of absorbance changes in time (at the limit of μs time range).
RESULTS AND DISCUSSION
Spectrophotometric and spectrofluorometric titrations are carried out under different temperature and ionic strength conditions (Figure 2).
The spectrophotometric experiments show hypochromic and bathochromic shifts by addition of DNA in the Pd(II)-porphyrin complex visible spectra in agreement with previously reported absorbance spectra [4]. This behaviour is in agreement with intercalative binding.

\[ \frac{C_D C_F}{\Delta A} + \frac{\Delta A}{2\Delta e^2} = \frac{1}{K\Delta e} + \frac{1}{\Delta e} \left( C_D + C_F \right) \]

Same equation is used also in case of the fluorescence data. The binding constants found are significantly high, close to quantitative reaction. This finding can be related to the high charge borne by the complex and show the importance of electrostatic attraction with the negatively charged DNA backbone for the interaction. Equilibrium data at different temperature enable, by Van’t Hoff analysis, \( \Delta H \) and \( \Delta S \) parameters for binding to be found. These values are in agreement with a very strong intercalative binding [5].
The T-jump relaxation curves are found to be monoeponential and were therefore analysed by means of a monoeponential equation. The analysis of the relevant time constants enables the kinetic parameters of the binding to be evaluated, according to equation (2)

\[ \frac{1}{\tau} = k_f ([D]+[P]) + k_d \]

where \( k_f \) and \( k_d \) denote respectively the forward and backward (dissociation) rate constants for Pd(II) complex binding to DNA and \([D]\) and \([P]\) are the unbound Pd(II) complex and unbound DNA concentrations (calculated iteratively using \( K = k_f/k_d \)). The kinetic data also concur to indicate high affinity of the planar porphyrin ring for the polynucleotide helix and high energy barrier to be overcome to dissociate from it.

CONCLUSIONS
The Pd(II)/5,10,15,20-tetrakis(1-methyl-4-pyridiyl) -porphyrin metal complex studied strongly interacts with DNA in an intercalative manner where the whole molecule inserts itself between the base pairs.

REFERENCES
flow rate was 1 mL/min. Analytes were detected with a diode-array detector at 225 nm. Carbamazepine was used as internal standard. Plasma samples were analyzed after a simple, one-step protein precipitation with acetonitrile. Separation time was 15 min including clean-up step. The proposed method was applied to quantify plasma concentrations of the analytes in rat plasma.

RESULTS AND DISCUSSION
Method was validated in terms of linearity, precision, accuracy, specificity, recoveries, matrix effect, stability and robustness. The method was found to be linear at the range of 0.065-130 μg/mL for QTP, 0.086-171 μg/mL for 7-OH QTP and 0.042-83.35 μg/mL for QTP-SF. Mean equations of the calibration curves in rat plasma for three injections were $y = (0.0154±0.0002)x + (-0.0223±0.0116)$, $r^2 = 0.9988$ for QTP and $y = (0.0156±0.0002)x + (-0.0006±0.0110)$, $r^2 = 0.9994$ for 7-OH QTP and $y = (0.0215±0.0002)x + (-0.0058±0.0053)$, $r^2 = 0.9997$ for QTP-SF (n = 8). The intra- and inter-day assay variability was less than 3% for the analytes. All validation parameters were within acceptable bioanalytical limits [4, 5].

Concentration data for QTP and its metabolites in rat plasma was determined by injecting 50 mg/kg QTP to six rats and collecting serum samples after 1.5 hours. Plasma levels of QTP, 7-OH QTP and QTP-SF were found in the range of 140.96-587.13 ng/mL, 373.32-909.87 ng/mL and 17.04-179.82 ng/mL, respectively.

CONCLUSIONS
A new, simple and specific method has been developed for the analysis of quetiapine and its two metabolites in rat plasma. The method was fully validated and it was successfully applied for the determination of analytes in rat plasma.

ACKNOWLEDGMENTS
The authors appreciate Research Council of Anadolu University for the support of the Project (Project No: 1302S023).

REFERENCES
volume with the same solvent and content was sonicated for 10 min. Ten milliliter portion of the resulting mixture was transferred into a polypropylene centrifuge tube and centrifuged to precipitate insoluble excipients. About two milliliters of the supernatant was taken and filtered through a 0.20-μm syringe filter. After subsequent dilution, the resulting solution was injected on to the column.

RESULTS AND DISCUSSION
Isocratic elution of Ceftiofur was successfully achieved with adequate retention, utilizing a mobile phase consisted of phosphate buffer, acetonitrile and water. Effects of main instrumental parameters on the analytical signal and retention were evaluated; moreover, comparison of system suitability parameters and chromatographic performances between the columns were performed. As a significant point, it was revealed that use of novel stationary phases with ultra high performance liquid chromatography instruments provides preferable performance characteristics in terms of efficiency and equilibration.

CONCLUSIONS
The method proposed in this study was found to be applicable for fast analysis of Ceftiofur in veterinary preparations.

REFERENCES

P-51: DETERMINATION OF TADALAFIL IN PHARMACEUTICAL PREPARATION BY LC WITH FLUORESCENCE DETECTION

S. Koyuturk, A.G.Dal
Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskisehir, TURKEY

A sensitive and specific method is described for the determination of tadalafil (TDF). The new method has been developed taking advantage of tadalafil nature fluorescence. This method show that it is possible to quite low limit of detection with FLD and can be used routine qualitative and quantitative TDF analysis.

INTRODUCTION
Tadalafil (TDF) is a selective reversible phosphodiesterase-5 (PDE5) inhibitor indicated for treatment of erectile dysfunction [1]. There are some analytical methods analysis of TDF such as HPLC-UV [2,3], LC/MS and CE. TDF analysis by LC with fluorescence detection is limited [1]. In this work, our objective is to purpose a new LC method with fluorometric detection for TDF, which has to nature fluorescence, and then apply to pharmaceutical tablet.

MATERIALS AND METHODS
The instrument was Agilent Technologies 1200 LC series. All chemical solutions were HPLC grade. Standard solution of TDF was dissolved in methanol. Granisetron (GRA) was used as internal standard and was dissolved in MilliQ water. Dilution of TDF and IS was made with mobile phase.

RESULTS AND DISCUSSION
For the optimization of method, buffer concentration, organic solvent ratio, pH, flow rate and injection volume were investigated. LC-FLD method was performed with fluorescence detection applying 275 nm excitation and 335 nm emission wavelength. 50% (v/v) acetonitrile containing 10mM acetate (pH 4.50) was used as mobile phase. Flow rate was 0.6 mL/min and injection volume was 10 μL. Separation was achieved with C8 column (4.6 x 150 mm, 5 μm) and analysis finished in 7 min (Figure 1). The developed method were validated for linearity, precision, LOD, LOQ, accuracy and stability. The validated method was applied pharmaceutical tablets. TDF tablet solution was prepared conveniently and was given to LC system.

CONCLUSIONS
A validated LC-FLD method is described for the analysis of TDF in pharmaceutical preparations. The method is simple, fast, sensitive and reproducible. The developed method was applied to tablet successfully and specific without any interference due to the other constituents present in the tablet. Briefly, the presented method can be used for the quality control of tadalafil in pharmaceutical preparations.

ACKNOWLEDGMENTS
This study was supported by Anadolu University Scientific Research Projects Commission under the grant no:1101S003.
REFERENCES

P-52: VOLTAMMETRIC DETERMINATION OF TENOXICAM ON MULTIWALLED CARBON NANOTUBE MODIFIED GLASSY CARBON ELECTRODE
S. Atal, F. Ağın
Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, Trabzon, Turkey

The electrooxidative behavior and determination of anti-inflammatory drug tenoxicam (TX) was investigated at multiwalled carbon nanotube (MWCNT) modified glassy carbon electrode (GCE) by cyclic, differential puls and square wave voltammetry. The dependence of the peak current and peak potentials on pH, concentration, nature of the buffer, and scan rate were examined. The best results were obtained for the quantitative determination of TX by differential puls and square wave voltammetry in 1 M acetate buffer at pH 5.5.

INTRODUCTION
Tenoxicam (TX) is a non-steroidal anti-inflammatory drug of the oxicams family. Tenoxicam is used to relieve inflammation, swelling, stiffness, and pain associated with rheumatoid arthritis, osteoarthritis, anklyosing spondylitis, tendinitis, bursitis, and periartthritis of the shoulders or hips [1]. Carbon nanotubes (CNTs) can be defined as a graphite sheet rolled up which are single walled carbon nanotubes (SWCNTs) or with additional graphene tubes around the core of SWCNTs which are multiwall CNTs (MWCNTs) [2]. Applications of CNTs as electrode materials or modifiers of conventional working electrodes in analytical chemistry can be found in the literature [3], because of a lower over potential and higher peak currents are observed in the voltammetric response at electrodes modified with CNTs [4].

MATERIALS AND METHODS
A three-electrode electrochemical cell was used for the experiments. It contained a GC electrode (BAS, φ: 3 mm diameter) as working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference. All measurements by CV, DPV and SWV were performed using a computer-controlled Autolab potentiostat/galvanostat with Nova 10.0 software (Metrohm-Autolab, The Netherlands).

RESULTS AND DISCUSSION
The voltammetric behavior and determination of TX was investigated by CV, DPV and SWV on MWCNT modified GC electrode in various buffer solutions at different pH values between 2.0–8.0. The oxidation process of TX exhibited irreversible and diffusion-controlled behavior in cyclic voltammetry. The best peak shape with peak current of TX for quantitative determination were obtained in 1 M acetate buffer at pH 5.5. The calibration curve was linear in the concentration range of 8.0×10^{-3}–8 μM for DPV and 0.2–10 μM for SWV.

CONCLUSIONS
The repeatability, reproducibility, selectivity, precision, and accuracy of proposed methods were investigated. These methods were successfully applied for the analysis of TX pharmaceutical dosage forms, without the need for separation, since there was no interference from the excipients.

REFERENCES

P-53: INTERACTIONS OF TRIMETHOPRIM AND SULFA METHOXAZOLE WITH AOT MICELLES BY SURFACE TENSION MEASUREMENTS
Z.B. Tamer, S. Göktürk
Marmara University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Istanbul, TURKEY
INTRODUCTION

Surfactants are composed of a hydrophilic surface and a hydrophobic core in aqueous media and their specific structure enables the micelles. These aggregates exhibit an interfacial region separating the polar bulk aqueous phase from the hydrocarbon-like interior. The interactions of drugs with surfactant micelles can be visualized as an approximation for their interactions with biological surfaces because surfactant micelles also have been used as mimetics for biomembranes. This provides an insight into more complex biological processes, such as the passage of drugs through cell membranes [1,2]. From this point of view the present study is an attempt to gain a better understanding of the characteristics of binding of trimethoprim (TMP) and sulfamethoxazole (SMX) to anionic AOT (Aeresol-OT) micellar system using surface tension measurements at 298 K. The chemical structures of SMX, TMP and AOT are shown in Fig.1, respectively.

MATERIALS AND METHODS

Surface tension of aqueous solutions of AOT in the absence and presence of fixed concentrations of TMP and SMX were measured by computer-controlled Wilhelmy-plate method in order to determine minimum area per molecule ($A_{min}$) and maximum surface excess concentration ($\Gamma_{max}$) by application of the Gibbs adsorption isotherm.

RESULTS AND DISCUSSION

We have focused our attention on the surface properties of AOT in order to gain better insight as to what happens at the interface during the interaction process. With this viewpoint, surface tension measurements have been performed on aqueous solutions of AOT in the presence of fixed concentrations of TMP and SMX. The effects of TMP and SMX on surface properties of anionic surfactant AOT are plotted by the surface tension ($\gamma$) versus concentration of AOT.

It was observed that the surface tension values of AOT in the presence of TMP are lower than those in the absence of TMP. On the contrary, the surface tension values of AOT in the presence of SMX is little higher than those in the absence of SMX.

CONCLUSIONS

The influence of TMP and SMX on surface properties of anionic surfactant AOT was evaluated using Gibbs Adsorption Isotherm and compared. The presence of TMP decreases the repulsion among head groups of AOT and more TMP molecules can be adsorbed at the interface which is also confirmed by the higher value of $A_{min}$ i.e. TMP increased the adsorption of AOT at the interface. The higher $\Gamma_{max}$ value and the lower $A_{min}$ value for AOT in the presence of SMX also explains that the electrostatic repulsion forces take place in the interaction of anionic SMX and anionic AOT molecules compared to a pure AOT solutions.

REFERENCES


P-54: SOLUTION BEHAVIOUR OF AMPHIPHILIC DRUG AMITRIPTYLINE HYDROCHLORIDE IN THE PRESENCE OF ADDITIVES

S. Göktürk, H.T.Karaçam, Z.B.Tamer, S.Bektaş

Marmara University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences
Istanbul, TURKEY

INTRODUCTION

Many drug molecules are amphiphilic and self-associate in a surfactant-like manner in aqueous environment to form small aggregates. Amitriptyline hydrochloride (AMT) is a cationic amphiphilic drug which self-associates in aqueous environment to form small aggregates. It belongs to a family of tricyclic antidepressant drugs and is actually used in the
management of neurogenic pain, attention-deficit, hyperactivity disorders, and phobic disorders. The molecular structure of AMT as below [1,2]:

The aim of this study is to examine micellization of the amphiphilic drug AMT in nature in water and the presence of various additives such as methanol, ethanol, acetone; dioxane, urea and sodium chloride by using conductometric measurements at 298 K.

MATERIALS AND METHODS
Conductometric measurements were successfully used in determination of critical micelle concentration (CMC) of AMT in the absence and presence of additives in aqueous media. The abrupt change in the plots of the conductivity of the solutions versus the concentration of AMT has been taken as indication of micelle formation.

RESULTS AND DISCUSSION
The effect of various concentrations of additives at various concentrations on micelle formation of AMT in aqueous solutions has been studied. From conductivity data the ionization degree (a) and counterion binding parameter (β) of micelles have been obtained [3]. It was observed that the presence of methanol, ethanol, acetone, dioxane and urea increased the CMC of AMT and diminished the micelle formation at the certain concentration. In order to study the effect of added salt the experiments were also carried out in the presence of sodium chloride which has the same counter ion of AMT.

Micellization of AMT totally inhibited when additive concentrations reached a certain value. It has been found that the CMC of AMT decreased with the increase in concentration of sodium chloride.

ACKNOWLEDGMENTS
This study was financially supported by Research Fund of Marmara University with the project numbers of SAG-CYLP-280214-0041.

REFERENCES

P-55: CHARACTERIZATION OF ENALAPRIL MALEAT BY RAMAN MAPPING

S.Yılmaz1, S. Ilbasmis-Tamer,2 H. Eksi1 I. H. Boyaci,3 U.Tamer1
Gazi University, Faculty of Pharmacy
1Department of Analytical Chemistry, 2Department of Pharmaceutical Technology, 3Department of Food Engineering, Faculty of Engineering, Hacettepe University, Beytepe, Ankara, TURKEY Ankara

INTRODUCTION
Raman spectroscopy has been actively used for analysis of solid dosage pharmaceutical formulations. Noninvasive Raman mapping may provide useful information on the composition and distribution of active ingredient in pharmaceutical formulations. Raman spectroscopy may also be implemented through a microscope to provide fine scale axial and lateral chemical maps.

The aim of this study is the characterization of formulations containing enalapril maleate and hydrochlorothiazide, ACE (Angiotensin converting-enzyme) inhibitor, used as an antihypertensive, by using Raman mapping.

MATERIALS AND METHODS
FT-Raman spectra were obtained using a Raman module mounted in the sample compartment of the Nicolet iS50 spectrometer (Thermo Fisher Scientific Co., Waltham, MA, USA). The excitation source was a 1064 nm diode laser fitted with an InGaAs detector. The Raman spectra of samples were collected at room temperature. The laser power on sample was set up to
500 mW with a spot size of approximately 50 microns. The area map was taken at 200 microns step. Spectral maps were analyzed using OMNIC™ Atlas software. The FT-Raman spectra of the samples were obtained at 8 cm⁻¹ resolution and 16 scans at each measurement point.

The pure Raman spectra of excipients in tablets containing, starch Mg-stearate, lactose, Aerosil 200 and enalapril maleate were taken individually in the range of 3700 and 200 cm⁻¹. Then, 100 mg and 150 mg tablets were prepared and compressed with pressure. Raman mapping spectra is collected from the flattened surfaces of solid dosage pharmaceutical formulations.

RESULTS AND DISCUSSION
In the present study, we prepared two different solid dosage formulations so-called formulation A and formulation B. Both formulations were prepared using different amount of enalapril maleate and other excipients such as lactose, magnesium stearate, starch, aerosil 200. The Raman spectra of active ingredient and each excipient were collected. Subsequently, Raman spectra of solid dosage pharmaceutical formulations were obtained by scanning the surface of samples and data analysis was performed to construct Raman mapping.

Fig. 1. Raman spectrum of solid dosage formulation containing (100 mg) of enalapril maleate and Raman mapping image/ the Raman spectrum was recorded using a 16 scans for each point at 500 mW power.

CONCLUSIONS
The cross section of a tablet containing a blend of excipients and a pharmaceutical active ingredient ((100 mg) of enalapril maleate) was examined in this study. Here, step-by-step mapping revealed the spatial distribution of the functional groups of enalapril maleate on the selected area.

ACKNOWLEDGMENTS
This study was supported by Gazi University Research Foundation (No: 02/2012-27).

P-56: LOOKING FOR HIGHEST SELECTIVITY OF ENANTIOSEPARATIONS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

T. Khatiashvili, R. Kakava, I. Matarashvili, B. Chankvetadze

Department of Physical and Analytical Chemistry, School of Exact and Natural Sciences, Tbilisi State University, Tbilisi, Georgia

INTRODUCTION
Separation selectivity makes the major contribution to the peak resolution in high-performance liquid chromatography [1, 2]. Therefore, it is preferable to operate with selectivity in order to improve the final result of chromatographic separation. At the same time improving enantioselectivity means to interfere with the thermodynamics of the separation process that is very challenging approach.

MATERIALS AND METHODS
The separation of enantiomers of chiral sulphoxides was studied on 4 different polysaccharide-based chiral columns in high-performance liquid chromatography with 2-propanol and the mixture of n-hexane and 2-propanol as mobile phases.

RESULTS AND DISCUSSION
In order to achieve the highest enantioselectivity one should optimize the structure of substance, chiral selector, mobile phase and the separation temperature. In the present study 2-(benzylsulfinyl) N-methyl benzamide, 3-(benzylsulfinyl) N-methyl benzamide, 4-(Benzylsulfinyl) N-methyl benzamide, 2-(4-nitrobenzylsulfinyl) N-methyl benzamide, 2-(4-trifluoromethylbenzylsulfinyl) N-methyl benzamide, 3-(benzylsulfinyl) benzamide and 2-(benzylsulfinyl) benzamide were used as chiral analytes. 4 cellulose-based chiral selectors, in particular, cellulose tris(3,4-dimethylphenylcarbamate), cellulose tris(3,4-dichlorophenylcarbamate), cellulose tris(3-chloro-4-methylphenylcarbamate) and cellulose tris(4-chloro-3-methylphenylcarbamate) were applied. As the mobile phase 2-propanol and the mixture of n-hexane
and 2-propanol were used. The separations were performed at 25°C, 15°C and 5°C.

CONCLUSIONS
The highest enantioselectivity ever reported in chiral HPLC (α=780) was obtained under following optimized conditions: Chiral analyte - 2-(benzylsulphinyl) benzamide, chiral selector - cellulose tris(4-chloro-3-methylphenylcarbamate), mobile phase – n-hexane/2-propanol-70/30 volume by volume and the separation temperature 5°C.

ACKNOWLEDGMENTS
Shota Rustaveli Georgian National Science Foundation (GNSF) and Georgian Research and Development Foundation (GRDF) are acknowledged for a partial financial support to this project.

REFERENCES

P-57: A SIMPLE AND RAPID HPLC-RF METHOD FOR THE DETERMINATION OF FLUOXETINE IN PLASMA SAMPLES
T. Recber1, E. Özkan1, E. Eren-Koçak2, M. Yılmaz2, E. Nemutlu2, S. Kir2

1Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey
2Hacettepe University, Institute of Neurological Sciences and Psychiatry, Ankara, Turkey

The simple and sensitive high performance liquid chromatographic (HPLC) method has been developed and validated for the quantification of fluoxetine in rat plasma. Fluorescence detector was used for quantification of fluoxetine. The method optimizations were performed in order to obtain short run-time without any interference from endogenous metabolites for quantification of fluoxetine in rat plasma.

INTRODUCTION
Fluoxetine is used for the treatment of major depressive disorder, obsessive–compulsive disorder, bulimia nervosa, panic disorder, and premenstrual dysphoric disorder. The aim of this study was to develop and validate a simple, rapid and sensitive HPLC method with fluorescence detection for quantification of fluoxetine in rat plasma samples exposed to orally or subcutaneously fluoxetine.

MATERIALS AND METHODS
Chromatographic conditions: The separation was made by C18 column (250 mm × 4.6 mm, 5 μm i.d.). Mobile phase included acetonitrile and 40 mM phosphate buffer (pH 2.5) in the ratio of 40:60 (v/v) at flow rate of 1.0 mL min⁻¹. Fluorescence detection was used for quantification of fluoxetine at excitation/emission 230/315 nm.

Sample preparation: 500 μl rat plasma sample was treated with 1000 μl acetonitrile in order to precipitate the proteins. After vortexing for 2 minutes, the sample was centrifuged at 15000 rpm for 5 min. Finally, 1000 μl solution was transferred to a clean vial to run on HPLC system.

RESULTS AND DISCUSSION
The chromatographic parameters, mobile phase organic phase ratio, pH and flow rate was optimized. The effect of pH on the chromatographic behaviour of the fluoxetine was tried pH 2.5, 4.3 and 7.0. The concentration of phosphate buffer was changed to 10 mM and 50 mM. The acetonitrile ratio of the mobile phase was tested between 30 and 40%. Finally, the effect of the flow rate of the mobile phase was evaluated at 0.8, 1.0 and 1.2 mL min⁻¹. The chromatogram of fluoxetine at the optimum conditions (acetonitrile and 40 mM phosphate buffer, pH 2.5, 40:60, v/v) at a flow rate of 1.0 mL min⁻¹) was shown in the Figure 1. At these conditions, the separation of the fluoxetine is completed in 9 min.

CONCLUSIONS
We developed and validated a fast, simple and sensitive HPLC-fluorescence method for the analysis of...
the fluoxetine. This method was successfully applied for the determination of fluoxetine in rat plasma.

REFERENCES

P-58: DEVELOPMENT OF A GRAPHENE-BASED ELECTROCHEMICAL DNA BIOSensor FOR MONITORING OF CISPLATIN-DNA INTERACTION

M. Vandeput, Y. Yardim, M. Çelebi, Z. Şentürk, J.-M. Kauffmann

1Free University of Brussels, Faculty of Pharmacy, Laboratory of Instrumental Analysis and Bioelectrochemistry, Brussels, BELGIUM
2Yüzüncü Ylı University, Faculty of Pharmacy and 3,4Science, Department of 2,4Analytical and 3Inorganic Chemistry, Van, TURKEY

INTRODUCTION
The development of inexpensive, simple, reliable and rapid methods of monitoring DNA–drug interaction is important in some areas such as pharmacology and medicine. Also, research on the interaction of the anti-cancer drug with DNA would be of a great significance on the carcinogenesis mechanism of drugs, the pharmacology and toxicity of anti-cancer drugs and the design and synthesis of some new drugs [1]. Cisplatin (cis-diaminedichloroplatinum(II)) is still one of the most frequently used cytotoxic agents. Graphene (GN) is the most recent member of carbon-material family. Considering the excellent properties of GN, such as large surface-to-volume ratio, high conductivity and electron mobility at room temperature, low energy dynamics of electrons with atomic thickness, robust mechanical and flexibility, it is an ideal material for electrochemical sensors [2]. In this study, glassy carbon (GC) electrode was modified with previously synthesized GN for the monitoring cisplatin-DNA interaction.

MATERIALS AND METHODS
Differential pulse voltammetric (DPV) measurements were carried out on with the aid of a µAutolab type III electrochemical analyzer with GPES 4.9 software package. A three electrode system in a 10-mL one-compartment voltammetric cell was employed consisting a GC or modified GC working electrode, a platinum wire counter electrode and Ag/AgCl reference electrode. The graphene oxide (GO) was firstly synthesized by a modified Hummers method [3]. GO was then reduced (to electrochemically reduced graphene oxide, ERGO) by a rapid and eco-friendly electrochemical method of repetitive cathodic cyclic potential cycling, without using any reducing reagents. The biosensing protocol at the DNA-modified electrode consisted of DNA immobilization, interaction with cisplatin, and its DPV transduction. The guanine and adenine oxidation peaks were used as the transduction signals. DP voltammograms were recorded after the transfer of the electrode into a blank background electrolyte.

RESULTS AND DISCUSSION
GO was characterized by UV/vis and FT-IR spectroscopies. The electrochemical behavior of the ds-DNA at the bare GC and the ERGO/GC was studied in 0.1 M acetate buffer (pH 4.7) by adsorptive transfer stripping differential pulse voltammetry (AdTS-DPV). The results showed that ERGO/GC combines the advantages of bare GC, such as high conductivity, large surface area and prominent electronic transport property, hence enhancing the electrocatalytic activity performance toward the oxidation of ds-DNA. Factors affecting the performance of the DNA immobilization such as the electrolyte pH, deposition time and potential, and DPV parameters were optimized. Under the optimal conditions of the experiment, the redox behavior of original DNA immobilized ERGO/GC exhibited oxidation processes of guanine around +0.93 V, mean response 2.08 ± 0.025 µA (n=3) and adenine around +1.10 V, mean response 1.15 ± 0.052 µA (n=3). The guanine and adenine signals were decreased about 51 and 49%, respectively, when the interaction occurred between DNA and cisplatin in solution phase.

CONCLUSIONS
The results showed that ERGO/GC could be applied successfully for monitoring cisplatin-DNA interaction.

REFERENCES
P-59: ADSORPTIVE STRIPPING VOLTAMMETRIC DETERMINATION OF SALBUTAMOL USING A BORON-DOPED DIAMOND ELECTRODE IN THE PRESENCE OF URIC ACID

H.S. Ali1, A.A. Abdullah1, Y. Yardım2, Z. Şentürk1

Yüzüncü Yıl University, 1Faculty of Science and 2Faculty of Pharmacy, Department of Analytical Chemistry, Van, TURKEY

INTRODUCTION
Salbutamol (SAL) [2-((r-butylamino)-1-(4-hydroxy-3-hydroxymethyl) phenylethanol] also known as albuterol, is a kind of selective β2-agonists widely used for the treatment of bronchial asthma, and other forms of allergic diseases. It is also applied as a tocolytic agent in humans as well as in veterinary medicine. Urinary SAL concentrations are frequently measured in competitive sports programs. Some efforts have been made for the determination of SAL either alone or in combination with other drugs by electrochemical techniques [1-5]. In this study, a simple and rapid method was developed for the determination of SAL, based on the oxidation of the compound at a boron-doped diamond (BDD) electrode.

MATERIALS AND METHODS
Stock standard solutions of SAL were prepared with deionized water. Four different supporting electrolytes, namely nitric acid (HNO3, 0.1 M), acetate buffer (0.1 M, pH 4.7), Britton-Robinson buffer (BR, 0.1 M, pH 2-10), and phosphate buffer (0.1 M, pH 2.5 and 7.4) solutions were used. All experiments of cyclic (CV) and square-wave adsorptive stripping (SW-AdSV) voltammetry were performed using a μAutolab type III electrochemical system driven by the GPES 4.9 software. The three-electrode system was immersed in a voltammetric cell containing required aliquot of the SAL working solutions and a selected supporting electrolyte at a desired pH. A selected accumulation potential was then applied to a BDD surface for a selected pre-concentration period, while the solution was stirred at 400 rpm. At the end of the accumulation period, the stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. Then, the voltammogram was recorded by scanning the potential toward to positive direction between +0.1 to +1.4 V using SW waveform.

RESULTS AND DISCUSSION
By using CV, the compound shows irreversible and adsorption-controlled oxidation peak at about +1.0 V in the BR buffer (pH 9.0) solution. Factors affecting the performance of the adsorptive stripping such as the electrolyte pH, accumulation time and potential, and SW parameters were optimized. Under the optimized experimental condition, using SW stripping mode, the compound yielded a well-defined voltammetric response in BR buffer, pH 9.0 at +0.95 V (vs. Ag/AgCl) (after 30 s accumulation at an open circuit condition). A linear calibration graph was obtained in the concentration range of 4 to 83 μg mL⁻¹ while holding the concentration of uric acid constant at 5 μg mL⁻¹. A detection limit of 1.1 μg mL⁻¹ was observed. The proposed voltammetric procedure was successfully applied to determine SAL in pharmaceutical formulation and spiked human urine samples in the presence of uric acid.

CONCLUSIONS
BDD electrode without any chemical electrode modification in combination of SW-AdSV could be allowed to develop a simple, novel and alternative electroanalytical method for SAL determination.

REFERENCES

P-60: SIMULTANEOUS QUANTITATIVE DETERMINATION OF HYDROCHLOROTHIAZIDE AND OLMESARTAN MEDOXOMIL IN TABLETS BY A NEW UPLC METHOD

Z.C. Ertekin, E. Dinç

Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, TURKEY

INTRODUCTION
The combination of HCT and OLM is a widely used strategy for the treatment of high blood pressure [1]. In previous studies, HCT and OLM were determined by bivariate and multivariate regression calibrations. [2]. The aim of this study was to develop a fast, easy and accurate method for the simultaneous
determination of HCT and OLM in tablets the routine analysis and quality control procedures.

MATERIALS AND METHODS
The chromatographic separation and quantitation of HCT and OLM was performed on an a Waters Acquity UPLC BEH C18 column (50 mm x 2.1 mm i.d., 1.7 μm) with a mobile phase consisting of acetonitrile : methanol : 0.1 M ammonium carbonate solution (20:40:40) at a constant 0.25 mL/min flow rate. Stock solutions and all samples were prepared in 0.05 M HCl and methanol mixture (50:50). Telmisartan (IS) was used as an internal standard with a constant concentration of 14 μg/mL for all samples.

RESULTS AND DISCUSSION
A good separation of the analytes was achieved using the above mentioned conditions with satisfactory suitability results. Retention times for HCT, OLM and IS were 0.51, 0.75 and 0.96 min, respectively and the optimum wavelength was determined to be 260 nm. The calibration curves were obtained by plotting the peak area ratios of the drug to IS. Linear regression lines were between 4-28 μg/mL for both drugs. LOD and LOQ values were found as 0.42 μg/mL and 1.41 μg/mL for HCT and 0.43 μg/mL and 1.44 μg/mL for OLM, respectively.

Validation studies were performed using synthetic test samples, inter-day intra-day samples, and standard addition samples. Recovery studies of the samples showed satisfactory results with high accuracy and precision. The standard addition studies indicated there was no effect of excipients on the analysis.

Fig. 1. UPLC chromatogram of HCT and OLM in their mixture with the presence of IS

The analysis results of the commercial tablets were found as 24.93 mg per tablet for HCT and 20.22 mg per tablet for OLM (Label claim : 25 mg HCT and 20 mg OLM per tablet).

CONCLUSIONS
A new UPLC method was developed and validated for the simultaneous determination of OLM and HCT in tablets. This rapid, accurate and precise method was successfully applied to commercial tablets. This method is suitable for the routine analysis and quality control of the tablets containing HCT and OLM.

ACKNOWLEDGMENTS
This work was done within the Chemometrics Laboratory of the Faculty of Pharmacy, and was supported by the scientific research project No. 10A3336001 of Ankara University.

REFERENCES

P-61: MELATONIN-LOADED PLGA NANOPARTICLES (NPs): PREPARATION AND IN VITRO EVALUATION

A. B. Ugur1, M. Cetin1, O. Ates2, A. Hacimuftuoglu3

Ataturk University
Faculty of Pharmacy, 1Department of Pharmaceutical Technology
Facility of Medicine, 2Department of Ophthalmology, 3Department of Pharmacology
Erzurum, Turkey

INTRODUCTION
Melatonin is a neuro-hormone and mainly produced and released by the pineal gland [1]. It regulates sleep/wake cycle and other circadian/seasonal rhythms [2]. Melatonin has potential for the treatment of various cancers and neurodegenerative diseases. It is important new drug delivery systems for melatonin due to its sensitivity to oxidation and its low bioavailability due to extensive first-pass metabolism [3]. The main objective of this study was to prepare and characterize melatonin-loaded PLGA NPs.

MATERIALS AND METHODS

Materials
Melatonin and Poly (D,L-lactide-co-glycolide) (PLGA) (75:25) were obtained from Sigma-Aldrich (USA). All other chemicals and reagents were of analytical grade and used as they were received.

Preparation of NPs
The organic phase (DCM) containing PLGA (100 mg) was introduced drop by drop into 12 mL of aqueous solution of PVA (3% w/v) and melatonin (15 mg) and emulsified by an ultrasonic probe (65% power; Sonoplus, HD 2070; Bandelin Electronics, Berlin, Germany) for 6 min. After the evaporation of organic phase under the reduced pressure at 45°C, this dispersion was centrifuged and obtained NPs were lyophilized for 24 hours. Nanoparticles were produced at least in triplicate.
**In vitro Evaluation of NPs**

**Surface Morphology**

The surface morphology of the nanoparticles was examined by scanning electron microscope (SEM) (NOVA NanoSEM 430, FEI, Czech Republic).

**Determination of drug content of NPs and In vitro release study**

Lyophilized nanoparticles (20 mg) in dichloromethane were sonicated for 15 minutes in ultrasonic bath and then mixed at 800 rpm for 30 minutes. To extract melatonin, phosphate buffer (PB; pH 7.4) was added into this mixture and mixed (800 rpm, 60 min.) After evaporation of organic solvent, the remaining aqueous dispersion was centrifuged at 12,000 rpm for 20 minutes. The drug content of each sample was then measured using a validated UV method at 222 nm.

In vitro release studies were performed using dialysis bag method. The freeze dried microparticles (15 mg) were suspended in PB pH 7.4 and sealed in a dialysis membrane (MWCO 14,000 Da). The sealed dialysis membrane was then placed in an amber vial containing 9 mL of PB (pH 6.8), and maintained at (37±0.5°C, 50 rpm). At predetermined time intervals, samples (3 mL) were withdrawn from the release medium and replaced with fresh buffer. Prior to analysis, all samples were centrifuged at 12500 rpm for 10 min, and their drug content was measured by means of a validated UV method at 222 nm.

**RESULTS AND DISCUSSION**

**Surface Morphology:** The SEM image of melatonin-loaded NPs was given in Fig. 1.

**Determination of drug content of NPs and In vitro release study:** Unfortunately, the low encapsulation efficiency (3±0.02%; mean ± SD, n=2) was observed for NPs. It may be due to the leakage of melatonin to the external medium during the preparation of NPs. Thus, more attempts (modifying the preparation method of NPs or using different methods/polymers) should be carried out to improve the encapsulation efficiency of melatonin. In PB (pH 7.4), about 15% and 89% of melatonin was released at 30 minutes and 48 hours, respectively (Fig. 2).

![Fig. 1. The SEM image of melatonin-loaded NPs](image1)

**Fig. 1. The SEM image of melatonin-loaded NPs**

![Fig. 2. In vitro release profile of melatonin from PLGA NPs (mean ± SD, n = 3)](image2)

**Fig. 2. In vitro release profile of melatonin from PLGA NPs (mean ± SD, n = 3)**

**CONCLUSION**

This study reports the preparations and in vitro evaluation of melatonin-loaded PLGA NPs. It might be useful to overcome the problems related to melatonin.

**ACKNOWLEDGMENTS**

This study was supported by Ataturk University Research Foundation (project number: 2013/012).

**REFERENCES**


**P-62: PREPARATION AND CHARACTERIZATION OF DEXKETOPROFENTROMETHAMOL LOADED KOLLIDON-SR NANOPARTICLES**

**A.A. Öztürk**, E. Yenilmez, Y. Yazan

1 Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskisehir, TURKEY

**INTRODUCTION**

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of musculoskeletal disorders such as osteoarthritis and rheumatoid arthritis [1]. Arylpropionic acid is among the NSAIDs which is currently produced in the racemic form. Dextroketoprofentromethamol (DT) is the dextrorotatory enantiomer of ketoprofensynthetized as tromethamine salt [2]. DT’s distribution half-life and elimination
half-life are 0.35 and 1.65 hours, respectively [3]. Kollidon SR (KSR) is a polyvinyl acetate and povidone based matrix retarding agent [4]. Polymeric nanoparticles prepared by spray-drying method for controlled analgesic delivery of oral use was aimed in this study.

MATERIALS AND METHODS
DT was a kind gift from Abdi Ibrahim (Istanbul, Turkiye). KSR was purchased from BASF, Germany and methanol from Sigma-Aldrich, Germany. Spray-drying was performed using Nano Spray Dryer B-90 (BÜCHI) with a long drying chamber. For the preparation of polymeric nanoparticles, KSR was dissolved in methanol prior to the addition of DT. Spray-drying conditions are given in Table 1 and formulations prepared are summarized in Table 2.

Table 1. Spray Dryer conditions

<table>
<thead>
<tr>
<th>Inlet Temperature</th>
<th>Outlet Temperature</th>
<th>Pump level</th>
<th>Spray level</th>
</tr>
</thead>
<tbody>
<tr>
<td>120°C</td>
<td>54°C</td>
<td>3</td>
<td>%100</td>
</tr>
</tbody>
</table>

Table 2. Formulations

<table>
<thead>
<tr>
<th>Code</th>
<th>KSR (g)</th>
<th>DT (g)</th>
<th>Methanol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSR 0</td>
<td>1</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>KSR 1</td>
<td>1</td>
<td>0.05</td>
<td>100</td>
</tr>
<tr>
<td>KSR 2</td>
<td>1</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>KSR 3</td>
<td>1</td>
<td>0.15</td>
<td>100</td>
</tr>
</tbody>
</table>

Particle size, zeta potential and polydispersity index (PI) values of formulations were analyzed by ZetasizerNanoZS (Malvern Instruments, UK). Possible temperature-dependent structure and crystallinity changes in the nanoparticles prepared were analyzed using differential scanning calorimetry (DSC-60, Shimadzu, Japan).

RESULTS AND DISCUSSION
Table 3. Particle size, zeta potential and PI values of nanoparticles prepared (mean ± SE) (n = 3)

<table>
<thead>
<tr>
<th>Code</th>
<th>Size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSR 0</td>
<td>92.74 ± 19.19</td>
<td>16.33 ± 0.12</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>KSR 1</td>
<td>110.60 ± 8.63</td>
<td>16.43 ± 0.45</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>KSR 2</td>
<td>226.10 ± 12.23</td>
<td>16.43 ± 0.45</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>KSR 3</td>
<td>357.90 ± 19.00</td>
<td>17.13 ± 0.17</td>
<td>0.39 ± 0.02</td>
</tr>
</tbody>
</table>

P-63: MAGNOLOL-LOADED MESOPOROUS SILICA NANOPARTICLES FOR MODIFIED DRUG DELIVERY APPLICATIONS

A. Stefanache1, A. Spac1, A. M. Tomioioaga2, L. Ochiuz1
1University of Medicine and Pharmacy “Grigore T. Popa” Faculty of Pharmacy, Iasi, Romania, 2“A.I.Cuza” University of Iasi, Romania

INTRODUCTION
Natural products are proved historically to be a promising pool for drug discovery and application that have reduced toxicity, less side effects and are significantly well tolerated by the human body [1]. Therefore, considerable attention is focused on development of new approaches to deliver the active ingredients of an herbal drug, in such a controlled manner to improve its therapeutic outcomes. The goal of the present study is to develop such novel delivery systems based on unique properties of mesoporous silica materials specifically designed for loading and sustained-release of magnolol. Mesoporous silica as MCM-41 and SBA-15 have ordered, non-connecting pore structure, well-defined pore geometry, and narrow pore size distribution. MCM-41 and SBA-15...
have the same chemical composition (SiO₂) and pore shape (cylindrical), and their function as drug hosts rely mainly on pore size and surface chemistry [2].

MATERIALS AND METHODS

Synthesis of MCM-41
MCM-41 was synthesized in alkaline media, under ultrasonic irradiation. The composition of the reaction mixture was TEOS:0.3CTAB:95EtOH:15NH₃:246 H₂O. The white solid was washed with water, filtered and dried at 60 °C overnight. Template removal was performed by calcination at 550 °C for 6h.

Synthesis of SBA-15
The SBA-15 mesoporous silica matrix SBA-15 support has been prepared by a procedure described earlier using triblock copolymer P123 as structure directing agent and tetraethyl orthosilicate (TEOS) as silica source. The synthesis gel has the following chemical composition: TEOS:0.017P123:5.87HCl:194 H₂O. The solid recovery and template removal was performed as described above.

Drug loading
The process involved mixing the components at a ratio of 50 mg m-SiO₂ matrix/50 ml of MGN solution (1 mg/ml). The tests were performed sonochemically for 120 min, using an ultrasonic generator SONICS VIBRA CellTM Model CV 33 (1.13 cm diameter Ti horn) with 750 W power, by applying a cycled periodic pulse of 3s US / 1s resting time.

RESULTS AND DISCUSSION

The structural, textural and morphological features of SBA-15 were investigated using XRD, N₂-sorption and SEM techniques before and after drug loading. The mesoporous silica matrix displays a XRD pattern with 3 diffraction peaks characteristic to hexagonal packing of mesopores with different sizes, typical for MCM41 and SBA15-type silica mesostructures. The hexagonal ordering is very well preserved after loading of drug molecules within the mesopores. On N₂-sorption both samples exhibit a type IV isotherm pattern in IUPAC classification, with a sharp inflection at relative pressures (P/P₀) typical for mesoporous MCM41 and SBA15-type silica mesostructures. The absorption equilibrium is reached in 15 min. The capacity of drug loading is of ~ 15% for MCM-41 and of ~ 10% for SBA-15 indicating the importance of pore surface chemistry and shape in the adsorption process.

Table 1. Porous features and drug loading of studied samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>S_{BET} (m²/g)</th>
<th>PV (cm³/g)</th>
<th>PD (nm)</th>
<th>Drug (mg/g matrix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCM-41</td>
<td>1244</td>
<td>0.979</td>
<td>2.35</td>
<td>-</td>
</tr>
<tr>
<td>MGN@MC M41</td>
<td>809</td>
<td>0.643</td>
<td>2.12</td>
<td>145</td>
</tr>
<tr>
<td>SBA-15</td>
<td>950</td>
<td>1.138</td>
<td>7.95</td>
<td>-</td>
</tr>
<tr>
<td>MGN@SB A15</td>
<td>675</td>
<td>0.778</td>
<td>7.28</td>
<td>107</td>
</tr>
</tbody>
</table>

Kinetic study was performed for nanoencapsulation of MGN within the mesopores of both silica-based matrices. The results are shown in figure 1. As observed, the adsorption equilibrium is reached in 15 minutes under ultrasonic irradiation, and the maximum amount of MGN adsorbed is of 145 mg, respectively 107 mg per 1 g of silica.

CONCLUSIONS

The present study reveals that MCM-41 and SBA-15 are a compatible matrix for MGN in preparing of sustained-release drug delivery system. Although, the pores are partially narrowed due to uniform spread-out of drug substance, XRD and N₂-sorption investigations indicate that the structural order is well preserved after MGN encapsulation. The absorption equilibrium is reached in 15 min. The capacity of drug loading is of ~ 15% for MCM-41 and of ~ 10% for SBA-15 indicating the importance of pore surface chemistry and shape in the adsorption process.

ACKNOWLEDGMENTS

This research was financed by the University of Medicine and Pharmacy “Gr. T. Popa” Iasi, Romania, and belongs to the Internal Research Program: Project no. 30889/2014.

REFERENCES

P-64: CHARACTERIZATION, PREPARATION AND CYTOTOXIC-APOPTOTIC EFFECTS OF CAFFEIC ACID LOADED SOLID LIPID NANOPARTICLES AND NANO LIPID PARTICLES

Lütfi GENÇ1,2, Gökhan DİKMEN3

1Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Tepebağ, 26470, Eskisehir-Turkey
2Anadolu University, Plant, Drug and Scientific Researches Center (AUBIAM), Tepebağ, 26470, Eskisehir-Turkey
3Eskisehir Osmangazi University, Central Research Laboratory (MERLAB), Odunpazarı, 26480, Eskisehir-Turkey

INTRODUCTION
Drug delivery systems such as liposomes, polymeric micelles, dendrimers, solid lipid nanoparticles (SLNs) and nano lipid particles (NLPs) provide controlled release of the drug in the desired time and the region. A lot of anti-cancerogen drugs such as ellagic acid exhibit various side effects due to especially high dosage. These side effects can be eliminated using drug delivery systems such as SLNs and NLPs [1-2].

MATERIAL AND METHODS
Firstly SLN and NLP formulations were prepared using hot homogenization method after then these formulations were characterized by Zeta Sizer, FT-IR, NMR, SEM, and DSC. Moreover, these formulations were compared with each other and advantages and disadvantages of them were shown. Cytotoxic and apoptotic effects will be examined on 3T3 and 5RP7 cells.

RESULTS
Caffeic acid loaded SLNs and NLP formulations were proven good stability (-30.4,-34.5 mV, respectively) and small size (nearly 190 nm and 150 nm). SLN and NLP formulations were compared with the freshly prepared formulations of caffeic acid and tween 80 by FT-IR spectroscopy and NMR spectroscopy. In FT-IR spectra, any chemical shift or deformation in the bands and any stability problems were not observed. In addition, nuclear magnetic resonance (NMR) spectra of SLN and NLP formulations have been compared to those of the freshly prepared samples of the Tween 80 and caffeic acid. According to NMR spectra, any new peaks were not observed for caffeic acid loaded SLN, NLP and placebo SLN, NLP. Moreover, SEM images were taken of these formulations.

CONCLUSIONS
This formulation may be suitable as a nano drug carrier system for cancer treatment and drug delivery.

REFERENCES

P-65: THE EFFECTS OF SOME FORMULATION PARAMETERS ON CLINDAMYCIN PHOSPHATE LOADED CHITOSAN FILM

M. Kilicarslan1, M. Gorgoz1, I. Erik2

Ankara University, Faculty of Pharmacy2, Department of Pharmaceutical Technology2, Ankara, TURKEY

INTRODUCTION
Periodontal disease includes conditions such as chronic periodontitis, aggressive periodontitis, systemic disease associated periodontitis, and necrotizing periodontitis. Clindamycin phosphate (CP) has been in use for treatment of periodontal disease as an antimicrobial agent and it is a unique antimicrobial that achieves high tissue concentrations even bone tissue. Because of the potential side effects of systemic antimicrobial therapy, recent treatments have been focused on the local antimicrobial drug delivery systems into the periodontal pocket [1-3]. The aim of this study is to prepare chitosan films containing clindamycin phosphate for local treatment of periodontitis and evaluation of the effects of some formulation parameters on the film characteristics.

MATERIALS AND METHODS
Materials
The following materials were used: Clindamycin phosphate (DEVA, Turkey), chitosan (low, medium, high molecular weight) (Aldrich, USA), acetic acid (Merck, Germany), propylene glycol (Aklar Chemicals, Turkey), acetonitrile (Sigma-Aldrich, USA).

Methods
Films were prepared by solvent casting technique [3]. The polymeric solution of low, medium or high molecular weight chitosan (LC, MC, HC respectively) was prepared using 1.5% (v/v) acetic acid then resulting solution was filtered and 1% (w/v) CP was added under constant stirring. Propylene glycol (5%, v/v) was used as plasticizer at all formulations. Therefore, films for each molecular weight of polymer were prepared with different drug-polymer ratio (1:1, 1:2 and 1:3). The viscosity of solution was determined (Brookfield Viskometer/RVTDV-2 USA) then poured into 5 cm diameter glass petri dish and dried at room
temperature. The drug amount adjusted to 1 mg for per disk. The discs with 3.5 mm diameter taken from different areas of films were weighted. Thickness was measured by manual micrometer. Content uniformity of films was performed as dissolving the film in pH 6.75 phosphate buffer. In vitro drug release studies were carried out as follow; films containing 1 mg CP placed in 1mL of pH 6.75 phosphate buffer in the dialysis bag, placed into 50 mL same buffer at 37 °C and shaken in a thermostatic horizontal shaker (Thermo scientific, MaxQ Mini 4450 Shaker) at 75rpm. 3mL of release medium were withdrawn at the predetermined time interval and replaced with fresh buffer. The drug concentration of the samples was determined by using HPLC (Agilent 1100 series HPLC, C18 (ACE5, C18, (150 x 4.6 mm id) column) at 210 nm [4]. The swelling degree of the films was evaluated by measuring the weight increase after contact with phosphate buffer at the same time intervals with in vitro dissolution test [5]. The percentage degree of swelling ratios was calculated using the following equation:

\[
\% S = \left( \frac{W_t - W_o}{W_o} \right) \times 100
\]

Where, \(W_t\) is the weight of the swollen film after time \(t\) and \(W_o\) is the original film weight at zero time.

**RESULTS AND DISCUSSION**

The thickness of the film were ranges from 515.0 ± 24.29 to 875.0 ± 37.82 µm, while the average weight of films were 5.78 ± 33 mg to 10.5 ± 0.88 mg. Viscosities of chitosan solution was increased with increasing polymer amount and the lowest incorporation efficiency was obtained with HC while it was higher with MC and LC with 1:1 drug polymer ratio. The lowest release and swelling degree were obtained from medium molecular weight chitosan films (Fig.1. and Fig.2). The highest buffer uptake was observed with film of low molecular weight chitosan.

**REFERENCES**


**P-66: OCULAR DELIVERY OF OFLOXACIN FROM POLOXAMER/CHITOSAN IN SITU GELLING SYSTEM**

A. Karatas, A. Bölük, A. H. Algan

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara- TURKEY

**INTRODUCTION**

Conventional liquid ophthalmic formulations are eliminated from the precorneal area immediately and only 1–10% of topically applied drug is absorbed. Also systemic absorption of the drug drained through the nasolacrimal duct may result in some side effects. Nowadays, in situ gelling systems are great of interest. They have the combined advantage of prolonged residence time of the gel in situ formed and reduced systemic side effects [1]. The objective of the present research was to develop a temperature triggered in situ
gelling system for ophthalmic delivery of Ofloxacin (OFL). For this purpose, Pluronic F127 (PF127) was used as in situ gelling agent in combination with Chitosan (CTS) as a natural, biodegradable and mucoadhesive polymer. The formulations were prepared using PF127 alone and in mixture with CTS. In situ gels were evaluated for clarity, pH, gelling temperature, rheological behaviors and in vitro drug release.

MATERIALS AND METHODS

Ofloxacin was obtained as a gift from Sanofi (Turkey). Pluronic F-127 and Chitosan (low molecular weight) were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals and solvents were of analytical grade.

In situ gelling formulations were prepared by a modified cold method [2]. All PF127 solutions used in this study (15, 18 % w/v) were prepared by mixing the polymer in cold (4°C) water or cold (4°C) pH 6 phosphate buffer. In formulations which CTS (1% w/v) was used, it was initially dissolved in aqueous acetic acid (2% v/v). The cold CTS solution was added as a mucoadhesive component to PF127 in situ gelling system.

Evaluation of physical properties of formulations:
Aqueous solutions of various concentrations of PF127 alone and PF127 together with CTS were evaluated for clarity, pH, gelation temperature and viscosity in order to identify the suitability of formulations for using as in situ gelling system.

In vitro drug release studies: In vitro release studies of in situ gel formulations were performed using a dialysis bag method [3]. Cold (4°C) drug formulations (100 μL) were transferred to the dialysis bags. Then dialysis bags were closed and dropped into 25 mL of pH 7.4 isotonic phosphate buffer at 35°C (ocular surface temperature). Equal aliquots of medium were withdrawn at different time intervals (5, 10, 15, 30, 60, 90, 120, 180 min) and equal volumes of fresh buffer medium were added to replace the withdrawn samples. Drug concentrations in the pH 7.4 isotonic phosphate buffer were measured by UV spectrophotometer (Shimadzu UV visible 1202) at 288 nm. The release profile of OFL was obtained by plotting the cumulative amount of drug released from each formulation against time. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Drug loaded formulations and physical properties are shown in Table 1 as FA, FB, FC, FD, FE.

Table 1. Combination and properties of formulations

<table>
<thead>
<tr>
<th>Formulation ingredients and physical properties</th>
<th>FA</th>
<th>FB</th>
<th>FC</th>
<th>FD</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin (% w/v)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>PF127 (% w/v)</td>
<td>18</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CTS 1% w/v mL</td>
<td>10</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water q.s. to mL</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>pH 6.0 buffer q.s. to mL</td>
<td>6.6±0.02</td>
<td>6.5±0.03</td>
<td>6.2±0.02</td>
<td>5.5±0.01</td>
<td>5.0±0.03</td>
</tr>
<tr>
<td>Gelation temperature (°C±SD)</td>
<td>30±1.2</td>
<td>34±0.9</td>
<td>30±1.2</td>
<td>30±0.8</td>
<td>33±1.4</td>
</tr>
<tr>
<td>Viscosity (Pa.s) at 10 rpm</td>
<td>90</td>
<td>31</td>
<td>55</td>
<td>62</td>
<td>66</td>
</tr>
</tbody>
</table>

Evaluation of physical properties of formulations:
Formulations were clear at room temperature. pHs were in a range of 5.0-6.7. Their gelling temperatures were ranged 30-34°C and they showed pseudoplastic behaviour. Especially for FE formulation OFL was successfully formulated in temperature induced in situ gelling system using PF127 (15% w/v) as a temperature triggered gelling agent in combination CTS (% 1 w/v) as a mucoadhesive hydrophilic copolymer. It was found that gelling system could flow easily at non-physiologic 4°C condition and undergo rapid gelation under physiological condition (33°C). The rheological behavior at its gelling temperature indicated that the formulation exhibited pseudoplastic flow mimicking tear fluid.

In vitro drug release studies: The comparison of in vitro drug release profiles are shown in Fig 1. FB and FC showed >90% drug release, while all the other formulations showed <80 % drug release after one hour. The release profiles indicated that as the concentration of PF127 increased from 15 % to 18 % the amount of the drug released decreased from 90% (FB, FC) to 80% (FA) after one hour (Fig 1). On the other hand, in the formulations containing CTS, about 70 % and 40 % of OFL was released after one hour from FD and FE formulations, respectively. For the FE, containing the highest amount of CTS, the slowest drug release was observed. At the end of 3 hours, only 60 % of the OFL was released.
CONCLUSIONS
In conclusion with all these properties FE formulation is considered as a promising vehicle for ocular delivery of OFL.

REFERENCES

P-67: POLYMERIC NANOPARTICLES FOR INTRA-ARTICULAR APPLICATION OF DICLOFENAC SODIUM

Z.A. Cankaya, E. Yilmaz, B. Kucukturkmen, U.C. Oz, A. Bozkir
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100 Tandogan/Ankara, TURKEY

INTRODUCTION
Diclofenac sodium, the sodium salt of o-(2,6-dichlorophenylamino)-phenyl acetic acid is a well known nonsteroidal anti-inflammatory drug (NSAID). Because of having anti-inflammatory, analgesic and antipyretic pharmacological effects, it is widely used in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Diclofenac sodium is absorbed quickly and almost completely with oral administration and also its half life is as short as 1-2 hours. This physicochemical properties brings with a couple of problems in terms of treatment process like the requirement of repeated peroral administrations. But unfortunately frequently repeated peroral administrations may cause adverse effects at gastrointestinal system. That is why the sodium salt of diclofenac is an appropriate candidate for intra-articular applications. The main purpose of this study is to investigate and regulate the optimization parameters of the diclofenac sodium loaded nanoparticulate systems and also its stability characteristics’ evaluation.

MATERIALS AND METHODS
Materials
Diclofenac sodium was kindly provided by Novartis®. Pluronic F68, Poly(lactide-co-glycolic acid) (50:50) (PLGA), Poly(ε-caprolactone) (PCL), Arlacel C (Sorbitan Sesquioleate), Dichloromethane was purchased from Sigma-Aldrich.

Methods
Preparation of nanoparticles was achieved by modified W/O/W emulsion-solvent evaporation technique which is quite common technique to encapsulate water soluble drugs [1,2]. Particle size, Polydispersity index and Zeta potential values were assessed with dynamic light scattering method. Encapsulation efficiency of the nanoparticle formulations were determined indirectly from supernatants via UV spectrophotometry. In-vitro release investigations were performed at incubated shaker via static method. Stability characteristics of the nanoparticles were observed at certain conditions such as 25°C, 60% relative humidity during one month. AFM images were taken in tapping mode.

RESULTS AND DISCUSSION
Nanoparticle structure with desired attributes was successfully achieved with modified W/O/W emulsion-solvent evaporation technique. A series of nanoparticle formulations were prepared to evaluate the effect of formulation parameters to optimize the quality attributes such as particle size, polydispersity index, zeta potential and also encapsulation efficiency for the final formulation. Particles with 190,8±3,136 nm size, 0,145±0,008 polydispersity index and -43,5±0,59 mV zeta potential were prepared successfully. Encapsulation efficiency of this formulation was found as 89,75±0,98%. In vitro release of diclofenac sodium was prolonged over 24 hours.
CONCLUSIONS
The results of the current research suggested the promising intra-articular application of diclofenac sodium loaded nanoparticles which may provide the avoidance from the side effects of peroral application.

REFERENCES

P-68: QUETIAPINE FUMARATE SUSTAINED RELEASE TABLET FORMULATION DESIGN BY USING INFORM V.4 ANN PROGRAM
Esher ÖZCELIK1, Burcu MESUT1, Buket AKSU2, Yildiz OZSOY1*
1Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Istanbul, TURKEY
2Santa Farma Pharmaceuticals, Istanbul, TURKEY

The purpose of this study is to find out the quality design of modified release tablet formulation of Quetiapine Fumarate using artificial neural networks and to determined a new formulation which is similar to the reference product (Seroquel XR tb).

INTRODUCTION
The obtained results and the reference product were used as outputs for the training of INForm V.4 ANN program of Intelligensys Ltd [1]. Accordingly, a design study was implemented within the scope of the QbD approach and twelve formulations were evaluated. Polymer type, polymer concentration as CPQ, mesh size as CPP parameter.

MATERIALS AND METHODS
Materials: Quetiapine Fumarate (Aurobindo Pharma Limited, Unit-XI), MCC 101 (FMC Biopolimer), Lactose monohydrate (DMV), Sodium sitrate (Merck), Carbopol 974P (Lubrizol, Parkoteks Chemistry), Xanthan gum (JUNGBUNZLAUER), Mg stearat (FACI).
Method: All formulations of Quetiapine fumarate were prepared by wet granulation method using Xanthan gum or Carbopol 974P. Internal phase were weighed, sieved and mixed then distilled water was slowly added. The wet granule dried at 45°C. Outer phase was added then tablets were compressed.

RESULTS AND DISCUSSION
It was observed that r2 values change between 74.31 as the lowest and 99.97 as the highest in all points of dissolution %, tablet weight, hardness, friability % and assay. So model was well built and the program was conditioned. INForm V.4 ANN program recommended the new optimized formulation which has the highest similarity to the reference product. According to this formula, Xanthan gum was chosen as a polymer matrix agent (20.35 % (w/w) and 1 mm granule size) by this program.

ACKNOWLEDGEMENTS
This study was supported by Research Fund of the Istanbul University: Project Number: 26586. It was applied to Turkish Patent Institute to get patent with the results of this study. Application number: TR 2013-G-316804

REFERENCES
1. Intelligensys Ltd. INForm Intelligent Formulation. UK, 2009.
P-69: PREPARATION AND EVALUATION OF RESPIRABLE MICROPARTICLES OF LEVOFLOXACIN

M. Alemdar1, B. Devrim1

1Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION

Lower respiratory tract infections (LRTIs) are responsible for the highest number of deaths in low-income countries, placing a considerable strain on their health economies [1]. A major concern with conventional antibiotic treatment of LRTIs is the requirement of a high dose to be delivered for effective eradication of the organism [2]. Hence, in the past decade increasing attention has been given to developing systems for delivery of antibiotics by means of inhalation directly to the respiratory epithelium [3,4].

The aims of this study were to prepare and evaluate levofloxacin-loaded PLGA microparticles for pulmonary delivery. PLGA microparticles were prepared using three different preparation methods such as oil-in-water (o/w), water-in-oil-in-water (w1/o/w2) and water-in-oil-in-water-in-water (w1/o/w2/w3) emulsion methods. Effects of preparation methods and formulation parameters on physicochemical properties of microparticles characterized in terms of the particle size distribution, entrapment efficiency, production yield and in vitro release were evaluated.

MATERIALS AND METHODS

Materials

Levofloxacin was kindly supplied by Koçak Farma (Türkiye). Poly(lactic-co-glycolic acid; PLGA) Resomer® RG 504 (50:50 lactic to glycolic acid ratio and a Mw=48 kDa, inherent viscosity 0.56 dl/g) was purchased from Boehringer Ingelheim (Ingelheim, Germany). Poly(vinyl alcohol) (PVA) (88 mol% hydrolysed, Mw=30 000 ± 70 000) and dichloromethane (99.9%, HPLC grade) were obtained from Sigma (Steinheim, Germany). All other chemicals used were analytical grade.

Preparation of microparticles: Levofloxacin-loaded PLGA microparticles were prepared by using oil-in-water (o/w), water-in-oil-in-water (w1/o/w2) and water-in-oil-in-water-in-water (w1/o/w2/w3) emulsion methods. In o/w emulsion solvent evaporation method, levofloxacin was added to organic solution of PLGA in dichloromethane. Then, organic phase was injected into the external aqueous phase consist of 60 ml of 2% PVA solution and homogenized by using a high-speed homogenizer (Ultra Turrax® T-25, Ika, Staufen, Germany) operating at 13500 rpm for 2 min. In w1/o/w2 emulsion method, the organic solution of polymer was emulsified with aqueous solution of levofloxacin containing 1% (w/v) Pluronic F-127® as surfactant. This primary emulsion (w1/o) was emulsified with PVA solution to form the secondary emulsion (w1/o/w2). Differently from this, w1/o/w2 secondary emulsion was transferred into 300mL of 0.5% (w/v) PVA solution (w3) and stirred with a mechanical stirrer in w1/o/w2/w3 emulsion method. Compositions used for levofloxacin-loaded microparticles are shown in Table 1.

Table 1. Composition of levofloxacin-loaded PLGA microparticles.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Preparation method</th>
<th>Amount of organic solvent (ml)</th>
<th>Amount of drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>w/o/w</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>F2</td>
<td>w/o/w</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>F3</td>
<td>w/o/w</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>F4</td>
<td>w/o/w</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>F5</td>
<td>w/o/w</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>F6</td>
<td>w/o/w/w</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>F7</td>
<td>o/w</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

Physicochemical characterizations of microparticles

To investigate the shape and morphology of microparticles, they were dispersed in a droplet of water, directly on a slide and observed under an optical microscope (Leica Model DM 4000B, Germany). The average size of microparticles was measured with a laser diffraction particle size analyser (Mastersizer 3000, Malvern Instruments, Germany). The drug encapsulation efficiency is determined from the ratio of the encapsulated levofloxacin to the levofloxacin initially added. Microparticles recovered at the end of preparation were weighed and the production yield (%) was calculated using equation (1).

\[
\text{Production yield (\%)} = \left( \frac{\text{Total microparticle amount (mg)}}{\text{Total solid material amount (mg)}} \right) \times 100
\]

In vitro release studies:

Microparticle samples were placed into dialysis bags and suspended in pH 7.4 phosphate buffer solution. Then, microparticles were shaken horizontally in a shaking incubator (JeioTech Shaking Incubator Model SI-300, Japan) at 50 rpm and 37±2°C. At various time points, samples were withdrawn from the release media and replaced with an equal volume of the corresponding fresh media. The samples were analyzed at 288 nm spectrophotometrically. The in vitro release experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The particle size, encapsulation efficiency and production yield results of microparticles are shown in Table 2.
<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean particle size (μm±SD)</th>
<th>Encapsulation efficiency (%±SD)</th>
<th>Yield of production</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>39.6±5.48</td>
<td>16.45±0.03</td>
<td>90.22</td>
</tr>
<tr>
<td>F2</td>
<td>4.08±0.00</td>
<td>8.95±0.07</td>
<td>97.64</td>
</tr>
<tr>
<td>F3</td>
<td>4.90±0.01</td>
<td>8.16±0.05</td>
<td>84.55</td>
</tr>
<tr>
<td>F4</td>
<td>4.13±0.29</td>
<td>6.99±0.09</td>
<td>89.99</td>
</tr>
<tr>
<td>F5</td>
<td>3.93±0.12</td>
<td>6.94±0.04</td>
<td>95.04</td>
</tr>
<tr>
<td>F6</td>
<td>5.34±0.12</td>
<td>10.10±0.18</td>
<td>96.13</td>
</tr>
<tr>
<td>F7</td>
<td>6.29±0.19</td>
<td>15.61±0.03</td>
<td>71.43</td>
</tr>
</tbody>
</table>

Figure 1 shows optical observation of levofloxacin-loaded microparticles. It is seen that these microparticles have spherical shape.

![Optical micrographs of microparticles.](image)

The release profile of levofloxacin-loaded PLGA microparticles is shown in Figure 2.

![Levofloxacin release profile from PLGA microparticles.](image)

CONCLUSIONS
Levofloxacin-loaded PLGA microparticles were prepared by using three different emulsion methods. The type of preparation method and formulation parameters affected physicochemical properties of microparticles. Higher encapsulation efficiency was obtained with o/w emulsion method. In vitro release profile of F7 coded formulation shown that levofloxacin-loaded microparticles exhibited a slow release profile. The results of our current study showed promising capabilities of levofloxacin-loaded PLGA microparticles for pulmonary drug delivery to treat respiratory infections.

REFERENCES
4. İmanoğlu, S.; Devrim, B.; Bozkır, A., Preparation and evaluation of levofloxacin-loaded PLGA microparticles for pulmonary delivery: effect of surfactant type and concentration on properties of microparticles. 17th International Pharmaceutical Technology Symposium, September 8-10, 2014, Antalya-Turkey

P-70: METFORMIN HYDROCHLORIDE-LOADED CHITOSAN-ALGINATE (CS-AL) BEADS
B. Server, A. B. Uğur, M. CETİN
Ataturk University, Faculty of Pharmacy
Department of Pharmaceutical Technology

INTRODUCTION
Metformin HCl (M-HCl) is an oral antidiabetic drug in biguanide class [1]. M-HCl is recommended by the American Diabetes Association as the first-line therapy for Type 2 diabetes. Its glucose-lowering action shows to reduce hepatic glucose output and lead to reduction in insulin resistance [2]. Its oral bioavailability is about %50-60. Conventional oral dosage forms should be used three or four times, and result in reducing patient compliance and/or enhancing the incidence of side-effects. M-HCl-loaded beads may be helpful to overcome these problems [3]. The aim of this study is to prepare and in vitro characterize the M-HCl-loaded chitosan-alginate beads for oral drug delivery.

MATERIALS AND METHODS

**Materials**
Sodium Alginate and chitosan (Protosan UP CL 113) were obtained from Sigma-Aldrich (USA) and FMC BioPolymer (Norway), respectively. M-HCl was a generous gift from Sandoz Ilaç Sanayi ve Ticaret AS (İstanbul, Turkey). All other chemicals were analytical grade.

**Preparation of the beads containing M-HCl**
Ionotropic gelation method was used for the preparation of beads. Sodium alginate was dissolved in distilled water and then metformin HCl (50 or 100 mg) was added in this aqueous solution (2%, w/v). The solution was dropped through a 26 gauge syringe...
needle into 3% (w/v) of calcium chloride containing chitosan (1%, w/v) chitosan solution (pH adjusted 4.5 with acetic acid). The formed beads were further mixed in gelling medium for an additional 1 hour at room temperature. After filtration, the prepared beads were lyophilized for 24 hours.

**Characterization of the beads containing M-HCl**

**Particle Size and Shape Evaluation**
The bead size in wet state was determined by vernier calipers. The shape evaluation of beads was carried out by using digital photographs.

**Drug content of beads**
15 mg of lyophilized beads in 10 mL of phosphate buffer (PB, pH 6.8) were mixed at 1000 rpm for 24 hours. Then, the dispersion was centrifuged at 12500 rpm for 10 minutes at 15 °C and the drug content of each sample was measured using a validated UV method at 232 nm.

**Swelling studies**
Swelling study of lyophilized beads was carried out in two aqueous media: PB pH 6.8 and HCl pH 1.2. Accurately weighed amounts of beads (15 mg) were immersed in 10 ml of medium at 37±0.5 °C. The swollen beads were periodically removed, wiped gently with paper and weighted. The weight change of the beads with respect to time was calculated according to the formula (Weight Change (%) = [(Ws-Wi)/Wi]x100; Ws: the weight of the beads in the swollen state and Wi: the initial weight of the beads.

**In vitro Release studies**
The release studies of dry beads (50 mg or 100 mg) were carried out in PB pH 6.8 (10 mL) at 37±0.5°C and agitated at 50 rpm by using a horizontal laboratory shaker. At predetermined time points, 2 mL of sample was withdrawn from the medium and replaced with fresh buffer (2 mL). The samples were centrifuged at 12500 rpm for 10 minutes, and their drug content was measured using the validated UV method at 232 nm.

**RESULTS AND DISCUSSION**

**Particle Size and Shape Evaluation**
The mean sizes of the 50 mg and 100 mg of drug loaded beads (sixty beads in wet state) were 1.59±0.18 mm 1.63±0.16, respectively. The digital photographs of dry and wet M-HCl-loaded beads were given in Figure 1.

![Fig.1](image1.jpg)

**Drug content of beads**
The encapsulation efficiency values of beads containing 50 and 100 mg of M-HCl were found 10.96±0.347% and 11.92±0.352%, respectively (mean ±SD, n= 3).

**Swelling Studies**

![Fig.2](image2.jpg)

**In vitro release studies**

![Fig.3](image3.jpg)
50% and ~90% of metformin HCl were released from both formulations within 4 hours and 2 days, respectively.

CONCLUSIONS

Alginate is an anionic natural polymer which forms a hydrogel in the presence of calcium. However, chitosan is a natural polymer with positive charge. Thus, chitosan-alginate polyelectrolyte complex are used to prepare modified release drug delivery systems [4]. In this study, the in vitro release profile showed a sustained release of M-HCl from the beads. The beads may be useful for metformin oral delivery.

REFERENCES


P-71: DEVELOPMENT AND EVALUATION OF A LIPID-POLYMER HYBRID NANOPARTICULATE SYSTEM

C.T. Sengel-Turk, C. Hascicek

Ankara University, Faculty of Pharmacy
Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION

Benign prostatic hyperplasia (BPH), a noncancerous enlargement of the prostate gland, is a common prostate disorder among older men. An estimated 50% of men have histological evidence of BPH by age 50 years and 80% by age 70 [1]. Lonidamine (LND) is a therapeutic agent with great clinical potential in the treatment of BPH as it acts to inhibit aerobic glycolysis and induce apoptosis via direct inhibition of hexokinase II. However, the clinical application of LND is impeded by the occurrence of residual liver toxicity [2]. The low bioavailability and liver toxicity demonstrated by LND in clinical trials make it an ideal candidate for nanoparticulate (NP) drug delivery. The focus of this research is to develop a new nano-sized lipid-polymer hybrid NP systems of LND and evaluate the in-vitro characteristics of the particles.

MATERIALS AND METHODS

The materials used were LND, PLGA 50:50 and lecithin (Sigma-Aldrich Chem Co., Germany) and also DSPE-PEG-COOH 2000 (Avanti Polar Lipids, USA). Lipid-polymer hybrid NP systems were prepared through a modified nanoprecipitation method [3]. In-vitro evaluation of the NP systems was performed in terms of entrapment efficiency, particle size, size distribution and surface charge. Transmission electron microscopy (TEM) analysis was used for the morphological characterization of the NP systems.

RESULTS AND DISCUSSION

In this research, LND encapsulated three different lipid-polymer hybrid NP systems were produced successfully by the modified technique. Lipid-PEG/Polymer ratio was kept constant at 0.1 molar ratio per formulation. The hybrid formulations were varied based on the three lipid-PEG/lipid ratio (17%, 34% and 68%) and coded as LPN1, LPN2 and LPN3 respectively. Table 1 displays the various characteristics of the hybrid systems.

<table>
<thead>
<tr>
<th>Code</th>
<th>EE±SD (%)</th>
<th>MPS±SD (nm)</th>
<th>PDI±SD</th>
<th>ZP±SD (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPN1</td>
<td>24.69±2.08</td>
<td>93.9±0.59</td>
<td>0.212±0.02</td>
<td>-29.8±1.08</td>
</tr>
<tr>
<td>LPN2</td>
<td>43.08±1.23</td>
<td>133.9±2.36</td>
<td>0.229±0.04</td>
<td>-32.9±0.64</td>
</tr>
<tr>
<td>LPN3</td>
<td>69.12±3.47</td>
<td>163.6±4.51</td>
<td>0.270±0.02</td>
<td>-36.8±1.11</td>
</tr>
</tbody>
</table>

EE: Entrapment efficiency, MPS: Mean particle size, PDI: Polydispersity index, ZP: Zeta potential, SD: Standard deviation

In our research, LND was encapsulated in the range of 24.69-69.12% into the particles. The particle size of all NP systems ranged from 93.9 to 163.6 nm with polydispersity in the range between 0.212-0.270 inhibited a narrow size distribution. The zeta potential values of the particles ranged between the -29.8 and -36.8 mV. The negative surface charge was resulted from the lipid layer on the particle surface and the surface charge was increased with increasing the lipid layer on the surface of the hybrid systems. It was seen that lipid-PEG/lipid ratio had a major effect on the properties of the final product. Generally, as the lipid-PEG/lipid ratio increased; EE, MPS and ZP were increased significantly (p<0.05). The formation of a more viscous inner phase might lead to increase EE and PS of the developed systems.
TEM images indicated that the investigated hybrid formulations consisted of sub-micron sized particles with spherical shape (Fig. 1.).

CONCLUSIONS
This research concluded that percentage of lipid-PEG/lipid ratio was one of the important parameters influencing on the in-vitro properties of LND-encapsulated lipid-polymer hybrid NP systems. In-vivo experiments are currently being conducted as the continuation of this study to evaluate the utilization of this developed technology in the treatment of BPH.

ACKNOWLEDGMENTS
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Grant No: 114S132.

REFERENCES

P-72: ANTITUMOR ACTIVITY OF GELS CONTAINING GEMSITABINE HCl-LOADED MICROSPHERES AND NANOPARTICLES ON BLADDER CANCER CELL LINES

INTRODUCTION
Cancer is a major public health problem in the world which causes millions of death each year. Bladder cancer is the most common type of tumor in the urogenital system. Approximately 75% of patients with bladder cancer present with non-muscle-invasive cancer, which is generally treated by transurethral resection and intravesical chemotherapy. Intravesical route is an important administration method to reduce the side effects caused by drugs given systemically, to escape from the first pass effect and to enable more effective treatment for local drug implementation [1]. Gemcitabine hydrochloride (GemHCl) is a water-soluble deoxycytidine analogue with a broad spectrum of antitumor activity and it has i.v. solution formulation for systemic administration in Turkey. Its characteristics make GemHCl is a promising candidate for intravesical therapy of non-muscle-invasive bladder cancer [2]. The aim of this study is to evaluate cytotoxic effects of intravesical gel formulations containing nano/micro particulate systems of GemHCl used for the treatment of the bladder cancer. Cytotoxicity assays were performed in two human bladder transitional cancer cell lines.

MATERIALS AND METHODS
Preparation of Chitosan and Poloxamer Gels: For the preparation of chitosan gel, 2% (w/w) chitosan was dissolved in lactic acid solution. In situ Poloxamer (Plx) gel was prepared according to cold method and 20% Plx 407 and 10% Plx 188 were dissolved in distilled water at 4°C. After the preparation of chitosan and Plx gels, GemHCl loaded nanoparticles (NPs) or microspheres (MSs) were suspended in gels.

Cell lines, culture conditions and chemicals: T24 and RT4 human bladder cancer cell lines were cultured in McCoy's 5a medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 2 mM L-glutamin (Gibco, USA). All cells were cultured as monolayers and maintained at 37°C in a humidified 5% CO2 incubator [3]. Cytotoxicity assay for gel formulations containing nanoparticles or microparticles: The cytotoxic effects of Plx and chitosan gel formulations containing NPs [chitosan or chitosan-thiolglycolic acid (chitosan-TGA)] or MSs were conventionally evaluated by living cell counting. For this purpose both RT4 and T24 cells were seeded in 12 well plates (1.5x10^5 cells/well), allowed to attach 24 hours, and then treated with different concentrations (0.1-100 μM) of aqueous GemHCl solution, equivalent amount of GemHCl NPs/MSs loaded gels and empty NPs/MSs gels (without GemHCl). Following the 48 hours exposure period, the treatments were removed and the cells were washed with fresh McCoy's 5a medium. Then the cells were trypsinized with 1xPBS (phosphate buffer solution), disaggregated through a pipet. The
harvested cells were counted under a microscope (OLYMPUS IX71, USA) by two independent investigators using a haemocytometer. For each concentration of GemHCl or gel formulations, the number of viable cells per millilitre were calculated. 

**Statistical analysis:**
The cells incubated in culture medium alone served as a control for cell viability. The cell-growth inhibition potency of formulations were expressed as IC₅₀ values, defined as the concentration of the drug necessary to inhibit the growth of cells by 50%. IC₅₀ was computed by nonlinear regression using Graph-pad PRIZM 5.0 software. The cell death caused by the maximum concentration (100µM) applied to cells were demonstrated as % inhibition. Data are the means±S.D. of three experiments.

**RESULTS AND DISCUSSION**
Intravesical chemotherapy for invasive bladder cancer is a treatment for preventing or delaying tumour recurrence after tumour resection. However, up to 70% of patients may fail and new intravesical agents with improved effectiveness are needed. GemHCl is a relatively new anticancer drug that has shown activity against bladder cancer [4]. Gels loaded with NPs and MSs formulations were studied *in vitro* on T24 (human bladder carcinoma) and RT4 (human bladder papilloma) cells. Gel formulations containing NPs loaded GemHCl did not exhibit 50% cell death on T24 cells, so IC₅₀ was not calculated (data not shown). Moreover, gel formulations containing NPs and MSs without GemHCl had no cytotoxic effects on both cells (data not shown).

IC₅₀ and inhibition% values of aqueous GemHCl solution and gel formulations containing GemHCl NPs were shown for RT4 (Table 1).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC₅₀</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GemHCl</td>
<td>0.18± 0.09</td>
<td>91.40</td>
</tr>
<tr>
<td>Chitosan gel containing ChitosanNPs (GemHCl)</td>
<td>0.40± 0.26</td>
<td>87.59</td>
</tr>
<tr>
<td>Plx gel containing ChitosanNPs (GemHCl)</td>
<td>22.35± 3.55</td>
<td>66.61</td>
</tr>
<tr>
<td>Chitosan gel containing chitosan-TGA NP (GemHCl)</td>
<td>26.72± 6.26</td>
<td>59.60</td>
</tr>
<tr>
<td>Plx gel containing chitosan-TGA NP (GemHCl)</td>
<td>---</td>
<td>29.92</td>
</tr>
</tbody>
</table>

MSs loaded with GemHCl in chitosan or Plx gel formulations showed potent cytotoxicity both T24 and RT4 cell lines. IC₅₀ values and % inhibition were given at Table 2 and Table 3.

**Table 2.** IC₅₀ and Inhibition% values of GemHCl and GemHCl loaded MSs gel formulations on T24 cells.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC₅₀</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GemHCl HCl</td>
<td>2.50± 0.90</td>
<td>75.55</td>
</tr>
<tr>
<td>GemHCl loaded microsphere in chitosan gel</td>
<td>71.75± 52.77</td>
<td>63.66</td>
</tr>
<tr>
<td>GemHCl loaded microsphere in Plx gel</td>
<td>26.84± 3.98</td>
<td>81.65</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**
GemHCl loaded NPs and MSs in gel formulations prepared for intravesical administration were evaluated in two different bladder cancer cell lines to assess the cytotoxic effects in this study. IC₅₀ and inhibition% values of aqueous GemHCl solution were different in two cells.

GemHCl was more potent on RT4 cells than T24 cells in terms cytotoxicity (IC₅₀ : 0.18± 0.09 and 2.50± 0.90, respectively). Gel formulations containing nanoparticles loaded with GemHCl did not exhibit an effective cell death on T24 cells. However, the cell-growth inhibition potency of chitosan gel containing chitosan nanoparticle loaded with GemHCl was so similar to aqueous GemHCl hydrochloride solution (IC₅₀: 0.18± 0.09 and 0.40± 0.26, respectively) on RT4. For this reason, this formulation is as potent as GemHCl on RT4 cells. Therefore it could be expected that *in vivo* application of this gel would be comparable conventional used GemHCl in patients suffer from bladder cancer.

As observed in NPs, the MSs formulations were more effective on RT4 than T24. MSs loaded GemHCl in Plx gel showed greater cytotoxicity than any other gel formulations in both cells.

In conclusion, both GemHCl loaded NPs and MSs gel formulations have greater cytotoxic effects on RT4 cells than T24 cells.
ACKNOWLEDGMENTS
This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-112/S/293). The authors would like to acknowledge Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use its laboratory instruments.

REFERENCES

P-73: CYTOTOXIC EFFECTS OF GEMSITABINE HCl-LOADED MICROSPHERES AND NANOPARTICLES ON BLADDER CANCER CELL LINES

1Gulnur SEVIN, 2Yalcin ERZURUMLU, 3Sinem Y. KARAVANA, 2Zeynep AY ŞENYİĞİT, 4Derya İLEM ÖZDEMİR, 4Çağrı ÇALIŞKAN, 3Esra BALOGLU

Ege University, Faculty of Pharmacy
1Department of Pharmacology, 2Department of Biochemistry, 3Department of Pharmaceutical Technology, 4Department of Radiopharmacy
35100 Izmir, TURKIYE

INTRODUCTION
Bladder cancer was the 9th most common cancer in the world, with 430,000 new cases diagnosed in 2012. It was three times more common in men compared with women. Smoking, infestations with schistosomes and exposure to industrial chemicals, such as aromatic amines are cause of this cancer [1]. Intravesical therapy for bladder cancer is important drug administration route for treating the cancer and stopping it from coming back or spreading into the deeper layers of the bladder. Gemcitabine hydrochloride (GemHCl) is a water-soluble deoxyctydine analogue with a broad spectrum of antitumor activity and it has i.v. solution formulation for systemic administration in Turkish Drug Market. Its characteristics make GemHCl a promising candidate for intravesical therapy of non-muscle-invasive bladder cancer [2]. This study investigates the cytotoxic effects of intravesical nanoparticles and microspheres formulations of GemHCl in bladder cancer cell lines. Cytotoxicity assay was performed with two cell lines.

MATERIALS AND METHODS

Cell lines, culture conditions and chemicals:
T24 (ATCC® HTB-4TM) and RT4 (ATCC® HTB-2TM) human bladder cancer cell lines were cultured in McCoy's 5a medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 2 mM L-glutamin (Gibco, USA). All cells were cultured as monolayers and maintained at 37°C in a humidified 5% CO2 incubator [3].

Cytotoxicity assay for nanoparticle formulations: The cytotoxic effects of nanoparticles were evaluated by WST-1 test (BioVision®, USA). Cells were seeded in 96-well plates (104 cells/well), allowed to attach 24 hours, and then treated with different concentrations (0.1-100 μM) of aqueous GemHCl solution, equivalent amount of GemHCl loaded nanoparticle formulations [chitosan, chitosan-thioglycolic acid (chitosan-TGA), protosan] and empty nanoparticles (without GemHCl). Following the 48 hours exposure period, the treatments were removed and the cells were washed with fresh McCoy's 5a medium and then individual wells were treated with a 100 μL of medium solution containing 10% WST-1 reagent for 1 and 2 hours at 37°C. At the end of the incubation period at 37°C for 1 and 2 hours, absorbance readings were performed at 440 nm using microplate reader (VERSAmax Molecular Devices Corporation, USA). Average absorbance was used.

Cytotoxicity assay for microsphere formulations: The cytotoxic effects of microspheres were conventionally evaluated by living cell counting. For this purpose both RT4 and T24 cells were seeded in 12 well plates (1.5x105 cells/well), allowed to attach 24 hours, and then treated with different concentrations (0.1-100 μM) of aqueous GemHCl solution, equivalent amount of Gem HCl loaded microsphere and empty microsphere (without GemHCl). Following the 48 hours exposure period, the treatments were removed and the cells were washed with fresh McCoy's 5a medium. Then the cells were trypsinized with 1xPBS (phosphate buffer solution), disaggregated through a pipette. The harvested cells were counted under a microscope (OLYMPUS IX71, USA) by two independent investigators using a haemocytometer. For each concentration of GemHCl or microsphere formulations, the numbers of viable cells per millilitre were calculated.

Statistical analysis: The cells incubated in culture medium alone served as a control for cell viability. Cell viability was calculated as a percentage of the
absorbance reading of compounds comparison to control reading. The cell-growth inhibition potency of formulations were expressed as IC₅₀ values, defined as the concentration of the drug necessary to inhibit the growth of cells by 50%. IC₅₀ was computed by nonlinear regression using Graph-pad PRIZM 5.0 software. The cell death caused by the maximum concentration (100μM) applied to cells were demonstrated as inhibition%. Data are the means±S.D. of six experiments.

RESULTS AND DISCUSSION
Intravesical chemotherapy for invasive bladder cancer is a treatment for preventing or delaying tumour recurrence after tumour resection. However, up to 70% of patients may fail and new intravesical agents with improved effectiveness are needed. GemHCl is a relatively new anticancer drug that has shown activity against bladder cancer [4]. Nanoparticle and microsphere formulations of it were studied in vitro on T24 (human bladder carcinoma) and RT4 (human bladder papilloma) cells. IC₅₀ and inhibition % values of aqueous GemHCl solution and GemHCl loaded nanoparticle formulations were given for T24 (Table 1) and RT4 (Table 2).

Table 1. IC₅₀ and inhibition% values of GemHCl and GemHCl loaded nanoparticle formulations on T24 cells.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC₅₀</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GemHCl</td>
<td>24.35± 1.05</td>
<td>76.81</td>
</tr>
<tr>
<td>GemHCl-ChitosanNP</td>
<td>67.26± 2.78</td>
<td>54.00</td>
</tr>
<tr>
<td>GemHCl-Chitosan-TGA NP</td>
<td>80.36± 7.35</td>
<td>51.00</td>
</tr>
<tr>
<td>GemHCl-Protosan NP</td>
<td>31.16± 3.40</td>
<td>56.00</td>
</tr>
</tbody>
</table>

Table 2. IC₅₀ and inhibition% values of GemHCl and GemHCl loaded nanoparticle formulations on RT4 cells.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC₅₀</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GemHCl</td>
<td>12.55± 0.62</td>
<td>88.89</td>
</tr>
<tr>
<td>GemHCl-ChitosanNP</td>
<td>28.07± 1.85</td>
<td>64.03</td>
</tr>
<tr>
<td>GemHCl-Chitosan-TGA NP</td>
<td>51.56± 3.45</td>
<td>51.96</td>
</tr>
<tr>
<td>GemHCl-Protosan NP</td>
<td>----</td>
<td>35.00</td>
</tr>
</tbody>
</table>

Table 3. IC₅₀ and inhibition% values of GemHCl and GemHCl loaded microsphere formulations on T24 and RT4 cells.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>IC₅₀</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T24 Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GemHCl</td>
<td>24.35± 1.05</td>
<td>76.81</td>
</tr>
<tr>
<td>GemHCl-Microsphere</td>
<td>15.88± 1.21</td>
<td>91.27</td>
</tr>
<tr>
<td>RT4 Cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GemHCl</td>
<td>12.55± 0.62</td>
<td>88.89</td>
</tr>
<tr>
<td>GemHCl-Microsphere</td>
<td>10.16± 1.10</td>
<td>96.30</td>
</tr>
</tbody>
</table>

CONCLUSION
GemHCl loaded nanoparticles and microspheres formulations prepared for intravesical administration were evaluated in two different bladder cancer cell lines to assess the cell-killing effect in this study. IC₅₀ value and % inhibition of aqueous GemHCl solution were different in two cells. GemHCl was more potent on RT4 cells than T24 cells in terms cytotoxicity (IC₅₀: 12.55± 0.62 and 24.35± 1.05, respectively). GemHCl loaded protosan nanoparticles exhibited the nearest cytotoxic effect to aqueous GemHCl compared with other formulations in human bladder carcinoma cells, T24. However, the gemcitabine HCl loaded chitosan nanoparticles had the nearest cytotoxic effect to aqueous GemHCl compared with other formulations in human bladder papilloma cells, RT4. Empty nanoparticles (without GemHCl) had no effect on cells.

It can be speculate that cytotoxic effect of GemHCl depends on cell type or nanoparticle formulation type. As the microsphere formulations, a comparison of cytotoxicity to human bladder transitional cancer cells (both T24 and RT4) indicated that the IC₅₀ values of microspheres showed more cytotoxicity than aqueous GemHCl solution. It means that microsphere formulations have high potency at the same dose compared with a commercial aqueous formulation.

ACKNOWLEDGMENTS
This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-112/S/293). The authors would like to acknowledge Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use its laboratory instruments.

REFERENCES
2. Dalbagni, G.; Russo, P.; Bochner B.; Ben-Porat, L.; Steinfield J.; Sogani, P.; Donat, M. S.; Herr H. W.; Bajorin D. Phase II trial of intravesical gemcitabine in Bacille Calmette-Guerin-Refractory transitional cell carcinoma of


**P-74: USE OF THE RYSHKEWITCH- DUCKWORTH EQUATION TO PREDICT THE TENSILE STRENGTH OF TABLETS FROM BINARY MIXTURES OF VARIOUS EXCIPIENTS AND MODEL APIS**

M. Lachmann, M. Weimer, Y. Ozalp, B. Tuncay, J. Khnast, M. Celik

1 Near East University, Faculty of Pharmacy, Pharmaceutical Technology, North Nicosia, North Cyprus
2 Research Center Pharmaceutical Engineering GmbH, Graz, Austria

The prediction of critical quality attributes of pharmaceutical formulations becomes ever more important. Tablet “hardness” is such a critical attribute and the presented work evaluates and analyses a predictive approach within a DOE framework.

**INTRODUCTION**

An approach to predict the true density of powder mixtures followed by the prediction of the tensile strength of compacts thereof has been published short of a decade ago [4]. In this study the approach is extended to a wider range of API / excipient mixtures.

**MATERIALS AND METHODS**

*Materials:* All materials were conditioned for at least one week at 22.5°C (+/- 2.5 °C) and 40 % (+/- 5 %) r.H. in a climate chamber (Binder; Germany). The following materials were used as provided by the manufacturers. Microcrystalline Celluloses (Avicel® PH 102; PH 105 and PH 200) was provided by FMC BioPolymer (USA); Alpha lactose monohydrate types (Granulac® 70, Granulac® 200 and SorboLac® 400) were provided by the Meggle Group (Germany). Calcium Hydrogen Phosphate Dihydrates (DI-CAFOS® D14, DI-CAFOS® D160) were from Chemische Fabrik Budenheim KG (Germany)

The APIs Naproxen, Phenylbutazone and Acetyl Salicylic Acid were obtained from Fagron GmbH & Co. KG (Germany). Calcium Hydrogen Phosphate with a median particle size of 87 μm was obtained by milling DI-CAFOS® D160 in a planetary ball mill (Retsch, Germany) followed by sieving off the fines fraction in a sieve shaker of the same company.

*Methods:* Particle size analysis was carried out on a QICPIC imaging system (Sympatec, Germany). Compaction of the powders was achieved using a Stylcomp 200 R compaction simulator (Medelpharm S.A.S, France) using 8 mm flat faced punches (Natoli Eng. Com. Inc, St. Charles, USA). Tablet diameters were analysed using a Digimatic Micrometer Series 293 (Mitutoyo Corp., Japan) and tablet height was measured using a dial gauge of the same company. The breaking force of tablets was measured using a PTB 311 E “Hardness” tester (Pharma Test AG, Germany).

True densities were measured with a helium pycnometer by Micromeritics (USA).

Tensile strengths of tablets were calculated by.

\[
\sigma_t = \left( \frac{2P}{\pi dh} \right)
\]

Equation: 1 [1]

where \(\sigma_t\) is the tensile strength of the compact, \(P\) is the breaking force of the tablet in Newton, \(d\) is the diameter of the compact and \(h\) its height respectively.

The tensile strength of tablets from mixtures was calculated using Equation 2 which is derived from the papers by Ryshkevitch and Duckworth [2, 3]

\[
\sigma_m = \sigma_{z1} \delta_1 + \sigma_{z2} \delta_2
\]

Equation: 2 [4]

where \(\sigma_{z1}\) and \(\sigma_{z2}\) are the tensile strengths of the materials at zero porosity, \(\delta_1\) and \(\delta_1\) are the volume fractions

The true density of powder mixtures was calculated using equation

\[
\frac{1}{\rho_m} = \frac{n_1}{\rho_1} + \frac{n_2}{\rho_2}
\]

Equation: 3 [4]

where \(\rho_{1,2}\) equal the density of the components and \(n_{1,2}\) the weight fractions

**RESULTS AND DISCUSSION**

Prediction of the combined true density of mixtures according to Equation 3 yields results with low deviation from the experimental values. The Ryshkevitch–Duckworth equation is a useful tool for predicting the tensile strength of tablets.

**CONCLUSIONS**

Equations to predict true density of mixtures and the tensile strength of tablets produced with these are a versatile tool for modern formulation efforts within a QbD framework.

**REFERENCES**


IN VITRO INCORPORATION STUDIES OF RADIOLABELED MUCOADHESIVE MICROSPHERES AT BLADDER CARCINOMA CELL LINES

Derya ılem Özdemir1, Sinem Y. Karavana2, Zeynep Ay Şenyiğit2, Meliha Ekinci2, Yalçın Erzurumlu2, Çağrı Çalışkan2, Makküle Akoğlu1, Esra Baloğlu2

1Ege University, Faculty of Pharmacy, Department of Radiopharmacy, 35100, Izmir, TURKEY
2Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100, Izmir, TURKEY
3Ege University, Faculty of Pharmacy, Department of Biochemistry, 35100, Izmir, TURKEY

INTRODUCTION

The role of gamma scintigraphy techniques is well established for designing new drugs, determining the biodistribution of promising pharmaceuticals. During the last 20 years, very important improvements have been achieved in drug development using radiopharmaceuticals as tracers [2]. Information about behavior of the pharmaceutical dosage forms can be obtained using radionuclides, in which a gamma ray emitting radionuclide is incorporated into the dosage form [3]. In the present study, mucoadhesive microspheres (MS), which were prepared with Gemcitabine hydrochloride (GHCl) for bladder cancer treatment, were radiolabeled and the incorporation studies performed in bladder carcinoma (T24) cell lines. For this purpose, MS were radiolabeled by 99mTc and the in vitro cell incorporation affinity to bladder carcinoma cell lines was investigated.

MATERIALS AND METHODS

Preparation of MS with GHCl

MS were prepared by solvent evaporation method (3). EE100 was dissolved in 8 mL acetone and Cp was added as a powder. 0.3 g of MS and 0.5 g GHCl in 4 mL acetone were prepared separately and added to the dispersion of polymers. The final dispersion was cooled to 5°C and poured slowly with stirring (750 rpm) into 80 mL of liquid paraffin, which was also cooled to 5°C. The obtained emulsion was stirred at 40°C for 50 min. The suspension of microspheres in liquid paraffin was filtered; microspheres were washed by 50 mL n-hexane five times and dried at room temperature. All samples of MS were sieved and the fraction 125 μm was used for further testing.

Radiolabeling Studies

99mTc labeled MS were prepared using 99mTc pertechnetate in the presence of a potent reducing agent stannous chloride. Quality controls of labeled compound were performed with radioactive thin layer chromatography (RTLC). In Vitro Stability

To test the stability of labeled formulation in cell medium at 37°C, 0.1 mCi radiolabeled complex was added to cell medium. The mixture was incubated at 37°C and radiochemical purity studies were performed up to 2 hours. For RTLC studies 5 μL sample was spotted on chromatographic papers and quality control studies were performed at 30 and 240 minutes after incubation.

Cell Culture Studies

The T24 cells were cultured in McCoy’s 5A supplemented with 10% fetal bovine serum. Cell monolayers were prepared by seeding cells on six wells. Cell culture was maintained at 37°C under 90 % humidity and 5 % CO2.

RESULTS AND DISCUSSION

Radiolabeling studies

The radiochemical purity of 99mTc-MM was found over 95% at room temperature immediately after incubation time.

In Vitro Stability

During incubation in cell medium, compound was stable as determined by RTLC. 99mTc-MM was not decreased from significantly within 2 hours.

Cell Culture Studies

In this study, the incorporation capacity of 99mTc-MS on bladder carcinoma cells has been observed. Incorporation percentage of 99mTc-MS and R/H 99mTc to T24 cell lines was increase significantly by the time. On the other hand, 99mTc-MS incorporation percentage was found 4.11 times higher than R/H 99mTc.

Evaluation of the results revealed that, 99mTc-MS has a good incorporation activity on T24 cell lines (around 35%).

Conclusion

In conclusion, 99mTc-MS was obtained in high radiochemical purity. 99mTc-MS is incorporated into the T24 cells. Consequently 99mTc-MS can be a candidate for bladder cancer diagnosis, offering potential for developing new selective therapies for this cancer.

ACKNOWLEDGMENTS

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-112/S/293). The authors would like to
acknowledge Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use its laboratory instruments. Also the authors thank to Ege University Nuclear Medicine Department to obtain the $^{99m}$Tc radionuclide.

REFERENCES
4. Burjak, M.; Bogatay, M.; Velnar, M.; Grabnar, I.; Mrhar, A. The study of drug release from microspheres adhered on pig vesical mucosa, Int. J. Pharm., 224, 123

P-76: RADIOLABELLED BIOADHESIVE MICROSPHERES LOADED CHITOSAN GEL FORMULATIONS INCORPORATION TO THE BLADDER PAPILLOMA AND CARCINOMA CELL LINES

D. İlem Özdemir1, S. Y. Karavana2, Z. Ay Şenyiğit2, M. Ekinçii, Y. Erzurumlu3, Ç. Çalışkan2, M. Aşköglü1, E. Baloğlu2

1 Ege University, Faculty of Pharmacy, Department of Radiopharmacy, 35100, Izmir, TURKEY
2Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100, Izmir, TURKEY
3Ege University, Faculty of Pharmacy, Department of Biochemistry, 35100, Izmir, TURKEY

INTRODUCTION
Gamma scintigraphy is one of the most popular methods to investigate the gastrointestinal performance of pharmaceutical dosage forms. This non-invasive technique gives information about integrity, dispersion or release characteristics of the radiolabeled delivery system. Choice of a suitable radionuclide for scintigraphic studies can be ascertained by considering factors such as the radiation energy, half-life, extent of particulate radiation, cost and availability. Technetium-99m is the most popular radionuclide with its versatile chemistry, near-ideal energy (140 keV), low radiation dose and short half-life (6 h) [1,2].

In the present study, bioadhesive microspheres (MS) were prepared in the absence and presence of the Gemcitabine hydrochloride (GHCI-MS). The MS and GHCI-MS were radiolabeled and the incorporation studies performed in bladder papilloma (RT4) and the carcinoma (T24) cell lines. For this purpose, MS and GHCI-MS were radiolabeled by $^{99m}$Tc and loaded to the chitosan gel (CG). The in vitro cell incorporation affinity to bladder papilloma and the carcinoma cell lines was investigated.

MATERIALS AND METHODS
Preparation of MS with GHCl-MS were prepared with CP and EE 100 polymers by solvent evaporation method according to our previous study (3). All MS samples were sieved and the fraction 125 μm was used for further testing. Prepared MS and GHCl-MS were added to the CG formulations (8mg/2g) (CGMS, CGGHCl-MS) which was prepared with 2% of high molecular weight CHI.

Radiolabeling Studies
Radiolabeled MS and GHCl-MS were prepared using $^{99m}$Tc pertechnetate. Radioactive thin layer chromatography (RTLC) studies were used for quality controls of labeled compound.

Cell Culture Studies
Cell monolayers were prepared by seeding cells on six wells. The RT4 and T24 cells were cultured in McCoy’s 5A supplemented with 10 % fetal bovine serum. Cell culture was maintained at 37°C under 90 % humidity and 5 % CO₂.

RESULTS AND DISCUSSION
Radiolabeling studies

The radiochemical purity of $^{99m}$Tc labeled MS and GHCl-MS was found over 95% at room temperature immediately after incubation time.

Cell Culture Studies
Incorporation percentage of formulations to RT4 and T24 cell lines for 30 and 240 minutes incubation periods are shown in Figure 1 and 2. Results revealed that CGGHCl-MS have a greater incorporation activity than both CGMS and CGGHCl-MS formulations for 30 and 240 minutes at 37°C. After incubation period the activities of cells and culture medium were counted by a gamma counter (Uniscaller). The cellular uptake was calculated as the percentage of the activity counted in the cells relative to the total activity.

Also a comparative incorporation study was designed to see the reduced/hydrolyzed Technetium loaded CG formulations (CGGHCl-MS) affinity to the cell lines. For this purpose the cells were incubated with 0.1 mCi CGGHCl-MS for 30 and 240 minutes at 37°C and the incorporation percentage was calculated as describe above.

RESULTS AND DISCUSSION
Radiolabeling studies

The radiochemical purity of $^{99m}$Tc labeled MS and GHCl-MS was found over 95% at room temperature immediately after incubation time.
formulation incorporation to the T24 cell lines was found 1.88 and 39.39 times higher than CG MS and CGRHTc-99m formulations respectively. In addition, CGGHCl-MS incorporation to the RT4 cell line was found 8.03 and 9.01 times higher than CGMS and CGR/HTc-99m respectively. Consequently, CGGHCl-MS formulation could be candidate for treatment of bladder cancer.

Figure 1: Incorporation percentage of formulations to T24 cell line.

Figure 2: Incorporation percentage of formulations to RT4 cell line.

ACKNOWLEDGMENTS
This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK-112/S/293). The authors would like to acknowledge Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use its laboratory instruments. Also the authors thank to Ege University Nuclear Medicine Department to obtain the 99mTc radionuclide.

REFERENCES

17th International Pharmaceutical Technology Symposium IPTS September 8-10 2014 Antalya Turkey.

P-77: ABSORPTION ENHANCING EFFECT OF TOTAL SAPONINS DERIVED FROM ACANTHOPYLLUMSQUARRUSOMAND QUILLAJASAPONARIAON NASAL PERMEATION OF GENTAMICIN SULFATE AND CARBOXYFLUORESCINE

E. Moghimipour1,2, S. A. Sajadi Tabassii, M. Ramezani3, R. Löbenberg4, S. Handali2

1Cellular and Molecular Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran
2Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
4Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada

INTRODUCTION
Several investigators have been focused on nasal route as a desirable drugs absorption site due to lack of first metabolism effect and less enzymatic activity in comparison to gastrointestinal tract. Many researchers are attempted to increase absorption of drugs through nasal mucosa [1]. The co-administration of absorption enhancing agents is one of the approaches to improve the uptake of poorly absorbable drugs [2, 3]. The objective of the study was to evaluate the influence of total saponin of Acanthopyllumsquarrusom (ATS) and Quillajasaponaria (QTS) as nasal permeation enhancers on the uptake of 5(6)-carboxyfluorescein (CF) and gentamicin sulfate (GS) as drug modelsincised nasal septum of rabbit.

MATERIALS AND METHODS
The roots of A. squarrusom were ground into powder, defatted in a soxhelet apparatus with petroleum ether (boiling range of 40-60 °C) for removing lipids and phenolic compounds. The air-dried powder was extracted with methanol for 48 h. Sodium lauryl sulfate (SLS) and benzalkonium chloride (BC) were utilized to compare their enhancing effect. Nasal mucosa was excised from the nasal septum of rabbits and mounted on a specially designed Franz-type diffusion cell. An aliquot of 100 μl of sample was withdrawn from the receptor compartment at 5, 10, 15, 20, 25 and 30 min and replaced with the same volume of phosphate buffer saline (PBS) to keep the volume constant. Concentration of GS and CF was determined using agar diffusion and spectrofluorimetry, respectively.
RESULTS AND DISCUSSION
The absorption enhancers increased the mucosal permeability of CF and GS across the nasal membrane. The results suggested that the saponins can potentially be used to increase the permeability of hydrophilic compounds through nasal epithelial membrane (Fig.1 and Fig.2). After 30 min, permeation of GS through nasal mucosa for control, ATS, QTS, SLS and BC was 3.25±0.03%, 19.63±0.02%, 22.88±0.06%, 26.00±0.03% and 25.97±0.03%, respectively. The cumulative permeated amount of CF for control, QTS, ATS, SLS and BC containing solutions after 30 min was 2.16±0.36%, 34±2.93%, 35.35±0.54%, 34.17±0.18% and 32.00±1.21%, respectively. By use of a suitable absorption enhancer, in the near future it is expected to see a range of novel nasal products reaching the market.

CONCLUSIONS
According to the results, co-administration of saponins and synthetic surfactants led to absorption enhancement of the hydrophilic drug models through nasal mucosa. By means of these ingredients, CF and GS may cross the epithelial cell membrane either by transcellular route or by paracellular route.

ACKNOWLEDGMENTS
The project was financially supported by Mashhad University of Medical Sciences, Mashhad, Iran.

REFERENCES

P-78: PREPARATION OF PLGA NANO PARTICLES CONTAINING NIFEDIPINE USING EMULSION-DIFFUSION METHOD

E. Özakar¹, M. Çetin¹, O. Ateş¹, A. Hacımuftuoğlu³
Ataturk University,
Faculty of Pharmacy, ¹Department of Pharmaceutical Technology,
Faculty of Medicine, ²Department of Ophthalmology,
³Department of Pharmacology
Erzurum, TURKEY

INTRODUCTION
Nifedipine (NF), a systemic calcium channel blocker, is a poorly water-soluble and photosensitive drug and also has low bioavailability due to first pass effect from liver [1, 2]. Conventional NF formulations have to be administered several times in a day. Thus, the sustained release dosage forms for NF have been developed [3]. The aim of this study was to prepare and characterize NF-loaded PLGA nanoparticles (NPs) using emulsion-diffusion method.

MATERIALS AND METHODS

Materials
NF and PLGA (75:25) (RESOMER® RG 756 S, Ave. Mw 76,000-115,000) were purchased from Sigma-Aldrich (USA). All other chemicals and reagents were of analytical grade and used as they were received.

Preparation of NPs
50mg PLGA and NF was dissolved in ethylacetate (EA). The organic phase was introduced drop by drop into the mixture of Pluronic® F-68 aqueous solution (2% w/v):EA (1:1:v/v) and emulsified using a homogenizer with 15000 rpm (5 min.)(Omni TH-02, Omni International, USA). Then, 45 mL of distilled water was added into this mixture and mixed for 30 minutes. The evaporation of EA was carried out under the reduced pressure in a rotary evaporator at 45°C. After centrifugation, NPs were re-suspended and then lyophilized for 24 h.

Surface Morphology of NPs
The surface morphology of NPs was examined by scanning electron microscope (SEM)(Inspect S50,
FEI, USA). The particle size of NPs obtained from SEM images.

**Drug content of NPs**

10 mg lyophilized NPs were added in DMSO and mixed on a magnetic-stirrer at 600 rpm for 30 minutes. After mixing, it was placed in ultrasonic bath for 10 minutes. Then, phosphate buffer (PB; pH 7.4) was added into this mixture and mixed for 10 minutes. This dispersion was centrifuged at 12000 rpm for 10 minutes. The drug content of each sample was then measured using a validated UV method at 238 nm.

**In vitro Release Studies**

NPs(10 mg) were suspended in 20 mL pH 7.4 PB in amber vials and release study was performed using horizontally shaking water bath (37±0.5 °C; 50 rpm). At predetermined time points, 3 mL of sample was withdrawn from the medium and 3 mL of fresh buffer was added into the medium. The samples were centrifuged at 12500 rpm for 10 minutes and their drug content was measured using the validated UV method at 238 nm.

**RESULTS AND DISCUSSION**

**Surface Morphology**

The images of NPs were observed using SEM (Fig.1). The particle size of NPs was 325±39nm.

**Drug content of NPs**

The NPs showed the encapsulation efficiency and drug loading values of 17.50±1.70% and 3.61±0.54%, respectively (mean±SD, n=3).

**In vitro Release Studies**

About 25% and ~90% of the loaded NF was released within 24 hours and 19 days, respectively in PB pH 7.4 (Fig.2).

---

**ACKNOWLEDGMENTS**

This study was supported by Ataturk University Research Foundation (project number: 2013/012).

**REFERENCES**


**P-79: IN VITRO EVALUATION OF PACLITAXEL INCORPORATED POLYMERIC NANOPARTICLES**

Ö. Atasoy1, E. Başaran2

1Sandoz Generic Pharmaceuticals Division of Novartis, Kocaeli, TURKEY
2Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, TURKEY

Paclitaxel (PTX) was successfully incorporated into Kollidon® SR based nanoparticles in order to enhance its oral bioavailability for the treatment of colorectal cancers.
INTRODUCTION
Paclitaxel (PTX) has been clinically used in the treatment of various cancer types especially breast and ovarian cancers due to its unique mechanism of action. However, bioavailability problems were reported during treatment [1, 2]. Therefore in this study Kollidon® SR based polymeric nanoparticles were formulated in order to enhance oral bioavailability of PTX for the treatment of colorectal cancers.

Materials and Methods
Formulation: Spray drying method (Büchi B-190, BüCHI Labotechnic AG, Switzerland) was used for the preparation of Kollidon® SR (BASF, Germany) based polymeric nanoparticles [3]. PTX (kindly gifted by Actavis, Istanbul) was used in different concentrations and selected compositions were given in Table 1.

Table 1. Compositions of the Formulations Prepared

<table>
<thead>
<tr>
<th>Code</th>
<th>KSR (g)</th>
<th>PTX (g)</th>
<th>Methanol (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPTX-PL</td>
<td>2</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>KPTX-05</td>
<td>2</td>
<td>0.1</td>
<td>150</td>
</tr>
<tr>
<td>KPTX-10</td>
<td>2</td>
<td>0.2</td>
<td>150</td>
</tr>
</tbody>
</table>

KSR: Kollidon® SR, PTX: Paclitaxel

Characterization: SEM, particle size, polydispersity index, zeta potential analyses were performed. In order to evaluate changes of the polymeric structure DSC, XRD, FT-IR and ¹³C NMR analyses were also performed. A validated HPLC method was used for the determination of incorporated PTX.

RESULTS AND DISCUSSION
Morphological analyses showed that particles are round in shape with small particle sizes (Figure 1). Particle size, polydispersity index, zeta potential analyses with incorporated PTX amount of the formulations were given in Table 2. Analyses results revealed that the particle sizes were in the nanometer range with homogenous size distribution (Table 2) with no changes in the polymeric structure (Fig. 2).

Table 2. Particle Size, Polydispersity Index, Zeta Potential Analyses with Incorporated PTX Amount of the Formulations Prepared (n=3, mean ±SE)

<table>
<thead>
<tr>
<th>Code</th>
<th>PS (nm)</th>
<th>PDI</th>
<th>Zeta (mV)</th>
<th>PTX Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPTX-PL</td>
<td>207.5±3.8</td>
<td>0.5±0.0</td>
<td>-</td>
<td>16.9±0.8</td>
</tr>
<tr>
<td>KPTX-05</td>
<td>240.2±22.9</td>
<td>0.5±0.0</td>
<td>-</td>
<td>19.5±1.5</td>
</tr>
<tr>
<td>KPTX-10</td>
<td>268.7±3.9</td>
<td>0.6±0.0</td>
<td>-</td>
<td>20.2±2.8</td>
</tr>
</tbody>
</table>

PS: Particle Size, PDI: Polydispersity Index

CONCLUSIONS
As a conclusion PTX incorporated polymeric nanoparticles were successfully formulated for the treatment of colorectal cancers.

ACKNOWLEDGMENTS
This study was financed by Anadolu University Scientific Research Project Foundation (No: 1403S065).

REFERENCES

P-80: DEVELOPMENT OF RADIOLABELING ALENDRONATE SODIUM MICROEMULSION WITH TECHNETIUM-99M

Yetkin Elitez, Meliha Ekinci, Derya Ilem-Ozdemir, Evren Gundogdu1, Makbule Asikoglu

1Faculty of Pharmacy, Department of Radiopharmacy, Ege University, Bornova, Izmir, Turkey
INTRODUCTION
Alendronate (ALD) is a bisphosphonate. It is a specific inhibitor of osteoclast mediated bone resorption (1). Microemulsions are mixtures of oils, surfactants and cosurfactants. Several studies have shown that compounds which have low permeability are better absorbed when administrated in microemulsion (2,3). 99mTc is radionuclide of choice by virtue of convenient half-life, ideal energy for imaging; short enough to minimize absorbed radiation dose (4). The aim of the present study was to develop a water/oil (w/o) microemulsion formulation of 99mTc ALD and to investigate the radiochemical purity of labeled ALD from microemulsion.

MATERIALS AND METHODS
ALD was obtained as a gift from Arylsa Company. All the other reagents and chemicals were reagents grade. 99mTc was eluted from Molybdenum-99 99mTc generator (Nuclear Medicine Department of Ege University).

Radiolabeling Studies
ALD was directly labeled by 99mTc with small modification on previously described (5). ALD was dissolved in saline. Reducing agent (stannous chloride) and antioxidant agent (ascorbic acid) were added to the solution. Radiolabeling was performed with freshly eluted 37 mBq 99mTc. The radiochemical purity was analyzed by RTLC.

Preparation of 99mTc-ALD Microemulsion
The pseudo-ternary phase diagram was constructed by titration of homogenous liquid mixtures of oil, surfactant (S) and cosurfactant (CoS) with water phase at room temperature. Surfactants were blended with oil phase. CoS was added in mixing ratios of 1:0.5, 1:1, 2:1, 3:1, 4:1 and 5:1 (S:CoS). The composition of microemulsion was shown in Table 1. The droplet size of microemulsion was measured by Zeta Sizer 3000. After the identification of microemulsion area in the phase diagrams, the microemulsion formulation was selected at desired component ratios. 99mTc-ALD was added into microemulsion and radiochemical purity of 99mTc-ALD microemulsion was analyzed by RTLC.

Table 1. Composition of ideal w/o microemulsion (% w/w)

<table>
<thead>
<tr>
<th>Composition of microemulsion</th>
<th>Soybean oil</th>
<th>Tween 80/Coliphror EL/Transcutol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>% amount of composition</td>
<td>7.75</td>
<td>60</td>
<td>32.75</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
The largest microemulsion areas were obtained with 1:1 ratio of S:CoS for microemulsion. The microemulsion existence areas with different surfactants were shown in Fig. 1. The droplet size of formulation was found 39.04±0.8.

CONCLUSIONS
The novel microemulsion system and simple method for radiolabeling of ALD microemulsion with 99mTc has been developed. Labeling efficiency of 99mTc-ALD microemulsion was assessed by RTLC. The complex was quite stable and labeling yield >90% was maintained for up to 6 hours.

REFERENCES
Rosuvastatin calcium (RCa) was successfully incorporated into Tripalmitin based solid lipid nanoparticles (SLNs) in order to enhance oral bioavailability of RCa.

**INTRODUCTION**

Rosuvastatin calcium (RCa) which is the member of the most effective cholesterol lowering drugs; Statins, is relatively high potent statin with good clinical efficacy with a long elimination half-life (approx. 20 hrs) [1, 2] however it has a low bioavailability due to low aqueous solubility because of its crystalline structure [3], therefore, RCa was incorporated into Tripalmitin (TP) based solid lipid nanoparticles (SLNs) in order to enhance its bioavailability in the treatment of dyslipidemia.

**MATERIALS AND METHODS**

**Formulation**: SLNs were prepared according to the hot homogenization method using high shear stirrers at 9500 rpm (Table 1) [4].

<table>
<thead>
<tr>
<th>Code</th>
<th>TP (%)</th>
<th>RCa (%)</th>
<th>Tween (%)</th>
<th>PC (%)</th>
<th>Water (q.s. mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTF3-PL</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>LTF3</td>
<td>4</td>
<td>0.3</td>
<td>2</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>LTF4-PL</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>LTF4</td>
<td>4</td>
<td>0.2</td>
<td>1</td>
<td>1</td>
<td>50</td>
</tr>
</tbody>
</table>

**Characterization**: Particle size, polydispersity index, zeta potential analyses were performed. In order to evaluate structural changes of the lipid DSC, XRD, FT-IR and NMR analyses results were used. A validated HPLC method was used for the determination of incorporated RCa. Release characteristics of the particles prepared were evaluated at 37°C±1°C in phosphate buffer solution (pH 6.8) during 24 hrs.

**RESULTS AND DISCUSSION**

Primary characterization analyses (particle size, polydispersity index, zeta potential analyses) with RCa amount of the formulations were given in Table 2.

Analyses results revealed that the particle sizes were in the nanometer range with homogenous size distribution. RCa was successfully incorporated into SLNs and encapsulation of RCa did not effect the the anionic character of the particles (Table 2). RCa was detected in the release medium up to 24 hrs showing incorporation of RCa into SLN has extended the RCa release as expected (Figure 1).

![Fig. 1. Release profiles of RCa from SLNs Prepared versus Pure RCa](image)

**CONCLUSIONS**

As a conclusion RCa incorporated SLNs were successfully formulated for the treatment of dyslipidemia.

**ACKNOWLEDGMENTS**

This study was financed by Anadolu University Scientific Research Project Foundation (No: 1404S289).

**REFERENCES**


**P-82: MODIFIED CHITOSAN NANOPARTICLES FOR ENHANCED VACCINE DELIVERY BY NASAL ROUTE**

G. Sinani1,2 M.K. Goker3, S. Ozgumus3, H.O. Alpar2,4 and E. Cevher2

1Faculty of Pharmacy, Istanbul University, Istanbul, 34116, Turkey; 2Faculty of Pharmacy, Istanbul Kemerburgaz University, Istanbul, 34217, Turkey; 3Faculty of Engineering, Istanbul University, Istanbul, 34320, Turkey; 4School of Pharmacy, University of London, London, WC1N 1AX, UK

**INTRODUCTION**

Nanotechnology offers the opportunity to design particulate delivery systems varying in composition, size, shape and surface properties for vaccine development [1]. In particular, biodegradable and biocompatible chitosan nanoparticles (NPs) have shown mucosal adjuvant activity [2]. In this study, new chitosan derivative NPs have been prepared and evaluated as nanocarrier system for nasal immunisation.

**MATERIALS AND METHODS**

**Preparation of nanoparticles**

Aminated derivative of chitosan (CSA) as a polymer was synthesised in house and NPs were prepared by ionotropic gelation of CSA with tripolyphosphate (TPP).

**Characterisation of nanoparticles**

The particle size and size distribution was determined by photon correlation spectroscopy and zeta potential was measured by Zetasizer Nano-ZS (Malvern Instruments, UK). The percentage yields of chitosan NPs were calculated after centrifugation at 15,000 rpm and following freeze-drying at -70°C. The morphology was examined by scanning electron microscopy. The entrapment efficiency of BSA in NPs was determined by Bradford assay (Sigma-Aldrich, Germany) and SDS-PAGE integrity of the encapsulated BSA. CSA particles containing BSA were compared with free BSA dispersions with the same dose of BSA administered as CSA particles. Serum BSA specific IgG titres for CSA particles at 13., 21. and 60. day were significantly different (p<0.05) than the equivalent dose of BSA solution.

**RESULTS AND DISCUSSION**

NPs with appropriate particle size, polydispersity and zeta potential for nasal delivery of vaccines were obtained. The CSA NPs showed good reproducibility and good stability at +4±1°C for 12 months (Table 1).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>CSA/ TPP (m/m)</th>
<th>Z-Average diameter nm±SD</th>
<th>Zeta potential mV±SD</th>
<th>Z-Average diameter nm±SD</th>
<th>Zeta potential mV±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1/1</td>
<td>aggregation</td>
<td>aggregation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>2/1</td>
<td>aggregation</td>
<td>aggregation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>3/1</td>
<td>125.2±5.5</td>
<td>31.4±4.5</td>
<td>144.8±0.7</td>
<td>33.7±4.6</td>
</tr>
<tr>
<td>F4</td>
<td>4/1</td>
<td>139.5±1.1</td>
<td>41.2±5.4</td>
<td>140.5±0.3</td>
<td>39.6±4.1</td>
</tr>
<tr>
<td>F5</td>
<td>5/1</td>
<td>146.7±4.0</td>
<td>42.4±5.4</td>
<td>127.1±6.6</td>
<td>40.4±5.4</td>
</tr>
<tr>
<td>F6</td>
<td>6/1</td>
<td>161.3±6.2</td>
<td>44.2±5.7</td>
<td>147.3±9.9</td>
<td>42.9±5.6</td>
</tr>
<tr>
<td>F7</td>
<td>7/1</td>
<td>177.3±10.2</td>
<td>47.8±7.2</td>
<td>168.3±4.2</td>
<td>46.5±4.9</td>
</tr>
<tr>
<td>F8</td>
<td>8/1</td>
<td>184.6±11.7</td>
<td>46.9±4.6</td>
<td>154.9±3.7</td>
<td>43.4±4.9</td>
</tr>
</tbody>
</table>

Formulation 3 was selected for the in vivo delivery of vaccines on the basis of high encapsulation efficiency (78.38%±5.73), small particle size, positive zeta potential and high yield (60.54%±9.82). These particles were spherical in shape, showed good cell viability up to concentrations as high as 2mg.ml⁻¹ in Calu-3 cell line and preparation process did not affect the SDS-PAGE integrity of the encapsulated BSA. CSA particles containing BSA were compared with free BSA dispersions with the same dose of BSA administered as CSA particles. Serum BSA specific IgG titres for CSA particles at 13., 21. and 60. day were significantly different (p<0.05) than the equivalent dose of BSA solution.

**CONCLUSIONS**

Modified chitosan NPs with suitable size, surface charge and stability for enhanced nasal vaccine delivery were successfully prepared and can be promising carrier systems for i.n. administration of vaccines.

**REFERENCES**

P-83: EVALUATION AND DEVELOPMENT OF CIPROFLOXACIN-LOADED NANOPARTICLES FOR ADMINISTRATION BY PULMONARY ROUTE

G. R. Çamca¹, B. Devrim¹, A. Bozkır¹
Ankara University, Faculty of Pharmacy, ¹Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION

Compared to pulmonary administration and the others, pulmonary route has many advantages such as large surface area for absorption, thin alveolar epithelium to allow speed absorption, the lack of first pass elimination, rapid start of effect, high bioavailability, fixing effect of active substance which is applicated local, reduction of unwanted systemic side-effects. In the treatment of various lung diseases directly to the lungs by inhalation of therapeutic benefits of supporting the findings are increasing from day to day. In this study we aimed to develop dry powder inhaler which contains ciprofloxacin-loaded nanoparticulate system. Multiple emulsion method was used during production of nanoparticles. The nanoparticles that we produced were used making composite microparticles. Partical size measurement, zeta potential analyse and encapsulation efficiency controlled on nanoparticles that we made.

MATERIALS AND METHODS

Materials:
Ciprofloxacin hydrochloride was kindly gift from Zentiva (Turkey). Aceton (AC), ethyl acetate (EA) and PEG 4000 were purchased from Merck (Germany). Dichloromethane (DCM), PCL (poly-caprolactone), PLGA 50/50 (MA 40000-75000) and PLGA 50/50 (MA 24000-38000) and Poly(vinyl alcohol) (PVA) (88 mol% hydrolysed, Mw=30 000±70 000) were obtained from Sigma (Germany). All the other chemicals used were of analytical grade.

Methods:
Nanoparticles were prepared by using water-in-oil-in-water (w₁/o/w₂) emulsion methods. Physicochemical properties of nanoparticles characterized in terms of the particle size distribution, zeta potential, entrapment efficiency, and in vitro release were evaluated.

RESULTS AND DISCUSSION

The particle size, zeta potential value and encapsulation efficiency results of nanoparticles are shown in Table 1.

<table>
<thead>
<tr>
<th>Code</th>
<th>Particle size (nm)</th>
<th>PI*</th>
<th>Zeta potential</th>
<th>Encapsulation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>200.98±1.4</td>
<td>5</td>
<td>0.13±0.02</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>228.77±2.8</td>
<td>1</td>
<td>0.17±0.04</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>202.10±1.5</td>
<td>5</td>
<td>0.09±0.06</td>
<td>-</td>
</tr>
<tr>
<td>F4</td>
<td>143.92±0.7</td>
<td>1</td>
<td>0.08±0.02</td>
<td>-</td>
</tr>
<tr>
<td>F5</td>
<td>195.65±45.2</td>
<td>33</td>
<td>0.18±0.01</td>
<td>-</td>
</tr>
<tr>
<td>F6</td>
<td>269.50±2.0</td>
<td>2</td>
<td>0.21±0.01</td>
<td>-</td>
</tr>
<tr>
<td>F7</td>
<td>212.47±1.6</td>
<td>9</td>
<td>0.09±0.01</td>
<td>-</td>
</tr>
<tr>
<td>F8</td>
<td>223.42±1.2</td>
<td>1</td>
<td>0.09±0.02</td>
<td>-</td>
</tr>
<tr>
<td>F9</td>
<td>220.82±2.1</td>
<td>5</td>
<td>0.12±0.02</td>
<td>-</td>
</tr>
<tr>
<td>F10</td>
<td>214.18±1.3</td>
<td>8</td>
<td>0.13±0.03</td>
<td>-</td>
</tr>
<tr>
<td>F11</td>
<td>205.75±2.2</td>
<td>7</td>
<td>0.07±0.01</td>
<td>-</td>
</tr>
<tr>
<td>F12</td>
<td>205.85±1.3</td>
<td>6</td>
<td>0.13±0.03</td>
<td>-</td>
</tr>
</tbody>
</table>

PI:Polydispersity index

CONCLUSIONS

The effects of type of the organic solvent, polymer type and ratios, quantity of active substance and excipients added to organic phase used to modify on particle size, encapsulation efficiencies, production yield, zeta potentials and drug release profiles were investigated. Our results showed that nanoparticles with appropriate properties for inhalation were prepared which include ciprofloxacin hydrochloride.

REFERENCES

P-84: PREPARATION AND CHARACTERISATION OF PROTEIN-LOADED POLYMERIC FILMS USING CASTING/SOLVENT EVAPORATION METHOD

G. Ozcelikay, B. Arica Yegin
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100 Shhije-Ankara, TURKEY

INTRODUCTION
Biodegradable film formulations incorporating model protein (BSA) were formulated by using different types of polymers such as PLGA and PCL. Biodegradable film formulations were prepared by casting/solvent evaporation technique. The purpose of the research was to evaluate the vitro characteristics such as film thickness, surface morphology and in vitro release profiles of the PLGA and PCL film formulations.

MATERIALS AND METHODS
PCL (Mw 65kDa, Sigma-Aldrich) and PLGA (75:25, Sigma-Aldrich) were dissolved in chloroform. The solution was cast into petri dishes and the organic solvent was evaporated. The films were removed and vacuum dried for a further 48 hours. Circular discs of 1cm in diameter were cut and washed in deionised water. The thickness of the film formulations were measured by using a Somet-Inox micrometer. For in vitro protein release, protein-loaded films were suspended in 1 ml phosphate buffer (pH 7.4). Release studies were maintained in a shaking water bath (37±0.5°C; 80 rpm). Amount of released protein was measured by micro BSA assay. The morphological properties of the PLGA and PCL film formulations were investigated by using Quanta 400F Field Emission SEM device, USA.

RESULTS AND DISCUSSION
The results were expressed in terms of mean ± standard deviation. The thickness of film formulations were 210±12.31 μm and 271±20.16 μm for PCL and PLGA film formulations, respectively. There was no significant difference found in thickness between the formulations with respect to their polymer types (p<0.05). The Scanning Electron Microscopy photographs clearly showed that no crystal like structures exist on the surface of the PCL and PLGA film formulations. In vitro release profiles were used to evaluate the effect of amount and type of polymers on release of BSA from film formulations. The total amount of BSA released was found as approximately 85% in 24 hours.

CONCLUSION
The preparation by solvent casting method is a very easy and simple method to obtain homogenous polymeric films without any protein precipitation. Similar protein release profiles were observed for all type of formulations.

REFERENCES

ACKNOWLEDGEMENT
This project was supported by a grant from The Scientific and Technological Research Council of Turkey (TÜBİTAK), (Project Number: 112S541)

P-85: ZETASIZER MEASUREMENTS OF POLY(MALEIC ANYDRATE-CO-VINYL ACETATE)-HYDROXYUREA POLYMER-DRUG SYSTEM IN SIMULATED BODY FLUIDS

N.M. CETIN1, D. SAKAR1, G. KARAKUS2
1Yildiz Technical University, Faculty of Arts and Sciences, Department of Chemistry, 34220 Esenler, Istanbul, TURKEY
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cumhuriyet University, 58140 Sivas, TURKEY

INTRODUCTION
Polymers have played an integral role in the advancement of drug delivery technology by providing controlled release of therapeutic agents in constant doses over long periods, cyclic dosage, and tunable release of both hydrophilic and hydrophobic drugs. Modern advances in drug delivery are now predicated upon the rational design of polymers tailored for specific cargo and engineered to exert distinct biological functions. The latest developments in polymers capable of molecular recognition or directing intracellular delivery are surveyed to illustrate areas of research advancing the frontiers of drug delivery [1]. This study involves highly reactive anhydride containing poly (maleic anhydride-co-vinyl acetate) (MAVA) copolymer as the drug carrier role for hydroxyure [2].

MATERIALS AND METHODS
Bioactive polymeric drug delivery system was synthesized by the ring opening reaction of poly (maleic anhydride-co-vinyl acetate) (MAVA) copolymer and hydroxyurea (HX) which is an anti-
cancer ("antineoplastic" or "cytotoxic") chemotherapy drug, labeled as MAVA-HX [2]. It was shown for the first time that Zeta sizer measurements such as zeta potential, mobility and particle size of MAVA-HX in different simulated body fluids (SBF) such as phosphate buffer saline (PBS) and dekstroz solutions were examined by Zeta Potential Analyzer. The stability and activity of MAVA-HX in SBFs were checked via UV Spectrophotometer.

RESULTS AND DISCUSSION
Zeta sizer measurements such as particle size (nm), polydispersity, mobility, Conductivity (µS) and also Zeta Potential (mV), as Smoluchowski and Huckel, was summarized as a function of time (h) depending in Table 1 and Table 2, respectively.

Table 1. Zeta sizer measurements of MAVA-HX conjugate in PBS solution

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Particle Size (nm)</th>
<th>Polydispersity</th>
<th>Mobility (µS)</th>
<th>Conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>734.1</td>
<td>0.396</td>
<td>-1.44</td>
<td>28100</td>
</tr>
<tr>
<td>0.5</td>
<td>845.4</td>
<td>0.449</td>
<td>-1.54</td>
<td>27733</td>
</tr>
<tr>
<td>1</td>
<td>1053.8</td>
<td>0.358</td>
<td>-0.9</td>
<td>27256</td>
</tr>
<tr>
<td>2</td>
<td>1182.3</td>
<td>0.389</td>
<td>-1.66</td>
<td>27656</td>
</tr>
<tr>
<td>4</td>
<td>639.1</td>
<td>0.347</td>
<td>-0.47</td>
<td>27512</td>
</tr>
<tr>
<td>6</td>
<td>656.4</td>
<td>0.411</td>
<td>-1.16</td>
<td>28182</td>
</tr>
<tr>
<td>24</td>
<td>262.8</td>
<td>0.376</td>
<td>-1.67</td>
<td>26927</td>
</tr>
</tbody>
</table>

Table 2. Zeta Potential of MAVA-HX conjugate in PBS solution

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Zeta Potential (mV)</th>
<th>Smoluchowski</th>
<th>Huckel</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-13.86</td>
<td>-27.56</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>-19.70</td>
<td>-29.55</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-11.49</td>
<td>-17.23</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-21.27</td>
<td>-31.91</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-5.98</td>
<td>-8.97</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-14.90</td>
<td>-22.36</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>-21.32</td>
<td>-31.98</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS
All of the Zeta sizer measurements showed that MAVA/HX polymeric conjugate was negatively charged and stable in all studied simulated medias.

ACKNOWLEDGMENTS
This work was supported by Scientific Research Project Coordination Center of Yildiz Technical University, Turkey (Project No: 2014-01-02-YL09).

REFERENCES
achieving uniform mixture, MgSt and talk were added and mixed for 5 minutes. ODTs were prepared with 10 mm single punch using hydraulic press at a pressure of 20 bars for 10 seconds.

**In vitro disintegration time**

900ml of medium was maintained at 37±1°C and Six ODTs were placed individually in each tube of the tablet disintegration time apparatus. The time was determined for all the tablets disintegrate completely.

**Table 1.** Composition of the tablets (mg)

<table>
<thead>
<tr>
<th></th>
<th>FB ODT</th>
<th>βCD-FB ODT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen</td>
<td>20</td>
<td>--</td>
</tr>
<tr>
<td>βCD-FB inclusion complex</td>
<td>--</td>
<td>113</td>
</tr>
<tr>
<td>MCC pH 102</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Crosscarmellose Sodium</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Aspartam</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mg-Stearat</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Talk</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Wetting time studies**

The wetting time of the ODTs was measured by using 0.1% w/v methylene blue solution. The time that the dye reached upper surface of the ODTs was determined.

**In vitro dissolution studies**

In vitro dissolution studies were performed using USP type II apparatus at 100 rpm. 900ml of pH 7.4 phosphate salin buffer at 37±1°C was used as medium. 5 ml samples were collected at appropriate time intervals and samples were analyzed by UV-Vis spectrophotometer at 247nm.

**RESULTS AND DISCUSSION**

The endothermic peak of the FB did not observed with the inclusion complex as seen in Figure 1. This result suggest that inclusion complex of FB with βCD was formed with kneading method successfully.

**CONCLUSIONS**

The present work revealed that ODT with βCD-FB could be useful oral delivery systems to enhance the onset of action of flurbiprofen for treatment pain rapidly.

**REFERENCES**

tia Pharmaceutica* 2009, 309-326.

**P-87: DEVELOPMENT AND IN VITRO EVALUATION OF γ-AMINOBUTYRIC ACID LOADED HALLOYSITE NANOTUBES**

G. Yurtdaş Kırmıloğlu¹, Y. Yazan¹

¹Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Eskişehir, TURKEY

**INTRODUCTION**

Epilepsy is one of the most common neurological diseases worldwide. Blockage in release of γ-aminobutyric acid (GABA) or postsynaptic reaction were determined to provoke epileptic convulsions [1]. Halloysite (HNT) is a clay mineral having a hollow tubular structure in submicron range. Nanotubular structure, good biocompatibility and very low cytotoxicity make HNT a promising carrier for drug delivery [2].

Incorporation of GABA into HNT was aimed in this study depending on the most recent theory related to GABA for epileptic brains. Development of nanosized, nontoxic, biocompatible, high affinity and
brain permeable formulations was the purpose of this study. In the present study, GABA loaded HNTs were prepared and in vitro characterization studies including particle size, zeta measurement, pH, DSC, XRD, FTIR analysis and drug loading studies were performed.

MATERIALS AND METHODS

Materials
GABA was purchased from Sigma (China). HNT was donated from Çanakkale Seramik. All other chemicals used were of analytical grade.

Preparation of GABA loaded HNTs
Different techniques were used for the preparation of GABA containing HNT. One of the loading methods was carried out under vacuum (HNT-GABA VAC). Vacuum (100 mmHg) applied to HNT alone prior to the addition of GABA solution. Vacuum was applied to the resulting HNT-GABA suspension (1:1 HNT:GABA molar ratio) for 30 minutes. This process was repeated two times in order to increase the loading efficiency. Heat treatment was used in the other method. GABA solution was added to HNT swollen in bidistilled water. Suspensions were washed with bidistilled water and centrifuged and dried at 50°C for 24 hr.

Formulations prepared were with 1:1 (HNT-GABA H1) and 1:2 (HNT-GAB, H2) (HNT:GABA) molar ratios. HNT-GABA H3 (1:1 HNT:GABA ratio) with no washing procedure was prepared using the same heat treatment method and the suspension obtained was lyophilized.

Characterization of HNTs
Mean particle sizes and zeta potential values of nanotubes were measured by Malvern Nano ZS (Zetasizer Nanoseries, England) analyzer and pH values were measured by WTW Profi Lab pH 597 (Germany) (n=3). DSC (Shimadzu, Japan) analyses were carried out at 50-550°C with an increment rate of 10°C.min⁻¹. XRD analyses were performed using RIKAGU D/Max-3C (Japan) at 2-50°C, over 20 with 40kV voltage and at current intensity level of 20 mA. FTIR spectra were recorded using Shimadzu IR Prestige-21.

Table 1. Particle size, PI, zeta potential, pH and % GABA content of nanotube formulations (n=3), (mean ± SE)

<table>
<thead>
<tr>
<th>Particle size (nm)</th>
<th>HNT-GABA VAC</th>
<th>HNT-GABA H1</th>
<th>HNT-GABA H2</th>
<th>HNT-GABA H3</th>
<th>HNT (pure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>554.2 ± 25.18</td>
<td>444.2 ± 2.36</td>
<td>530.7 ± 5.06</td>
<td>807.7 ± 10.7</td>
<td>446.8 ± 23.7</td>
</tr>
<tr>
<td>PI ± SE</td>
<td>0.670 ± 0.02</td>
<td>0.265 ± 0.10</td>
<td>0.445 ± 0.07</td>
<td>0.677 ± 0.09</td>
<td>0.417 ± 0.06</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-32.7 ± 0.58</td>
<td>-28.8 ± 0.38</td>
<td>-25.3 ± 2.78</td>
<td>-30.2 ± 2.44</td>
<td>-22.3 ± 0.09</td>
</tr>
<tr>
<td>pH value</td>
<td>7.28 ± 0.01</td>
<td>7.42 ± 0.01</td>
<td>7.45 ± 0.00</td>
<td>7.29 ± 0.01</td>
<td>7.38 ± 0.00</td>
</tr>
<tr>
<td>GABA loading %</td>
<td>-</td>
<td>1.72 ± 0.03</td>
<td>0.73 ± 0.01</td>
<td>8.54 ± 0.11</td>
<td>-</td>
</tr>
</tbody>
</table>

DSC thermograms obtained are given in Figure 1. The endothermic peak of GABA at 202.78°C was diminished in formulations prepared with heat treatment while a broad endothermic peak was observed at 300°C. XRD profiles are demonstrated in Figure 2. Profiles of HNT-GABA H1 and HNT-GABA H2 are almost consistent with patterns of pure HNT. This indicates that the whole process of preparation does not damage both the structure of HNT and the interaction between HNT and GABA.

RESULTS AND DISCUSSION

Mean particle sizes, polidispersity index (PI), zeta potential and pH values and % GABA loading of HNTs are presented in Table 1.
Figure 2. XRD spectra of pure GABA, pure HNT and nanotube formulations

FTIR spectra were demonstrated in Figure 3. Spectral results of HNT-GABA H1 showed a shift at inner –OH groups and Si-O-Si stretching bands and characteristic peaks of GABA at 1574.55 cm⁻¹ were disappeared.

Figure 3. FTIR spectra of pure GABA, pure HNT, physical mixture and nanotube formulations

CONCLUSION
GABA could be incorporated successfully into HNTs. Particle size of all HNTs was in the nanoscale range and possessed negative zeta potential values. pH values were found to be in the range of 7.28-7.45. Drug loading into HNTs was found to differ according to the preparation method. DSC, XRD and FTIR studies indicated that HNT-GABA H1 showed the highest interaction between GABA and HNT and seems to be a better candidate for further studies. Surface morphology, cytotoxicity, in vitro GABA release from HNTs and in vivo studies are under investigation.

ACKNOWLEDGMENTS
Çanakkale Ceramics Research and Development Center is acknowledged for providing HNT and Prof. Dr. Alpagut Kara and Eda Küçüköglu from Ceramic Research Center for professional advice. This study was financed by Anadolu University Scientific Research Foundation (No: 1206S106).

REFERENCES

P-88: NEW DRUG LOADING SYSTEMS: USING TWO-COMPONENT GELLING STRATEGY

M. Çolak1, M. Evcil2, D. B. Cebe2, H. Högören1
1Dicle University, Faculty of Science Department of Chemistry,
2Department of Chemistry University of Batman

The thermo-reversible property of the organogels has generated much interest for the potential use of the organogels as drug delivery system. The aim of this study was to investigate two component gelling strategy as a drug carrying system.¹

INTRODUCTION
In contrast to hydrogels, in which the gelator is normally a polymer, most of organogelators are relatively small molecules and they have been called low molecular weight organogelators. Initially, organogels were developed using various nonbiocompatible organogels which rendered the organogels noncompatible. Of late, research on organogels using various biocompatible constituents has opened up new dimensions for the use of the same in various biomedical applications. These have been an exponential rise in exploring the possibility of the use of organogels as a drug delivery vehicle. This has been greatly motivated due to longer shelf life, ease of preparation and thermo-reversible nature of the organogels- based formulations.²

MATERIALS AND METHODS
For this purpose, seven new two-component gelling system as complex salts have been prepared (PLELL, PLELA, PLELFA, PLEPFA, PLEMFA, MLEPFA, MLELFA) by using N°-alkanoyl-L-Lysine ethyl esters (N°-Lauroyl-L-Lysine ethyl ester, N°-myristoyl-L-Lysine ethyl ester, N°-palmitoyl-L-Lysine ethyl ester) as base component; N-alkanoyl-L-amino acids (phenylalanine, alanine and leucine) as
acid component. These organic salt compounds have good organogelation ability for suitable pharmaceutical liquids, such as liquid paraffin, isopropyl myristate, ethyl laurate, ethyl myristate, isopropyl laurate. Naproxen was chosen as a model drug for drug loading experiments.

RESULTS AND DISCUSSION
Naproxen and its different salt forms such as sodium and N'-alkanoyl-L-Lysine ethyl esters salts were used as drug loading experiments. The later form of naproxen has given high loading capacity in the PLELL gel than others. PLELL gel will be interesting as sustained-release depot preparations.

CONCLUSIONS
PLELL was found to be the best one among tested 7 organogelators and was used in the pharmaceutical loading test.

ACKNOWLEDGMENTS
We thank the research Project Council of Dicle University (DÜBAP-13-FF-71) and Technological Research Council of Turkey (TUBITAK) (Project no: 113Z142) for their financial support.

REFERENCES

P-89: RELEASE PROPERTIES OF IN SITU- GELLING THERMOSENSITIVE OPHTHALMIC FLURBIPROFEN FORMULATIONS

P. Adısanoğlu¹, I. Özgüney², and T. Güneri²

¹Abdi İbrahim Pharmaceutical Industry, Sanayi Mahallesi, Tunç Caddesi, No:3 Esenyurt, Istanbul, Turkey
²Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 35100 Bornova, Izmir, Turkey

INTRODUCTION
Several in situ gel forming systems have been developed to prolong the precorneal residence time of drug and improve ocular bioavailability. In this study, ophthalmic thermogelling systems containing flurbiprofen (FB) solid dispersions (SDs) were developed using poloxamer and different bioadhesive polymers. FB SDs were prepared to improve the solubility of FB. The release profiles of FB and the effect of bioadhesive polymers on FB release were investigated. The results thus obtained were evaluated kinetically and the mechanism of release of FB from ophthalmic thermogelling systems was analyzed.

MATERIALS AND METHODS
Preparation of ophthalmic thermogelling systems
SDs were prepared using poloxamer 407 (P407) and FB according to melting method at 140°C and SDs containing require amount of FB were completely dissolved in HPLC grade water and bioadhesive polymers with different concentrations were added and then cooled down to 4°C. The mixture of P407 and Poloxamer 188 (P188) was then slowly added to the solution with continuous agitation. The liquid solution was left at 4°C through the night. pH was adjusted to 7.4 ± 0.5. Finally, the volume was increased to 5mL with HPLC grade water at pH 7.4. Gelation temperatures of the formulations prepared with and without bioadhesive polymers were determined according to the method of Choi, H.G. et al.¹ and found between 32.4-33.7 °C (Table 1).

Table 1. Composition and gelation temperatures of ophthalmic formulations

<table>
<thead>
<tr>
<th></th>
<th>P407/P188 %15/26.5</th>
<th>FB (%)</th>
<th>CP (%)</th>
<th>CMC (%)</th>
<th>Gelation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>33.7</td>
</tr>
<tr>
<td>F2</td>
<td>+</td>
<td>+</td>
<td>0.2</td>
<td>-</td>
<td>33.3</td>
</tr>
<tr>
<td>F3</td>
<td>+</td>
<td>+</td>
<td>0.4</td>
<td>-</td>
<td>32.6</td>
</tr>
<tr>
<td>F4</td>
<td>+</td>
<td>+</td>
<td>0.6</td>
<td>-</td>
<td>32.4</td>
</tr>
<tr>
<td>F5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.2</td>
<td>33.2</td>
</tr>
<tr>
<td>F6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.4</td>
<td>33</td>
</tr>
<tr>
<td>F7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>0.6</td>
<td>32.9</td>
</tr>
</tbody>
</table>

(FB: Flurbiprofen; CP: Carbopol 934 P; CMC: Carboxymethylcellulose)

In vitro drug release from ophthalmic thermogelling systems
In vitro drug release of FB from formulations was evaluated by a dialysis method using a dialysis bag with a molecular weight cutt of 6-8kD (spectra/Por 1). Release medium was 200 mL phosphate buffer solution which maintained at 34±0.5°C under constant magnetic stirring at 300 rpm. Samples were assayed spectrophotometrically at 248 nm.
Kinetic Evaluations

The obtained results were evaluated kinetically by zero-order, first-order, Higuchi, and Hixson-Crowell equations. The determination coefficients ($r^2$) and the residuals were calculated. The release mechanism of FB from formulations was analyzed using the following equations:

$$\frac{M_t}{M_0} = kt^n$$  \hspace{1cm} (1)

$$\log \left( \frac{M_t}{M_0} \right) = \log k + n \log(t)$$  \hspace{1cm} (2)

$M_t/M_0$ is the fraction of released drug at time $t$, $k$ is a release characteristic constant of the formulation, and $n$ is a release exponent indicative of the release mechanism.

RESULTS AND DISCUSSION

As to the results of drug release studies, CP and CMC have no effect on release rate below the concentration of 0.6%. However, the decrease of release rate for the formulations containing CP and CMC in the concentration of 0.6% was 23 and 18% respectively during 8 hours (Figure 1 (a), (b)).

![Graph showing release of FB from ophthalmic formulations containing (a) Carbopol 934 P (b) CMC (0-0.6 %) (n=3)](image)

According to kinetic evaluation as to $r^2$, the formulations show better fit with first order and Hixson-Crowell kinetic models and these results fit with results of release mechanism analyse (Tables 2, 3).

Table 2. Release kinetic parameters of FB from ophthalmic formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>1st Order</th>
<th>2nd Order</th>
<th>Higuchi</th>
<th>Hixson-Crowell</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r^2$</td>
<td>$r^2$</td>
<td>$r^2$</td>
<td>$r^2$</td>
<td>$r^2$</td>
</tr>
<tr>
<td>F1</td>
<td>0.976</td>
<td>107.7</td>
<td>0.981</td>
<td>0.991</td>
</tr>
<tr>
<td>F2</td>
<td>0.979</td>
<td>15.0</td>
<td>0.994</td>
<td>0.996</td>
</tr>
<tr>
<td>F3</td>
<td>0.981</td>
<td>34.6</td>
<td>0.989</td>
<td>0.995</td>
</tr>
<tr>
<td>F4</td>
<td>0.985</td>
<td>43.9</td>
<td>0.967</td>
<td>0.982</td>
</tr>
<tr>
<td>F5</td>
<td>0.977</td>
<td>24.6</td>
<td>0.993</td>
<td>0.996</td>
</tr>
<tr>
<td>F6</td>
<td>0.973</td>
<td>46.1</td>
<td>0.986</td>
<td>0.991</td>
</tr>
<tr>
<td>F7</td>
<td>0.989</td>
<td>20.2</td>
<td>0.987</td>
<td>0.994</td>
</tr>
</tbody>
</table>

* $\Sigma$(Residual)$^2$/ (n-2)

Table 3. n exponent assessments of release data of FB from ophthalmic formulations

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>n</th>
<th>k</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.0148</td>
<td>1.009</td>
<td>0.9836</td>
</tr>
<tr>
<td>F2</td>
<td>1.0044</td>
<td>0.9829</td>
<td>0.9882</td>
</tr>
<tr>
<td>F3</td>
<td>1.0281</td>
<td>0.9644</td>
<td>0.9899</td>
</tr>
<tr>
<td>F4</td>
<td>1.0506</td>
<td>0.7744</td>
<td>0.986</td>
</tr>
<tr>
<td>F5</td>
<td>0.9323</td>
<td>1.072</td>
<td>0.9911</td>
</tr>
<tr>
<td>F6</td>
<td>1.0351</td>
<td>0.9713</td>
<td>0.9873</td>
</tr>
<tr>
<td>F7</td>
<td>1.0404</td>
<td>0.8657</td>
<td>0.9957</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Thermosensitive ophthalmic formulations of FB were successfully prepared. According to the results of drug release studies, it was concluded that CP and CMC in the concentration of 0.6% have a significant effect on release rate of FB. Formulations F2, F5 and F6 were found to be promising formulations as new ophthalmic dosage forms for effective anti-inflammatory therapy.

REFERENCES


P-90: CHARACTERIZATION STUDIES OF FREEZE-DRIED QUERCETIN/METHYL-ß-CYCLODEXTRIN COMPLEX

K. Güleç¹, M. Demirel²

¹Anadolu University, Graduate School of Sciences, Program in Nanotechnology
Quercetin (Qu), a polyphenolic flavonoid, is one of the most effective plant originated antioxidants. Despite the potential use of Qu in cancer treatment, low water solubility, stability problems and the scarcity of cellular bioavailability limit its applications. In this scope, inclusion complex of Qu with Methyl-β-cyclodextrin (Me-β-CD) was prepared by freeze-drying method.

**INTRODUCTION**

CD inclusion complexes have been shown to improve the stability, solubility, dissolution rate, and bioavailability of the drugs.

**MATERIALS AND METHODS**

*Phase solubility studies and preparation complex.* Phase solubility studies were performed according to the method of Higuchi and Connors [1]. A validated HPLC method was used for the determination of Qu. Qu/Me-β-CD complex prepared by freeze-drying method with 1:1 molar ratios determined by the phase solubility diagram studies (Fig. 1).

![Fig. 1. Phase Solubility Diagram of Qu/Me-β-CD System in Ethanol:Water (1:4) at 25°C (n=3, mean±SE)](image)

*Characterization:* Aqueous solubility tests carried out at 25°C. The antioxidant properties were evaluated by determination of the scavenging activity towards DPPH radical [2]. The complex was characterized by FT-IR, DSC, XRD, NMR analyses.

**RESULTS AND DISCUSSION**

The phase solubility diagram showed a linearity between enhanced solubility of Qu with increased amount of Me-β-CD (r²=0.992) (Fig. 1). The curve obtained can be classified as type Aα [1]. Complexation strongly increases the water solubility of Qu from 0.3 μg/ml to 83.9 μg/ml, at 25°C (n=3). In addition, the antioxidant activity of the complex was more effective than pure Qu on DPPH scavenging test (Fig. 2).

![Fig. 2. Antioxidant Activity of Pure Qu and Qu/Me-β-CD Complex (n=5, mean±SE)](image)

The formation of inclusion complex with Me-β-CD in the solid-state were confirmed by DSC, XRD, NMR and FT-IR analyses.

**CONCLUSIONS**

As a conclusion, formation of Qu/Me-β-CD inclusion complex has enhanced the aqueous solubility of Qu in a great extend as well as increased antioxidant activity of Qu was detected.

**ACKNOWLEDGMENTS**

This study was financed by Anadolu University Scientific Research Project Foundation (No: 1501S059).

**REFERENCES**

microcryogels. The microcryogels were characterized thoroughly by performing optical microscopy, swelling tests, scanning electron microscopy (SEM), injectability tests with the conventional needle–syringe.

INTRODUCTION
Implantable biomaterials have been investigated for locally deliver of therapeutic molecules such as antineoplastic agents and hormones [1]. However, surgical implantation may cause risks and complications. Therefore, minimally invasive methods are gaining importance [2]. Cryogel is a kind of hydrogel formed via cryogenic treatment. Because the macroporous and tissue-like structure, cryogels offer unique 3D scaffolds as implantable biomaterials for local delivery of therapeutic molecules [3]. Herein, we report injectable microcryogels for a local administration of drug and protein molecules.

MATERIALS AND METHODS
HEMA and MBAam were obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4 °C until use. N,N,N’,N’-tetramethylethlenediamine (TEMED) and ammonium persulfate (APS) were obtained from Fluka (Buchs, Switzerland). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). Water used in all the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system.

Microcryogels were prepared in a microstencil by free radical polymerization in the moderately frozen state of HEMA and MBAam in the presence of APS/TEMED as initiator/activator pair.

RESULTS AND DISCUSSION
Microcryogels presented complete shape recovery (i.e. shape memory) features. They collapsed dramatically when compressed, and when rehydrated, return their previous shape within just 1–2 s. Specific surface area, swelling degree, and macroporosity of PHEMA microcryogels were presented in Table 1. The microscopic and SEM images of PHEMA microcryogels are shown in Fig. 1. Microcryogels have highly macroporous structures, which allow the effective migration of drug molecules (i.e. diffusion) owing to easy contact of tissues with injected microcryogels [3].

CONCLUSIONS
In this study, we prepared low cost microcryogels via cryogenic treatment in a microstencil for local delivery of therapeutic agents via injection. Results showed that microcryogels have extraordinary mechanical and diffusional properties to be applied for the implantation.

ACKNOWLEDGMENTS
K. Çetin thanks to The Scientific and Technological Research Council of Turkey (TUBITAK) for funding Ph.D. fellowship supplied by Science Fellowships and Grant Programs Department (BIDEB).

REFERENCES

P-92: DEVELOPMENT AND EVALUATION OF THE ANTISTAPHYLOCOCCAL ACTIVITY OF ERYTHROMYCIN-β-CYCLODEXTRIN-LACTIDE COMPLEX

M. Hortolomei1, L. Ochiuz2, I. Popovici1, M. Mares2, A. Stefanache1, C. Peptu3

1University of Medicine and Pharmacy “Grigore T. Popa” Faculty of Pharmacy, Iasi, Romania, 2 Ion Ionescu de la Brad, University of Agricultural Science Veterinary
INTRODUCTION

Erythromycin (E), a hydrophobic macrolide antibiotic, is prescribed for the treatment of acute bacterial infections, such as respiratory, urine, skin, and mouth infections. Cyclodextrins (CD) ability to form inclusion complexes and hence to alter the physical-chemical properties of guest molecules has been exploited in order to increase the water solubility of hydrophobic drugs. Specifically to optimize the physical inclusion of specific guests, the polarity of native CD molecules should be adjusted. Generally, this is achieved by attaching small alkyl or hydroxalkyl substituents through ether linkages. Our study aiming at improving the stability and tolerance of E in topical products developed a complex of E with \( \beta \)CD-LA_E. The antibacterial activity of the synthesized complex was assessed against \( S. \) aureus strains.

MATERIALS AND METHODS

Erythromycin, \( \beta \)-CD, mechlorethamine, (Sigma Aldrich, Germany). All chemicals used were of reagent grade.

Preparation and characterization of CD-LA_E complex

The \( \beta \)-CD derivative modified with lactide (LA) oligoesters was prepared through ring-opening polymerization of LA initiated solely by \( \beta \)-CD as previously published [1]. The structure of the obtained CD-LA derivatives was demonstrated by electrospray ionization mass spectrometry (ESI MS). High-resolution MS spectra were acquired on an AGILENT 6520 QTOF mass spectrometer (Agilent Technologies) equipped with an ESI source. The CD-LA_E complex was prepared as previously described for \( \beta \)-CD-Erythromycin complexes [2]. The preparation procedure consisted in kneading the appropriate amounts of CD-LA solution in water (0.125 g/mL) and E solution in ethanol (0.25 g/mL) in a 1/1 molar ratio. The E solution was slowly added drop by drop into the former, while mixing for 30 min and the mixture was continuously stirred until most of the solvents were evaporated (4 hours) and a pasty product was obtained. To elucidate the formation of a CD-LA_E complex, morphologic observation was performed using high-resolution SEM.

Antibacterial activity assay

We tested the susceptibility to CD-LA_E of 20 \( S. \) aureus strains: two reference strains (\( S. \) aureus ATCC 25923 and meticillin resistant \( S. \) aureus ATCC 43330) and 18 clinically isolated strains, previously identified by specific methods (cultivation on Chapman agar, catasa test, and citrate plasma clotting assay). The antimicrobial susceptibility assay was performed using the diffusion method, on Mueller-Hinton agar.

RESULTS AND DISCUSSION

The spectrum presented in fig. 2 proves that LA has been covalently attached to the CD molecule as depicted in fig. 1.

![Fig. 1. Synthesis and structure of CD-LA](image)

![Fig. 2. ESI MS spectrum of CD-LA derivatives](image)

The \( \beta \)-CD was functionalized with a variable number of lactide units from 1 (m/z = 1296) to 6 (m/z = 2016). For example, the peak found at m/z = 1728 corresponds to a cyclodextrin molecule bearing 4 lactide units which can be rationalized as follows: \( 1728 = 1134(CD)+4\times144(LA)+18(NH_4^+) \). A monoclinic morphology of the CD-LA powder was observed under SEM and this was consistent with literature reports while the E powder was shown to be a semicrystalline precipitate. As SEM revealed, the kneading processes facilitated the interaction of CD and E by altering their original crystalline structures and amorphization during freeze-drying. The CD-LA_E complex proved antimicrobial activity against the reference strain \( S. \) aureus ATCC 25923 while meticillin resistant \( S. \) aureus ATCC 43330 was not susceptible to the complex. Furthermore, we noted that 16 of \( S. \) aureus isolated strains were susceptible to CD-LA_E complex having a minimal inhibitory concentration in the range of 0.5-1 mg/L.

CONCLUSIONS

The CD-LA_E synthesized complex was formed as a result of kneading process of CD-LA and E. CD-LA_E revealed a promising antistaphylococcal activity.

REFERENCES

P-93: RADIOLABELING STUDIES OF CHITOSAN NANOPARTICLES WITH TECHNETIUM-99M

M. Ekinci, D. Ilem-Ozdemir, E. Gundogdu, M. Asikoglu
Ege University, Faculty of Pharmacy, Department of Radiopharmacy, Izmir, TURKEY

INTRODUCTION
Nanoparticles (NPs) usually refer to particles of sizes smaller than 100 nm [1]. NPs have accessibility in the body and can be transported to different body sites through systemic circulation of blood [2]. Therapeutic efficacy of drugs can be enhanced by targeting the drug through polymeric NPs as carrier. Methotrexate (MTX) is one of the most widely and effectively use anticancer drug in human malignancies and also has a therapeutic effect on many types of cancer cells [3]. During the last 20 years, very important improvements have been achieved in drug development using radiopharmaceuticals as tracers [4]. Technetium-99m (99mTc) is employed for about 85% of single-photon imaging procedures performed in nuclear medicine because of its favorable gamma energy and lack of particulate radiation [5]. The aim of this study is to radiolabel MTX loaded chitosan (CS) NPs and make quality control studies with suitable process.

MATERIALS AND METHODS
Materials: MTX was obtained from Kocak Pharma (Turkey). Stannous tartrate was purchased from Sigma-Aldrich (USA) and ascorbic acid was purchased from Sigma-Aldrich (United Kingdom). CS and Tripolyphosphate (TPP) were used to prepare NPs. 99mTc-sodium pertechnetate was obtained from Nuclear Medicine Department of Ege University. Preparation of MTX loaded CSNP: MTX loaded CSNP was prepared by using ionic gelation process. CS was dissolved in 1% acetic acid solution. TPP was prepared with distilled water and MTX solution was added in TPP solution. Then, TPP solution was added drop wise under constant stirring to CS solution. An opalescent suspension was formed. Radiolabeling studies: MTX loaded CSNP was radiolabeled by 99mTc using stannous tartrate and ascorbic acid which were used as reducing and antioxidant agents respectively. Quality control studies: The labeling efficiency of 99mTc-MTX-CSNP was assessed by a gamma counter. For this purpose, 1 mL of 99mTc-MTX-CSNP was taken to eppendorf. Then, it was separated by centrifugation at 2822 rpm for a period of 10 minutes at 25 °C. The supernatant was taken to another eppendorf. The activities of MTX loaded CSNP and supernatant were counted by a gamma counter. The % radioactivity of CSNP was calculated from the following equation.

Radioactivity of CSNP = 100 x [Radioactivity of CSNP / (Radioactivity of CSNP + Radioactivity of supernatant)]

RESULTS AND DISCUSSION
Labeling efficiency of 99mTc-MTX-CSNP was calculated according to equation and found >90%. Also 99mTc-MTX-CSNP was quite stable up to 6 hours.

CONCLUSIONS
Quality control of 99mTc-MTX-CSNP was assessed by gamma counter. 99mTc-MTX-CSNP was obtained in high radiochemical purity and high yield labeling capacity.

ACKNOWLEDGMENTS
The authors thank to Ege University, Scientific Research Projects Comission (Project number: 14/ECZ/037) and Aliye Uster Foundation for financial supports.

REFERENCES
P-94: RADIOLABELING AND QUALITY CONTROL OF METHOTREXATE WITH TECHNETIUM-99M

E. Ozgenc, M. Ekinci, D. Ilem-Ozdemir, E. Gundogdu, M. Asikoglu

Ege University, Faculty of Pharmacy, Department of Radiopharmacy, Izmir, TURKEY

INTRODUCTION

Methotrexate (MTX) is one of the most widely and effectively used anticancer drug in human malignancies such as acute lymphoblastic leukemia, malignant lymphoma, osteosarcoma, breast cancer and neck cancer [1]. Technetium-99m (99mTc) is the radionuclide of choice by virtue of convenient half life, ideal energy for imaging, short enough to minimize absorbed radiation dose [2].

The aim of this study is to radiolabel MTX with 99mTc in a simple radiochemical method.

MATERIALS AND METHODS

MTX was obtained from Kocak Pharma (Turkey). Stannous tartrate was purchased from Sigma-Aldrich (USA) and ascorbic acid was purchased from Sigma-Aldrich (United Kingdom) which were used as reducing and antioxidant agents respectively. 99mTc was eluted from the Molybdenum-99 (99Mo)/99mTc generator (Nuclear Medicine Department of Ege University).

Radiolabeling studies: The radiolabeling of 99mTc-MTX was tested with different concentrations of reducing and antioxidant agent. Radiochemical purity (RP) was determined with Radio Thin Layer Chromatography (RTLC) analysis.

RTLC studies: Whatman 3MM papers and instant thin layer chromatography-silica gel coated plates (ITLC-SG) were used as stationary phases. Free 99mTc was determined by using Whatman 3MM papers as stationary phase and acetone as the mobile phase. Reduced/Hydrolyzed (R/H) 99mTc was determined by ITLC-SG plates which developed in Acetonitrile/Water/Trifluoroacetic acid (ACN/W/TFA; 50/25/1.5) solvent system. The radioactivity on plates was measured using a TLC scanner and RP (%) of 99mTc was calculated from the following Equation by subtracting from 100 the sum of measured impurities percentages.

\[ \text{RP} \% = 100 - \left[ \text{Free } 99mTc \% + \text{R/H } 99mTc \% \right] \]

RESULTS AND DISCUSSION

RTLC results showed that MTX was radiolabeled by 99mTc with high labeling efficiency (>90%). Maximum RP was obtained with 1000 µg stannous tartrate and 0.1 mg ascorbic acid including formulations (Figure 1). Also radiolabeled complex was found stable in saline and human serum at room temperature up to 24 hours.

![Fig. 1. The labelling efficiency (%) of 99mTc-MTX](image)

CONCLUSIONS

MTX is a well-known adjuvant for the treatment of various cancers. Simple method for radiolabeling of MTX with 99mTc has been developed and standardized. Labeling efficiency of 99mTc-MTX was assessed by RTLC. The resulting complex was quite stable and labeling yield >90% was maintained for up to 6 hours. 99mTc-MTX can be candidate for cancer diagnosis.

ACKNOWLEDGMENTS

This study was supported by 14-ECZ-36.

REFERENCES


P-95: PREPARATION AND CHARACTERIZATION OF TRANSFEROSOMES LOADED WITH MULTIPLE ANTIWRINKLE AGENTS

M. Macit1, M. A. Rouf1, I. Aslan2, C. Macit3, S. Ozdemir1

1 Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology Istanbul, TURKEY
2 Yeditepe University, YUKOZ Istanbul, TURKEY
3 Yeditepe University, Faculty of Pharmacy, Department of Clinical Pharmacy Istanbul, TURKEY

INTRODUCTION

Transferosomes are deformable vesicles that can penetrate through much smaller pores than their own size. Their penetration capability into the skin has been reported [1]. Antiwrinkle agents when applied singly or in conventional cosmetic forms like gel or cream are not very effective. Therefore, we planned to load both
water soluble and lipid soluble antiwrinkle agents into the internal water compartment and lipid bilayer of transfersomes, respectively.

MATERIALS AND METHODS

Hydrogenated phosphatidylcholine (Phospho-lipon 90H) and Tween 80 were used to form the transfersome. Cholesterol, instead of Tween 80, was used for the control liposome. Glycolic acid and Na-EDTA were used as water soluble antiwrinkle agents and vitamin E as the lipid soluble agent. Transfersome and liposomes were prepared by thin film method [2]. Vitamin E, lipid and Tween 80 were dissolved in a 2:1 mixture of chloroform and methanol. The organic solvents were evaporated in a rotavapor (Heidolph, Germany) to form a thin film which was kept overnight at 1 mbar vacuum to remove the residual solvents. Then, glycolic acid and Na-EDTA were dissolved in pH 5.5 phosphate buffered saline (PBS). After that, this solution was added to the thin film with moderate hand shaking. These milky multilamellar large vesicles were ultrasonicated for 10 min (Cycle 9, power 60%, Bandelin, Germany). Later, the formulations were extruded (Liposofast, Avestin, Canada) successively through 800, 400 and 200 nm pore size polycarbonate filter (Whatman, UK) 15 times each. Finally, vesicles were purified by ultracentrifugation (Beckman Coulter, USA) at 150000 g for 1 h.

Vesicle size and zeta potential were characterized by dynamic light scattering method (Zetasizer Nano ZS, Malvern, UK). Vitamin E entrapment efficiency was calculated using a validated UV-Vis spectrophotometric method (Fig. 1). Elasticity was measured by using 100 nm polycarbonate filters at 2.5 bar pressure [3].

RESULTS AND DISCUSSION

Transfersomes with average diameter of less than 300 nm (Fig. 2, PDI<0.3) were produced. The vesicles were elastic compared to their liposome counterpart. Vitamin-E entrapment efficiency was calculated using a validated UV-Vis spectrophotometric method (Fig. 1). Elasticity was measured by using 100 nm polycarbonate filters at 2.5 bar pressure [3].

CONCLUSIONS

Due to their penetration capability and ability to carry both hydrophilic and lipophilic substances, transfersome seems to be an interesting tool to deliver multiple antiwrinkle agents to the target region of the skin. We have prepared and characterized suitable transfersome for this purpose. Their in vivo efficiency will be evaluated in our ongoing studies.

REFERENCES

PREVENTS POSTERIOR CAPSULAR OPA-CIFICATION. *Molecular Vision* 2012, 18, 1701-1711.

**P-96: DEVELOPMENT OF NANO-PARTICULATE SYSTEMS FOR OCULAR DRUG DELIVERY OF MELOXICAM**

O. Sahin¹, N.O. Sahin¹, Ç.E. Ozdemir¹, E. Szubert¹,²

¹Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Yenisehir Campus, mersin 33169, Turkey. ²Jagellonian University, Faculty of Pharmacy, Krakow, Poland

**INTRODUCTION**

Non-steroidal anti-inflammatory drugs have been used for the treatment of pre-operative inflammation and pain in the field of ophthalmology¹. However, some obstacles should have been overcome in order to create the desired therapeutic effect at the site of action in ocular therapy. Current topical medications are prepared in the form of conventional dosage forms and applied to eye(s) several times in a day. This reduces patient compliance and hence, effectiveness of the therapy. Meloxicam is a non-steroidal anti-inflammatory drug with a significant potential to be used in treatment of inflammation and pain of eye². With a few clinical trials in ophthalmology, the goal of this study was set to develop a topical dosage form of meloxicam for ophtalmic inflammation and pain threatment.

Niosomes, nanoparticles systems, were selected to be used as controlled release topical dosage forms in this study because of their biodegradable and biocompatible nature, their better chemical stability, low toxicity, enhanced bioavailability, and patient compliance³,⁴.

**MATERIALS AND METHODS**

Meloxicam was a gift from Drogsan Pharm. Co., Ankara, Turkey. Span20 and Brij 76 were provided from Merck (Germany) and Sigma Aldrich (St. Louis, USA), respectively. All other chemicals were in analytical grade.

Meloxicam bearing niosomes were prepared with film rehydration method and characterized. The effect of surfactant type and concentration, drug concentration and cholesterol on encapsulation efficiency were investigated. The best formulation was selected. In vitro drug release was studied using Franz diffusion cell system.

**RESULTS AND DISCUSSION**

Niosomes prepared with Span 20 showed the highest encapsulation capacity (% 41.3) and the smallest size (523 nm). Higher the surfactant concentraton, lower the size of niosomal vesicles formed.

*Fig. 1. The release profile of meloxicam (MX) from different niosome formulations in comparison to the solution (= meloksikam çözelti) of MX.*

**CONCLUSIONS**

In vitro release kinetics of meloxicam from niosomes best fitted to Higuchi model (Figure 1). Sustained release of the drug encapsulated was obtained. In conclusion, niosomes of meloxicam may be promising drug delivery systems for ocular delivery.

**REFERENCES**


**P-97: FORMULATION OF NOVEL FLURBIPROFEN LOADED NLC BASED OCULAR INSERTS**

E. Homan Gökçe¹, N. Üstündağ Okur²

¹Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ege, 35100 Bornova, Izmir, Turkey
²Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Istanbul Medipol, 34810 Istanbul, Turkey

**INTRODUCTION**

The inflammatory response of the ocular tissues is a common side-effect associated to ophthalmic surgery. Flurbiprofen can antagonise the pupillary constriction during the intra-ocular surgery, by inhibiting the cyclooxygenase (1). Therefore the aim of this study was to formulare novel flurbiprofen loaded nano structured lipid carrier (NLCs) based inserts for ocular delivery.

**MATERIALS AND METHODS**

Preparation of NLC and NLC based inserts

NLCs were prepared with high shear homogenization and inserts were developed with these NLCs by means of solvent casting. Unloaded NLC (N1) consisted of weight/volume oleic acid (1.5%), Compritol (0.75%), Tween 80 (0.75%) and distilled water (97%). The
modified NLC (N2) was prepared by adding sodium alginate (0.75%) into the aqueous phase of N1. To obtain inserts sodium alginate (0.75%) was added to N1 and mixed with 5% glycerine (Ins1) or 5% PEG 400 (Ins2) as plasticizers. These mixtures were dried also loaded with 0.3% of flurbiprofen.

Table 1: Composition of the inserts

<table>
<thead>
<tr>
<th>Code</th>
<th>NLC</th>
<th>alginate (%)</th>
<th>plasticizers (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins1</td>
<td>N1</td>
<td>0.75</td>
<td>Glycerin</td>
</tr>
<tr>
<td>Ins2</td>
<td>N1</td>
<td>0.75</td>
<td>PEG 400</td>
</tr>
</tbody>
</table>

**In-vitro characterization studies**

Flurbiprofen loaded N1 and N2 were evaluated for particle size (PS), zeta potential (ZP), pH, viscosity, encapsulation efficiency (EE%) and drug loading. Inserts (Ins1 and Ins2) were evaluated for drug content, thickness uniformity, weight uniformity, moisture absorption % and moisture loss %.

**Stability**

NLCs and inserts were stored at 4±1°C, 25±2°C (relative humidity (RH) 60%) and 40±2°C (RH 75%) for 3 months. Flurbiprofen content and appearance were evaluated for inserts. NLCs were also evaluated for the changes in PS, ZP, viscosity and pH.

**RESULTS AND DISCUSSION**

NLCs were prepared by high shear homogenization method and PS and PDI values pointed out the homogeneity of particles. NLC based ocular inserts were prepared successfully by solvent casting technique. The characterization of flurbiprofen loaded NLCs are given in Table 2.

Table 2: Characterization parameters of flurbiprofen loaded NLCs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NLCs</th>
<th>N1Flurbiprofen</th>
<th>N2Flurbiprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS (nm)</td>
<td></td>
<td>241.7 ± 15.8</td>
<td>383.6 ± 29.61</td>
</tr>
<tr>
<td>PDI</td>
<td></td>
<td>0.135 ± 0.01</td>
<td>0.306 ± 0.017</td>
</tr>
<tr>
<td>ZP (mV)</td>
<td></td>
<td>-3.74 ± 0.08</td>
<td>-8.84 ± 0.27</td>
</tr>
<tr>
<td>viscosity(cP)</td>
<td></td>
<td>13.9 ± 0.003</td>
<td>19.22 ± 0.025</td>
</tr>
</tbody>
</table>

Inserts had homogeneous appearances and could be easily removed from the petri dishes. The drug content of inserts is very important in terms of homogeneity of production. The novel production method developed in this study was regarded as an appropriate method to obtain uniform flurbiprofen loaded inserts, since flurbiprofen content in each insert was found to be almost the same without any loss (Table 3).

**Table 3**: Characterization parameters of inserts

Flurbiprofen content and physical characteristics of formulations did not show any change for 3 months.

**CONCLUSIONS**

The formulation of flurbiprofen as NLC or insert for ophthalmic delivery is achievable. The use of ophthalmic inserts developed on the basis of NLC could be an alternative way for the delivery of drugs especially having short biological half-lives, reduction of systemic side effects and improvement of the efficiency by an effective drug concentration in the eye over an extended period of time.

**ACKNOWLEDGMENTS**

The authors would like to thank to FABAL.

**REFERENCES**


---

**P-98: MODIFICATION OF SOLID LIPID NANOPARTICLES: POLYETHYLENE GLYCOL VERSUS CHITOSAN OLIGOSACCHARIDE LACTATE**

N. Üstündağ-Okur¹, A. Yurdasiper², E. Gündoğdu³, E. Homan Gökçe²

1 Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Istanbul Medipol, 34810 Istanbul, Turkey
2 Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ege, 35100, Izmir, Turkey
3 Department of Radiopharmacy, Faculty of Pharmacy, University of Ege, 35100, Izmir, Turkey
The aim of this study was to formulate solid lipid nanoparticles (SLN) and modify with chitosan oligosaccharide lactate (COL) or polyethylene glycol (PEG) for development of oral drug delivery.

MATERIALS AND METHODS
SLNs were produced with homogenization method as optimized in our previous study (1). Compritol, poloxamer and lecithin amounts were varied for pre-formulation studies (Table 1). COL was dissolved in the aqueous phase and PEG was added to the lipid phase used in SLN preparation step at the concentrations of 0.1-0.4% (w/v). Ideal % concentration of COL/PEG was chosen according to particle size (PS), PDI and zeta potential (ZP) values of SLNs.

RESULTS AND DISCUSSION
S5 formulation was selected and was modified with lecithin at different amounts and 10 mg of lecithin (S19) to lead to a more uniform system (Table 2). After S19 formulation was determined as optimum formulation, it was modified with COL or PEG at the concentrations of 0.1-0.4 % and characterized as seen in Table 3

Addition of COL or PEG showed concentration dependent effects on physicochemical properties. COL changed ZP. PS was reversely proportional to PEG concentration.

CONCLUSIONS
PEG concentration was reversely proportional to particle size and due to its steric stabilization, zeta potential did not alter. COL at 0.1 % and PEG at 0.4% gave the optimized results. The findings of this study revealed the suitability of PEG modified SLN. The presented delivery system could provide a new promising strategy for enhancing the oral bioavailability of such drugs with poor hydrophilicity.

ACKNOWLEDGMENTS
This study was supported by TUBITAK Project No: 112S292. The authors would like to thank to FABAL for homogenization facilities.

REFERENCES
P-99: CONTROLLED RELEASE OF METHYLPREDNISOLONE FROM CHITOSAN MICROSPHERES 1: EFFECT OF SOME FORMULATION PARAMETERS

S. Evirgen, J. Şahin, N. Özdemir

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100 Tandoğan, Ankara-TURKEY

INTRODUCTION
In this study, investigations have been carried out to prepare implantable controlled release system of methylprednisolone, which is a synthetic glucocorticoid, requires frequent administration specially for long term therapy of pain and edema. For this aim, a natural biodegradable polymer, chitosan was used. Chitosan, (β-(1-4)-2-amino-2-deoxy-D-glucose) is produced by deacetilation of chitin which is the second most frequently found polymer in nature after cellulose. Because of they are biocompatible and biodegradable, they are very useful as a vehicle for controlled drug delivery (1). We used ionotropic gelation method in which electrostatic interaction between chitosan and tripolyphosphate (TPP) due to reversible physical crosslinking instead of chemical crosslinking to avoid possible toxicity and undesirable effects (2).

In the present study, it was aimed to prepare implantable controlled release chitosan microspheres including methylprednisolone. It was also aimed to investigate the effects of Molecular weigh and amount of polymer, the amount of active material and ratio of internal phase:external phase.

MATERIALS AND METHODS

**Materials:** Methylprednisolone was supplied by Mustafa Nevzat İlaç (İstanbul, Turkey) in addition the following were used in the study Chitosan (HMw, MMw, LMw) (Sigma-Aldrich Co.), tripolyphosphate (TPP) (Sigma Co.).

**Methods:** Methylprednisolone was dispersed in a solution of chitosan (1% w/v) in acetic acid (1% v/v). This dispersion was dropped through a syringe (0.45 mm in diameter) into a gently agitated TPP solution. The chitosan microspheres were filtered and washed with 20 ml distilled water at least three times and dried at 37°C for 24 hours.

Table 1. Codes and variables of formulations

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug Content (%w/w)</th>
<th>Type of Polymer (%1w/v)</th>
<th>Ratio of internal:external phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL1</td>
<td>50</td>
<td>LM</td>
<td>10/100</td>
</tr>
<tr>
<td>FM1</td>
<td>50</td>
<td>MM</td>
<td>10/100</td>
</tr>
<tr>
<td>FH1</td>
<td>50</td>
<td>HM</td>
<td>10/100</td>
</tr>
<tr>
<td>FH10</td>
<td>50</td>
<td>HM</td>
<td>10/200</td>
</tr>
<tr>
<td>FH11</td>
<td>50</td>
<td>HM</td>
<td>10/300</td>
</tr>
<tr>
<td>FH12</td>
<td>25</td>
<td>HM</td>
<td>10/100</td>
</tr>
<tr>
<td>FH13</td>
<td>75</td>
<td>HM</td>
<td>10/100</td>
</tr>
</tbody>
</table>

The drug content of microparticles was determined spectrophotometrically after dissolving the weighted amount of microspheres in 100 ml 0.1 N HCl, at a wavelength 247.5 nm. The particle size and size distribution of microspheres were determined by Sympatec Laser Diffraction Particle Sizer.

Drug release from microspheres was tested using stational method in phosphate buffer solution (pH 7.4) at 37 °C for 140 hours (n=3). At predetermined time intervals samples were taken and analyzed spectrophotometrically.

RESULTS AND DISCUSSION

SEM images of microspheres were conducted to examine the surface morphology. They showed that the shapes were smooth and spherical.

The loading capacity increased when the Mw and concentration of polymer and the amount of active material increased, but it was observed that when the internal:external phase ratios were increased this capacity was decreased.

The particle size of microspheres were varying between 700-1500 μm. The particle size decreased as the Mw and concentration of polymer and the ratio of internal:external phase increased.

The particle size increased as a result of increasing active substance concentration and decreasing chitosan amount in the dribble of the same volume. The rapid release of drug from FL1 formulation was observed. There was no significant difference of release rates between FH1 and FM1 formulations containing high molecular weight and medium molecular weight of chitosan, respectively.

At the end of the study; the effect of ratio of internal and external phase on active material release was investigated. Drug release from microspheres were also decreased with increasing the ratios of internal and external phase.

The drug release results were found to give fits to Q→vt and zero order equation.

CONCLUSION

Methylprednisolone containing chitosan microspheres were successfully prepared by ionotropic gelation method. Controlled drug release rate could be
achieved by changing different formulation parameters.

REFERENCES

P-100: MODELLING OF DEXKETOPROFEN TABLETS BY USING QUALITY BY DESIGN APPROACH

Ö. Demir¹, B. Aksu², Y. Özsöy³

¹Mustafa NevzatlaçSanayi, Department of Research&Development
²Santa FarmalllaçSanayi, Department of Corporate Affairs
³Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technolog

INTRODUCTION
Quality by design (QbD) encompasses designing and developing formulations and manufacturing processes which ensures predefined product specifications. An important part of QbD is to understand how process and formulation parameters affect the product characteristics and subsequent optimization of these parameters should be identified in order to monitor these parameters online in the production process [1]. In this study, development, understanding, control strategy and specifications of formulation and manufacturing process of immediate-release film coated tablets containing DexketoprofenTrometamol were determined by using Quality by Design approach. Design of Experiment (DoE) was formed for formulation development via Minitab software. Results of the experiments were firstly analyzed by Minitab software capable of linear regression modelling. These results were then assessed via Artificial Neural Network (ANN) and Gene Expression Programming (GEP) modules of INForm software capable of non-linear modelling.

MATERIALS AND METHODS
Dexketoprofen trometamol, Starch 1500-LM, Avicel PH102, Aerosil 200, Primojel, Precirol ATO 5, Compritol 888 ATO, sodium stearyl fumarate (PRUV) and Opadry White 03J280000 coating mixture were used as formulation ingredients. GEA SP 15 mixer, Korsch eccentrically tablet pressing machine, Glatt SIEB GS 60 milling granulator, 30 and 40 mesh manual sieve, Glatt GMPCI Minicoater coating machine were used as process equipments. Minitab 17.1.0 (LEAD Technologies, Inc) and INForm V5.1 (Intelligensys) were used as evaluation softwares.

Formulations were produced via dry granulation (slugging) and direct compression as well as using general full factorial Design of Experiment with 3 factors and total 18 formulation trials. INForm ANN and GEP modules optimization parameters utilized to find optimum formulation were submitted in the Table 1.

RESULTS AND DISCUSSION
As a result of modelling studies, it was determined that slug compression should be implemented as a process parameter, glyceryl dibehenate should be used as lubricant type. The amount of lubricant should be between %2.03 - %2.50 to provide an optimized formulation. But, it was observed that sodium stearyl fumarate was the unique lubricant solved the punch sticking problem during experiment studies. As this problem must be solved to produce an acceptable product in the pharmaceutical industry, it is decided to use sodium stearyl fumarate as lubricant type.

CONCLUSIONS
As response variables measured were found to be non-linear to changing factors in reality, INForm software capable of non-linear modelling was deemed to be more successful with comparison to Minitab software. Optimum formulas found as a result of the modelling studies, were appropriate in terms of chemical and physical tests and solved the sticking problem. Sodium stearyl fumarate was required to be between %2.03 and %2.50 and slug compression was needed for that optimum formulation.

ACKNOWLEDGMENTS
There is no acknowledgement to be declared on this study.

REFERENCES
P-101: COMPARATIVE EVALUATION OF GRANULAC®-STARCH GRANULES PRODUCED BY WET GRANULATION METHOD USING PVP BINDER IN A LOW SHEAR MIXER GRANULATOR

O. Gun, A.D. Ergin, M. Gorgoz, G.R. Camca, S. Arisoy, N. Yuksel
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION
The production of high quality tablets requires excellent properties of granules. A granulation before tableting has been and still is the common procedure to ensure good properties in the tablettability. Although the granulation process has been extensively studied, it is still needed investigation of the effects of fillers and binders on granule properties. The purpose of this study was to evaluate the effect of fillers with different particle size and different binder concentrations on the flow properties, compressibilities and consolidation behaviors of the granules which were prepared by the wet granulation method in a low shear mixer granulator.

MATERIALS AND METHODS
Materials: As fillers, two special lactose grades for wet granulation (Granulac® 230 and Granulac® 70, Meggle Excipients&Technology, Germany) were used. Corn starch (Kimetsan, Turkey) was used as an internal disintegrant and PVP K30 (Luviskol® K30, BASF, Germany) was evaluated as wet binder.

Methods: Formulations of the granules were given in Table 1. After mixing Granulac® and corn starch in a cube mixer (Erweka, Germany) for 25 min, the powder mixture was transferred to a sigma blade mixer granulator and mixed for 5 min. Aqueous solution of PVP K30 was added dropwise with portions of 10 mL. The wet mass was granulated by a wet granulator with oscillating rotor employing the sieve 1200µm (Erweka, Germany); the produced wet granules were dried at 45°C to give a moisture content of < 2%, determined on a moisture measurement equipment (Ohaus.MB45, Switzerland).

Particle size, flow rate, angle of repose, consolidation, and compressibility behaviors of the dried granules were investigated before and after applying friability test to the granules [1-3].

Table 1. Formulation for the granulation

<table>
<thead>
<tr>
<th>Ingredients (g/batch)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulac® 70</td>
<td>350</td>
<td>350</td>
<td>350</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Granulac® 230</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>350</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>PVP K30* (dry)</td>
<td>3.5</td>
<td>7.0</td>
<td>10.5</td>
<td>3.5</td>
<td>7.0</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*PVP K30 was used as binder in the form of aqueous solution of 5 – 10 – 15 % (w/v).

RESULTS AND DISCUSSION
Friability has been used to evaluate granule strength. Therefore, granule properties were determined before and after friability test. Particle size specifications of Granulac® 230 and Granulac® 70 are <63 µm (≥90%) and <400 µm (≥95%), respectively. It was seen that in terms of particle size and then flow rate, there are statistically significant differences between the formulations F4 and F6 which were prepared using Granulac® 230 with smaller particle size before and after friability test. Conversely, there are no differences between the formulations regarding the values of angle of repose (Fig. 1). Consolidation properties were determined according to Carr’s index and Hausner ratio (Table 2). When considered Carr’s index and Hausner ratio before friability test flow properties were poor, but after friability test these values were passable [4]. Compressibility of the granules was evaluated according to Heckel equation [1]. When considered Py-yield stress values, the formulations F4 - F6 have smaller Py values with lower variability before and after friability test indicating better densification and easier compression of the granules.

Figure 1. Comparison of the flow rate, particle size, angle of repose, and Py values of the granules made with different formulations (B.F: before friability test and A.F: after friability test)
Table 2. Comparison of the Hausner ratio and Carr’s index of the granules made with different formulations

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>AF</th>
<th>BF</th>
<th>AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1.42</td>
<td>1.32</td>
<td>29.5</td>
<td>24.5</td>
</tr>
<tr>
<td>F2</td>
<td>1.53</td>
<td>1.26</td>
<td>34.5</td>
<td>20.5</td>
</tr>
<tr>
<td>F3</td>
<td>1.43</td>
<td>1.29</td>
<td>30.0</td>
<td>22.5</td>
</tr>
<tr>
<td>F4</td>
<td>1.56</td>
<td>1.31</td>
<td>36.0</td>
<td>23.5</td>
</tr>
<tr>
<td>F5</td>
<td>1.64</td>
<td>1.47</td>
<td>39.0</td>
<td>32.0</td>
</tr>
<tr>
<td>F6</td>
<td>1.47</td>
<td>1.36</td>
<td>32.0</td>
<td>26.0</td>
</tr>
</tbody>
</table>

CONCLUSIONS
The results of this study reveals that concentration of the binder and particle size of the filler significantly influence the granule properties and resultant tablet properties. According to particle size, flow, consolidation and compressibility properties, F5 formulation which contains Granulac-230 and PVP 10% (w/v) showed optimum granule properties.

REFERENCES

P-102: EFFECT OF SODIUM ALGINATE AND HYDROXYPROPYL METHYLCELLULOSE ADHESIVE POLYMERS ON POLOXAMER BASED MELOXICAM GELS

O. Inal¹, F. Uzunlu²
Ankara University, Faculty of Pharmacy², Department of Pharmaceutical Technology¹, 06100 Tandoğan, Ankara, TURKEY

INTRODUCTION
In this study, two different adhesive polymers; sodium alginate (Na-ALG) and hydroxypropyl methyl cellulose (HPMC) were combined in the same concentration with two different concentrations of P407-MLX gels for modifying the gel texture and in vitro drug release characteristics. The performance of gel bases were evaluated by means of gelation temperature, texture properties, in vitro gel erosion and in vitro drug release studies.

MATERIALS AND METHODS
Poloxamer 407 (P407; Pluronic F-127®, Sigma,USA), Hydroxypropylmethyl cellulose (HPMC; Methocel K100 PRM LV, Dow Chemical Company, Germany; 100 cP), Sodium Alginate (Na-Alg; Sigma; 250 cP), Meloxicam (MLX; Fargem,Turkey).

Preparation of transdermal gels
Transdermal gel formulations were prepared on a weight percentage basis using the "cold method". Briefly, weighed amount of polymers were slowly added to cold water and then stored in a refrigerator (4-5 °C) for 48 hours and mixed with a gentle mixing (100 rpm) in cold environment for 10-15 min at every 12 h periods. MLX was added by weight percentage to the polymeric mixture (Table 1).

Table 1: Contents of gels (% w/w)

<table>
<thead>
<tr>
<th>Code</th>
<th>P407</th>
<th>HPMC</th>
<th>Na-ALG</th>
<th>MLX</th>
<th>Water (q.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>20</td>
<td>0.5</td>
<td>-</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>F2</td>
<td>20</td>
<td>-</td>
<td>0.5</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>F3</td>
<td>26</td>
<td>0.5</td>
<td>-</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>F4</td>
<td>26</td>
<td>-</td>
<td>0.5</td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

Texture profile analyses (TPA)
TPA was carried out with TA.XT Plus Texture Analyzer (Stable Micro Systems, UK) in TPA mode, with P/0.5 probe, depth of 10 mm at a rate of 2 mm/sec (for all test speeds) and delay period of 10 sec between the two compressions (n=3). Gel texture characteristics of the prepared gels were defined from the resultant force-time plot of TPA graph (Table 2) [1].

Table 2: Texture properties of gels

<table>
<thead>
<tr>
<th></th>
<th>H (N)</th>
<th>A (Nsec)</th>
<th>C (Nsec)</th>
<th>Ch</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.400±0.023</td>
<td>1.200±0.152</td>
<td>1.439±0.231</td>
<td>0.924±0.045</td>
<td>0.990±0.008</td>
</tr>
<tr>
<td>F2</td>
<td>0.391±0.012</td>
<td>1.105±0.033</td>
<td>1.354±0.020</td>
<td>0.943±0.015</td>
<td>0.993±0.014</td>
</tr>
<tr>
<td>F3</td>
<td>0.838±0.014</td>
<td>2.438±0.122</td>
<td>2.734±0.124</td>
<td>0.952±0.019</td>
<td>0.992±0.001</td>
</tr>
<tr>
<td>F4</td>
<td>0.786±0.051</td>
<td>0.610±0.026</td>
<td>1.779±0.096</td>
<td>0.921±0.062</td>
<td>0.991±0.001</td>
</tr>
</tbody>
</table>

H: Hardness; A: Adhesiveness; C: Compressibility; Ch: Cohesiveness; E: Elasticity

In vitro gel erosion/drug release studies
In vitro gel erosions and in vitro drug release of MLX were studied with a membraneless method [2] by using 1 gram polymeric solution (+4°C) which was gelled at 37°C in an oven. Results were given with Figure 1 and Figure 2.

RESULTS AND DISCUSSION
Lower values of cohesiveness, indicating spreading of the formulation, and increased adhesion on the
application surface are preferred properties in the in situ gel formulations [4] which was obtained by F3 amongst the studied formulations. All the formulations underwent erosion within 6 hours; however, F3 was the lowest. Also the slowest release was achieved with this formulation. This was attributed to swelling characteristic of HPMC which causes slower release of drug by forming a barrier layer on the surface of gel [3].

CONCLUSIONS
This study indicated that a release of MLX at least for 6 hours, could be achieved with both P407:Na-ALG and P407:HPMC based gel formulations. However, because of better adhesive properties with swelling characteristic which causes slower release of drug by forming a barrier layer on the surface of gel, F3 formulation was found to be more promising for further studies.

REFERENCES

P-103: SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND AMLODIPINE IN PHARMACEUTICALS AND APPLICATION ON DRUG DISSOLUTION STUDIES FOR KINETIC PARAMETERS

C. Kose Ozkan\(^1\), O. Esim\(^1\), S. Kurbanoglu\(^2\), A. Savaser\(^1\), S.A. Ozkan\(^2\), Y. Ozkan\(^1\)

\(^1\)Gulhane Military Medical Academy, Department of Pharmaceutical Technology, Etlik, 06018 Ankara, Turkey
\(^2\)Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Tandogan, 06100 Ankara, Turkey

INTRODUCTION
Statins are the drugs commonly used to treat several forms of hypercholesterolemia. They have potent cholesterol-lowering effects and they could reduce morbidity and mortality associated with coronary heart disease significantly. The fixed dose combination which contains the antihypertensive agent Amlodipine and the statins. Atorvastatin is the first combination of its kind designed to treat two risk factors for cardiovascular disease (1). The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. For highly soluble and rapidly dissolving drug Amlodipine (BCS class 1), a single-point dissolution test specification of NLT 85% (Q=80%) in 60 minutes or less is sufficient as a routine quality control test for batch-to-batch uniformity. For slowly dissolving or poorly water soluble drugs Atorvastatin (BCS class 2), a two-point dissolution specification, one at 15 minutes to include a dissolution range and the other at a later point (30, 45, or 60 minutes) to ensure 85% dissolution, is recommended to characterize the quality of the product. For the BCS class II drugs, the oral absorption is predominantly limited by the solubility and/or dissolution in gastrointestinal (GI) tract. In such case, it would be value of to have an in vitro dissolution test that could be used to predict the in vivo behaviour during the various steps in formulation development. The quantitative interpretation of the values obtained in the dissolution assay is facilitated by the usage of a generic equation that mathematically translates the dissolution curve in function of some parameters related with the pharmaceutical dosage forms (2). The aim of this study is to develop new simple and accurate method, for the simultaneous determination of the combined drug in tablet and in drug dissolution studies. It is suggested that the proposed HPLC procedures could be used for routine quality control
and enlighten the development of new dosage forms studies without any interference from the excipients.

MATERIALS AND METHODS
Amlodipine (AML), Atorvastatin (ATOR) and CADUET® were obtained from Pfizer Pharmaceutical Company (İstanbul, Turkey). All other reagents were either of analytical grade or chromatographic grade. The commercial brand of amlodipine-atorvastatin tablet tested was CADUET® 5/20 mg tablet (AML/ATOR) (Lot.36047) as the test product.

LC system consisting of the following components: a Hewlett-Packard (Avondale, USA) Model 1100 series with a Model Agilent series G-13159 UV detector and Model Agilent 1100 series G-1329 ALS auto sampler and HP chemstation. The Zorbax (100 mm, 4.6 mm, 3.5 μm particle size) column was used for chromatographic separation using an isocratic elution. The mobile phase components were phosphate buffer (pH 5, 0.2%):acetonitrile:methanol (46:27:27, v/v/v) at a flow rate of 1.2 mL min⁻¹. Detector was set at 236 QP$QLQMHFWLRQYROXPHRI—/ZDVXVHGIRUVWXGLHV

Rosuvastatin (ROS) was used as an internal standard. The following parameters were evaluated for dissolution medium; potassium phosphate buffer (pH 6.8), Apparatus 2 (paddle) at a rotation speed of 75 rpm, where these rotation speeds were preset for each apparatus according to U.S. Pharmacopeia and the FDA Guide (2,3). The dissolution medium was heated to 900 mL, and stabilization of solution temperature was 90°C ± 0.5°C and the aliquots were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min. The release data were evaluated according to the different kinetic models, namely zero-order, first-order, Hixson-Crowell, Weibull distribution (RRSBW) function and Peppas equation.

RESULTS AND DISCUSSION
The high selectivity and efficiency of the chromatographic column employed is proven through the system suitability tests such as theoretical plate results, which is approximately 4636 for ROS, 4618 for AML, 6585 for ATOR; the tailing factor 1.08 for ROS, 1.72 for AML, 1.11 for ATOR, selectivity to previous peak 2.67 for AML, 1.61 for ATOR, selectivity to next peak 2.67 for ROS, 1.61 for AML, resolution to previous peak 10.90 for AML, 7.22 for ATOR, resolution to next peak 10.96 for ROS, 7.22 for AML. It was chosen to work with the first concentration level of 5.00 μg/mL of AML and ATOR for being representative of ±5% of the drug dissolved in the dissolution medium, presenting the method to be capable of detecting, AML and ATOR within the linearity range, from 6.11x10⁻⁷ M to 2.45x10⁻⁵ M for AML and 4.48x10⁻⁷ M to 4.48x10⁻⁵ M for ATOR with LOD values of 6.95x10⁻⁸ M for AML and 3.76x10⁻⁸ M for ATOR.

Weak base drugs might exhibit bioequivalence (BE) using existing in vitro dissolution methods like USP dissolution apparatus II. Consequently, the in vitro dissolution results could be used for BCS class II drugs, for newly developed combined dosage forms of Atorvastatin and Amlodipine.

REFERENCES

P-104: SIMULTANEOUS DETERMINATION OF IMATINIB MESYLATE AND DEXKETOPROFEN TROMETAMOL USING UPLC
O. Coban, G.M. Demir, S. Tuna Torunoğlu, Z. Degim, I.T. Degim

Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

A new, simple and precise UPLC (Ultra Performance Liquid Chromatography) method was developed and validated for the simultaneous determination of Dextetoprofen trometamol (DEX) and Imatinib mesylate (IMA) in samples. Proposed method was developed using BEH C-18 column (100 mm×2.1 mm, 1.7 μm) with isocratic elution. A mixture of methanol:acetonitrile (80:20) and pH 9.5, 0.05 M ammonium acetate were (70:30) used as a mobile phase. Detection was carried out at 258 nm, eluation time was 3 minutes. The method was validated considering linearity, accuracy, precision and repeatability.

INTRODUCTION
DEX is used as an analgesic and anti-inflammatory agent [1]. IMA inhibits certain protein tyrosine kinases which are disregulated and overexpressed in number of human cancer cells [2]. UPLC system enables to separate small particles that result in uniquely superior performance with significant improvements in resolution, sensitivity and speed [3]. In this study it was aimed to develop an UPLC method for simultaneous analysis of DEX and IMA in a sample.

MATERIALS AND METHODS
The analysis of the sample was carried out on a Waters Acquity UPLC system equipped with a binary solvent
and a photo diode array detector. 10 mg DEX and 10 mg IMA were weighted, transferred into volumetric flask and add up to 100 ml with water or pH 7.4 phosphate buffer and stirred for 10 minutes.

A variety of mobile phases were used during the development stage of the method for the analysis of DEX and IMA. A mixture of methanol:acetonitrile (80:20) and 0.05 M ammonium acetate (pH 9.5 adjusted with ammonium hydroxide) (70:30) was found to be the most suitable mobile phase [4]. The flow rate of the mobile phase was set to 0.3 ml/min and the column temperature was maintained at 30°C. The detection of the drug was carried out at 258 nm [5]. The total run time was 3 min.

RESULTS AND DISCUSSION
Under these optimized chromatographic conditions, the retention time of DEX and IMA was 0.974 min and 1.362 min in aqueous samples and 1.015 min and 1.481 min in phosphate buffer, respectively. In both of medium, a typical chromatogram was depicted in Figure 1 and Figure 2.

All the validation parameters were obtained and calculated for six replicates. Results were given as means±standart deviations (SD). The calibration curve of analytical method was obtained by considering concentrations versus peak areas. Quite high correlation was obtained ($r^2=0.999$ for both of samples in both of medium) with the concentration range of 0.1-20 μg/ml for DEX and IMA. The accuracy was 80%, 100% & 120% and recovery was found within the acceptable range. System precision was also calculated for ten samples. Intermediate precision was also determined by analyzing the samples different days. Precision values were found to be suitable. Limit of quantitation (LOQ) and limit of detection (LOD) for DEX and IMA were found to be suitable. For all parameters, relative standart deviations (RSD) were lower than 1%.

CONCLUSIONS
A rapid, isocratic UPLC method developed for quantitative analysis of DEX and IMA in samples which is found to be precise, accurate, linear and fast. Satisfactory results were obtained and it is thought to be useful for further studies.

ACKNOWLEDGMENTS
This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, No. 213M675).

REFERENCES

P-105: THE PREPARATION AND IN-VITRO CHARACTERIZATION OF DAIDZEIN-LOADED PLGA NANOPARTICLES
R. Sevinc Ozakar, M. Cetin
Atatürk University, Faculty of Pharmacy
Department of Pharmaceutical Technology, Erzurum, TURKEY

INTRODUCTION
Daidzein (DA) mainly isolated from leguminous plants, is a water-insoluble isoflavone and used in the treatment of hypertension, coronary heart diseases, cerebral thrombosis, and menopause syndrome. Recent studies demonstrated that daidzein could
inhibit the growth of cancer cells by activating a cell death pathway and prevent the onset of diabetes [1]. Our aim was to prepare and characterize DA-loaded PLGA nanoparticles (DA-NPs).

MATERIALS AND METHODS

Materials: Daidzein (LC Laboratories®, USA), PLGA (75:25; RESOMER® RG 756 S, Sigma-Aldrich®, Germany).

Preparation of DA-NPs: The DA-NPs were prepared by emulsion-diffusion-evaporation method. PLGA and DA were dissolved in 2.5 mL of dichloromethane:ethyl acetate (DCM:EA, 1.5:1). The organic phase was dropped (26-G needle) into 1% (w/v) of polyvinyl alcohol (PVA) solution as stabilizer and stirred at 1000 rpm. The resulting emulsion was homogenized at 15000 rpm (10 min) and then, distilled water poured into this emulsion to facilitate diffusion. After evaporation under the the reduced pressure at 40°C, DA-NPs suspension were centrifuged and lyophilized (24 h) [2].

**Drug Content of DA-NPs**

The lyophilized DA-NPs (10 mg) in 15 mL of 1:2 ratio of DCM/DMSO:PB [dimethyl sulphoxide:phosphate buffer pH 7.4; 1:1 (v/v)] in amber vials were stirred for 4 h and later, the organic solvent was evaporated at 45°C. Then, this mixture was centrifuged at 12500 rpm (10 min). The drug content in supernatant of each sample was then measured using a validated UV method at 259 nm. Then, the encapsulation efficiency (EE) and drug loading (DL) were calculated.

**In vitro Release Studies:** An incubation method was used for investigation of DA release from NPs. Lyophilized DA-NPs (10 mg) were suspended in 20 mL of PB (pH 7.4) kept in a shaker water bath with agitation at 50 rpm at 37±0.5°C. At predetermined time intervals, samples (3 mL) were withdrawn from the release medium and replaced with fresh buffer. Prior to analysis, all samples were centrifuged at 12500 rpm (10 min), and their drug content was measured using a validated UV method at 259 nm.

**Surface Morphology and Particle Size:** The surface morphology of DA-NPs was examined by SEM (LEO 440, England). The particle size and zeta potential of DA-NPs were determined by Zetasizer 3000HS (Malvern Instruments, UK).

**RESULTS AND DISCUSSION**

The results of characterization studies are summarized in Table 1. The prepared DA-NPs had almost spherical morphology (Fig. 1). Emulsion-diffusion-evaporation method is suitable for preparation of DA-NPs with high encapsulation efficiency.

<table>
<thead>
<tr>
<th></th>
<th>EE% (n=3)</th>
<th>DL% (n=3)</th>
<th>Particle Size (nm) (n=6)</th>
<th>Zeta Potential (mV) (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>45.00</td>
<td>4.23</td>
<td>672.78</td>
<td>-3.83</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.19</td>
<td>0.01</td>
<td>70.95</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The obtained results demonstrated that nanoparticles made up of PLGA can entrap DA providing a sustained drug release.

**REFERENCES**


**P-106: STUDIES ON POLY (ETHYLENE OXIDE) ELECTROSPUN NANOFIBERS WITH VARIOUS POLYMERS III: SODIUM ALGINATE**

S. Tort, F. Acartürk

Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY
INTRODUCTION
Electrospinning is a method to fabricate nanofiber mats, which have a range of many pharmaceutical applications. Nanofiber mats are useful for tissue engineering, wound healing and drug delivery applications. Alginates, which are naturally occurring substances, found in brown algae and has a protective effect on the mucous membranes. As one of the most extensively studied FDA approved polymer for electrospinning, poly (ethylene oxide) (PEO) has been widely used with/without the addition of different polymers. The aim of this study was to produce PEO/sodium alginate nanofibers via electrospinning and to characterize them for mucoadhesive applications.

MATERIALS AND METHODS
PEO (POLYOX WSR-205, Mw 600,000) and sodium alginate (Protanal LF10/60) were gift from Colorcon and FMC Biopolymer, respectively. Electrospinning process was performed using NE-100 Laboratory Scale Electrospinning Unit, Inovenso LTD, Turkey. PEO nanofibers were prepared from 5%, 6% and 7% PEO solutions. Sodium alginate was used at a constant concentration of 2%. PEO and sodium alginate solutions were mixed at 70:30 volume ratio and stirred for 24 hours at room temperature. The composition of the polymer solutions are shown in Table 1.

Table 1. Composition of polymer solutions for electrospinning

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Polyox WSR-205 (5%)</th>
<th>Polyox WSR-205 (6%)</th>
<th>Polyox WSR-205 (7%)</th>
<th>Protanal LF10/60 (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>S1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>PS1</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>PS2</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 2. Experimental Parameters for Electrospinning Nanofibers

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Feed Rate (mL/h)</th>
<th>Distance of Needle Tip to the Collector (cm)</th>
<th>Applied Voltage (kV)</th>
<th>Process Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0,1</td>
<td>10</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>P2</td>
<td>0,1</td>
<td>10</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>P3</td>
<td>0,1</td>
<td>15</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>PS1</td>
<td>0,3</td>
<td>15</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>PS2</td>
<td>0,5</td>
<td>15</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

Before electrospinning, the viscosity and conductivity of polymer solutions were measured to characterize the solution properties. The viscosity of solutions were measured at room temperature using the Brookfield DV-II Rheometer with spindle type CPE-41. The conductivity of solutions were measured with Hanna Instruments HI-9033 conductivity meter at room temperature. Process parameters for electrospinning of each formulation are shown in Table 2. The morphologies and the mean diameter of the fibers were determined through scanning electron microscopy (SEM). Surface contact angles of nanofibers were measured using sessile drop observation (Attension, Theta Lite). Tensile strength, elongation at break values and mucoadhesion properties of nanofibers were measured with a Texture Analyzer.

RESULTS AND DISCUSSION
Viscosity values of 5%, 6%, 7% PEO solutions were found 3009, 6524, 8770 cPs, respectively. Viscosity increased with increasing of PEO concentration. Viscosity values of PS1 and PS2 solutions were 1547, 3797 cPs, respectively. Viscosity values were decreased after adding of sodium alginate to PEO solutions. Sodium alginate was used at a constant concentration of 2%. PEO and sodium alginate solutions were mixed at 70:30 volume ratio and stirred for 24 hours at room temperature. The composition of the polymer solutions are shown in Table 1.

Viscosity values were increased after adding of sodium alginate solution. PEO was required for electrospinning, because nanofibers could not be obtained from pure sodium alginate solution (S1). Nanofiber diameter of P1, P2, P3 were found 182.31, 194.72 and 277.6 nm, respectively (Pic.1). The electrospinning of 5% (w/v) PEO solutions resulted non-homogenous fibers. Mean diameters of nanofibers PS1 and PS2 were found 193.6 and 211 nm, respectively (Pic.2,3).

Pic 1. SEM images of P2 nanofibers (A:2000x, B:8000x, C:60000x)

Pic 2. SEM images of PS1 nanofibers (A:2000x, B:8000x, C:60000x)

Pic 3. SEM images of PS2 nanofibers (A:2000x, B:8000x, C:60000x)
Elongation at break and tensile strength values of P2 were found to be 144% and 1.32 mPa, whereas these values were found to be 73.6% and 1.01 mPa for PS1. Work of mucoadhesion value of P2 was 0.160 mJ/cm², whereas that value was 0.143 mJ/cm² for PS1. Addition of sodium alginate to PEO solution reduced the mechanical strength and mucoadhesion properties of nanofiber mats.

CONCLUSIONS
The results showed that nanofiber mats, which prepared from sodium alginate and polyethylene oxide, have a potential system for mucoadhesive drug delivery system. Further studies on drug loading to PS1 nanofibers will be investigated.

ACKNOWLEDGMENTS
This study was supported by a “Short Term R&D Funding Program” (112S399) from the Scientific and Technical Research Council of Turkey (TUBITAK).

REFERENCES

P-107: CARBON NANOTUBE MEMBRANE TO STUDY SKIN PENETRATION OF COMPOUNDS

S. Ilbasmis-Tamer, F. Tugcu-Demiroz, I.T. Degim

Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION
In present study, we aimed to develop a synthetic composite membrane which mimics the human stratum corneum (sc) barrier properties which can be used to estimate transdermal delivery and to determine the permeability coefficient of model active substances. The usefulness of this membrane for transdermal applications and the performance were also aimed to be determined. Knowing that sc has lipophilic characteristics involving many transition pathways [1]. We prepared carbon nanotube composite membranes for the estimation of transdermal delivery of compounds through sc. Due to the similarity with sc and some fats and/or protein based ingredients were also added to the CNT membrane to make its composition more similar to the sc.

MATERIALS AND METHODS
MWCNTs (Nanocyl 3101) were purchased from Nanocyl. All other reagents and chemicals were of analytical grade. Ultra performance liquid chromatography (Waters Acquity, UPLC system (MA, USA)) system was used to analyze the amount of the active ingredient which passes through CNT membranes. We selected Dexketoprofen as a model substance. To mimic composition and barrier properties of sc, a variety of lipids (cholestrol, L-Alpha-Dipalmitoyl phosphatidylcholine (DPPC) etc) and different proteins (bovine serum albumin) were added to the CNT membranes. Penetration properties of dexketoprofen were determined using Franz type diffusion cells.

CNT membrane preparation for transdermal application with CNT
20 mg MWCNT was mixed with 1 mL triton X, 100 mL distilled water was added and waited for two minutes in ultrasonic homogenizer. The mixture was then filtered using cellulose nitrate membrane under vacuum and washed with distilled water. Then CNT membrane was formed and inserted in acetone and cellulose nitrate membrane was dissolved. The CNT membrane was dried at 40°C. The prepared CNT membrane was shown in Figure 1.

RESULTS AND DISCUSSION
The membrane used in this study was used as four layer as depicted below.

Penetration of dexketoprofen from CNT membrane, dialysis membrane, selofan membrane, tuffryn membrane, strat-m membrane was determined. Permeability coefficients of model active substance from prepared CNT membrane was compared(Fig 2).
The release of dexketoprofen passed through different membrane from saturated dexketoprofen solution for two days (n=3, mean ±SD).

Active drug was successfully penetrated through to the membrane with a controlled rate. These material was found to be promising being an alternative membrane for the estimating the transitions of chemical through the skin.

CONCLUSIONS
In conclusion in the present work the artificial membranes have been developed to mimic the barrier properties of stratum corneum. After development of suitable membrane, the skin permeability of the prepared formulations can be estimated in advance and researchers will be able to get faster results in future studies. There is numerous works regarding the administration of drugs through the skin therefore this results were found to be helpful.

ACKNOWLEDGMENTS
This work was supported by the Scientific and Technological Research Council (TUBITAK) grant number 114S133.

REFERENCES

P-108: UTILIZATION OF BACTERIAL AND ARCHEAL LIPIDS FOR FORMULATION OF A NEW GENERATION OF LIPOSOMES

Eskandar Moghimipour1,2, Zahra Ramezani2, Mohammad Kargar3, Abdulgani Ameri4, Mahmoood Hashemitabar3, Sadegh Saremy1, Somayeh Handali2*

1Cellular and Molecular Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran
2Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.

INTRODUCTION
Liposomes are spherical vesicles composed of concentric phospholipid bilayers that can entrap drugs. Due to easy access to microorganisms all year round, ability of growth on various substrates and being economic, bacterial lipids can be suitable candidates for preparation of liposomes [1, 2]. Archaeosomes are a novel generation of liposomes that are made from polar ether lipids extracted from the Archaea [3,4]. The aim of the study was to evaluate the exploitation of bacterial liposomes and archaeosomes as drug carriers for the cytoplasmic delivery of molecules in cancer cells.

MATERIALS AND METHODS
This study was performed experimentally on E.coli, Acidianus brierleyi and Sulfolobus acidocaldarius. The lipids were extracted from theses microorganism using chloroform and methanol and analyzed by High Performance Thin-Layer Chromatography (HPTLC). Film method was used for preparing of nano-systems and methylene blue was used as a drug model. Then they characterized by Differential Scanning Calorimetry (DSC), and their particle sizes were determined using particle sizer. The release and permeation of methylene blue was carried out using dialysis membrane and rat skin. Also, trailing them in cancer cells was evaluated by using carboxyfluorescein (CF).

RESULTS AND DISCUSSION
HPTLC analysis of the extracted lipids revealed that the glycerol ether was the major lipids with more than 70 percent probability. The average particle size of E.coli liposomal and archaeosome of A. brierleyi and S. acidocaldarius was 338, 109.83 and 158.33 nm, respectively. The results of DSC indicated the possible interaction of methylene blue with lipids of liposomes and archaeosomes during the preparation. Encapsulation efficiency was 53.3±2.88%, 81.66 ± 2.88% and 61.66±2.88% for bacterial liposome and archaeosome of A. brierleyi and S. acidocaldarius, respectively. Also, there was about 97.54%± 0.00 and 100% release after 24 h for liposomes and archaeosomes, respectively. Liposomes and archaeosome could deliver the carboxyfluorescein to the cancer cells (Fig.1).
CONCLUSION
It is concluded that bacterial and archaean lipids are suitable substitutes for synthetic lipids in formulation of nano-drug delivery systems.

ACKNOWLEDGEMENT
The work was financially supported by Nanotechnology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, grant NO. N-57. We gratefully thank National Iranian copper Industries Co. Sarcheshmeh, Kerman, Iran for their kind cooperation in donating A. brierleyi and S. acidocaldarius.

REFERENCES

P-109: AVAILABILITY OF ARGAN OIL AS LIQUID LIPID IN NLC FORMULATIONS TO IMPROVE SKIN HYDRATION

T.Sen1, U. Badlli1, Ö.İnal1, G.Amasıa2, Ç.Öztürk1, K. Çakır3, N. Tarımcı1

Ankara University, Faculty of Pharmacy, 1Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION
Nanostructured lipid carriers (NLC) are colloidal carrier systems which composed of biocompatible and biodegradable lipids stabilized with surfactants which has the advantage of occlusive properties on the skin (1).
Argan oil, consisting 99 % w/w of triglycerides, is an antioxidant and acts as a free radical scavenger. It has the skin moisturizing activity and claimed to hydrate the skin, neutralize free radicals, heal acne blemishes, help reduce scars, revitalize and improve skin elasticity (2).

The aim of our study was to achieve a synergistic effect of argan oil and NLC on skin. Thus, NLC formulations were prepared by using argan oil as a liquid lipid and Tween 80 as surfactant. Three different concentrations of Tween 80 as 0.5, 1 and 2% and two different argan oil concentrations as 20 and 40% were used. The physicochemical characteristics of NLC formulations were studied. Stability of NLC formulations were also investigated for 1month period.

MATERIALS AND METHODS
Materials: Glycerol tripalmitate (Tripalmitin) was purchased from Sigma Aldrich (USA). Argan oil was a gift from Mecitefendi Cosmetics (TURKEY). Tween 80 was purchased from Loba Chemie Chemicals (India). All the other chemicals and reagents were of the highest purity grade and commercially available.

Preparation of NLC formulations: Tween 80 was added to 30 ml purified water and heated to 70°C in a water bath. Tripalmitin and argan oil were also heated to 70°C. Both water and lipid phase were emulsified by high shear homogenizer (UltraTurrax, IKA) for 5 min. The hot pre-emulsion was immediately homogenized using high-pressure homogenizer (Microfluidics M-110L, USA) applying 1000 bar and five homogenization cycles. Composition of NLC formulations were given in Table 1.

<table>
<thead>
<tr>
<th>Table 1: Composition of NLC formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Tripalmitin (mg)</td>
</tr>
<tr>
<td>Argan oil (mg)</td>
</tr>
<tr>
<td>Tween 80 (mg)</td>
</tr>
<tr>
<td>Purified water (mL)</td>
</tr>
</tbody>
</table>

Physicochemical Characterization of NLC: Zeta potential, mean particle size and polydispersity index (PDI) of the lipid nanodispersions were measured by Zetasizer Nanoseries Nano-Zs (Malvern Instruments, UK), (n=5). Samples were diluted with purified water before analysis.

Stability: Samples were left into climate cabinet for 30 days at 25°C and 60% relative humidity for stability tests.

RESULTS AND DISCUSSION
Zeta potential, PDI and mean particle size results were given in Table 2. As PDI values were found between 0.130±0.015 and 0.244±0.008; all formulations were interpreted to give narrow particle size distribution.
The aim of this study is to achieve a 200 nm particle size. According to the particle size results, only two formulations coded F1 and F3, which consist of 20% argan oil were found suitable. Both F1 and F3 formulations were in proper zeta potential range for stability.

Table 2. Mean particle size, PDI and Zeta potential of NLC formulations

<table>
<thead>
<tr>
<th></th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>181.8 ± 2.69</td>
<td>0.188 ± 0.007</td>
<td>-26.12 ± 0.91</td>
</tr>
<tr>
<td>F2</td>
<td>170.4 ± 1.58</td>
<td>0.211 ± 0.006</td>
<td>-25.80 ± 0.46</td>
</tr>
<tr>
<td>F3</td>
<td>184.1 ± 2.88</td>
<td>0.244 ± 0.008</td>
<td>-30.06 ± 0.97</td>
</tr>
<tr>
<td>F4</td>
<td>177.4 ± 0.94</td>
<td>0.130 ± 0.015</td>
<td>-37.12 ± 0.89</td>
</tr>
<tr>
<td>F5</td>
<td>161.8 ± 1.66</td>
<td>0.180 ± 0.004</td>
<td>-34.14 ± 1.67</td>
</tr>
<tr>
<td>F6</td>
<td>148.6 ± 1.17</td>
<td>0.195 ± 0.008</td>
<td>-24.88 ± 0.96</td>
</tr>
</tbody>
</table>

All the formulations were subjected to a stability test in their dispersion form, and no significant difference was found for the studied parameters after 1 month of stability test.

CONCLUSIONS
As a conclusion, having the nearest mean particle size to our objective, F3 coded formulation containing 20% argan oil and 2% Tween 80 was found to be the most appropriate NLC formulation for topical, dermal and transdermal administration of cosmetics and pharmaceuitics.

REFERENCES

P-110: OCCLUSIVE AND MECHANICAL CHARACTERISTICS OF ETOFENAMATE ENCAPSULATED SEMISOLID SLN FORMULATIONS


Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION
Topical route is one of the major application way for the treatment of not only skin diseases but also the systemic delivery of the several therapeutic agents. Nano-sized drug delivery systems such as solid lipid nanoparticles (SLN) are the most rapidly developing research area today to provide a reduction on the systemic side effects and achieving site specific skin targeting. For these reasons, in recent years, topical form of nano-sized particles have gained much attention for dermal administration [1,2]. In the light of this phenomenon, the purpose of this research is to evaluate the occlusive and the mechanical properties of the developed semisolid SLN formulations of etofenamate (EFM), which is an active non-steroidal anti-inflammatory agent, used several inflammation diseases including rheumatoid arthritis and osteoarthritis.

MATERIALS AND METHODS
EFM was a gift of Santa Farma Drug Company (Istanbul, TURKEY). Compritol 888 ATO and Precirol ATO 5 were kindly provided by Gattefosse (Saint-Priest, France). Semisolid SLN formulations of EFM were prepared through a novel one-step production process as previously described [2]. Two different semisolid SLN formulations were produced by using Compritol 888 ATO and Precirol ATO 5 as different kinds of lipids. Compritol 888 ATO was used at 10% into the SSLN1 coded formulation whereas Precirol ATO 5 was used at 10% into the SSLN2 coded formulation. The developed formulations were characterized by means of entrapment efficiency, particle size, polydispersity index, and surface charge. Occlusive effects and mechanical properties of semisolid SLN formulations and commercial gel product (CGP) of EFM were also investigated. Mechanical properties of SLNs and the CGP were evaluated by using TA-XT plus texture analyzer.

RESULTS AND DISCUSSION
The entrapment efficiencies of EFM in semisolid SLNs were found 99.97±0.65% for SSLN1 and 99.88±0.23% for SSLN2. The mean particle sizes of the semisolid SLNs were obtained 321.4±4.1 and 287.8±5.0 nm with a PDI value of 0.296 and 0.400 for SSLN1 and SSLN2 formulations, respectively. Zeta potential values were determined nearly -30 mV both of the formulations which is considered a stable dispersion. The mechanical properties of the SLNs and the CGP is summarized in Table 1. The texture properties of the topical developed systems informed that the CGP and SSLN1 were have similar hardness, compressibility and adhesiveness behaviours (p>0.05). When two semisolid SLN formulations were compared with each other, SSLN1 showed better mechanical properties by means of cohesiveness and elasticity.
Table 1. Texture profile analysis (TPA) results of the SLNs and CGP (Mean±SD).

<table>
<thead>
<tr>
<th>Mechanical Properties</th>
<th>Investigated Products/Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CGP</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>0.208±0.01</td>
</tr>
<tr>
<td>Compressibility (N.mm)</td>
<td>1.081±0.05</td>
</tr>
<tr>
<td>Adhesiveness (N.mm)</td>
<td>-</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.916±0.01</td>
</tr>
<tr>
<td>Elasticity</td>
<td>0.962±0.01</td>
</tr>
</tbody>
</table>

Fig. 1. In-vitro occlusive effects of CGP and the semisolid SLN formulations.

The occlusive properties of semisolid SLN formulations were investigated by in-vitro occlusion test. As seen in Figure 1, the occlusion factors of SSLN1 and SSLN2 were found to be significantly higher than CGP (p<0.1). The highest occlusive effect was obtained with SSLN1 coded formulation which was prepared using Compritol 888 ATO as lipid (Fig.1).

CONCLUSIONS
It was concluded that the semisolid SLN formulation which was prepared by Compritol 888 ATO showed the best occlusive effect and suitable mechanical characteristics. It can be said that SSLN1 coded formulation is directly relevant to clinical practice.

REFERENCES

P-111: COMPARISON AND EVALUATION OF IN VITRO DIGESTION STUDY OF THE DEVELOPED SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS AND THEIR COMPONENTS

Ş. Akkuş Arslan¹, F. Tırnakızı²

¹Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

INTRODUCTION
Our aim is to formulate two oil/water self-nanoemulsifying drug delivery systems (SNEDDSs) with digestible and non-digestible components composed of oil, surfactant and co-surfactant; to perform in vitro lipolysis experiments on these formulations and the components of the formulations (oil, surfactant and co-surfactant), using bio-relevant media; to understand whether the structures were affected from pancreatic enzymes or not; and to interpret whether the components and formulations could maintain their integrity in vivo.

MATERIALS AND METHODS
Medium chain triglyceride (Labrafac Lipophile WL 1349), polyethoxilated caprylic/capric glyceride (Labrasol) and glycerol mono linoleate (Maisine 35-1) was a generous gift from Gattefosse, France. Monodiglycerides of caprylic acid (Capmul MCM C8) was kindly obtained from ABITEC, USA. PEG-40 hydrogenated castor oil (Cremophor RH40) was kindly supplied by BASF, Germany. Polyoxyethylene oleyl ether (Brij O10), trizma maleate (T3128) and pancreatin (P1750) were purchased from Sigma, USA. Bile salt (B8756) was purchased from Fluka, New Zealand. Egg lecithin (Lipoid E80) was purchased from Lipoid GmbH, Germany. The used water was bidistilled water (Barnstaed Mes UP-1104, Germany). All other chemicals and solvents were of analytical grade and used without further purification.

Lipolysis studies were performed with the components of formulations (each one of the oils, surfactants and co-surfactants) and with the formulations themselves (A and B), to understand whether the structures were affected from pancreatic enzymes or not, and to interpret whether the components and formulations could maintain their integrity in vivo. For this purpose, in vitro lipolysis model of Fatouros et al. [1] was used. Lipolysis experiments were performed in a vessel, which was on a hot plate with magnetic stirring (Snijders-Gemini, Holland). The temperature of the medium maintained at 37±0.5°C throughout the experiment. The pH of the medium was kept constant at pH 6.5 (Jenco6173 pH, China).
RESULTS AND DISCUSSION
The amount of consumed NaOH for digestible components and formulation A was more than the amount of consumed NaOH for non-digestible components and formulation B. The hydrophilic surfactant Cremophor RH40 was thought to have the effect of reducing lipid digestion. Besides, Cremophor RH40 and Brij O10 have an inhibitor effect for lipolysis. As a result of these, formulation A was protected from lipolysis in the gastro intestinal tract (GIT). The formed droplets would maintain their integrity without being digested. Formulation B was digested with lipase in GIT, its droplets were crashed and aceclofenac broke out from the droplets. Especially in lower pH values, a ceclofenac crystallization could recur, and absorption of aceclofenac could be affected negatively. However, oil droplets of formulation A, kept aceclofenac in soluble state in GIT. Lipase could not disrupt these oil droplets. Because the components of formulation A were non-digestible.

CONCLUSIONS
The non-digestible SNEDDS (formulation A) was found more effective than the digestible SNEDDS (formulation B).

REFERENCES

P-112: EFFECTS OF DIFFERENT PREPARATION METHODS ON POLYMERIC MICELLE FORMATION
Z. Sezgin, A.D. Ergin, N.Yuksel
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, TURKEY

Polymeric micelles are colloidal drug carrier systems with unique properties that modify the stability and in vivo efficiency of poorly water soluble drugs [1]. Among a wide range of micelle forming block copolymers polyethylene glycol-lipid derivatives (PEG-PE) are unique because they can form polymeric micelles at very low concentrations and preserve this structure upon in vivo dilution [2]. There are several polymeric micelle preparation methods, this study is aimed to compare these methods and find an optimum protocol for preparing PEG-PE micelles.

MATERIALS AND METHODS
PEG-PE (PEG2000–DSPE) and dialysis membrane (MWCO: 12–14,000) were purchased from Avanti Polar-Lipids (Alabaster, AL/USA) and Spectra/Por (Houston, TX, USA). All other chemicals were of analytical grade preparations.

Preparation of PEG-PE polymeric micelles
Film formation method: A solution of PEG2000–DSPE in chloroform was prepared (Table 1). The organic solvent was removed under vacuum by rotary evaporator and a polymer film was obtained. Micelles were formed by extensively vortexing of this film in water or HEPES buffer (HBS) at 60°C for 15-20 min (FZ1). This formulation was modified by mixing the mixture at 500 rpm in room temperature or 60°C for 2 h (FZ4 -FZ5). Dialysis method: PEG–DSPE was dissolved in dimethyl sulfoxide (DMSO) (Table 1) and dialyzed against ultrapure water for 3 days to remove DMSO and provide micelle formation. Nanoprecipitation method: The polymer solution in tetrahydrofuran (THF) was injected into 2 ml of distilled water stirring at 500 rpm at 35-40°C. The system was mixed overnight to evaporate THF (FZ2). This method was modified by the addition of probe sonication at the dropwise polymer solution addition and heating the system to 60°C for 2 h. The rest of the protocol was followed as given for FZ2. The measurement of particle diameter for polymeric micelles was performed by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK). The mean particle size and polydispersity index (PDI) values were recorded.

Table 1. Preparation methods of the micelle formulations
<table>
<thead>
<tr>
<th>Code</th>
<th>Preparation Method</th>
<th>Copolymer Amount</th>
<th>Dispersion Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>FZ1</td>
<td>Thin film method</td>
<td>15 mg</td>
<td>Water</td>
</tr>
<tr>
<td>FZ2</td>
<td>Nanoprecipitation</td>
<td>15 mg</td>
<td>Water</td>
</tr>
<tr>
<td>FZ3</td>
<td>Dialysis method</td>
<td>20 mg</td>
<td>Water</td>
</tr>
<tr>
<td>FZ4</td>
<td>Thin film (modified)</td>
<td>40 mg</td>
<td>HBS</td>
</tr>
<tr>
<td>FZ5</td>
<td>Thin film (modified)</td>
<td>40 mg</td>
<td>HBS</td>
</tr>
<tr>
<td>FZ6</td>
<td>Nanoprecipitation (modified)</td>
<td>40 mg</td>
<td>Water</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
The formation of polymeric micelles was confirmed by particle size measurements. Micelle formation was observed in modified thin film and nanoprecipitation methods. The micelle size for FZ5 and FZ6 formulations were 163.1±0.613 nm and 128.8±0.407 nm respectively. The PDI values were 0.124±0.011 and 0.160±0.007 for these formulations indicating the narrow size distribution. For the other formulations appropriate size measurements were not obtained. Besides the consequences of micelle preparation methods, the hydration temperature and mixing step were found to be critical process parameters in micelle formation.

CONCLUSION
This study revealed the importance of the preparation method on the formation and size distribution of the...
polymeric micelles. Modified nanoprecipitation method is suggested as a simple, inexpensive and easy method.

ACKNOWLEDGMENTS
This study was financially supported by a grant (112S529) from TUBITAK-Scientific and Technological Research Council of Turkey.

REFERENCES

P-113: THE ASSESSMENT OF EFFECTS TO CELL INVASION, CELL PROLIFERATION AND COLONY FORMATION IN THE BREAST CANCER CELL LINES OF SHVEGF CONTAINING CHITOSAN NANOPLEXES

E. Salva¹, A. Kaymaz²

Inönü University, Faculty of Pharmacy, ¹Department of Pharmaceutical Biotechnology, Malatya, TURKEY

INTRODUCTION
The breast cancer is the most frequent cancer in women. The increase of VEGF level in breast tumors is related to tumor proliferation, invasion, angiogenesis and metastasis. Therefore, treatment towards VEGF is a promising strategy in cancer gene therapy. RNAi is a highly promising technology for gene therapy application in treatment of cancers and shRNAs targeted against VEGF for breast cancer treatment are encouraging [1,2]. The aim of this study is to examine of effects to cell invasion, proliferation and colony forming of shVEGF containing chitosan nanoplexes in breast cancer cell lines.

MATERIALS AND METHODS
shVEGF containing chitosan nanoplexes were prepared in the different N/P ratios (0.5/1, 1/1, 2/1, 5/1, 10/1) and controlled with agarose gel electrophoresis. The in vitro characterization were made (zeta potential, particle size, stability and morphology studies). In the in vitro cell culture studies (MCF-7 and MDA-MB-231), invasion or migration study by invasion assay, colony forming by clonogenic assay, cell proliferation by BrdU assay were examined.

RESULTS AND DISCUSSION
Fullcomplexation was observed at chitosan/shRNA nanoplexes after 0.5/1 N/P ratio. The size and surface charge of nanoplexes were measured around 250 nm and 15 mV. Chitosan efficiently protected the shVEGF from serum and enzyme degradation. In invasion assay, shVEGF containing nanoplexes showed a significant decrease in cell invasion and migration. In BrdU study, cell proliferation by shVEGF containing nanoplexes decreased compared to control. In clonogenic assay, colony number in cells given of chitosan/shVEGF nanoplexes was decreased 65% compared to control.

CONCLUSIONS
We demonstrated growth and invasion inhibiting effects of shVEGF containing chitosan nanoplexes. These results suggested that the delivery of shVEGF by chitosan nanoplexes would be a promising approach for suppression of tumor invasion and proliferation.

ACKNOWLEDGEMENT
This study was supported by TUBITAK (1919B011403542).

REFERENCES

P-114: NANO-CELECOXIB WITH ENHANCED ANTI-INFLAMMATORY EFFICACY

G. Sonmez¹, A.S. Buyuk¹, Fatma Kazdal², Fatemeh Bahadori³

¹Bezmialem Vakif University Faculty of Pharmacy, Undergraduated Student
²Bezmialem Vakif University, Institute of Health Sciences, Biotechnology Master’s Program
³Bezmialem Vakif University Faculty of Pharmacy, Department of Pharmaceutical Biotechnology

INTRODUCTION
Celecoxib (CLX) (Fig. 1) (4-[5-(4-Methylphenyl)-3-(trifluromethyl)pyrazol-1-yl]benzenesulfonamide) is a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID) which inhibits the prostaglandins (PG) especially PGI2[1]. However, PGI2 is a kind of anti-aggregation molecule and the blocking of this pathway results in thrombus and consequently coronary artery disorders or strokes may occur.
Micelles are spherical particles obtained from single or branched chained materials with both hydrophobic and hydrophilic edges called amphiphilic materials. Self assembly of amphiphilic materials in aqueous media results in obtaining aspherical particles consisted of hydrophobic core and hydrophilic shell, where, poorly water soluble drug molecules are being entrapped physically by hydrophobic core of the micelle.

1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG 2000)[2] is a lipid based amphiphilic material which forms Sterically Stabilized Micelles (SSM) in aqueous media (Fig. 2). Drug uploaded nano micelles with a size less than 200 nm can target side of inflammation due to leaky microvascular structure of this side using Enhanced Permeability and Retention (EPR)[3] effect. Sending CLX molecules specifically to the inflamed cells would decrease side effects and enhance its efficacy.

RESULTS AND DISCUSSION
CLX uploaded SSM resulted in formation of particles with 15 nm size. According to our results, the most stable formulation was obtained from uploading 300 µg CLX to 1 mM SSM. The release profile of formulation was calculated by following the amount of CLX released from 3 ml formulation in dialysis tubing membrane inserted in the 300 ml of PBS media. As a result, SSMs are able to carry 50% of CLX for 4 and 80% for 12 hours and the rest is released the 24th hour of the release assay.

CONCLUSIONS
CLX-SSM formulation is a novel formulation for the anti-inflammatory drug Celecoxib which possess ideal size and stability to target the inflammation side. Further investigations are required to evaluate efficacy and toxicity of this formulation in vivo.

REFERENCES
P-115: THE IMPORTANCE OF USING RAMAN SPECTROSCOPY IN PHARMACY

G. Acikgoz1, G. Bora2, B. Hamamci3

1 Mustafa Kemal University, Hatay Vocational Health School of Health Services
2Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology

INTRODUCTION
Spectroscopy techniques play an important role in determining of components in pharmaceuticals. Raman spectroscopy is a versatile tool in pharmaceutics, bio pharmaceutics, and drugs and is ideal for components. Raman spectroscopy is also used in the pharmaceutical analytical laboratory in a variety of ways. However, Raman Spectroscopy is a method which isn’t requires any sample preparation, only a small sample is sufficient of the measurement and not damages to the samples [1].

Spectroscopy operates based on Raman scattering and Infrared absorption processes for detect of the vibration of molecules. These are widely used that to identification of substances from characteristic spectral pattern (“fingerprints”), to provide information about the chemical composition and physical form of the materials and to determine the amount of a substance in a sample quantitatively or semi-quantitatively [2].

MATERIALS AND METHODS
Renishaw in Via Raman Spectrometry was used with a 785 nm laser operating and the CCD (Charge-Coupled Device) detector in this study. Measurements were obtained with the same parameters. These parameters are 785 nm edges, 10 s acquisition times, 10mW laser power, 5x objectives and 1 s exposure time. Raman spectra were measured at range from 100 cm\(^{-1}\) to 4000 cm\(^{-1}\).

RESULTS AND DISCUSSION
Raman Spectroscopy researches provide important advantages in pharmaceutical development such as monitoring of drug concentrations, liquid samples and is an effective way to learn about non-heterogeneous samples with two or three-dimensional mapping [3, 4]. For this purpose we want to examine ethanol and methanol. Parameters for this liquid samples are ideal but it must change for solid samples or drugs. Between 1500 and 400 cm\(^{-1}\) is known the fingerprint region. We found 880 cm\(^{-1}\) (C-O stretching) for ethanol (Fig. 1) and 1030 cm\(^{-1}\) (C-C stretching) for methanol (Fig. 2).

CONCLUSIONS
With the presented fingerprint results, it is feasible to acquire detailed information on the pharmacokinetics of different drugs with the Raman spectroscopic technique.

REFERENCES

P-116: MAGNETIC HALF GENERATION PAMAM DENDRIMERIC NANOPARTICLES FOR TARGETING DELIVERY OF GEMCITABINE

Maryam PARSIANa, Pelin MUTLUP, Serap YALCINc, Aysen TEZCANERa,d, Ufuk GUNDUZa,e

a Department of Biotechnology, Middle East Technical University, Ankara, Turkey
INTRODUCTION
Antitumor activity of Gemcitabine (2’,2’difluorodeoxycytidine), a nucleoside analogue, has been reported in a variety of human tumors, including breast cancer in both experimental and clinical studies. Conventional chemotherapeutic agents are unspecifically distributed all over the body where they affect both cancerous and normal cells. They cause major systemic toxicities and drug resistance, which also restricts drugs therapeutic efficacy. Recent advances in nanotechnology have explored new targeting strategies for enhancing intra-tumoral drug concentrations while limiting the systemic toxicity and side effects. Controlled release of Gemcitabine within cancer cells could be achieved by PAMAM coated magnetic nanoparticles (DcMNPs) which could be targeted to the tumor tissue.

MATERIALS AND METHODS
Bare magnetic nanoparticles were synthesized by co-precipitation methods and were coated by APTS as described by Gupta et al.[1]. The first step involves addition of methylacrylate methanol solution (20%, v/v) to the G0 DCMNPs, and mixing at room temperature for 7 h by ultrasonic water bath or mechanical stirrer [2]. The first step produces a half generation. Various amounts of Gemcitabine in methanol solution were incubated with G4.5, G5.5, G6.5, generation. Various amounts of Gemcitabine in methanol solution were incubated with G4.5, G5.5, G6.5, G7.5 DcMNPs (2.5 mg/ml) for 24 h at room temperature. After the incubation period, Gemcitabine-conjugated DcMNPs were separated by magnetic field and conjugation efficiency was quantified by measuring the absorbance of the supernatant at 269 nm by a UV spectrophotometer. The amount of Gemcitabine conjugated was calculated with the help of standard curve. The amount of Gemcitabine conjugated was confirmed by FTIR, XPS and Z- potential analyses. Cytotoxicity of bare and Gemcitabine conjugated DcMNPs was determined using XTT cell proliferation assay kit.

RESULTS
In this study, we developed half-generation PAMAM magnetic nanoparticles (G5.5), which have carboxyl end groups. As a result these dendrimeric nanoparticles are less toxic. The zetapotential analysis indicated positive charge for full generation DcMNPs and negative charge in half generations. Besides these anionic surface charge of half-generation DcMNPs increased after Gemcitabine conjugation. There was no significant cytotoxic effect of the of bare G5.5 DcMNPs on SKBR-3 and MCF-7 cells in the XTT analysis. On the other hand, as it was previously reported full generation bare DcMNPs have some toxicity (more than 250 μg/ml concentration). Figures 1 demonstrate the dose dependent anti-proliferative effects of Gemcitabine loaded G5.5 DcMNPs on SKBR-3 and MCF-7 cells lines. In MCF-7 cells, IC50 values of Gemcitabine and Gemcitabine conjugated nanoparticles were found as 3.9 μM and 1.1 μM, respectively. IC50 values of Gemcitabine and Gemcitabine conjugated nanoparticles were found to be about 6.5 μM and 1.2 μM for SKBR-3 cells, respectively.

CONCLUSIONS
Gemcitabine conjugated half generation DcMNPs were found as nearly 6 and 3 fold more toxic on SKBR-3 and MCF-7 cells respectively compared to free Gemcitabine. These results showed that Gemcitabine conjugated DcMNPs are more effective over breast cancer cell lines.

REFERENCES

P-117: THE EFFECTS OF DRUG-RESISTANT MCF7 SUBLINES ON CELLULAR PROLIFERATION AND GENE EXPRESSION PATTERN OF DRUG-SENSITIVE MCF7 CELLS

E. G. Seza*, L. Yapındı*, Ç. Urfalı-Mamatoğlu, U. Gündüz
Middle East Technical University, Department of Biological Sciences, Ankara, TURKEY
* Authors contributed equally.
INTRODUCTION
The resistance of tumor cells to structurally and functionally unrelated drugs is named as multidrug resistance (MDR) [1]. Tumors consist of cells with different genetic make-ups. Due to the tumor heterogeneity, some of the cells in a tumor could be drug-sensitive whereas the others could be intrinsically resistant or acquire resistance during the course of chemotherapy. This heterogenous nature of tumors could cause the failure in chemotherapy. The aim of the current study is to investigate the effects of drug-resistant sublines on drug-sensitive MCF7 cells.

MATERIALS AND METHODS
Doxorubicin and docetaxel were kindly obtained from Gülhane Military Medical School, Ankara, Turkey. Zoledronic acid was provided by Novartis, Switzerland. Cell culture: MCF7 cell line was donated by SAP Institute, Ankara, Turkey. Doxorubicin, docetaxel and zoledronic acid resistant MCF7 sublines were developed previously in our laboratory by stepwise selection of cells in increasing drug concentrations [2,3,4]. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS and 0.1% gentamycin at 37°C in a humidified atmosphere with 5% CO2. Drug-resistant cells were treated with corresponding drugs at proper concentrations to maintain resistance. Growth curve analysis: The effects of drug-resistant MCF7 cells on the growth rate of drug-sensitive MCF7 cell line were investigated by mimicking co-culture conditions. To this end, 2x10^5 drug-resistant and drug-sensitive MCF7 cells were separately seeded in 6-well plates. Medium was collected from drug-resistant cells, centrifuged at 1000 rpm for 5 min and supernatant, also called as conditioned medium, was used to treat drug-sensitive MCF7 cells. At each 24 h interval, drug-sensitive MCF7 cells were trypsinized and counted to plot a growth curve. The results were analyzed by one-way ANOVA followed by post-hoc Tukey's test in GraphPad Prism 5.0 software (GraphPad Inc, USA).

RESULTS AND DISCUSSION
The growth rate of drug-sensitive MCF7 cells significantly increased when they were treated with conditioned medium obtained from doxorubicin and docetaxel resistant MCF7 sublines. Cellular proliferation was significantly higher even in the absence of FBS which is essential for cell growth. However, the growth rate of MCF7 cells was not significantly affected when they were treated with conditioned medium obtained from zoledronic acid resistant MCF7 cells.

CONCLUSIONS
Drug-resistant MCF7 cells could secrete various growth stimulators such as growth factors and cytokines, into the medium which may stimulate the growth of drug-sensitive cells and increase the expression of multidrug resistance related genes. Further analyses are required to identify the exact nature of biomolecules of resistant cells in the secretions which can potentially enhance cellular proliferation of others.

REFERENCES

P-118: INVESTIGATION OF THE EFFECT OF VIMENTIN ON MULTIDRUG RESISTANCE IN CANCER
Ö. Akbulut, H. İzgi, N. Çakmak, Ç. Urfal-Mamatoğlu, U. Gündüz
Middle East Technical University, Department of Biological Sciences, Ankara, TURKEY

INTRODUCTION
Multidrug resistance (MDR) is the resistance of tumor cells to structurally and functionally unrelated anticancer agents [1]. MDR could be developed via several mechanisms, however, the most important MDR mechanism is the increased drug efflux by ATP-binding cassette (ABC) transporter proteins. The ABC transporters act as pumps and prevent the anticancer drugs to reach lethal concentrations in the cells. The most important ABC transporters are P-glycoprotein (P-gp/MDR1) and MRP1. Vimentin is a Type III intermediate filament. Vimentin is overexpressed in breast cancer cell lines and associated with the increased migratory and invasive activities of cancer cells [2,3]. In doxorubicin resistant MCF7 (MCF7/Dox) cells, the expression levels of vimentin and MDR1 are elevated [3]. In the current study, the effect of the silencing of vimentin gene, VIM, on the expressions of MDR1 and MRP1 was investigated.

MATERIALS AND METHODS
Doxorubicin was kindly obtained from Gülhane Military Medical School, Ankara, Turkey. MCF7 cell line was donated by SAP Institute, Ankara, Turkey. Doxorubicin resistant MCF7 (MCF7/Dox) subline...
was developed previously in our laboratory by stepwise selection of cells in increasing drug concentrations [4]. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS and 0.2% gentamycin at 37°C in a humidified atmosphere with 5% CO₂.

MCF7/Dox cells were transfected with either VIM siRNA (Qiagen, Germany) or control siRNA (Santa Cruz Biotechnologies, USA). After transfection, total RNA was isolated from MCF7/Dox cells, cDNA was synthesized and VIM, MDR1 and MRP1 gene expression levels were examined by qRT-PCR. The results were analyzed by 2⁻DDCt method [5]. The effect of vimentin silencing on doxorubicin resistance in MCF7/Dox cells was examined by XTT cell proliferation assay after transfection with VIM or control siRNA. The results were analyzed by one-way ANOVA followed by post-hoc Tukey's test in GraphPad Prism 5.0 software (GraphPad Inc, USA).

RESULTS AND DISCUSSION
Gene expression analyses showed that siRNA transfection successfully silenced the VIM expression in MCF7/Dox cells. It was observed that the silencing of VIM gene did not have any effect on MRP1 expression. However, MDR1 expression was significantly elevated after VIM silencing. Cell proliferation analyses demonstrated that the silencing of vimentin did not alter IC₅₀ value of doxorubicin in MCF7/Dox cells, indicating that vimentin expression was not directly related with the doxorubicin resistance.

CONCLUSIONS
Vimentin expression in MCF7/Dox cells is associated with the increased invasive and migratory activity, but not drug resistance.

ACKNOWLEDGEMENTS
This project is supported by TUBITAK 2209/A Undergraduate Students Research Support Programme.

REFERENCES

P-119: THE EFFECT OF THYROID HORMONE-INSULIN INTERACTION ON DIABETIC RAT AORTA
Arioglu-Inan E., Ozakca I., Kayki-Mutlu G., Karaomeriouglu I., Altan V.M.

Ankara University, Faculty of Pharmacy, Department of Pharmacology, Ankara, TURKEY

INTRODUCTION
Diabetes has been shown to cause diminished beta adrenergic responses both in heart and vasculature [1,2]. Insulin treatment has been found to reverse this impairment in diabetic rat heart only in the presence of adequate thyroid hormone [3]. On the other hand, this interaction of insulin-thyroid hormone has not been studied in diabetic aorta. Thus, the aim of this study was to determine the effect of insulin-thyroid hormone interaction in chronic diabetic rat thoracic aorta in terms of beta adrenergic responsiveness.

MATERIALS AND METHODS
Male Sprague-Dawley rats were divided into 7 groups; Control (C), Diabetic (D), Thyroidectomized Diabetic (TxD), Insulin treated Diabetic (D1), Insulin treated Thyroidectomized Diabetic (TxD-I), low dose T₃ treated Thyroidectomized Diabetic (TxD-I-T₂.₅), high dose T₃ treated Thyroidectomized Diabetic (TxD-I-T₅). First, thyroidectomy was conducted, then diabetes was induced with 38mg/kg streptozotocin. At the end of 6-week-diabetes period some groups (D-I, TxD-I) were treated with insulin (5-20U/kg/day, subcutan), some groups (TxD-I-T₂.₅, TxD-I-T₅) insulin + thyroid hormone combination (2.5 or 5ug/kg/day T₃ via osmotic minipump) for 2 weeks. Beta adrenoceptor mediated effects were studied in thoracic aorta. The aorta strips were constricted with Phenylephrine (0.01uM-30uM). The relaxation reponses were evaluated by using acetylcholine (0.01uM-30uM), isoprenaline (0.001uM-30uM) and BRL 37344 (0.01nM-1uM).

RESULTS AND DISCUSSION
Phenylephrine mediated contraction response was augmented in diabetic group, however, it was diminished significantly in TxD groups (Emax (mg): 75 TxD-I-T₂.₅, 75 TxD-I-T₅). These results are not suprising, since the effect of thyroid hormone on contractile response has been reported before. Isoprenaline mediated relaxation was markedly decreased in diabetic groups (Emax (%): C, 96.2±1.6; D, 482±48; TxD-I, 613±19; TxD-I-T₂.₅, 752±94). The response was normalized in D-I and TxD-I-T₃ groups (D-I, 1018±39; TxD-I-T₃, 943±51). These results are not suprising, since the effect of thyroid hormone on contractile response has been reported before.

Isoprenaline mediated relaxation was markedly decreased in diabetic groups (Emax (%): C, 96.2±1.6;
D, 82.0±2.3; Tx-D, 61.8±4.9; TxD-I, 74.1±5.6; TxD-I-T2.5, 81.7±2.1). This response was improved in in D-I and TxD-I-T5 groups (D-I, 85.6±1.6; TxD-I-T5, 92.7±2.2). These findings show that beta 2 adrenoceptor mediated relaxation could not be corrected with insulin in the absence of thyroid hormones. Beta 3 adrenoceptor preferential agonist BRL 37344 caused dose dependent relaxation in all groups. The response was increased in diabetic groups (Emax (%): C, 19.9±1.8; D, 32.5±1.3; Tx-D, 30.8±6.8; TxD-I, 30.8±3.5; TxD-I-T2.5, 26.9±9.6). Although it was normalized in D-I and TxD-I-T5 groups (D-I, 22.4±3.1; TxD-I-T5, 20.7±3.1), the response was not statistically significant. The role of beta 3 adrenoceptor mediated responses in the diabetic rat aorta and the effect of insulin-thyroid hormone interaction on it could not be explained with these data, thus more studies should be performed.

CONCLUSIONS
These results show that adequate thyroid hormone levels are essential for improving effects of insulin on beta adrenoceptor mediated relaxation response in diabetic rat aorta.

ACKNOWLEDGMENTS
This study was supported by a grant from TUBITAK (110S179).

REFERENCES

P-120: EFFECTS OF TRIMEBUTINE MALEATE ON VASCULAR SMOOTH MUSCLE OF THE ISOLATED RAT THORACIC AORTA
E.N. Gazioglu1, M. Saglam2, S. Engin1, M. Kadioglu-Duman2
1Karadeniz Technical University, Department of Pharmacology, Faculty of Pharmacy, 61080 Trabzon, TURKEY
2Karadeniz Technical University, Department of Pharmacology, Faculty of Medicine, 61080 Trabzon, TURKEY

INTRODUCTION
Trimebutine maleate (TMB) has been efficiently used for the treatment of functional gastrointestinal disorders, such as irritable bowel syndrome [1,2]. The effect of TMB on gastrointestinal tracts is mediated by its calcium and potassium channels modulating action [3]. Effect of TMB on thoracic aorta contraction is not clarified. In the present study, we aimed to investigate the action of TMB on phenylephrine-induced vascular smooth muscle contractions of the isolated rat thoracic aorta.

MATERIAL AND METHODS
The rat thoracic cavity was opened under anesthesia. The descending thoracic aorta was immediately dissected and the adherent tissues were removed. Strips of about 0.5 cm wide and 0.7 cm long were prepared form each aorta. The strips were immediately mounted in an organ bath containing 30 mL Krebs-Henseleit physiological solution. Each of the thoracic aorta preparations was suspended with one end tied to a holder and the other end attached to an isometric force transducer (MAY FDT10A) connected to BIOPAC MP 100 system. The tissues were incubated in water-jacketed organ baths containing Krebs-Henseleit solution at a temperature of 37°C and bubbled with 95% O2 and 5% CO2 throughout the experiments. The tissues were allowed to equilibrate for 60 minutes with a resting tension of 1 g. At the end of duration period; Phenylepherine (PE, 10-5 M) was used to test the contractility of tissues. Cumulative acetylcholine (10-8 – 10-3 M) dose-response relaxation curves were obtained on precontracted tissues with PE to investigate whether the endothelium is intact or denuded. At the first stage of the experiments, different TMB doses (10μM and 50μM) were added to the bath to test effects of TMB on the precontracted tissues with PE. At the next stage of the experiment, TMB (10μM, 50μM and 100μM) were added to the organ baths. After the incubation period with different TMB doses, PE (10-6 – 10-4 M) was added to the bath to test inhibitory effect of TMB on PE induced contractions.

RESULTS AND DISCUSSION
TMB (10μM and 50μM) caused relaxations on PE precontracted strips in endothelial intact preparations (11% and 44%, respectively). The drug also caused relaxations on PE precontracted strips in denuded preparations. Our findings suggest that TMB have relaxant effect on rat thoracic aorta tissues. As the relaxations induced by TMB is observed in both endothelial intact and denuded preparations, it is thought that this effect is independent from endothelium. The drug (10μM, 50μM and 100μM) also caused a decrease in the cumulative PE induced contractions dose dependently (36%, 71% and 90%, respectively). This vasorelaxant effects may be due to its adrenergic receptor blocking action as TMB.
attenuated the contractions caused by PE via α1 adrenergic receptors. This effect also can be due to calcium channel blocking action of TMB.

CONCLUSIONS
Hypertension, which is defined as chronically high blood pressure, is a major risk factor for cardiovascular morbidity and mortality [4]. Vasorelaxant agents have been efficiently used for treatment of hypertension. Therefore, TMB may be evaluated as effective drug for the treatment of hypertension. However further investigations is needed to reveal the certain vasorelaxant mechanism of TMB.

REFERENCES

P-121: THE EFFECTS OF IN VIVO L-NAME TREATMENT ON CARDIAC TROSPONIN I PHOSPHORYLATIONS IN RATS
L.Ozakça, M. Cicek, V.M. Altan, A.T. Ozcelikay
1Ankara University, Faculty of Pharmacy, Department of Pharmacology, Ankara, TURKEY

INTRODUCTION
It is well known that systemic long term (>4 weeks) Nω-nitro-L-arginine methyl ester (L-NAME, nonselective NOS inhibitor) treatment induces an experimental hypertension model which depends on the inhibition of nitric oxide/cyclic GMP (NO/cGMP) signaling pathway [1]. Vasodilator-stimulated phosphoprotein (VASP) and troponin I (Tn I) both have target phosphorylation sites for protein kinase G (PKG) which is activated by NO/cGMP signaling pathway [2, 3]. It is hypothesized that PKG inhibition induced by in vivo L-NAME treatment can be detected by the decrease in phosphorylated VASP and Tn I on PKG-regulated sites. In the present study, the effects of short term (14 days) L-NAME treatment on the phosphorylation levels of VASP and Tn I were examined to assess the cardiac NOS inhibition more directly.

MATERIALS AND METHODS
Male 8-weeks-old Sprague-Dawley rats randomized into four groups according to the treatment with L-NAME (20, 50 and 100 mg.kg⁻¹.day⁻¹ in drinking water; L20, n=4; L50, n=4; L100, n=5) or placebo (C, n=5) for 14 days. Systolic blood pressure measurements were performed by tail-cuff method at the beginning (0D) and at the end of the treatment (14D). At the end of the in vivo protocol, rats were sacrificed and the left ventricular tissue samples were isolated. The alterations of phosphorylation status of VASP from Ser239 and of TnI from Ser23/24 were detected from heart lysates by western blot analysis.

RESULTS AND DISCUSSION
L-NAME treatment increased the systolic blood pressure in a dose-dependent manner indicating the inhibition of NOS activation in vivo (Fig. 1A). Any dose regimens of L-NAME treatment for 14 days did not induce any change in the ratio of left ventricle weight to total heart weight which is used as an index for cardiac mass (C, 0.54±0.02; L20, 0.52±0.04; L50, 0.54±0.04; L100, 0.54±0.04, p>0.05). The levels of Ser239 phosphorylation of VASP were found to be unchanged compared to control in all L-NAME treated groups (C, 1.00±0.09; L20, 0.95±0.11; L50, 1.00±0.10; L100, 0.95±0.09, p>0.05). Surprisingly, the Ser23/24 phosphorylation of Tn I levels increased significantly in response to L-NAME treatment in a dose-dependent manner (Fig. 1B).

CONCLUSIONS
These data suggest that the duration (14 days) of in vivo L-NAME treatment may not be sufficient to detect the inhibition of cardiac NOS and/or PKG activity by the assessment of pVASP/VASP and pTnI/TnI. In addition, based on the Ser23/24 phosphorylation of Tn I by both protein kinase A (PKA) and PKG, it can be suggested that the possible alterations induced by 14-day L-NAME treatment in cardiac excitation-contraction coupling can be compensated by the effects of different kinases including PKA.
ACKNOWLEDGMENTS
This study was supported by a grant from TUBITAK (114S563).

REFERENCES

P-122: INHIBITION OF MAMMALIAN TARGET OF RAPAMYCIN REVERSES LIPOPOLYSACCHARIDE-INDUCED HYPOTENSION AND INFLAMMATION IN RATS
M. Temiz1, S.P. Senol1, D.S. Guden1, P. Cecen1, A.N. Sari1, B. Tunctan2, A. Gorur3, L. Tamer3, C.K. Buharalioglu2, S. Sahan-Firat1

1 Mersin University, Faculty of Pharmacy, Department of Pharmacology, Mersin, TURKEY
2 International Cyprus University, Faculty of Pharmacy, Department of Pharmacology, Nicosia, CYPRUS
3 Mersin University, Faculty of Medicine, Department of Biochemistry, Mersin, TURKEY

INTRODUCTION
The mammalian target of rapamycin (mTOR) is a serine-threonine kinase that regulates both cell growth and cell cycle progression through its ability to integrate signals from nutrient and growth factor stimuli. Dysregulated mTOR signaling has been implicated in major diseases including cancer, neurodegeneration, diabetes and inflammation [1]. Septic shock is a systemic inflammatory response, which is mostly due to Gram-negative bacteria, and has been reported as the primary cause of mortality in critical care units [2]. Lipopolysaccharide (LPS) is a well-characterized pathogen-associated molecular pattern (PAMP) found in the outer membrane of Gram-negative bacteria that interacts with toll-like receptor (TLR)-4 and is used to induce experimental septic shock model [3]. Although the role of mTOR in TLR-4 signalling is known in different cell types [5], the role of this enzyme in LPS signalling still remains unclear in vivo. The aim of this study is to determine the contribution of mTOR to LPS-induced hypotension and inflammation associated with production of vasodilator and pro-inflammatory mediators.

MATERIALS AND METHODS
Male Wistar rats were randomly divided into saline (n=8), LPS (n=8), rapamycin (n=8) and LPS+rapamycin (n=8) groups. Endotoxic shock was induced in rats as previously described by Tunctan et al [4]. Saline (4 ml/kg, i.p.) and LPS (10 mg/kg, i.p.) was administered at time 0 and rapamycin (1 mg/kg, i.p.) was administered 1 h after saline or LPS injection. At time 0 and 1, 2, 3 and 4 h after injection of saline or LPS mean arterial pressure (MAP) and heart rate (HR) were measured using a tail-cuff device. At the end of the experiments, rats were sacrificed and blood, kidney, heart, thoracic aorta and superior mesenteric artery were collected. Nitrite, nitrotyrosine, TNF-α and 6-keto-PGF1α levels and myeloperoxidase (MPO) enzyme activity were measured in tissue samples and/or sera.

RESULTS AND DISCUSSION
LPS caused a gradual fall in MAP and an increase in HR in rats. MAP fell by 34 mmHg and HR rose by 120 bpm in rats treated with LPS. After 4 h from LPS administration nitrite, nitrotyrosine, TNF-α, 6-keto-PGF1α levels and MPO activity were increased in sera and/or kidney, heart, thoracic aorta and superior mesenteric arteries. These changes caused by LPS reversed with a specific mTOR inhibitor, rapamycin administration. Rapamycin alone had no effect either on MAP and HR or on nitrite, nitrotyrosine, TNF-α and 6-keto-PGF1α levels and MPO activity.

CONCLUSIONS
Our results demonstrated that LPS-induced decrease in blood pressure, increase in HR and inflammation presumably due to enhanced production of nitric oxide, peroxynitrite, prostacycline, TNF-α levels and MPO activity were mediated by mTOR activation.

ACKNOWLEDGMENTS
Financial support was provided by grant from the Research Foundation of Mersin University (BAP-SBE FB (MT) 2014-1 YL).

REFERENCES
P-123: P-COUMARIC ACID INDUCES ANTIINOCICEPTION IN MICE

N. Bektas1, R. Arslan1, D. Nemutlu1

1Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskisehir, Turkey

INTRODUCTION

Although there are many drugs to relieve pain, many patients apply to herbal treatment as complementary or alternative treatments. The interest in the management of pain with herbal medicines that have less adverse effects and stronger therapeutic effects is growing in modern medicine. In this respect, many plants that comprise phenolic compounds draw attention in pain management. p-Coumaric acid (p-CA) is a plant secondary metabolite and found in considerable amounts in the human diet. The studies about its analgesic effect are too limited and conflicting [1;2]. In this sense, the central analgesic effect of p-CA at the doses of 25, 50 and 100 mg/kg (p.o.), has been evaluated with tail-immersion and hot-plate tests time dependently, peripheral analgesic effect in acetic acid-induced writhing test in mice. The effectiveness of p-CA compared with chlorogenic acid (CGA) at the same doses, which one of the most investigated phenolic

MATERIALS AND METHODS

Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals and approved by the Local Ethics Committee of Anadolu University, Eskisehir.

Tail-immersion: An area of the tail of mice (Swiss albino) was immersed in the water bath (52.5° ± 0.2°C), (Heto-Holten, Allerod, Denmark). The withdrawal time of the tail from hot water was noted as reaction time. The maximum cut-off time for immersion was 15 s [3].

Hot-plate test: The mice were put on the surface of Hot Plate Analgesia Meter (No. 7280, Ugo Basile Instruments, Comerio, Italy), set to 55 ± 0.5 °C. The latency of hind paw licking, hind paw flicking, or jumping was measured as reaction time. The cut-off time was taken as 20 s [3].

Acetic acid-induced writhing test: Mice were injected with 10 ml/kg of 0.6% acetic acid solution (i.p.) 45 min after the administration of the phenolics. 5 min after the administration of acetic acid, the number of writhes was counted for 10 min [3].

RESULTS AND DISCUSSION

p-CA at the doses of 50 mg/kg (6.351±1.937; P<0.05;45min; 6.397±1.309; P<0.01;60min) and 100 mg/kg (6.727±1.108; P<0.05;30min; 9.101±1.275; P<0.001;45min; 9.563±1.753; P<0.001;60min) enhanced the response latency against thermal stimulus significantly in tail-immersion test and reduced the writhing number (13.33±1.174; P<0.01, 8.167±2.272; P<0.001) as CGA. However only the dose of 100 mg/kg p-CA succeeded (8.350±0.8609; P<0.05;45min; 7.609±0.9715; P<0.05;60min) to enhance the thermal threshold and not as potent as CGA in hot-plate test. All doses of CGA showed effectiveness in all methods [(25 mg/kg: P<0.05;45min; P<0.01;60min), (50 mg/kg: P<0.01;45min: P<0.001;60min), (100 mg/kg: P<0.05;30min; P<0.01;45 min; P<0.001;60min; P<0.01;90min) in tail-immersion test. (25 mg/kg: P<0.05;45min; P<0.01;60min), (50 mg/kg: P<0.01; 45 and 60min, P<0.05; 90min), (100 mg/kg: P<0.001; 30, 45, 60 and 90min) in hot-plate test. (25mg/kg: 14.71±1.742; P<0.05, 50mg/kg; 12.71±1.190; P<0.01, 100mg/kg; 9.571±1.066; P<0.001) in writhing test]. Moreover, the acting time seems to be much longer by administration of CGA.

According to results, p-CA shows analgesic activity centrally and peripherally. This analgesic action of p-CA similarly potent as CGA, however, the effectiveness may not reach the same degree in the hot plate test that assesses the supraspinal organized response of pain [4]. Thereby it is possible to say that these phenolics utilize dissimilar pathways and/or mechanisms to induce analgesia. As a consequent, p-CA is hopeful for developing natural originated analgesics and may be useful in the management of various acute or chronic pain conditions. However, further investigations are necessary to lighten the mechanism of action and to evaluate in which conditions p-CA may be used.

CONCLUSIONS

These claims may be proved by further studies and also their pharmacological effect profile can be determined via elucidation of the mechanism of peripheral antinociceptive action.
ACKNOWLEDGMENTS
This study is a part of the project which is supported by the Anadolu University Scientific Research Projects Unit (Project No: 1105S085), Anadolu University, Eskisehir, Turkey. The investigations are still in process by our team.

REFERENCES

P-124: PHARMACOGENOMICS OF CANTHARIDIN IN TUMOR CELLS

O. Kadioglu1, N. Salehi Kermani2, G. Kelter3, U. Schumacher2, H.-H. Fiebig3, H.J. Greten4,5, T. Efferth1

1Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Pharmaceutical Biology Department, Mainz, Germany
2Department of Anatomy and Experimental Morphology, University Hospital Hamburg-Eppendorf, Hamburg, Germany
3Oncotest GmbH, Institute of Experimental Oncology, Freiburg, Germany
4Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal
5Heidelberg School of Chinese Medicine, Heidelberg, Germany

INTRODUCTION
Cantharis vesicatoria (blister beetle) has been categorized as highly toxic in the Chinese pharmacopeia. Cantharis patches have been used in Chinese medicine and in Europe since ages to treat various skin-related diseases.

MATERIALS AND METHODS
We investigated the cytotoxicity of the Cantharis ingredient, cantharidin, in 41 tumor cell lines (Oncotest panel) and compared the results with those of 60 cell lines of the National Cancer Institute, USA. Using a PCR array for 84 apoptosis genes, we checked the deregulated genes upon cantharidin treatment. In silico molecular docking analyses were performed on protein phosphatase 1β and 2A.

RESULTS AND DISCUSSION
We found profound activity at low micromolar concentrations (log_{10}IC_{50} values between -6.980 and 5.009 M). Cantharidin bound to protein phosphatase 2A (PDB ID: 3DW8) with higher affinity (~8.12 kcal/mol) than to protein phosphatase 1β (PDB ID: 1S70) (~6.25 kcal/mol) in molecular docking analyses. Using a PCR array for 84 apoptosis genes, cantharidin treatment up-regulated gene expression of caspase-1 and nerve growth factor receptor, but down-regulated mRNA expression of Bcl-2 like protein 10, Fas ligand, and tumor necrosis factor-α. By using COMPARE analysis of microarray-based transcriptome-wide mRNA expressions, 21 genes were found to significantly correlate with response of 60 tumor cell lines to cantharidin. As shown by hierarchical cluster analysis and chi-squared test, the distribution of cell lines in the dendrogram according to their gene expression profiles predicted sensitivity or resistance to cantharidin (P = 6.482 × 10^{-5}). The compassionate use of Cantharides patches in two patients suffering from basalioma and Mycosis fungoides, respectively, considerably improved the diseases without signs of toxicity (Fig. 1).

CONCLUSIONS
In conclusion, these results indicate that cantharidin may be a useful candidate to develop novel strategies for cancer therapy.

ACKNOWLEDGMENTS
We are grateful to the Ph.D. position of the Johannes Gutenberg University (Mainz, Germany) for Onat Kadioglu.

REFERENCES

P-125: EFFECTS OF AGMATINE ON THE EXTINCTION AND REINSTATEMENT OF NICOTINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

O. Allahverdiyev1, S. Özyazgan2, G. Akkan2

Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology2, Van, TURKEY
Istanbul University, Cerrahpasa Medical Faculty, Department of Medical Pharmacology2, Istanbul, TURKEY

INTRODUCTION
Traditional pharmacological treatments for drug dependence aim to reduce three most important aspects: craving, relapse and withdrawal syndrome. Pharmacological treatments presently available for the treatment of tobacco smoking are able to alleviate withdrawal symptoms but are not adequately effective in reducing craving and occasional effective to prevent relapse. Lately smoking (nicotine) is considered as most important health problem in the world (1). Conditioned place preference (CPP) continues to be one of the popular models to study the motivational effects of drugs and non-drug treatments in experimental animals. The aim of the present study was to examine the effect of agmatine on nicotine induced-CPP in male Sprague Dawley rat. It is a polyamine that is produced via decarboxylation of l-arginine by the enzyme arginine decarboxylase. Agmatine has several mechanisms. It binds to various receptors and has been accepted as a novel neurotransmitter in brain. In experimental studies, agmatine indicated antinociceptive, anxiolytic anticonvulsant and antidepressant like actions (2). It also binds with high affinity to all subclasses of 2-adrenoceptors and imidazoline binding sites (3). It has also been found that agmatine inhibits nitric oxide synthase (NOS) in rodents (4). In this study, agmatine’s effects on nicotine dependence, extinction, reinstatement and locomotor activity were evaluated.

MATERIALS AND METHODS
The day after the pre-conditioning session, the conditioning phase took place, which was designed as an unbiased, counterbalanced, CPP schedule that included eight 40-min daily conditioning sessions. Conditioned place preference procedure consisted of 6 phases: Habituation (5 minutes), pre-test (15 minutes), conditioned (40 minutes; sessions were conducted twice each day with an interval of 6–8 h for 3 consecutive days; three saline and three drug pairing), post-test (15 minutes), extinction (15 minutes) and relapse (15 minutes) phases. Nicotine (0.175 mg/kg, s.c.), agmatine (0.75 or 10 mg/kg, s.c.) and saline were administered on day 12th, 16th and 20th. Animals were tested for 15 minutes on 12th, 16th and 20th day. On day 21st the control and nicotine rats were given saline whereas, remaining groups followed agmatine (0.75 or 10 mg/kg, s.c) 15 minutes prior to single dose of nicotine (0.175 mg/kg, s.c) and were tested for 15 minutes. For all of these phases, the mice were tested during the same time period each day (0900–1400 hours). Rats showing a strong unconditioned preference (>66 % of the session, i.e. 600 s) or aversion (<33 % of the session, i.e. 300 s) for either compartment were excluded from the study. Locomotor activity was assessed at the end of the experiment (5). The statistical analyses were performed using repeated measure analysis of variance (ANOVA), post hoc comparisons were carried out with Newman–Keuls tests. Statistical significance was set at p<0.05.

RESULTS AND DISCUSSION
Nicotine alone produced significant CPP and increased the locomotor activity (p<0.05). High-dose agmatine precipitated extinction and also prevent reinstatement (10 mg/kg, p<0.05). Neither dose of agmatine alone produced CPP nor change the locomotor activity (p>0.05). Agmatine inhibited nicotine-induced locomotor activity (10 mg/kg, p<0.05).

CONCLUSIONS
Agmatine showed increase in elimination of nicotine induce extinction and prevented reinstatement. In subsequent studies, agmatine using NOS activators, alpha 2-adrenergic receptor and imidazole receptor antagonists may be apposite to identify mechanisms involved in nicotine addiction.

REFERENCES
P-126: THE ROLE OF VITEXIN AND QUERCETIN IN THE CARRAGEENAN-INDUCED TAIL THROMBOSIS MODEL IN MICE

R. Arslan1, E. Sümbül1, N. Bektas1, Ö. Özkan1,2

Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskisehir
Ege University, Faculty of Pharmacy, Department of Clinical Pharmacy, Izmir
TURKEY

INTRODUCTION

Thrombosis is a major cause of cardiovascular diseases (CVDs) like ischemia, myocardial infarction and angina. In the past decade, these diseases has been the most important causes of death in the world (1; 2). Various medicinal plants have been used for the treatment of CVDs and these plants involve some bioactive compounds like flavonoids which are responsible for their pharmacologic activities. It is informed that flavonoids show analgesic, antioxidant, anti-inflammatory, antiplatelet, antithrombotic and anti-allergic effects (3, 4). The present study is focused on investigating the effects of antithrombotic effects of vitexin and quercetin.

MATERIALS AND METHODS

Antithrombotic effects of vitexin and quercetin were investigated by using carrageenan-induced (1% type I) tail thrombosis model. After carrageenan administration, the vitexin and quercetin were injected (ip.) at the doses of 50, 100 and 200 mg/kg. Heparin (100 IU) was used as the reference drug. The lengths of tail thrombosis were measured at 24-72 h interval (5). The statistical analyses were carried out using GraphPad Prism version 5.0. Data obtained from H\[SHULPHQW SUHVHQWHG DV PHDQ"6. E.M Statistical differences between the treatments and the control was evaluated by one-way ANOVA, followed by Dunnett’s multiple comparison tests.

RESULTS AND DISCUSSION

Findings obtained in the present study have indicated that vitexin and quercetin decreased carrageenan-induced mouse tail thrombosis length when compared to the control groups at 24-72 h interval. All doses of vitexin showed significant inhibition of tail-thrombosis at all days (1st day: 50 mg/kg P<0.001; 100 mg/kg: P<0.001; 200mg/kg P<0.05), though just in 200 mg/kg dose of quercetin (1st day: P<0.001) showed significantly inhibition of tail-thrombosis.

CONCLUSIONS

Carrageenan-induced tail thrombosis model is used to determine the novel antithrombotic drugs in vivo. Our results showed that administration of vitexin (and quercetin has antithrombotic effect. They can be used seperately or together as therapeutic agents or complementary treatment against thrombosis.

ACKNOWLEDGMENTS

Our work is financially supported by Scientific Research Projects Foundation of Anadolu University, Eskisehir, Turkey (Project code no: 1306F254). Our project studies still in process.

REFERENCES


P-127: REDUCING EFFECT OF APIGENIN AND HYPEROSIDE ON TAIL THROMBOSIS IN MICE

E. Sümbül1, R. Arslan1, N. Bektas1

Anadolu University, Faculty of Pharmacy, Department of Pharmacology, Eskisehir, TURKEY
INTRODUCTION
Cardiovascular diseases (CVDs) are among the most important diseases in the world. Atherosclerosis and thrombosis are two of the most common causes of CVDs such as ischemia, myocardial infarction, deep vein thrombosis and angina (1; 2). Thereby, many researchers are focused on this topic and also traditional medicine are used for treatment of thromboembolic diseases. Flavonoids are valuable polyphenolic compounds of traditional medicine and their benefits for health are reported in various epidemiological studies (3). In present study, we aimed to investigate the antithrombotic effects of two flavonoids: apigenin and hyperoside.

MATERIALS AND METHODS
Carrageenan-induced tail thrombosis model was used to evaluate the antithrombotic effects of apigenin and hyperoside. The right hind paw of each mouse was injected subplantar with 1 % Type I carrageenan to induce thrombosis in vivo. After carrageenan administration, hyperoside and apigenin were injected at the doses of 50, 100, and 200 mg/kg (i.p.). Heparin (100 IU) was used as reference drug. The length and appearance ratio of wine-colored tail thrombus were observed at 1-3 days periods (4). The results were expressed as the mean±S.E.M and significance level was set at P<0.05. The resulting values were subjected to one-way analysis of variance with Dunnett’s multiple comparison tests (GraphPad Prism version 5.0.).

RESULTS AND DISCUSSION
The results indicate that hyperoside and apigenin decreased the length of wine-colored tail thrombus when compared to control groups at 24-72 h interval. The carrageenan induced-tail thrombosis length was decreased by apigenin at all doses when compared to control group (1st day: 50 mg/kg P<0.001; 100 mg/kg: P<0.001; 200mg/kg P<0.01), however hyperoside exhibited this action just at 200mg/kg dose (1st day: P<0.001). It is possible to conclude that hyperoside and apigenin have potentially antithrombotic activities.

CONCLUSIONS
Hyperoside and apigenin may be useful for reducing or eliminating the risks of thromboembolic diseases and can be used separately or together as therapeutic agents or complementary treatment against thrombosis.

ACKNOWLEDGMENTS
Our work is financially supported by Scientific Research Projects Foundation of Anadolu University, Eskisehir, Turkey (Project code no: 1306F254). Studies of project is under way.

REFERENCES

P-128: EFFECTS OF TRIMEBUTINE MALEATE ON ISOLATED RAT DETRUSOR MUSCLE CONTRACTILITY
S. Engin1, M. Saglam2, E.N. Gazioglu1, M. Kadioglu-Duman2
1Karadeniz Technical University, Department of Pharmacology, Faculty of Pharmacy, 61080 Trabzon, TURKEY
2Karadeniz Technical University, Department of Pharmacology, Faculty of Medicine, 61080 Trabzon, TURKEY

INTRODUCTION
Trimebutine maleate (TMB) has been commonly prescribed since 1969 for the treatment of both hyperrmotility and hypomotility of gastrointestinal tract, such as irritable bowel syndrome and dyspepsia [1,2]. TMB is known to have spasmolytic activity and mechanism of action is complicated. Spasmolytic activity of TMB might be related to its local anesthetic, antimuscarinic and weak mu opioid agonist effects [3]. No research has been performed on the effects of TMB on detrusor muscle contractility. As for antimuscarinic properties of this drug, we aimed to elucidate the action of TMB on acetylcholine-induced contractions in isolated rat detrusor muscles.

MATERIAL AND METHODS
The rat urinary bladder was removed from the animals under anesthesia, and placed in cold Tyrode’s solution.
The urinary bladder was carefully dissected from the adherent tissues and detrusor muscle strips (5–6 mm long and 2–3 mm wide) were prepared. Each of the urinary bladder preparations of detrusor muscle was suspended with one end tied to a holder and the other end attached to an isometric force transducer (MAY FDT10A) connected to BIOPAC MP 100 system. The tissues were incubated in water-jacketed organ baths containing Tyrode solution at a temperature of 37°C and bubbled with 95% O₂ and 5% CO₂ throughout the experiments. The tissues were allowed to equilibrate for 60 minutes with a resting tension of 1 g. At the end of duration period to test the effect of TMB on baseline tension of preparations, three different doses of TMB were organ baths. After the incubation period with different TMB doses; cumulative dose-response curves were obtained with acetylcholine. At the next stage, acetylcholine (AcH, 10⁻⁸M-1 0⁻³M) were used to produce contractions cumulatively and then effect of three doses of TMB were tested on these cholinergic contractions.

RESULTS AND DISCUSSION
TMB alone caused neither contraction nor relaxation in baseline tension of preparations. TMB (10, 50 and 100μM) induced a concentration-dependent decrease in AcH-contracted strips (15%, 22% and 48%, respectively) when the tissues were incubated with TMB. It is thought that the decrease in cholinergic contractions of detrusor muscle may be due to antimuscarinic effect of TMB. Moreover, cumulative AcH-induced contractions were significantly attenuated when TMB (10, 50, 100 μM) was added when the maximum contraction of AcH was obtained (35%). TMB may also affect some ion channels activity which involved in regulation of detrusor muscle contractions. Further investigations are required to exhibit certain effect of TMB on urinary bladder contractility.

CONCLUSIONS
Pharmacological treatment of overactive bladder is essentially based on blocking the excessive contractions of the detrusor smooth muscle with antimuscarinic drugs [4]. Our findings suggest that TMB can be an effective drug for the treatment of overactive bladder because of its antimuscarinic effects.

REFERENCES
1. Sinniger, V.; Mouchet, P.; Bonaz, B., Effect of nor-trimebutine on neuronal activation induced by a noxious stimulus or an acute colonic inflammation in the rat. Life Sciences 2005, 77, 2927-2941.
Age and Gender: Most of the adults are between 21 and 30 years of age group (39.1 %), and overall female to male ratio is 1.6 : 1.0. The percentages of females and males in 21 and 30 years of age group are 40.0 and 38.4, respectively.

Major groups of agents: Pharmaceutical drugs were main offenders (54.4 %) in all years followed by household products and pesticides with percentages of 10.8 and 10.6, respectively. Among pharmaceuticals, antidepressants with 13.3 % ranked first. These results are compatible with some other reports from Turkey and other countries [1-4]

Mode of poisoning: Major mode of poisoning is suicidal attempts (47.5 %). The percentage of accidental exposures are 41.1. While females are dominant (71.2 %) in self harm cases, accidental exposures affected both sexes almost equally. The general economic and social difficulties in our country have strongly affected the intentional poisonings especially in more sensitive groups such as females and young adults [5]

Route of Exposure: While in 76.2 % of the cases oral ingestions caused poisonings, inhalations came second with 13.8 %.

Distribution of professions and locations: The majority of the callers are physicians (75.0 %), and the locations of them are mostly state hospitals including former social security hospitals (41.3 %).

CONCLUSIONS
The education for both health professionals and public about safe use and storage of all chemicals is essential in preventing poisonings at home and environment. It is another obligation to check safe use and storage of chemicals by manufacturers and relevant authorities to prevent occupational poisonings in workers. In the more sensitive groups, such as females and young adults, economical and psychological supports and close observation by health authorities and governmental bodies are of utmost importance.

Poison information centers / units may play vital role in providing education for people as well as health professionals and in lessening morbidity, mortality and economical burden due to poisonings.

REFERENCES

P-130: THE ANALYSIS OF DRUG INFORMATION ENQUIRIES OF NURSES BETWEEN 2004 AND 2013 IN HACETTEPE DRUG AND POISON INFORMATION UNIT

A. Çeliker1,2, G. Gürdemir3

Hacettepe University, Ankara, TURKEY
1Hacettepe Drug and Poison Information Unit,
2Department of Clinical Pharmacy, Ankara, TURKEY

INTRODUCTION
After the emergence of Clinical Pharmacy component of pharmacy education, the establishment of drug information centers have provided a valuable tool in patient oriented pharmacy practice. Ever after, besides pharmacists and physicians, nurses also, have been one of the main groups of enquirers of drug information centers in order to get accurate, unbiased and updated information for their routine services. Being an experienced one in Turkey, Hacettepe Drug and Poison Information Unit (HIZBIB) is in service for 23 years with 2 pharmacists in working-hour basis. The objective of this study is to analyze the extent and characteristics of consultations to HIZBIB by nurses.

MATERIALS AND METHODS
The enquiries directed from nurses between January 1, 2004 and December 31, 2013 were assessed retrospectively and analyzed with IBM SPSS Statistics 21®.

RESULTS AND DISCUSSION
The percentage of overall nurses’ drug related calls’ was 14.7 (total drug calls: 10857) in the selected ten years’ experience of HIZBIB. Although annual distribution showed a periodical unsteadiness, the last point is over three times of the percentage of 2004. Hours: Calls received mostly between 10:31-12:30 (29.0 %) and 13:31 - 15:30 (28.9 %) hours. Distribution of Calling Sites: The majority (95.1 %) of the calls received from nurses working in Hacettepe University Hospitals. Types of Enquiries: Half of the enquiries (55 %) were about stability and almost one quarter were about incompatibility in intravenous (iv) admixtures. The percentage of dose related enquiries ranked in third place with 7.5 %
Answering Time: While 85.8 % of enquiries were answered by HIZBIB in less than 5 minutes, in 0.4% of all calls took more than 1 day to gather and tailor necessary information that requiring more literature search.
CONCLUSIONS

- Pharmacists have been consulted by nurses frequently (1-3) throughout the world. As of Drug Information Centers, this fact means that this service provided by pharmacists is of importance for nurses because of its rapidity, accuracy and currency.
- The preparing iv admixtures that has been left to nurses’ responsibilities in practice in our country can be taken over by pharmacists since this service has been fulfilled by “drug experts”, namely pharmacists in many countries (2, 4).
- Drug Information Centers can provide not only emergent information for nurses but also education through seminars and conferences in some topics they need. This will be of use to improve patient care and rational drug usage provided by nurses.

REFERENCES


MATERIALS AND METHODS

This was a prospective and randomized control study conducted between December 2012 and April 2013. 200 patients who has been prescribed with topical corticosteroid drugs were recruited. The DLQI (Dermatology Life Quality Index) was used to assess the quality-of-life. DLQI is based on a scoring range of 0 to 30, and consists of 10 questions, each with a score of up to three. Patients’ demographic data, medical history and results of their physical examination were also recorded by the researchers, and the patients filled the DLQI on their first day of examination. After two weeks, medication adherence was evaluated by comparing the initial and final quantities of the creams and ointments, and the patients completed the DLQI again in order to provide an estimate of the quality of life changes. Both verbal and illustrated written education methods were employed for the patients in the study group. Written education in the form of a brochure concerning the disease and its treatment was offered immediately following the oral education. The control group received routine information about prescription details and advice from the physician.

RESULTS AND DISCUSSION

This study suggested that there was a significant improvement in quality of life of the patients with dermatitis based on DLQI scores (p<0.05). The average DLQI value from the first examination in dermatitis patients was 8.29. After two weeks of treatment the average DLQI value was 2.08. The quality of life of the patients with psoriasis according to DLQI scores also showed an improvement but no statistically significant changes were recorded. The average DLQI value from the first examination was 7.97. After two weeks of treatment the average DLQI value was 5.08. Although compliance measures increased in the study group no statistically significant changes were recorded compared with the control group. No significant relationship between patients’ knowledge of the medication and compliance was observed.

CONCLUSIONS

Adherence to treatment in dermatological patients is highly variable. Patient education on how to use topical corticosteroids has beneficial effects on...
Coordinated efforts of dermatologists, dermatology nurses and pharmacists may endure an effective treatment for the patients [2].

REFERENCES
POSTER SESSION II
(Poster 132 - 274)
June 10, 2015
P-132: ENZYMATIC ANTIOXIDANT DEFENSE IN OVERWEIGHT/OBESE POSTMENOPAUSAL WOMEN

A. Klisic1, N. Kavaric1, M. Jovanovic1, M. Matic2,3
1Primary Health Care Center, Podgorica, Montenegro; 2Faculty of Medicine, University of Belgrade, Serbia; 3Institute of Medical and Clinical Biochemistry, Belgrade, Serbia

INTRODUCTION
Obesity is characterized by increased oxidative stress [1]. However, discrepant results were obtained when antioxidant defense was measured, both in children and adults [2-5]. Therefore, we aimed to determine the antioxidant defense measured by glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in overweight/obese postmenopausal women.

MATERIALS AND METHODS
A total of 100 sedentary overweight/obese SRVWPHQRSDXVDOZRPHQPHDQDJH”, and 50 age-matched normal-weight controls were included in this cross-sectional study. All women were non-smokers, without diabetes, thyroid dysfunction or cardiovascular disease, and were not using hormonal therapy or any other medication. Biochemical parameters including GPx, SOD, fasting glycemia and lipid parameters were determined spectrophotometrically. Body weight and height were measured. Body mass index (BMI) was calculated. Statistical analysis was performed using SPSS statistical package (version 15.0 for Windows, SPSS, Chicago, IL, USA). Data are presented as mean ± standard deviation. Differences between groups were evaluated with a Student’s t test for normally or Mann-Whitney test for non-normally distributed parameters. A correlation analysis by Spearman's (p) correlation coefficient was used to determine the relationships between enzymatic antioxidant defense (GPx and SOD) and other variables.

RESULTS AND DISCUSSION
Overweight/obese postmenopausal women displayed lower GPx activity, as compared with the normal weight group (143.19±84.65 vs. 188.49±139.00 U/L, p=0.045). However, the despite fact that overweight/obese women exhibited higher mean SOD activity, this increase did not reach statistical significance (47.52±18.63 vs. 43.21±18.65 Ux103/L, p=0.184). Furthermore, GPx inversely correlated with BMI, but only in the normal weight group (p= -0.274, p=0.050). There were no correlations with fasting glycemia or any of the lipid parameters with GPx and SOD activity in each group. These discrepancies in decrease in GPx, but not in SOD activity may be explained by duration of obesity, considering that the different antioxidant enzymes act in a temporal order by increasing expression and activity of antioxidant enzymes at the onset of obesity, but which become depleted in chronic long-term obesity [1-3].

CONCLUSIONS
Decreased antioxidant defense measured by GPx, but not by SOD, was observed in overweight/obese postmenopausal women. Inverse association of GPx with BMI was detected only in the normal weight group, suggesting that duration of obesity may influence on GPx activity. Therefore, weight loss programs may be of benefit in increasing antioxidant defense in postmenopause, especially in increasing of GPx activity.

REFERENCES

P-133: ANGIOTENSIN CONVERTING ENZYME INSERTION/DELETION POLYMORPHISM HAS NO EFFECT ON LIVER FIBROSIS

NK. Turhan1, S. Uygun Ilikhan2, AC. Hamamcioglu3, Y. Ustundag3, A. Dursun4, F. Kokturk5

Ataturk State Hospital, 1Internal Medicine Clinic, Bulent Ecevit University; 2Department of Internal Medicine, School of Medicine, 3Department of Biochemistry, School of Pharmacy, 4Department of Medical Genetics, School of Medicine, 5Department of Statistics, School of Medicine, Zonguldak, TURKEY

INTRODUCTION
Chronic viral hepatitis B (HBV), chronic viral hepatitis C (HCV), non-alcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), autoimmune hepatitis (AH), primary biliary cirrhosis (PBC) and secondary biliary cirrhosis (SBC) are important health issues worldwide. Experimental studies demonstrated an association between angiotensin-converting enzyme gene insertion/deletion (ACE gene I/D)
polymorphism and liver fibrosis [1]. However, the results of clinical studies are controversial [2, 3]. We aimed to investigate a possible association between ACE gene I/D polymorphism and liver fibrosis in a large group of Turkish patients from western Black Sea region.

MATERIALS AND METHODS
The ACE I/D polymorphisms, detected by polymerase chain reaction (PCR) and serum ACE levels, detected spectrophotometrically were investigated in 418 patients with HBV, HCV, NASH, ALD, AH, PBC and SBC.

RESULTS AND DISCUSSION
The distribution of “DD”, “ID”, “II” genotypes of ACE gene were 32.5%, 48.8% and 18.7% in mild to moderate fibrosis group (N=246, F: 1-3 according to Ishak’s score) and 39.0%, 44.2%, 16.9% in advanced fibrosis group (N=172, F: 4-6 according to Ishak’s score) respectively. No significant association was found (Fig. 1). However, there was a significant correlation between serum ACE levels and ACE gene alleles (p <0.001). Serum ACE levels of patients with D alleles were higher than the patients with I alleles [(44 (min 7-max 101) versus 29 (min 7-max 96)]. Between the two fibrosis groups, HAI, MELD and Child-Pugh scorings were also found to be significantly associated (p <0.001). Patients with advanced fibrosis were older than the patients with mild to moderate fibrosis groups (p <0.001). No significant association was noted between the sex of the patients and the fibrosis groups.

CONCLUSIONS
ACE I/D polymorphism do not associate with the fibrosis stage of hepatic patients from the western Black Sea region of Turkey. Therefore, ACE gene (I/D) polymorphism cannot be considered as a marker of the severity of liver fibrosis and/or as an alternative to liver biopsy. We believe that large scale studies comparing different races are needed to define the effects of ACE I/D polymorphism on hepatic fibrosis and more environmental factors such as alcohol and tobacco consumption should be explored.

REFERENCES

P-134: DECREASED NIDOGEN-2 CONCENTRATIONS AND IMPAIRED OXIDANT/ANTIOXIDANT BALANCE IN PATIENTS WITH ENDOMETRIAL CANCER

A. E. Aççi1, A. Gönenç2, F. Atalay2, I.S. Gürcan3, M. Torun1

1Department of Biochemistry, Faculty of Pharmacy, Gazi University, Ankara, Turkey
2Department of Gynecologic Oncology, Ankara Oncology Education and Research Hospital, Ankara, Turkey
3Department of Bioistatistics, Faculty of Veterinary, Ankara University, Ankara, Turkey

INTRODUCTION
Endometrial cancer is the most common malignancy of the female genital tract in the world and the seventh cause of death from cancer in women in Europe [1]. Basement membranes are thin pericellular protein matrices that control a large number of cellular activities. They contain a tissue-specific composition of extracellular matrix components containing nidogen. The loss of nidogen-2 expression (NID-2) in basal membrane has been shown to have a potential pathogenic role in colon and stomach tumorigenesis [2]. Also, reactive oxygen species and the coupled oxidative stress have been associated with cancer formation. We aimed to measure NID-2, lipid hydroperoxides (LPO), nitrotyrosine (NT) and total antioxidant capacity (TAC) levels in gynecological cancer, and evaluate oxidant and antioxidant status in these disease.

MATERIALS AND METHODS
We studied 83 patients with newly diagnosed gynecological cancer and 30 age-matched control subjects at Ankara Oncology Educational and
Research Hospital. NID-2 and oxidative stress parameters were measured in 28 patients with endometrial cancer, 45 patients with ovarian cancer, other gynecological cancers including cervical, vulvar and vaginal and 30 matching healthy controls. NID-2 and NT were quantitated by the ELISA method. LPO and TAC were assayed with spectrophotometric methods.

RESULTS AND DISCUSSION
Our data showed a significant decrease of NID-2 levels in patients with endometrial cancer as compared with controls ($p<0.01$). When compared to control group, the following were higher: LPO levels in endometrial cancer ($p<0.05$), ovarian cancer ($p<0.01$) and others ($p<0.01$); NT levels in endometrial cancer ($p<0.01$). When compared to endometrial cancer group, the following were higher: LPO levels in ovarian cancer ($p<0.01$), others ($p<0.01$); NT levels in ovarian cancer ($p<0.01$), others ($p<0.01$); TAC levels in ovarian cancer ($p<0.05$). When compared to ovarian cancer group, NT levels were higher in others ($p<0.05$).

There are conflicting results about NID-2 concentrations in cancer. UlaZZi et al. suggest that loss of nidogen expression may favor invasion and metastasis of cancer cells by loosening cell interaction with basal membrane, first barrier from the connective vascularized matrix [3]. However, Kuk et al. [4] reported that increased NID-2 concentrations in serum of ovarian cancer patients in comparison to healthy controls. Unfavorable side effects occur when there is an imbalance between overproduction of ROS and decrease of antioxidant molecules in body. Increased LPO levels were found in various cancer patients. Pejic et al. showed that patients with premalignant and malignant lesions had enhanced LPO and altered uterine antioxidant status than patients with benign uterine diseases [5].

CONCLUSIONS
These findings support the idea that loss of NID-2 and impaired oxidant/antioxidant balance may play an important role in the pathogenesis of endometrial cancer. This study shows a decrease of serum NID-2 concentrations and an increase of lipid peroxidation and protein oxidation in endometrial cancer. Also, there is an increase oxidative stress in patients with ovarian cancer and other gynecologic cancer.

ACKNOWLEDGEMENTS
The work was supported by Gazi University Research Foundation (Project No 02/2011-37).

REFERENCES

P-135: PROTEIN- POLYSACCHARIDE RATIO AND PH EFFECT ON GCSF POLYSACCHARIDE CONJUGATION
A. G. Kaya Özsan¹, A. F. Öner²
Hacettepe University, Faculty of Pharmacy, ¹Department of Pharmaceutical Biotechnology, Ankara, TURKEY

INTRODUCTION
Recombinant human granulocyte colony stimulating factor (rGCSF) is a first line protein drug for the treatment of neutropenia occurred in cancer patients during chemotherapy [1]. Due to the short half-life in blood, parenterally administered filgrastim is rapidly cleared from blood stream and used in repeated doses. Second generation polymer conjugated protein drugs have been developed by different ways such as site-specific mutagenesis, protein fusion, chemical and enzymatic modifications. Among these enzymatic modification has advantages in the pharmaceutical biotechnology field as being less toxic and more environment friendly than chemical modification. In this study, rGCSF conjugation is studied by using chitosan. Polysaccharide/protein ratio, pH and enzyme amount are evaluated as factors affecting rGCSF polymer conjugation by the way of enzymatic coupling.

MATERIALS AND METHODS
Low molecular weight chitosan is purchased from Sigma and depolymerized. Recombinant GCSF is produced in the E.coli cells as N-methylated form. 

Conjugation Protocol
10g LMWC (low molecular weight chitosan) is dissolved in 2 M 1 L HCl. The solution was heated for 3.5h under reflux. After cooling, 3 L of ethanol (96%) was added and the precipitate was washed thoroughly with ethanol and freeze dried for 24 h [2].
Conjugations were made with both non-depolymerized and depolymerized LMWC. A wide range of pH values (pH 4.0-7.0 with 0.5 unit intervals and 7.4) were studied. Studies are conducted at 25°C where the drug can stay stable and the enzyme work well. A solution of rGCSF in a 0.004% polisorbate and 5% sorbitol containing 10 mM pH 4.0 sodium acetate buffer is used. Under those circumstances, different ratios of enzyme, biopolymer and drug are mixed for 24 hours and the resultant solution is analyzed by SDS-PAGE. Experiments were carried out to analyze the covalent coupling between LMWC and rGCSF for different conditions of polysaccharide/protein ratio, pH and enzyme amount. SDS-PAGE analysis was carried out using a polyacrylamide gel (8% stacking gel, 12% running gel) prepared according to Laemmli [3].

RESULTS AND DISCUSSION
Figure 1 shows conjugates formed at different pHs. Some protein bands visualized at the stacking gels, indicating the presence of materials with high molecular weight developed after attachment between the polysaccharide and the protein. Isoelectric point of rGCSF is 5.61. The protein bands at the stacking gels appeared well above that pH. Different polysaccharide/protein ratio is studied with both non-depolymerized and depolymerized LMWC. The blue color of the bands ascribed to rGCSF gradually decreased with increasing polymer ratio, especially for conjugates produced with depolymerized LMWC (Figure 2). Different enzyme amount is also studied. Both non-depolymerized and depolymerized LMWC are used at pH 4.5 and 5.5. The behavior was the same, independent of the enzyme amount (Figure 3).

CONCLUSIONS
According to the results obtained, protein-polysaccharide conjugates can be prepared by the enzymatic method. Further studies for conjugation conditions are necessary to show complete attachment.

ACKNOWLEDGEMENTS
This study was supported by Turkish Scientific and Research Council (TUBITAK) Project No: 114S759.

REFERENCES
2. Elsayed A, Al-Remawi M, Farouk A, Badwan A. Insulin-chitosan polyelectrolyte nanocomplexes: Preparation,

P-136: OXIDATIVE MODIFICATIONS IN PATIENTS WITH REPLACEMENT THERAPIES

B. BABA1, C. M. TOKAR1, K. ÖNEÇ.2, M. BALİ1, A. HACIŞEKİ1

1Gazi University, Faculty of Pharmacy, Department of Biochemistry, 2Faculty of Medicine, Department of Nephrology, Ankara, TURKEY

INTRODUCTION

Hemodialysis (HD) and continuous ambulatory peritoneal dialysis (PD) are the most commonly used replacement therapies in the patients with end stage renal failure[1]. Oxidativestress (OS) is defined as an imbalance between the production of pro-oxidant substances and the action of the antioxidant system, in favor of production. Renal replacement therapy is responsible for the exacerbation of this pro-oxidant status[2]. We aimed to investigate the role of the oxidative stress in renal failure pathogenesis by measuring the total antioxidant capacity (TAC), protein oxidation and lipid peroxidation products in patients with HD and PD treatment.

MATERIALS AND METHODS

This study was conducted with eighty five patients with chronic renal failure (55 men, 30 women) and 30 healthy individuals (6 men, 24 women) with the mean age 37.20±1.28 years. Patients were divided into two groups: first group whose receiving HD therapy (36 men, 19 women) with the mean age being 44.53±1.74 years and second group whose receiving PD therapy (19 men, 11 women) with the mean age being 45.33±2.65 years. The malondialdehyde (MDA) levels and TAC were assayed with spectrophotometric method. The protein carbonyl (PCO) and advanced oxidation protein products (AOPP) levels in serum as the markers of protein oxidations were measured by using kits.

RESULTS AND DISCUSSION

In this study, we found that there were statistically significantly higher serum PCO and MDA levels (p<0.000) while no differences were observed in AOPP and TAC levels in total patients as compared with healthy subjects. A significant decrease of TAC levels and a significant increase of AOPP levels were observed in patients on HD before when compared to HD after treatment (p=0.000). Significant differences were found among HD before, PD patients and healthy controls according to MDA and PCO levels (p<0.01). Significant differences were found between HD after and controls according to all parameters (p<0.05) and between PD patients and HD after treatment according to PCO and AOPP levels (p=0.014, p=0.017). A significant decrease of TAC levels was observed in PD patients as compared with HD before treatment (p=0.011). We observed that PCO levels in HD patients with high parathormone (PTH) (> 300) were significantly higher than those of the patients with low PTH (≤ 300). A significant decrease of AOPP levels was observed in HD patients with high PTH when compared to HD patients with low PTH. There were no statistically significant differences in OS parameters in PD patients according to PTH levels. Several experiments have shown increased OS in renal insufficiency. There is also evidence for carbonyl stress in uremia that is characterized as reactive carbonyl compounds overload [3,4]. There are conflict findings about TAC in CRF[5].

CONCLUSIONS

There is strong experimental evidence indicating that chronic kidney disease (CKD) patients and especially those undergoing dialysis treatments are exposed to enhanced OS. However, there is little evidence to suggest how early OS starts during the progression of CKD and how renal replacement by regular HD or PD influences the intensity of OS that patients experience. In our study showed that lipid peroxidation and protein oxidation products were increased and TAC was mildly decreased. Despite the limitations of this study, further studies are required to investigate the clinical applicability of OS markers in a larger population. Further investigations on the intensity of the OS after replacement therapies and its influence on the stage of the renal disease will elucidate the clinical relevance of the phenomenon.

ACKNOWLEDGEMENTS

This study was supported by the Research Fund of Gazi University (02/2010-44).

REFERENCES


P-137: PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF LICHEN *PERTUSARIA SP.*

B. Barut¹, S. Yıldırım², A. Ozel¹, A. Yasar²

¹Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, 61080 Trabzon, TURKEY
²Karadeniz Technical University, Faculty of Pharmacy, Department of Analytical Chemistry, 61080 Trabzon, TURKEY

INTRODUCTION

Lichens are symbiotic organisms of fungi, algea and cyanobacteria. Lichens are valuable plant resources used as medicines, food, fodder, dyes perfume, spice and for other purposes [1]. About 18,500 different lichens species have been described all over the world. [2]. The pharmacological and biological activities of lichens and their substances can be divided into the following categories based on knowledge; antimicrobial, antitumoral, antiviral, enzyme inhibitor activity, antiherbivare, antibacterial and antioxidant activities. Lichens contain a variety of secondary metabolite which has strong antioxidant activity [3, 4]. These substances have high ability to scavenge toxic free radicals due to phenolic compounds.

The aim of this study was to determine *in vitro* antioxidant capacity and total phenolic content by spectrophotometric methods and phenolic compounds by reverse phase-high performance liquid chromatography (RP-HPLC).

MATERIAL AND METHODS

The lichen *Pertusaria sp.* was collected at an altitude of 500 m above sea level in November 2014 from Of, Trabzon, Turkey. Dried and powdered material was extracted with dimethylformamide (DMF). Phenolic compounds (gallic acid, proto-catechuic acid, proto-catechuic aldehyde, p-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, syringic aldehyde, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid) were determined by RP-HPLC. Ferric reducing antioxidant power (FRAP) assay, superoxide radical scavenging (SOD) assay, ferrous ion-chelating effect assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay were performed to determine *in vitro* antioxidant activities of the DMF extract. The aliquots mixtures included 125, 250, 500 and 1000 µg/mL of extract for antioxidant activities. Also the total phenolic content was determined by Folin Ciocalteu method as gallic acid equivalent (GAE).

RESULTS AND DISCUSSION

76.56% inhibition was observed by measuring Superoxide radical scavenging activity (SOD) with 500 µg/mL lichen extract. However, DPPH scavenging activity was determined as 40.00% with 1000 µg/mL. In the ferric reducing antioxidant power (FRAP) assay, the lichen extract had moderate reducing activity on ferric ion. On the other hand, the lichen extract was found to be significantly active (46% inhibition with 1000 µg/mL of extract) by ferrous ion-chelating test compared to ascorbic acid (6.09% inhibition with 1000 µg/mL of ascorbic acid) and BHA (21.81% inhibition with 1000 µg/mL of BHA), the reference compounds. The total phenolic content of the extract was reported as gallic acid equivalent and the lichen extract was determined to have high (80 mg/mL GAE) amount of phenolic compounds.

Gallic acid, proto-catechuic acid, proto-catechuic aldehyde, p-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, ferulic acid, benzoic acid were determined in DMF extract of *Pertusaria sp.* especially ferulic acid was the highest.

CONCLUSIONS

*Pertusaria sp.* extract has significantly antioxidant activity and contains phenolic compounds especially ferulic acid. The lichen can be used against several diseases caused by free radicals. Ferulic acid, which has an important role on preventing DNA damage and cancer, can be isolated.

REFERENCES


P-138: ENHANCEMENT OF THE RESPONSE TIME OF THE IMMOBILIZED GLUCOSE OXIDASE ENZYME

E. Hasanoglu Özkan¹, N. Kurnaz Yetim¹,², N. Sarı¹

¹Gazi University, Faculty of Science, Department of Chemistry, Ankara, 2Kırklareli University, Faculty of Art and Science, Department of Chemistry, Kırklareli, TURKEY
INTRODUCTION
From 1960s till today, there has been curiosity in developing sensors for the continuous measurement of glucose in human blood [1]. The methods used to assay blood and urine samples for glucose have undergone an evolution from general chemical assays for reducing sugars, through the more specific biochemical approach, to the present versatile proposals for biosensors [2].

MATERIALS AND METHODS
Kinetic parameters were studied for immobilized supports [(APS-SchBr-Pt^{2+}-GOx), (APS-SchBr-Pt^{4+}-GOx), (APS-SchCl-Pt^{2+}-GOx), (APS-SchCl-Pt^{4+}-GOx)] of the substrate concentration on the reaction rate was examined, using unfunded concentrations (0.2-50 mM-D-glucose substrate). The Michaelis-Menten constant ($K_m$) and the maximum reaction rate ($V_{max}$) of immobilized GOx were calculated from the Lineweaver-Burk plots.

RESULTS AND DISCUSSION
We known that the $V_{max}$ value defines the maximum velocity when all of enzyme is saturated with substrate. $K_m$, the substrate concentration at which an enzyme reaches ½ $V_{max}$ reflects the effective characteristic of the enzyme and depends upon both partitioning and diffusion [3]. The determined $K_m$ values which are shows the affinity of the enzyme to substrate, for immobilized GOx were found to be about 4.24 / 7.61/ 3.42/ 4.64 mM (for optimum conditions) APS-SchBr-Pt^{2+}-GOx / APS-SchBr-Pt^{4+}-GOx/ APS-SchCl-Pt^{2+}-GOx/ APS-SchCl-Pt^{4+}-GOx respectively. $V_{max}$ values were calculated from Lineweaver-Burk plots for immobilized GOx to the APS-SchBr-Pt^{2+}-GOx /APS-SchBr-Pt^{4+}-GOx / APS-SchCl-Pt^{2+}-GOx / APS-SchCl-Pt^{4+}-GOx supports, $V_{max}$ 2.11 / 1.81/ 7.86/ 9.45 mMmin^{-1}, respectively.

CONCLUSIONS
In the present study, the conversion of glucose to gluconic acid response time were examined in terms of varying substituents and metal ions whose superiorities are compared with each other. While the interest of complexes containing chloride ions (APS-SchCl-Pt^{2+}-GOx /APS-SchCl-Pt^{4+}-GOx ) to the enzyme ($K_m$) were increased, these complexes’ response time to the reaction was shortened. This improvement APS-SchBr-Pt^{2+}-GOx side gave better results in terms of metal atoms which complexes containing bromine ions. We hope that, the study will shed on light the preparation of glucose sensors which can respond quickly and use in industry (eg: food industry, health service...).

REFERENCES

P-139: ATTACHING THE SUPPORT OF PLATINUM (II) COMPLEX AND ANTIFUNGAL ACTIVITIES
E. Hasanoğlu Özkan¹, N. Kurnaz Yetim¹,², N. Sari¹, O. Eren¹, H. Öğütçü³
¹ Gazi University, Faculty of Science, Department of Chemistry, Ankara, ² Kırklareli University, Faculty of Art and Science, Department of Chemistry, Kırklareli, ³ Ahi Evran University, Faculty of Arts and Science, Department of Biology, TURKEY

INTRODUCTION
Platinum is an essential trace element in the anti-cancer research. So, numerous derivatives of Pt(II) complexes have been prepared in recent years [1]. Since 2000, increased research on Platinum-complexes linked with polyamine ligands [2]. The amine groups of the polyamine linkers is groups capable of hydrogen bond formation with DNA atoms such as the O6 of guanine or the O3 of thymine [3].Antifungal polymers are gaining the attention of pharmaceutical makers and industrial design.

MATERIALS AND METHODS
Nanospheres-Polymers attached Platinum (II) complexes (nanospheres involving Schiff bases derived from (aminomethyl) polystyrene and four substitute salicylaldehyde (2-hydroxy benzaldehyde, 5-fluoro-2-hydroxy benzaldehyde, 5-kloro-2-hydroxy benzaldehyde, 5-bromo-2-hydroxy benzaldehyde) have been investigated antifungal activities. These activities of polymer attached Schiff bases and their Pt (II) complexes were checked against two fungal strains such as A. fungus, and C. albicans. For activities measurement, the media used were Mueller- Hinton agar for bacteria and Potato Dextrose agar for the fungi. At the end of the incubation period (72 h at room temperature), the diameter of the zone of inhibition around the wells was measured.
RESULTS AND DISCUSSION

As shown in Table I, the results of antifungal screening indicated that the Pt(II) derivatives with nano-spheres showed more activity than the other studied nano-spheres. All of the polymers with nano-sphere were active against *Candida albicans* and *A. fumi.* As shown in Table I, the [APS-SchF-Pt2+] that showed a significant activity against *C. albicans* and *A. fumi.* Generally, the Pt(II) derivatives with nano-spheres are more antifungal than their ligands. This enhancement in activity may be explained on the basis of chelation theory [4].

Table 1. Antifungal activity of polymer attached Schiff bases and Pt (II) complexes (0.018 g/ml) (Diameter of zone inhibition (mm))

<table>
<thead>
<tr>
<th>Component</th>
<th>APS-SchF-Pt²⁺</th>
<th>APS-SchF-Pt²⁺</th>
<th>APS-SchF-Pt²⁺</th>
<th>APS-SchF-Pt²⁺</th>
<th>APS-SchF-Pt²⁺</th>
<th>APS-SchF-Pt²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em> (F.1350-MH)</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>22</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td><em>A. fumi.</em></td>
<td>19</td>
<td>20</td>
<td>25</td>
<td>18</td>
<td>16</td>
<td>20</td>
</tr>
</tbody>
</table>

CONCLUSIONS

We know that, *Aspergillus fumi.* is an emerging worldwide problem with major epidemiological and clinical implications. *Aspergillus fumi.* test results of polymeric-spheres exhibited excellent activity. The Pt(II) complexes showed much better activity than ligands. We can say that this compound merits further investigation as an alternative drug. *Aspergillus fumi.* test results of Pt(II) derivatives with nanospheres compounds are especially noteworthy.

ACKNOWLEDGMENTS

This work was supported by the Gazi University Research Fund (Project number: 05/2012-53 and 05/2014-02).

REFERENCES


RESULTS AND DISCUSSION
The cell growth was inhibited 82.98% in treatment with β-elaterin-IM combination whereas the inhibition levels were 61.69% for IM and 43.34% for β-elaterin, alone at 100 μM concentration.
The apoptotic effect of β-elaterin-IM combination on MCF cells was performed by automated Muse cell analyser. The percentage amount of Annexin V binding was 32.17% for β-elaterin and 41.23% for IM at 100 μM concentration.
The late-apoptotic cell amount was measured as 80.11% for β-elaterin-IM combination.

CONCLUSIONS
The results indicate that β-elaterin synergistically increased the effect of imatinib mesylate on apoptosis of MCF breast cancer cells.

ACKNOWLEDGEMENTS
The study was supported by Scientific and Technological Research Council of Turkey Grant, Tubitak-SBAG-114S871.

REFERENCES
mechanisms may involve cell cycle arrest and the induction of apoptosis.

CONCLUSIONS
Since capsaicin is the main component found in hot pepper, the consumption of hot pepper may contribute to the maintenance of body weight and prevent the development of obesity.

REFERENCES

P-142: ANTIMICROBIAL ACTIVITIES OF ACTINOBACTERIA ISOLATED FROM ALGERIAN SAHARA SOILS

M. Harir1, Bellahcene M2, Fortas Z1

1 Es-Senia University, Faculty of Sciences, Department of Biotechnology, Oran, ALGERIA

INTRODUCTION
Actinomycetes naturally inhabit soils and have a great importance in biotechnological processes due to their ability to produce a large number of antibiotics and other bioactive secondary metabolites. The search for such substances of microbial origin is largely based on the isolation, from diverse sources, of different strains. In addition, one of the strategies for enhancing the probability of obtaining particular isolates and secondary metabolites is to analyse ecosystems subjected to extreme conditions and consider genera of actinomycetes poorly studied in the past. Several researchers found that extreme environments were inhabited by micro-organisms especially adapted to these ecological niches. So, a lot of these micro-organisms may represent new taxa and, thus, can provide a valuable resource for their use in future biotechnological processes [1]. In the last years, the discovery of new actinomycetes and new metabolites found in microbiologically poorly explored areas worldwide suggests that a careful exploration of new habitats might continue to be useful for the above-mentioned purposes [2].

In the present work, we describe the isolation of actinomycetes strains from Sahara soil samples, their antimicrobial activity against pathogenic microorganisms.

MATERIALS AND METHODS
Thirty-two actinomycetes were isolated from 6 soil samples collected from Algerian Sahara soils in searching for new antimicrobial secondary metabolites producers. All the isolates were further subjected to antimicrobial screening against pathogenic bacteria, yeast and fungi. After that the 0) active isolates named (C; Ms Iand 10) were identified by using morphological, biochemical and physiological methods.

In addition, the production of antimicrobial secondary metabolites from the selected isolates (i.e. C, MS1 and 10) was investigated.

RESULTS AND DISCUSSION
The obtained results indicated that three of the isolates (named C, MS1 and 10) showed antimicrobial activity against most of the pathogenic bacteria tested, the yeasts and the phytopathogenic fungi Fusarium culmorum, Fusarium oxysporum f sp albidinis and Verticillium dahliae. Consequently, the three promising isolates were identified as Streptomyces by morphological, biochemical and physiological methods. Most antimicrobial tests showed that the three isolates had antimicrobial activity against different Gram-positive and Gram-negative pathogenic bacteria and three phytopathogenic fungi. After fermentation in ISP2 medium and extraction the crude extract of isolate C was tested by the well-diffusion method on Muller Hinton medium (MHA) and results revealed that the maximum zone of inhibition was recorded against C. albicans (17 mm) followed by M. luteus and K. pneumoniae (15 mm).

For the strain MS1, its crude extract was also active against bacteria and fungi but much less active than strain C. The strain 10 exhibited an antimicrobial activity higher than the strains C and Ms1, especially against P. fluorescens (36 mm). These differences can be attributed to their different chemical structures, their disintegration during the extraction process and environmental factors (temperature and pH of the crude extract). It is worthy to mention that the crude extracts showed antimicrobial activity against Gram-negative bacteria since, in general, they are more resistant to antimicrobial compounds than the Gram-positive bacteria [3].

Key words: Actinobacteria; antimicrobial activities, Saharan soils, strain identification, fermentation

CONCLUSIONS
The obtained results highlight the use of actinomycetes, in particular extremophile actinomycetes, as a source of novel antimicrobial compounds which could be likely used as new antibiotics after proper pharmacological evaluation. Future research will be required to indentify the produced antimicrobial compounds, which will involve their purification and the use of different chemical analysis such as HPLC, spectroscopy and other sophisticated techniques. This will be pursued in the next future in our laboratory.
REFERENCES

P-143: MACROLIDE, LINCOSAMIDE, STREPTOGRAMIN B (MLSB) RESISTANCE PHENOTYPE IN STAPHYLOCOCCAL ISOLATES

H.Bal1, S.Yildiz2

1Cumhuriyet University, Faculty of Pharmacy Department of Pharmaceutical Microbiology, Sivas TURKEY
2Ankara University, Faculty of Pharmacy Department of Pharmaceutical Microbiology, Ankara, TURKEY

INTRODUCTION
Staphylococcus aureus (S. aureus) is increasingly recognized as a cause of hospital associated (HA) and community associated (CA) infections. Macrolide, lincosamide and streptogramin B (MLSB) antibiotics are commonly used in treatment of staphylococcal infections. Widespread use of MLSB antibiotics has led to an increase in resistance to these antibiotics especially clindamycin, amongst staphylococcal strains[1-3].Macrolide, lincosamide and streptogramin B (MLSB) antibiotics have different structure, but similar mode of action. These antibiotics inhibit bacterial protein synthesis by binding to 23s rRNA in 50S ribosomal subunits [4]. The macrolide antibiotic resistance in S. aureus is usually caused either by ribosomal modification mediated by 23s rRNA methylases encoded by erm genes, or by active efflux of the antimicrobial agent by an ATP-dependent pump encoded by msrA gene. Methylyases confer inducible (iMLSB) or constitutive (cMLSB) resistance, while the efflux mechanism affects only macrolides and type B streptogramins (M/MSB). Other more rare macrolide resistance mechanisms include ribosomal mutations and antibiotic inactivation by specific hydrolases or phosphotransferases [5]. In this study, the MLSB resistance of S. aureus strains isolated from the pharmacist and pharmacy employees was investigated.

MATERIALS AND METHODS
Specimens were obtained from 300 pharmacists and pharmacy employees between June 2014 - September 2014. Nasal swabs were collected from all the participants by using sterile cotton swabs, by rotating the swabs in both the anterior nares consecutively. The swabs were processed immediately by inoculating the samples from them onto sheep blood agar plates. The plates were incubated aerobically at 37°C for 24-48 hours. Identification of the isolates as S. aureus was based on colony and microscopic morphology, growth on mannitol salt agar and fermentation of mannitol, and production of catalase, coagulase, and deoxyribonuclease. The reference strain S. aureus ATCC 25923 was used as a quality control strain. 64 S. aureus isolated from 300 volunteers. MLSB phenotype resistance pattern was determined according to the method advised by Clinical and Laboratory Standards Institute (CLSI). Briefly, an overnight culture of each isolate was adjusted to 0.5 McFarland (108 cf/mL) and spread on unsubplemented Mueller-Hinton agar. The following antibiotic disks were applied on an inoculated media erythromycin (E-15 μg), and clindamycin (Cl-2 μg) disks were placed by hand to provide distances of 15-26 mm from edge to edge. Following incubation for 16 to 18 hours at 35°C, zone diameters were measured in the usual manner; any flattening or blunting of clindamycin zone shape (D-shape), indicating iMLSB, while resistance to both erythromycin and clindamycin indicated cMLSB. Lack of a D-shaped zone in erythromycin resistant and clindamycin-susceptible isolates were interpreted as M/MSB. [5]

RESULTS AND DISCUSSION
During the 4 months study period, 64 S.aureus strains were isolated from 300 pharmacist and pharmacy employees. In disk diffusion testing, 8 strains showed resistance to erythromycin (12.5%) and 56 strains to erythromycin resistant strains of staphylococci are especially clindamycin susceptible. In D-testing, 3 strains indicated cMLSB resistance phenotype.[1] In another study at University of Texas Health Science Centre, 29% of 114 S. aureus isolates showed inducible resistance while 34% showed constitutive resistance to clindamycin. [1]

Clindamycin it serves as common in infections caused by S. aureus. If iMLSB resistance to clindamycin susceptibility investigated to detect false and treatment will fail.

CONCLUSIONS
When the routine antibiotic susceptibility tests applied to erythromycin resistant strains of staphylococci are detected, must be evaluated in terms of the MLSB phenotype of these strains. D test methods will contribute to appropriate and effective treatment of infections.
REFERENCES

P-144: APOPTOTIC EFFECT OF NOVEL BENZIMIDAZOLE DERIVATIVES BEARING PYRIDYL/PYRIMIDINYL PIPERAZINE MOIETY

G.A. Çiftçi1, H. E. Temel1, Leyla Yurttas2
Anadolu University, Faculty of Pharmacy
1Department of Biochemistry, 2Department of Pharmaceutical Chemistry, Eskişehir, TURKEY

INTRODUCTION
Benzimidazoles have been known to act as potential anticancer. Recently, different authors have reported antitumor, antiproliferative or anticancer potential of benzimidazole. Cancer is a chronic disease with a striking significance in the world which is the second leading cause of death, after cardiovascular diseases. Therefore researchers to seek more effective anticancer agent for cancer treatment. Chemotherapeutic drugs used in the cancer treatment are cytotoxic agents. Ideally, anticancer drugs should be selectively cause toxicity to cancer cells with minimal toxic effects to normal cells. Given the toxic side effect, development of resistance, and lack of broad spectrum treatments, there is a continuing need for the development of novel molecules for improving cancer chemotherapy. Most of cancer chemotherapeutics and chemopreventives exert their effects by triggering apoptotic cell death. Methyl-2-benzimidazole carbamate is an anticancer agent that induces apoptosis of cancer cells [1-3]. In this study, we aimed to investigate the cytotoxic and apoptotic properties of novel benzimidazole derivatives bearing pyridyl/pyrimidinyl piperazine moiety against C6 glioma cells.

MATERIALS AND METHODS
C6 glioma cell lines were used in the studies. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Detection of apoptosis was performed using Annexin V-FITC apoptosis detection kit BD, Pharmingen according to the manufacturer’s instruction. All measurements were performed on a FACS-calibur cytometer [4].

RESULTS AND DISCUSSION
The IC50 values of the compounds were determined for C6 cell line. Compounds 6,7,12 and 14, which were including 4-trifluoromethylphenyl and 4-nitrophenyl on pyridine ring; 4-fluorophenyl and 4-nitrophenyl on pyrimidine moiety, had significant cytotoxic activity with IC50 values lower than 51.67±16.07 μg/mL. Compound 14 showed the highest cytotoxic activity with a IC50 value of 28.2 μg/mL, whereas cisplatin IC50 values were 15±3 μg/mL against C6 cells. Cytotoxic activity of compound 6,7 and 12 with a IC50 value were 48±2, 51.67±16.07 and 30±2 μg/mL, respectively. Compound 6 showed the highest population of early apoptotic cells (14%) of the tested compounds which was 2.26-fold higher than for cisplatin. Compound 7 and 12 produced a comparable population of apoptotic cells with a percentage of 11.8% and 13.9 %, respectively according to cisplatin’s percentage of 6.2 %.

CONCLUSIONS
It was determined that synthesized compounds 6,7,12 and 14 had considerable anticancer activity against C6 cell lines compared to cisplatin. Compound 14 including 4-nitrophenyl on pyrimidine ring was the most cytotoxic compound against the C6 cell line. Our study results demonstrated that compound 6, 7, 12 affected C6 cells by the apoptotic pathway.

ACKNOWLEDGMENTS
The project is funded by Anadolu University (BAP, Project no: 1404S113)

REFERENCES
P-145: NOVEL MORPHOLINE DITHIOCARBAMATE DERIVATIVES BEARING BENZIMIDAZOLE MOIETY INDUCES APOPTOSIS

H.E. Temel¹, G. Akalın Çiftçi¹, L. Yu’tu’at²

Anadolu University, Faculty of Pharmacy
¹Department of Biochemistry, ²Department of Pharmaceutical Chemistry

INTRODUCTION
Cancer is a group of diseases characterized by abnormal and uncontrolled growth of cell and being a second leading cause of mortality worldwide from decades and day by day population of patients with cancer. So there is an urgent need to design and develop novel molecules for improving cancer chemotherapy. Benzimidazoles are the privileged components of many bioactive components and benzimidazole derivatives have attracted particular interest due to their potential anticancer activity in discovering novel drugs. Moreover, advances in understanding the mechanisms of cell proliferation, differentiation and apoptosis together with the mechanism of action of anticancer drugs allow us to pursue cancer chemotherapy at cellular and molecular levels [1,2]. In this present study, we aimed to investigate the possible underlying apoptotic mechanism for the cytotoxicity of new morpholine dithiocarbamate derivatives bearing 1-(2-aryl-2-oxoethyl)-2-substituted benzimidazole moiety on A549 lung carcinoma.

MATERIALS AND METHODS
A549 (human lung adenocarcinoma cells) cell lines were used in the studies. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Detection of apoptosis was performed using Annexin V-FITC apoptosis detection kit BD, Pharmingen according to the manufacturer’s instruction. All measurements were performed on a FACS-calibur cytometer [2].

RESULTS AND DISCUSSION
The IC50 values of the compounds were determined for A549 cell line. Compounds 2, 9, and 10, which were including 4-methyl, 3-chloro, and 3-fluoro substituents on phenyl acetyl moiety, had significant cytotoxic activity with IC50 values lower than 48.33± 7.64 µg/mL. Compound 2 showed the highest cytotoxic activity with a IC50 value of 16.67±3.06 µg/mL, whereas cisplatin IC50 values were 14±2 µg/mL against A549 cells. Cytotoxic activity of compound 9 and 10 with a IC50 value were 48.33 ± 7.64 and 31.67 ± 9.07 µg/mL, respectively. Compound 2 showed the highest population of early apoptotic cells (23.2%) of the tested compounds which was 3.09-fold higher than for cisplatin. Compound 10 and 9 produced a comparable population of apoptotic cells with a percentage of 13.7% and 9.8%, respectively according to cisplatin’s percentage of 7.5%.

CONCLUSIONS
It was determined that synthesized compounds had considerable anticancer activity against A549 cell lines. However compound 2 including 4-methyl substituent was the most active compound against the A549 cell line. Also our study results showed that compound 2, 9, 10 affected A549 cells by the apoptotic way.

ACKNOWLEDGMENTS
The project is funded by Anadolu University (BAP, Project no: 1404S116)

REFERENCES

P-146: MALATYA REGION OF 5 DIFFERENT APRICOT FRUIT OF METHANOL EXTRACT ANTIOXIDANT CAPACITY

Türkan Kutlu¹, Nida Topal¹, Işıl Yıldırım²

Inonu University, Faculty of science and arts
Department of Chemistry/Biochemistry, 44280, Malatya, TURKEY

INTRODUCTION
Antioxidants are substances that neutralize free radicals or their actions. Apricot is from Rosaceae family. This work performed that comparison of antioxidant capacity of Malatya region of 5 different apricot fruit of methanol extract.

MATERIAL AND METHOD
In this work were used Sekerpare, Sogancı, Kabaası, Hacihaliloglu, Çöloglu apricot fruit. Apricotson 2 July2010were collected from the Inonu University ofgardeniculture. Later the same day, all chopped, bagged -85C were kept stored until analysis time. Extract was prepared with methanol. Antioxidant capacity were determined by DPPH[1], β-carotene bleach[2] power reduction[3], ABTS[4], deoxyribose[5], and total antioxidant phenolic content[6] methods. All analysis were...
determined Shimadzu UV-1600 mark. As a standard ascorbic acid, Trolox and α-tocopherol were used.

RESULT AND DISCUSSION

Table 1. 200μg/ml values for apricot methanol extracts

<table>
<thead>
<tr>
<th>Apricot species</th>
<th>DPPH % inhibition</th>
<th>β-carotene</th>
<th>ABTS % inhibition</th>
<th>DPPH scavenging</th>
<th>Trolox</th>
<th>Total phenolic content of GAE/100g of fruit</th>
<th>GAE/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sekerpare</td>
<td>81.55</td>
<td>68.55</td>
<td>78.07</td>
<td>83.87</td>
<td>0.318</td>
<td>22.106</td>
<td></td>
</tr>
<tr>
<td>Kabaasi</td>
<td>72.99</td>
<td>70.99</td>
<td>69.10</td>
<td>82.18</td>
<td>0.230</td>
<td>12.778</td>
<td></td>
</tr>
<tr>
<td>DDDVÕ</td>
<td>57.12</td>
<td>71.71</td>
<td>74.80</td>
<td>79.22</td>
<td>0.450</td>
<td>9.087</td>
<td></td>
</tr>
<tr>
<td>Hicazlısu</td>
<td>84.00</td>
<td>70.05</td>
<td>70.45</td>
<td>67.72</td>
<td>0.380</td>
<td>12.979</td>
<td></td>
</tr>
<tr>
<td>Çolıgolo</td>
<td>98.00</td>
<td>77.12</td>
<td>75.78</td>
<td>66.87</td>
<td>0.394</td>
<td>12.425</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>67.80</td>
<td>55.20</td>
<td>66.79</td>
<td>55.56</td>
<td>0.270</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In a result the highest antioxidant capacity was determined Cöloğlu apricot fruit (88%) for DPPH method. In β-carotene bleach method a difference was observed between theapricot methanol extract. The best capacity was observed in the methanol extract of Sekerpare (0.318%). The Kabaasi (0.359) was followed to it for power reduction method (Analysis, lower absorbance indicates a higher activity). The highest antioxidant capacity was determined in the methanol extract of Sekerpare for ABTS method (78.08%). The highest antioxidant capacity was determined in the methanol extract of Sekerpare (83.67%) and Kabaasi (83.18%) for deoxyribose method. Total antioxidant phenolic content of 22.196 mg GAE/100g was methanol extract of Sekerpare.

CONCLUSION

Apricots are rich in other antioxidants, too, including polyphenolic antioxidants like flavonoids. Diets rich in flavonoids and the other types of polyphenols found in apricots have been linked to reductions in heart disease in humans, as well as other potential health benefits.

REFERENCE

Consequently; as based on the dose, methanol extract of this plant exhibited partially cytotoxicity against LS174t colon carcinoma cells.

CONCLUSION
Currently have been many studies on the anticancer effect of garlic. This endemic species of garlic will be useful to study the anticancer activity on different cancer cells.

REFERENCE

INTRODUCTION
Phenylketonuria (PKU) is a disease that characterized by an inability to metabolize the amino acid L-phenylalanine[1]. PKU is caused by absent or virtually absent phenylalanine hydroxylase enzyme activity. Children born with this disease, cannot convert essential amino acid phenylalanine to tyrosine[2]. Phenylalanine which is taken with food in PKU patients, piles up in blood and goes into cerebrospinal fluid. This case causes to mental and neurological retardation in individuals [2]. Accumulating phenylalanine produces irreversible brain damage. PKU is usually diagnosed by measuring blood-phenylalanine concentration with newborn screening test[2]. In this study, we report a new L-phenylalanine dehydrogenase (PHD) and Toluidine Blue O (TBO) based amperometric L-phenylalanine biosensor for the determination of L-phenylalanine.

MATERIALS AND METHODS
In this study, an amperometric L-phenylalanine biosensor with immobilization of TBO (as a mediator), PDH and nicotinamide adenine dinucleotide (NAD+) onto polypyrrole-polyvinylsulphonate (PPy-PVS) film was accomplished on the surface of a platinum electrode. PDH, TBO and NAD+ were immobilized by a glutaraldehyde/bovine serum albumin (BSA) crosslinking procedure on to PPy-PVS film after the electropolymerization processes. The effects of substrate concentration, pH and temperature on the response of the L-phenylalanine biosensor were investigated. The stability and reproducibility of the biosensor was also studied.

RESULTS AND DISCUSSION
A novel amperometric L-phenylalanine biosensor with PDH, TBO and NAD+ on PPy-PVS film was accomplished. TBO was used as a mediator; it could facilitate the electron transfer that involved a chemical interaction between the electrode surface and NADH. Determination of L-phenylalanine was carried out by the oxidation of TBO at -0.195 V vs. Ag/AgCl. The optimum working conditions with respect to the substrate concentrations were investigated. The effects of pH and temperature were investigated and optimum parameters were found to be 7.0 and 60.0°C, respectively. The storage stability and operational stability of the enzyme electrode were also studied.

CONCLUSIONS
In this study, we developed an amperometric biosensor for direct, effective, and rapid detection of L-phenylalanine. The experimental results clearly showed that the biosensor exhibited perfect performance for the determination of L-phenylalanine. It was seen that the L-phenylalanine biosensor was highly sensitive and selective and its operational stability and long-term storage stability were found to be good. This biosensor was also easy to prepare and was highly cost effective. In addition, PPy-PVS could supply a biocompatible and electrochemical microenvironment for immobilization of the enzyme, making this material a good candidate for the fabrication of highly sensitive and selective L-phenylalanine biosensors.

REFERENCES

P-149: EFFECTS OF AQUEOUS EXTRACT OF MYRTUS COMMUNIS L. LEAVES ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

H, Baz1, M. Gulaboglu1, L. Gozc1, G.M. Demir1, D. Canayakin1, H. Suleyman2, Z. Halici3, N. Kiliç Baygutalp4

1 Ataturk University, Faculty of Pharmacy, Department of Biochemistry, Erzurum, TURKEY
2 Erzincan University, Faculty of Medicine, Department of Pharmacology, Erzincan, TURKEY
3 Ataturk University, Faculty of Medicine, Department of Pharmacology, Erzurum, TURKEY
4 Ataturk University, Faculty of Pharmacy, Department of Pharmacy Management, Erzurum, TURKEY

INTRODUCTION
Diabetes mellitus (DM) is the known most common chronic disease world-wide. Leaves of a Mediterranean plant, Myrtus communis L. (MC), is traditionally used in the treatment of diabetes mellitus (1). Therapeutic effects of leaves, oil and fruit of MC on diabetes mellitus have been investigated in many studies (2,3). There are few studies in the literature investigating the antioxidant effect of aqueous extract from MC leaves on diabetes (4). AST, ALT and ALP are liver enzymes considered to be associated with diabetes risk (5). In this study, we investigated the antidiabetic and antioxidant effects of the aqueous extract of MC leaves on normal and streptozotocin-induced diabetic rats.

MATERIALS AND METHODS
Materials
Plant material: The aerial parts of MC was collected from Antalya, a city located in the Mediterranean region of Turkey, in August 2012. The powdered leaves of herb was percolated in pure water. The solvent was filtered and evaporated under vacuum using a rotary evaporator. The obtained water extract was stored after lyophilization.

Chemicals: Streptozotocin (C9H12N3O7) (STZ) was purchased from Sigma Chemical Co. (Germany).

Methods: Thirty Sprague Dawley male rats were randomly divided into six groups as each composed of five rats (Table 1). Diabetes mellitus was induced by a single 40 mg/kg dose of STZ. Aqueous extract of MC leaves was administered orally to the groups 150, 300 and 600 mg/kg (I, II and III doses, respectively) for 14 days, starting the third day of STZ administration. Animals were sacrificed and the livers were rapidly removed to determine antioxidant levels tissue homogenate. Blood glucose levels were measured by Accu-Chek Active blood glucose monitor with disposable dry reagent strips which is suitable for glucose oxidase technique. Superoxide dismutase (SOD) activity, glutathione (GSH) and malondialdehyde levels (MDA) from each sample supernatant were measured by commercial ELISA kits according to manufacturer’s instructions. Serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) levels were measured by biochemical and immune-enzyme analyzer GBG ChemWell 2990 (USA) with respective standard kits.

Statistical analysis: Results was expressed as mean ± standard deviation (SD). Differences among the groups were determined by Fisher’s least significant difference (LSD) test and p values less than 0.05 was considered significant, using SPSS 20.0 software.

RESULTS AND DISCUSSION
The aqueous extract of MC at the dose of 600 mg/kg provided significant decrease in blood glucose levels (p<0.001) (Table 1).

MC administration reduced serum AST, ALT and ALP levels in all diabetic groups (Table 2). SOD activity and GSH level were found to be increased and MDA level were found to be significantly decreased in MC administration groups compared to control group (p<0.05). This antioxidant activity was dose dependent and found to be highest at the 600 mg/kg dose of MC aqueous extract. MDA and GSH levels and SOD activity measured by administration of MC at the dose of 600 mg/kg were similar to the levels in the normal control group (Table 3).

Table 1. Effects of MC administration on blood glucose levels in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level before MC administration (mg/dL)</th>
<th>Blood glucose level after MC administration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>80.290 ± 4.438</td>
<td>81.000 ± 4.795**</td>
</tr>
<tr>
<td>Diabetic Control (n=5)</td>
<td>262.600 ± 10.358**</td>
<td>459.400 ± 17.812**</td>
</tr>
<tr>
<td>MC 600 mg/kg (n=5)</td>
<td>89.210 ± 9.294</td>
<td>88.400 ± 9.294**</td>
</tr>
<tr>
<td>DM+MC 150 mg/kg (n=5)</td>
<td>250.000 ± 8.860**</td>
<td>252.600 ± 9.338**</td>
</tr>
<tr>
<td>DM+MC 300 mg/kg (n=5)</td>
<td>260.400 ± 9.813**</td>
<td>217.400 ± 8.876**</td>
</tr>
<tr>
<td>DM+MC 600 mg/kg (n=5)</td>
<td>264.200 ± 11.344**</td>
<td>161.000 ± 8.916**</td>
</tr>
</tbody>
</table>

Results are means±SD. MC: Myrtus communis L aqueous extract, DM: Rats with diabetes mellitus comparisons: **control vs other groups **: p<0.001, ** diabetic control vs other groups ++: p<0.001
Table 2. Effects of *Myrtus communis* L. administration on serum ALT, AST and ALP levels in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>28.43 ± 1.154**</td>
<td>49.86 ± 6.559**</td>
<td>93.73 ± 6.518**</td>
</tr>
<tr>
<td>Diabetic Control (n=5)</td>
<td>44.75 ± 0.482</td>
<td>98.85 ± 8.312</td>
<td>151.31 ± 10.107</td>
</tr>
<tr>
<td>MC 500 mg/kg (n=5)</td>
<td>25.74 ± 0.922**</td>
<td>45.03 ± 6.434**</td>
<td>80.10 ± 4.464**</td>
</tr>
<tr>
<td>DM + MC 150 mg/kg (n=5)</td>
<td>42.18 ± 1.906*</td>
<td>88.16 ± 6.914*</td>
<td>141.53 ± 5.995*</td>
</tr>
<tr>
<td>DM + MC 300 mg/kg (n=5)</td>
<td>36.41 ± 2.146**</td>
<td>82.84 ± 4.596**</td>
<td>122.38 ± 7.435**</td>
</tr>
<tr>
<td>DM + MC 600 mg/kg (n=5)</td>
<td>29.21 ± 1.571**</td>
<td>72.42 ± 15.108**</td>
<td>106.23 ± 5.459**</td>
</tr>
</tbody>
</table>

Results are means±SD. MC: Myrtus communis L. aqueous extract, DM: Rats with diabetes mellitus, comparisons: diabetic control group vs other groups, *p<0.05, **p<0.001

Table 3. Effects of *Myrtus communis* L. administration on liver tissue SOD activity and GSH, MDA levels in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg tissue)</th>
<th>GSH (µM/mg tissue)</th>
<th>MDA (µM/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>0.238 ± 0.013**</td>
<td>112.303 ± 7.901**</td>
<td>6.113 ± 1.168</td>
</tr>
<tr>
<td>Diabetic Control (n=5)</td>
<td>0.392 ± 0.005</td>
<td>84.964 ± 6.832</td>
<td>11.119 ± 1.13</td>
</tr>
<tr>
<td>MC 500 mg/kg (n=5)</td>
<td>0.250 ± 0.016**</td>
<td>116.782 ± 5.495**</td>
<td>7.123 ± 1.525</td>
</tr>
<tr>
<td>DM + MC 150 mg/kg (n=5)</td>
<td>0.141 ± 0.012</td>
<td>94.440 ± 8.756</td>
<td>9.579 ± 0.735</td>
</tr>
<tr>
<td>DM + MC 300 mg/kg (n=5)</td>
<td>0.176 ± 0.009**</td>
<td>130.166 ± 5.519**</td>
<td>9.149 ± 0.685</td>
</tr>
<tr>
<td>DM + MC 600 mg/kg (n=5)</td>
<td>0.201 ± 0.012**</td>
<td>126.613 ± 7.173**</td>
<td>8.294 ± 1.287</td>
</tr>
</tbody>
</table>

Results are means±SD. MC: Myrtus communis L. aqueous extract, DM: Rats with diabetes mellitus, comparisons: diabetic control group vs other groups, *p<0.05, **p<0.001

CONCLUSIONS
The results of this study suggest that aqueous extracts of MC leaves at the doses of 150, 300 and 600 mg/kg decrease blood glucose, serum ALT, AST and ALP levels. Besides, all extracts have antioxidant effects being highest at 600 mg/kg dose.

ACKNOWLEDGMENTS
This work was supported by Scientific Research Projects Unit of Ataturk University (grant number: 2009/314).

REFERENCES

P-150: ELECTROMAGNETIC FIELD EFFECTS ON OXIDATIVE STRESS PARAMETERS IN RAT LIVER AND KIDNEY TISSUES

B.A. Mamur1, N. Aras1, M. Berkoz2, Ü. Comelekoglu3, M. Yildirim4, S. Yalin4

Mersin University, Faculty of Medicine, 1Department of Medical Biology, 3 Department of Biophysics, Faculty of Pharmacy, 4Department of Biochemistry, Mersin, TURKEY

INTRODUCTION
Extremely low frequency electromagnetic fields (ELF-EMF) have been common in daily life all over the world. EMF represents one of the environment factor that influence animal organism that that conduct the organism to stress. It is known that a powerful stress is associated with metabolic modifications, including the entire complex of redox processes which facilitate the adaptable processes of the living organisms. An important link in oxide-reducing homeostasis maintenance is due to cell antioxidant enzymes. In this study we determined the oxidative stress parameters from rat liver and kidney tissues that were exposed to the Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1800MHz.

MATERIALS AND METHODS
We divided female mature albino rats of wistar strain in three groups two of which were control (Group I) and sham (Group II). Third group was exposed to the RF-EMF for 2 h/day for 8 weeks (Group III). At the end of the study, the rats in all groups were sacrificed by cardiac punction under ketamine and xylazine anesthesia. Liver and kidney tissues were separated and kept at −80 °C until superoxide dismutase (SOD) and catalase (CAT) activities and glutathione (GSH) malondialdehyde (MDA) levels were measured. The CAT activities of tissues were determined in accordance with the method introduced by Aebi. SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O2 generated by the xanthine/xanthine oxidase system. The reduction in NBT levels by superoxide anion to blue formazan was measured at 560 nm. Virtually all of the nonprotein sulphydryl compounds of tissue were existing in the form of GSH. 5,5′ Dithiobis (2-nitro benzoic acid) (DTND) is a disulfide compound readily reduced by sulphydryl compounds that form a highly colored yellow anion by the method described by Beutler et al. The levels of MDA as an index of LPO were determined in tissue homoginates by thiobarbituric acid reaction by using the method of Yagi.

Tissue protein contents were measured according to the method developed by Lowry et al. using bovine...
serum albumin as standard. Statistical analysis was performed using SPSS 10.0 for windows software. The obtained data were presented as mean ± SE (standard error) unless otherwise specified. The differences were considered as statistically significant when p<0.05.

RESULTS AND DISCUSSION
SOD activities were found in Group I, Group II and Group III as 86.62±10.81 U/mg protein, 81.92±11.54 U/mg protein and 51.93±8.46 U/mg protein in liver tissue and 53.27±6.49 U/mg protein, 49.27±6.06 U/mg protein and 34.2±5.88 U/mg protein in kidney tissue, respectively. Catalase activities were found in Group I, Group II and Group III as 56.22±6.27 U/mg protein, 51.07±7.42 U/mg protein and 24.01±4.31 U/mg protein in liver tissue and 44.02±7.81 U/mg protein, 45.91±8.11 U/mg protein and 22.2±4.06 U/mg protein in kidney tissue, respectively. GSH levels were found in Group I, Group II and Group III as 0.81±0.06 μmol/mg protein, 0.84±0.06 μmol/mg protein and 0.53±0.04 μmol/mg protein in liver tissue and 0.67±0.08 μmol/mg protein, 0.66±0.09 μmol/mg protein and 0.41±0.06 μmol/mg protein in kidney tissue, respectively. MDA levels were found in Group I, Group II and Group III as 91.06±10.04 nmol/mg protein, 94.04±10.47 nmol/mg protein and 147.83±19.61 nmol/mg protein in liver tissue and 78.71±12.63 nmol/mg protein, 76.81±11.87 nmol/mg protein and 106.93±8.08 U/mg protein in kidney tissue, respectively. The electromagnetic field led to a significant increase in malondialdehyde (MDA) levels and significant decrease in SOD and CAT levels in the liver and kidneys tissue of rats (p<0.05). There was no significant difference in GSH levels in the same tissues (p>0.05).

CONCLUSIONS
In conclusion, electromagnetic field emitting from mobile phone might produce impairments in some oxidative stress parameters in the liver and renal tissue of albino rats.

REFERENCES

P-151: INFLUENCE OF SUBLETHAL CHLORPYRIFOS EXPOSURE ON OXIDATIVE STRESS AND ACETYLCHOLINESTERASE ACTIVITY IN CARP (CYPRINUS CARPIO)

M. Berköz1, S.G. Gunduz2, F.Ö. Yılmaz2, S. Yalın3, A.O. Hunt4, M. Yıldırım3

Yuzuncu Yıl University, Faculty of Pharmacy, 1Department of Pharmaceutical Biotechnology, Van, Turkey, Mersin University, Faculty of Fisheries, 2Department of Basic Sciences, 3Department of Aquaculture, Faculty of Pharmacy, 4Department of Biochemistry, Mersin, Turkey

INTRODUCTION
The commonly used pesticides in agriculture may react with macromolecules and may cause enzyme inactivation and DNA damage. Furthermore, they may also initiate peroxidation of poly-unsaturated fatty acids (PUFA) due to their deposition in fatty tissues by the generation of reactive oxygen species (ROS) as by-products. In the course of these events they can lead to oxidative stress. The objective of our study was to determine the oxidative and neurotoxic potential of sub-lethal concentrations (0.26 ppm and 0.52 ppm) of chlorpyrifos which is extensively used as a pesticide in Turkish agriculture in brain tissue at the 96th and 240th hours.

MATERIALS AND METHODS
In order to detect the levels of oxidative stress in brain tissue, glutathion levels were detected by using superoxide dismutase possessing antioxidant features. Moreover, malondialdehyde (MDA) levels and acetylcholine esterase (AChE) levels were examined for the determination of levels of lipid prexodiation and neurotoxic effect, respectively. Acetylcholine esterase activity in cerebral cortex was performed by utilizing the spectrophotometric method of described by Ellman, Courteney, Andres, and Featherstone. The levels of tissue lipid peroxidation products such as thiobarbituric acid (TBA)-malondialdehyde (MDA) adducts were measured spectrophotometrically by the method described by Yagi. Virtually, all of the nonprotein sulfhydryl compounds of tissue were H[LVWLQJLQWKHIRUPRI*6+ƍ'LWKLRELV-nitro benzoic acid) (DTND) is a disulfide compound readily which is reduced by sulfhydryl compounds that form a highly colored yellow anion by the method described by Beutler et al. The optical density of this yellow substance is measured at 412 nm. SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O2 generated by the xanthine/xanthine oxidase system. The contents of tissue protein were measured in accordance with the method developed by Lowry et al. by using bovine serum albumin as standard. Statistical analysis was performed using SPSS 10.0 for windows software. The obtained data were
presented as mean ± SE (standard error) unless otherwise specified. ANOVA and Tukey multiple range tests were used to analyze differences between the groups. The differences were considered as statistically significant when p<0.05.

RESULTS AND DISCUSSION

AChE, SOD and GST activities and GSH and MDA levels were shown in the table.

<table>
<thead>
<tr>
<th></th>
<th>Control 90th hour</th>
<th>0.26 ppm 90th hour</th>
<th>0.52 ppm 90th hour</th>
<th>Control 240th hour</th>
<th>0.26 ppm 240th hour</th>
<th>0.52 ppm 240th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE (U/mg prt.)</td>
<td>0.14±0.02</td>
<td>0.08±0.01</td>
<td>0.06±0.01</td>
<td>0.13±0.02</td>
<td>0.07±0.01</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>SOD (U/mg prt.)</td>
<td>41.17±5.22</td>
<td>38.11±6.23</td>
<td>21.61±5.01</td>
<td>40.23±6.17</td>
<td>35.96±5.12</td>
<td>22.33±4.74</td>
</tr>
<tr>
<td>GST (U/mg prt.)</td>
<td>38.24±4.07</td>
<td>34.52±5.74</td>
<td>31.49±6.42</td>
<td>35.04±5.01</td>
<td>32.96±6.41</td>
<td>17.04±8.43</td>
</tr>
<tr>
<td>GSH (µmol/mg prt.)</td>
<td>0.08±0.01</td>
<td>0.07±0.01</td>
<td>0.04±0.01</td>
<td>0.07±0.01</td>
<td>0.07±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>MDA (µmol/mg prt.)</td>
<td>15.71±4.32</td>
<td>24.45±7.21</td>
<td>29.1±4.04</td>
<td>16.81±4.54</td>
<td>18.26±3.51</td>
<td>19.54±3.07</td>
</tr>
</tbody>
</table>

AChE esterase activity was reduced in both concentrations over the time (p<0.5). MDA levels were increased at the 96th hour for both concentrations (p<0.05). The levels of SOD and GSH were elevated at the 240th hour for both concentrations (p<0.5). GST activity reduced at 240th hour for only the concentration of 0.52 ppm (p<0.05).

CONCLUSIONS

We were able to observe an induced oxidative stress and significant inhibition of AChE in the brain tissues of *Cyprinus carpio* exposed to chlorpyrifos. These findings manifest that sub-lethal concentration of chloropyrifos leads to significant systemic toxicity in the brain tissues of *Cyprinus carpio*.

REFERENCES


P-152: IRON STATUS IN ADOLESCENT GIRLS-RELATION TO OBESITY AND INFLAMMATION

N. Kavarić 1, A. Klisic 1, T. Injac 2, D. Bozovic 3, N. Gligorović-Barhanovic 3

1 Primary Health Care Center, Podgorica, Montenegro

INTRODUCTION

Obesity is associated with chronic low-grade inflammation [1]. Moreover, recent studies reported iron deficiency in overweight/obese adolescents [2, 3]. On the contrary, some other reports suggested that adiposity was sufficient to cause chronic inflammation but not to impair iron status [4]. Therefore, we aimed to determine some iron status biomarkers and to examine their potential association with anthropometric and inflammation parameters in normal weight and overweight/obese adolescent girls.

MATERIALS AND METHODS

A cross-sectional study was performed in a primary care setting. A total of 22 overweight/obese adolescent girls (mean age 17.5±0.34 years) and 16 age-matched normal weight controls were included. Biochemical and haematological parameters of iron status: serum iron, soluble transferrin receptor concentration (sTfR), transferrin, ferritin, red blood cell count (RBC), haemoglobin, haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured. Inflammation was determined by high sensitivity C-reactive protein (CRP). Body weight and body height were obtained. Body mass index (BMI) z-score was calculated [5]. All the participants completed a questionnaire including dietary habits, somatic illnesses, medications use, and lifestyle habits. Adolescent girls younger than 16 years, and older than 19 years, as well as participants who had diabetes mellitus, renal, hepatic or thyroid dysfunction, cardiovascular disorders, with signs and symptoms of acute inflammatory disease and CRP > 10 mg/L, with a history of alcohol consumption and smoking, and those who used any medications were excluded from the study. Using the World Health Organization growth reference 5-19 years [5] adolescents were categorized as normal weight (-2SD ≤ BMI z-score ≤ +1SD), and overweight (+1SD < BMI z-score < +2SD) or obese (BMI z-score ≥ +2 SD).

RESULTS AND DISCUSSION

Overweight/obese girls displayed higher serum CRP, sTfR and ferritin levels (p<0.001, p=0.013 and p=0.034, respectively), but lower MCHC (p=0.008) as compared with normal weight group. However, there was no difference in serum transferrin and iron level, RBC, haemoglobin, Hct, MCV and MCH between groups. In all girls, serum ferritin correlated positively with body weight and BMI z-score (p=0.043 and p=0.030, respectively). sTfR correlated positively with body weight and BMI z-score (p=0.023 and p=0.029, respectively), RBC (p=0.005), and negatively with MCV, MCH and MCHC (p=0.011,
p=0.002, p=0.002, respectively). Serum CRP correlated positively with body weight and BMI z-score (p<0.001), ferritin (p=0.042), and negatively with serum iron level (p=0.048), hemoglobin (p=0.043), and MCHC (p=0.009).

Results of the current study suggest that not only overweight/obese adolescent girls exhibited higher inflammation level, but they also displayed higher sTfR (the best clinical measure of iron status), which is not an acute-phase protein, unlike ferritin. Our results are discordant with some previous reports [4].

CONCLUSIONS
Though relatively small sample size of study groups, our results show that overweight/obese adolescent girls displayed poorer iron status as measured by higher sTfR and ferritin levels and both were correlated with body weight and BMI z-score. Moreover, beside expected association between CRP and anthropometric indices, this inflammation marker correlated with many iron status indices, suggesting a complex association between obesity, obesity-related inflammation and alternation of iron biomarkers in adolescent girls.

REFERENCES

INTRODUCTION
Retinol-binding protein 4 (RBP4) has been proposed as an emerging cardiometabolic risk factor in adults[1, 2]. Yet, there are discrepancies regarding some of the possible metabolic roles of RBP4 in children and adolescents [3, 4, 5]. Therefore, we aimed to evaluate serum RBP4 and examine its potential association with cardiometabolic parameters in Montenegrin adolescent girls.

MATERIALS AND METHODS
A total of 90 adolescent girls (ages between 16-19 years) were included. Of them, 45 were normal-weight and 45 were overweight/obese. Biochemical parameters: RBP4, glycemia, insulin, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides were determined. Body weight, body height, waist circumference (WC), and blood pressure were measured. Body mass index (BMI) z-score and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated. All the participants completed a questionnaire including somatic illnesses, medications use, and lifestyle habits. Girls younger than 16 years, and older than 19 years, as well as participants who had diabetes mellitus, renal, hepatic or thyroid dysfunction, cardiovascular disorders, with signs and symptoms of acute inflammatory disease, with a history of alcohol consumption and smoking, and those who used any medications were excluded from the study. A correlation analysis by Pearson’s (r) correlation coefficient was used to determine the relationships between serum RBP4 level and other variables.

RESULTS AND DISCUSSION
In all girls, RBP4 correlated positively with BMI z-score (r=0.297, p=0.004), WC (r= 0.379, p<0.001), triglycerides (r=0.469, p<0.001), insulin (r=0.247, p=0.019), HOMA-IR (r=0.257, p=0.014), and negatively with HDL-cholesterol (r= -0.387, p<0.001). After stepwise multiple linear regression analysis, only triglycerides (Beta=0.317, p<0.001) were independently associated with RBP4 (R² = 0.335, p<0.001). Results of the current study suggest that only triglycerides independently correlated with RBP4 in adolescent girls, presuming that RBP4 may be involved in the pathophysiology of hypertriglyceridemia, and that previously reported associations between RBP4 and insulin resistance may be influenced primarily by triglyceride levels. Our results are discordant with some earlier reports which confirmed this association predominantly in boys, but not in girls [4]. Moreover, some others did not observe the association between RBP4 and triglycerides at all [5].

P-153: ASSOCIATION OF RETINOL-BINDING PROTEIN 4 WITH CARDIOMETABOLIC RISK FACTORS IN MONTENEGRIN ADOLESCENT GIRLS

N. Kavaric1, A. Klisic1, Tanjaljac1, D. Bozovic3, N. Gligorovic-Barhanovic 3
1 Primary Health Care Center, Podgorica, Montenegro
2 Primary Health Care Center, Niksic, Montenegro
3 Center of Clinical-Laboratory Diagnostics, Clinical Center of Montenegro, Podgorica, Montenegro
CONCLUSIONS
Higher serum RBP4 levels were associated with unfavorable cardiometabolic profile in adolescent girls, but triglycerides remained the only independent predictor of RBP4.

REFERENCES

RESULTS AND DISCUSSION
Statistically significant increases were found in all caspase-3, -8, and -9 enzymes; antioxidant capacity and lipid peroxidation were evaluated by measurement reduced glutathione (GSH) content and superoxide dismutase (SOD) enzyme activity and the level of malondialdehyde (MDA), respectively. Besides nitric oxide (NO) production was evaluated by measurement of nitrite content in lung tissues. Histopathological investigations were also carried out.

CONCLUSION
The data suggested that iloprost is a cytoprotective agent by reducing apoptosis and lipid peroxidation on I/R injury in lung as a distant organ.

MATERIALS AND METHODS
Wistar albino rats were divided into five groups as sham, ischemia, I/R, iloprost (10 μg/kg) and I/R with iloprost (10 μg/kg), including 12 rats in each group. A 4 h reperfusion procedure was carried out after 4 h of ischemia. 10 μg/kg iloprost was administered to the rats in 1 mL of saline from the tail vein in iloprost group. Rats received 10 μg/kg iloprost in the same way 10 min before reperfusion in I/R with iloprost group. Apoptosis was evaluated by western-blotting analysis of caspase-3, -8, and -9 enzymes; antioxidant capacity and lipid peroxidation were evaluated by measurement of reduced glutathione (GSH) content and superoxide dismutase (SOD) enzyme activity and the level of malondialdehyde (MDA), respectively. Besides nitric oxide (NO) production was evaluated by measurement of nitrite content in lung tissues. Histopathological investigations were also carried out.

ACKNOWLEDGMENTS
This research was supported by research Foundation of Mersin University, Mersin, Turkey. (Grant No: BAB-ECZ F TEB (NC) 2007)

REFERENCES
P-155: INVESTIGATION OF ANTIMICROBIAL EFFECT FOR NOVEL THE POLYMERS WITH NANO-SPHERE

N. Kurnaz Yetim1,2, E. Hasanoğlu Özkân1, N. Sari1, O. Eren1, H. Öğütçü1

1 Gazi University, Faculty of Science, Department of Chemistry, Ankara, 2 Kırklareli University, Faculty of Art and Science, Department of Chemistry, Kırklareli, 3 Ahi Evran University, Faculty of Arts and Science, Department of Biology, TURKEY

INTRODUCTION

Antimicrobial polymers are gaining the attention of pharmaceutical makers and industrial design [1,2]. Nanospheres-Polymers attached Platinum (II)/(IV) complexes have been synthesized to investigate antimicrobial activities. They have the advantage that they are chemically stable and do not permeate through skin. Furthermore, they play an important role in reducing the incidences of infections caused by biomaterial implant. As far as we know, no studies have been carried out on nanosphere including Platinium-Schiff bases complexes. This study aimed to fill in this gap.

MATERIALS AND METHODS

Nanospheres involving Schiff bases synthesized from (aminomethyl)polystyrene and two substitute salicylaldehyde (5-kloro-2-hydroxy benzaldehyde, 5-bromo-2-hydroxy benzaldehyde). Secondly, polymers attached Platinum(II) / (IV) complexes have been prepared by means of template method. For the bacterial subcultures, L. monocytogenes 4b ATCC19115, S. aureus ATCC25923, E. coli ATCC1230, S. typhi H NCTC-901.8394, Br. abortus RSKK03026, S. epidermis sp., M. luteus ATCC 9341, S. dysenteria type 10 NCTC 9351, B. cereus sp were chosen. For activities measurement, the media used were Mueller- Hinton agar for bacteria. At the end of the incubation period (24 h at 37 oC), the diameter of the zone of inhibition around the wells was measured.

RESULTS AND DISCUSSION

All the synthesized compounds and antibiotic exhibited varying degree of inhibitory effects on the growth of different tested strains. The results of antifungal and antibacterial screening indicated that the Pt(IV) derivatives with nano-spheres showed more activity than the other studied nano-spheres. All of the polymers with nano-sphere were active against S.aureus and B. cereus.

Table I. Antimicrobial activity of polymer attached Schiff bases and Pt(II) / Pt(IV) complexes (0.018 g/ml) (Diameter of zone inhibition (mm))

<table>
<thead>
<tr>
<th>Compound</th>
<th>[APS-SchCl-Pt2+]</th>
<th>[APS-SchCl-Pt4+]</th>
<th>[APS-SchBr-Pt2+]</th>
<th>[APS-SchBr-Pt4+]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (RSLC-07055)</td>
<td>13</td>
<td>10</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Sh. albus ip 10</td>
<td>11</td>
<td>11</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>L. monocytogenes 4b (ATCC 15115)</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>E.coli (ATCC 1293)</td>
<td>14</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>S.epidermis (NCTC 901.8394)</td>
<td>11</td>
<td>-</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>S.epidermis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Br. abortus (RSKK03026)</td>
<td>20</td>
<td>15</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>M.luteus</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B.cereus</td>
<td>19</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The new Pt(II) / Pt(IV) derivatives with nanospheres compounds ([APS-SchCl-Pt2+], [APS-SchCl-Pt4+], [APS-SchBr-Pt2+], [APS-SchBr-Pt4+]) prepared in our study seems to have interesting biological activity. The Pt(IV) derivatives with nano-spheres are more potent bactericides than the Pt(II) derivatives with nanospheres. We know that bacteria needs folic acid to synthesize DNA and repair DNA. Bacteria have to make their own folic acid. Pt(IV) is kinetically more inert than Pt(II). So, Pt(IV) drugs are more stable to acidic media.

ACKNOWLEDGMENTS

This work was supported by the Gazi University Research Fund (Project number: 05/2012-53 and 05/2014-02).

REFERENCES


P-156: THE RESPONSE TIME IN THE ENZYME STUDIES OF THE POLYMER SUPPORTS INCLUDING DIFFERENT SUBSTITUENTS

N. Kurnaz Yetim1,2, E. Hasanoğlu Özkân1, N. Sari1

1 Gazi University, Faculty of Science, Department of Chemistry, Ankara, 2 Kırklareli University, Faculty of Art and Science, Department of Chemistry, Kırklareli, TURKEY

INTRODUCTION

Enzymes are used as biocatalysts in the chemical, pharmaceutical and food industries and as specific
ligands in clinical and chemical analyses [1]. Glucose oxidase (GOx) is a commercially important enzyme, which has applications in the pharmaceutical industry as a biosensor for the enzymatic determination of glucose in the fermentation of liquor and in the food industry for the removal of glucose and shelf life of various products [2,3].

MATERIALS AND METHODS

The activities of the immobilized enzymes with various substrate concentrations were plotted as Lineweaver-Burk graphs to calculate $V_{\text{max}}$ and $K_m$ values. The $V_{\text{max}}$ value defines the maximum velocity when all of enzyme is saturated with substrate. $K_m$, the $V_{\text{max}}$ reflects the effective characteristic of the enzyme and depends upon both partitioning and diffusion [4].

RESULTS AND DISCUSSION

$V_{\text{max}}$ values were calculated from Lineweaver-Burk plots for immobilized GOx to the [APS-SchF-GOx], [APS-SchCl-GOx] and [APS-SchBr-GOx] supports, $V_{\text{max}}$=72.99, $V_{\text{max}}$=49.01 and $V_{\text{max}}$=8.99 mMmin$^{-1}$, respectively.

![Figure: Kinetic parameters for [APS-SchCl-GOx], [APS-SchBr-GOx], [APS-SchF-GOx]]

CONCLUSIONS

The $V_{\text{max}}$ values are compared that the highest value of the [APS-SchF-GOx] support. In addition, when [APS-SchF-GOx] support is used, the time for the oxidation of glucose to gluconic acid is shortened.

REFERENCES


P-157: SYNTHESIS, ANTIBACTERIAL, ANTIUREASE AND ANTI OXIDANT ACTIVITIES OF SOME NEW 1,2,4-TRIAZOLE SCHIFF BASE AND AMINE DERIVATIVES

N. Gumrukuoğlu1, B. Bilgin Sokmen2, H. Sahin2, Y. Sagkal2, S. Ugras3, H. I. Ugras4

Vocational School of Health Sciences, Trabzon, TURKEY

INTRODUCTION

There is an increasing demand for the preparation of new antibacterial agents due to the developing resistance towards conventional antibiotics. The synthesis of 1,2,4-triazole derivatives has attracted widespread attention due to their diverse biological activities, including antibacterial, anti-inflammatory, analgesic, and antitumoral [1]. In this present study, we have synthesized some new 1,2,4-triazole compounds and antiurease, antioxidant activities for the newly synthesized compounds have evaluated.

MATERIALS AND METHODS

Melting points were determined on a Barnstead Electrothermal melting point apparatus and are uncorrected. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian-Mercury 200 MHz spectrophotometer using tetramethylsilane as the internal reference. The IR spectra were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. Antioxidant activities of samples were determined in a spectrophotometer (UV-1240, Shimadzu, Japan). The synthesized compounds were tested individually against 11 gram-positive and gram-negative bacteria species. The bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC). Urease inhibitory activity was determined according to Van Slyke and Archibald [2]. The antioxidant activities of the samples were determined by FRAP assay. The method is based on the measurement of the iron reducing capacities of the compounds. Radical scavenging activity of samples against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was spectrophotometrically at 517 nm. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants. Radical scavenging activity was measured by using Trolox, BHT as standards and the values are expressed as SC50, the concentration of the samples that causes 50% scavenging of DPPH radical.

RESULTS AND DISCUSSION

In this study, the compound 2 was synthesized from the reaction of ethyl benzimidate hydrochloride 1, which were obtained by a published method [3], with furan-2-carboxyhydrazide. The compound 3 was obtained by treatment of the compound 2 with
hydrazine hydrate. The reaction was carried out in 1-propanol at refluxing temperature for 24 h and the desired 4-amino-3-furan-2-yl-5-phenyl-1,2,4-triazole 3 was yielded. The compound 3 was converted to its Schiff bases (4a-c) by refluxing with different aldehydes in acetic acid. Previously, we obtained the Schiff bases of 1,2,4-triazole derivatives. The synthesis of compounds (5a-c) was performed by the reduction of only the exocyclic azomethine bond of the Schiff bases (4a-c) (Figure 1). Antibacterial activity was measured using the standard method of agar well diffusion, compound 2 has a moderate activity against E. faecalis, P. vulgaris and B. subtilis, but it has higher activity against E. coli and K. pneumonia. Furthermore, compound 3 and 5c have a moderate activity against S. typhimurium and P. vulgaris, too. Almost all compounds showed moderate to good urease inhibitory activity.

Fig. 1. Synthesized all ligands

In this study, eight compounds were studied and also evaluated antioxidant activity by two assays which were Ferric reducing antioxidant power (FRAP) and scavenging of free radical (DPPH). In 4 samples that were 2, 3, 4a and 5c antioxidant activity was determined. Result ranges of FRAP assay were 43.43-869.65 μmol FeSO4.7H2O/g sample and about DPPH; 4376.08±26.551 μM. According to the results, 2 and 3 compounds had the highest activity among others. Due to radical effect, 50% scavenging activity was determined in DPPH assay. The best value of SC50 was measured in compound 3. Because of the low SC50 value of samples show a high antioxidant effect.

CONCLUSIONS
In our study, new 1,2,4-triazole derivatives have been synthesized, and their antibacterial, antiurease and antioxidant activities were evaluated. The results showed that the synthesized new 1,2,4-triazole derivatives had antioxidant, antibacterial and antiurease activities. For reason, new 1,2,4-triazole derivatives may be considered as a main urease inhibitory and free radical scavenger. Therefore, these compounds could be used as a source of antioxidant, antibacterial and antiurease in pharmaceutical and agriculture industries.

REFERENCES
1. Palaska, E.; Sahin, G.; Kelicen, P.; Durlu, N. T.; Altinok, G. Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. *II Farmaco* 2002, 57, 101-107.

P-158: SYNTHESIS OF NEW 3,5-DISUBSTITUTED-1,2,4-TRIAZOLES AND EVALUATION OF ANTIBACTERIAL, ANTIUREASE AND ANTIOXIDANT ACTIVITIES

N. Gumrukcuoglu1, B. Bilgin Sokmen2, H. Sahin2, Y. Sagkal3, S. Ugras3, H. I. Ugras4

Vocational School of Health Sciences, Trabzon, TURKEY

INTRODUCTION
Azole compounds are one of two major groups of clinically important antifungal drugs. Fluconazole, terconazole, voriconazole and posaconazole may be examples for this group. In our studies we have reported that 1,2,4-triazole ligands showed ion extraction selectivity[1]. In this present study, we have synthesized some new 1,2,4-triazole compounds and antiurease, antibacterial and antioxidant activities for the newly synthesized compounds have evaluated.

MATERIALS AND METHODS
The synthesized compounds were tested individually against 11 gram-positive and gram-negative bacteria species. The bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC). Urease inhibitory activity was determined according to Van Slyke and Archibald [2]. The antioxidant activities of the samples were determined by FRAP assay. The method is based on the measurement of the iron reducing capacities of the compounds. Radical scavenging activity of samples against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was spectrophotometrically at 517 nm. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants. Briefly, various concentrations 0.75 mM of compound extracts were mixed with 0.75 mM of a 0.1 mM of DPPH in methanol. Radical scavenging
activity was measured by using Trolox, BHT as standards and the values are expressed as SC50, the concentration of the samples that causes 50% scavenging of DPPH radical.

RESULTS AND DISCUSSION
In this study, Chemical shift values consistent with the literature [1]. Furthermore, [M+] ion peaks were observed at the related m/z values supporting the proposed structures. In addition, these compounds gave reasonable elemental analysis data. The newly synthesized compounds 2–5 were evaluated via agar well diffusion assay for their antibacterial activities. The compound 2, 4a and 4b have not shown inhibition activity against all test bacteria. Also, the other compounds have a moderate high inhibition activity against test bacteria. Results of the antibacterial activity screening test indicated that compounds as 4c and 5a-c exhibited good antibacterial activity and we think that they can very important for inhibition of pathogen bacteria as S. aureus. Almost all compounds showed moderate to good urease inhibitory activity. The inhibition was increased with increasing triazole concentration. Lower IC50 values indicate higher enzyme inhibitor activity. Compound 4a aproved to be the most potnet showing an enzyme inhibition activity with an IC50= 0.0435±0.0072 μM. The least active compound4c had an IC50=0.1064±0.0037µM.

![Fig. 1. Synthesized all ligands](image)

In the light of these methods, eight synthesized compounds were studied for the evaluation of antioxidant activity. Compounds 2, 3, 4b, 4c, and 5a showed in varying range activity values, while compounds 4a, 5b, and 5c showed no activity. Result ranges of FRAP were 11.61-174.87 μmol FeSO4.7H2O/g sample and ranges of DPPH; 7.914 - 51.485(*103) µM. When compared the results, some flexible differences can be seen in both tests. For example, compound 5a showed the highest antioxidant value about FRAP, but also compound 3 had the most antioxidant effect on DPPH. Because of the low SC50 value of samples show a high antioxidant effect. Especially, tree dimension structures and their steric effects of synthesized compounds are so important on DPPH methods. Some complex compounds, it is difficult for H atom donor to reach the radical active center of DPPH. The fact that it is possible that activities can be determined as a slightly lower than usual values and minor deviations can be occurred about the value correlation.

CONCLUSIONS
In our study, the synthesis of some 1,2,4- triazole derivatives (3–5) were performed, and their structures were confirmed by IR, 1H-NMR, 13C-NMR, Mass spectroscopic, and elemental analysis techniques. In addition, the newly synthesized compounds were screened for their antibacterial and antiurease activities. Many of them were found to high inhibition activity on S. aureus, K. pneumonia, B. subtilis, and S. epidermidis. Furthermore, we can say that all the compounds exhibited moderate-to-good antioxidant, and antiurease activities. For reason, 1,2,4- triazole derivatives may be considered as a main urease inhibitory and free radical scavenger. Therefore, these compounds could be used as a source of antioxidant, antibacterial and antiurease in pharmaceutical and agriculture industries

REFERENCES

P-159: EFFECTS OF METABOLIC SYNDROME ON ANTIOXIDANT ENZYMES ACTIVITIES OF MASSETER MUSCLE FROM MALE RATS

O. Alptekin1, H.C. Tukel2, B. Turan3

1Department of Biochemistry, Faculty of Pharmacy, Cukurova University, Adana, TURKEY
2Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cukurova University, Adana, TURKEY
3Department of Biophysics, Faculty of Medicine, Ankara University, Ankara, TURKEY

INTRODUCTION
Metabolic syndrome (MetS), being an important risk factor for development of type-2 diabetes and cardiovascular disorders, is a disease characterized by obesity, hyperinsulinemia, hyperlipidemia and hypertension. Its incidence, even among young population, is increasing and becoming a serious health problem during present century in all population. MetS is basically characterized by insulin
resistance, abdominal obesity, glucose intolerance, diabetes mellitus, dyslipidemia, hypertension and systemic pro-inflammatory and pro-coagulant pathologies [1]. Recent evidences have demonstrated that there are close relation between oxidative stress and the development of MetS in mammalians. Oxidative stress occurs when the net amount of reactive oxygen species (ROS) include free radicals as well as non-radical species exceed the antioxidative capacity. To eliminate the harmful effects of ROS, preventive antioxidant enzymes; like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) operate to detoxify or to scavenge the ROS. Antioxidant enzymes are considered as important markers of oxidative status of tissues.

Masticator muscles are devoted to the movement of the jaw, mainly, in relation to food consumption and mastication. There are large amounts of spindles in these muscles, which are critical in determining muscle contraction and mandibular movement in response to any stimulation [2]. The structural and functional features of the muscle fibers in masticator muscle can vary dependent on our life-style, diet and eating habits. Masseter muscle, being main part of the masticatory muscles, contains more proprioceptors compared to the others. In the literature, although there were some data on MetS-induced alteration in antioxidative enzymes activities of serum, liver, kidney and heart of rats [3,4], there are no information about these enzyme activities in masseter muscle. Therefore, the aim of this study is to evaluate the enzyme activities of antioxidative defense mechanism in masticatory muscle of rats with MetS. Our data in this model study showed that MetS-induced alterations in masseter muscle are mostly related through the altered levels of antioxidative metabolism.

**MATERIALS AND METHODS**

Young-male Wistar rats (200–220 g) were randomly separated into two-groups; control rats (n=6) and rats with MetS (n=10). MetS was induced by supplementation with 32% (w/v) sucrose in their drinking water besides the standard rat chow [4]. At the 16th week, animals were tested to determined their obesity, glucose tolerance and insulin resistance and then were anesthetized with pentobarbital sodium (30 mg/kg body weight). Masseter muscles of rats were removed by sharp and blunt dissection. Masseter muscles were weighed and each sample were homogenized (1/10, w/v) in ice-cold buffer containing 100 mM potassium phosphate buffer (pH 7.4), 100 mM KCl and 1 mM EDTA at 9500 rpm for 1.0 min. Homogenates were centrifuged at 10,000 g for 30 min (+4 0C) and supernatants were used as enzyme source. SOD, CAT, GPx, GR and GST activities were measured by spectrophotometrically.

**RESULTS AND DISCUSSION**

The body weight, blood glucose level and blood insulin level were significantly increased in MetS group as 26%, 150% and 35% compared to that of control group, respectively. The glucose-insulin index of MetS-group was significantly greater (136%) than control, which indicated the decline in insulin sensitivity in tissues associated with insulin resistance. SOD, CAT, GPx, and GR activities in masseter muscle were significantly decreased in MetS group as 32%, 26%, 33%, and 16% to that of controls, respectively. However, GST activity did not change significantly in masseter muscle of MetS rat with respect to that of controls. The decreased antioxidant enzyme activities may be due to acceleration of ROS production acting as inhibitors of antioxidant enzyme or enhancing protein glycation or reduced antioxidant reserve or their combinations.

**CONCLUSIONS**

Our results show that MetS altered antioxidant defense mechanism in masseter muscle of rats. Decreased antioxidant enzymes activities, indicating an imbalance between the production and inactivation of ROS, may be correlated with the initiation of the muscle fatigue and/or muscle injury processes.

**REFERENCES**


**P-160: PREPARATION OF A NEW AMPEROMETRIC BIOSENSOR WITH IMMOBILIZATION ON POLYPYROLE-POLYANILINE FILM OF GLUCOSE OXIDASE**

S. Cete1, Rabia E.Keles2

1Gazi University, Faculty of Science
2Department of Chemistry, 2Institute of Science, Ankara, TURKEY
INTRODUCTION
Conducting polymers have been applied to biosensors since they can be used both as immobilization matrices and as redox systems for the transfer of electrical charge [1,2]. Also, conducting polymer based enzyme electrodes have been predicted to exhibit notable operational stability and rapid response time. The significance of glucose in human metabolism is well known, as is the fact that the defects in glucose level lead to complications of diabetes.

MATERIALS AND METHODS
Instrumentation and Reagents: The electrochemical studies were carried out by using Epsilon EC electrochemical analyzer with a three-electrode cell. The working electrode was a Pt plate (0.5 cm²). Auxiliary and reference electrodes were Pt wire and Ag/AgCl electrode (3.0 M KCl), respectively. The pH values of the buffer solutions were measured with an ORION Model 720 A pH/ionmeter. Temperature control was achieved with Grant GD120 thermostat. The supporting electrolyte sodium perchlorate, pyrrole, aniline and sodium dodecylbenzene sulphonate were supplied by Aldrich. All other chemicals were obtained from Sigma. All the solutions were prepared by the use of twice-distilled water.

Preparation of Pt/PPy–Pani–DBS Film Electrode: The surface of the Pt plate electrode was cleaned [3] and covered with PPy-Pani-DBS(sodium dodecylbenzene sulphonate) film by electropolymerization of aniline and pyrrole in an anionic dopant containing. The Pt plate electrode was immersed in 10.0 mL solution containing 0.15 M pyrrole, 0.15 M aniline and 5.0 mL of sodium dodecylbenzene sulphonate. The electropolymegramerization of pyrrole and aniline upon the electrode surface was performed by the cyclic voltammetric scans between -1.2 and 1.0 V at a scan rate of 20 mV/s. After electropolymerization, PPy-Pani-DBS film was rinsed with deionized water to remove the unreacted pyrrole monomer.

RESULTS AND DISCUSSION
In this study, a PPy-Pani-DBS(sodium dodecylbenzene sulphonate) electrode sensitive to hydrogen peroxide was prepared for the use as a biosensor. The optimum conditions for the enzyme electrode were found to be the pyrrole concentration, 0.15 M; aniline concentration 0.15 M the DBS concentration, 0.1 M; the working potential, 0.4 V; 4 and 10 cycles aniline and pyrrole respectively the number of cycles, 10. On account of the increased pore size of the DBS-doped PPy-Pani film which is suitable for the immobilization of enzymes. It is shown that enzyme electrode was very sensitive against to glucose. This enzyme electrode can be used for determination of glucose in blood and urine. This film electrode can be used for preparing of enzyme electrodes to determine of the hydrogen peroxide.

CONCLUSIONS
In this work, glucose oxidase was successfully immobilized on a poly(pyrrole)-polyaniline-DBS(sodium dodecylbenzene sulphonate) (PPy–Pani-DBS) composite film. The experimental results showed clearly that the biosensor exhibited good performance in the determination of glucose. The glucose biosensor has high sensitivity and good selectivity. Operational stability and long term storage stability are good. In addition, PPy–Pani-DBS can provide a biocompatible and electrochemical microenvironment for immobilization of enzyme, making this material a good candidate for the fabrication of highly sensitive and selective glucose biosensors.

ACKNOWLEDGMENTS
Any funding sources or additional contributions should be declared under this title.

REFERENCES

P-161: PREPARATION OF BIOSENSOR FOR GLUCOSE DETERMINATION IN BIOLOGICAL FLUIDS
S. Cete¹, Tuce Y.Uzumer²

Gazi University, Faculty of Science
¹Department of Chemistry, ²Institute of Science, Ankara, TURKEY

INTRODUCTION
The most important endocrine disorder of carbohydrate metabolism is diabetes mellitus. Diabetes mellitus is the most frequent cause of heart disease, kidney failure, and blindness, and widely recognized as one of the leading causes of death and disability in the world. The significance of glucose in human metabolism is well known, as is the fact that the defects in glucose level lead to complications of diabetes [1,2]. Biosensors containing enzymes, which have unique ability to recognize target molecules quickly and accurately in a complex system, have been widely applied in chemistry and biology [3]. Oxido-reductase enzyme electrodes are a great group of biosensors,
accounting for over 90% of the existing amperometric enzyme-based biosensors.

MATERIALS AND METHODS

Instrumentation and Reagents: The electrochemical studies were carried out by using Epsilon EC electrochemical analyzer with a three-electrode cell. The working electrode was a Pt plate (0.5cm²). Auxiliary and reference electrodes were Pt wire and Ag/AgCl electrode (3.0 M KCl), respectively. The pH values of the buffer solutions were measured with an ORION Model 720 A pH/ionmeter. Temperature control was achieved with Grant GD120 thermostat. All other chemicals were obtained from Sigma. All the solutions were prepared by the use of twice-distilled water.

Preparation of Pt/PPy–Pani-PSS Film Electrode: The surface of the Pt plate electrode was cleaned [4] and covered with PPy-Pani-PSS(poly(sodium-4-styrene sulphonate)) film by electropolymerization of pyrrole and aniline in an anionic dopant containing. The electropolymerization of pyrrole and aniline upon the electrode surface was performed by the cyclic voltammetric scans between -0.8 and -0.8 V at a scan rate of 20 mV/s. The electrode was washed with buffer solution after the coating process.

RESULTS AND DISCUSSION

In this study, a PPy-Pani-PSS (polypyrrole-polyaniline-poly(sodium-4-styrene sulphonate)) electrode sensitive to hydrogen peroxide was prepared for the use as a biosensor. The amperometric determination was based on the electrochemical detection of H₂O₂ generated in enzymatic reaction of glucose. Determination of glucose was carried out by the oxidation of enzymatically produced H₂O₂ at 0.4 V vs.Ag/AgCl. The effects of pH and temperature were investigated and optimum parameters were found to be 7.5 and 65°C, respectively. The effect of working potential was investigated and optimum potential was determined to be 0.4 V.

CONCLUSIONS

In this work, glucose oxidase was successfully immobilized on a poly(pyrrrole)-polyaniline-poly(styrene sulphonate) (PPy–Pani-PSS) composite film. The experimental results showed clearly that the biosensor exhibited good performance in the determination of glucose. The glucose biosensor has high sensitivity and good selectivity. Operational stability and long term storage stability are good. In addition, PPy-Pani-PSS can provide a biocompatible and electrochemical microenvironment for immobilization of enzyme, making this material a good candidate for the fabrication of highly sensitive and selective glucose biosensors.

ACKNOWLEDGMENTS

Any funding sources or additional contributions should be declared under this title.

REFERENCES

1. Adlan, MA.; Bondugulapati, LN.; Premawardhana, LD.; Glucoseintolerance and diabetes mellitus in endocrine disorders – two casereports and a review. Curr Diabetes. 2010, 6(5),266–73.


P-162: AN AMPEROMETRIC BIOSENSOR FOR FISH FRESHNESS DETECTION

S. Cete1, Y. Azizli2

Gazi University, Faculty of Science
1Department of Chemistry, 2Institute of Science
Ankara, TURKEY

INTRODUCTION

Biosensors are analytical devices which tightly combine biorecognition elements and physical transducers for the detection of target compounds. In the enzyme-based biosensors, the biological element is the enzyme which reacts selectively with its substrate. It is well known that the response of a biosensor to the addition of a substrate is determined by the concentration of the product of the enzymatic reaction on the surface of the sensor. The reaction is controlled by the rate of two simultaneous, i.e. the enzymatic conversion of the substrate and the diffusion of the product from the enzyme layer [1]. The development of a sensor for xanthine (X) and hypoxanthine(Hx) is of medical and biological importance [2], because the levels of these compounds are generally used in the food industry as an index for evaluating meat or fish freshness . The level of hypoxanthine in fish muscle either alone, or in conjunction with the levels of other ATP metabolites, is used as an indicator of quality.

MATERIALS AND METHODS

Instrumentation and Reagents: All electrochemical experiments carried out using an Epsilon EC electrochemical analyzer A conventional three-electrode system was equipped with a Pt plate (0.5 cm²) as the working electrode, an Ag/AgCl electrode (3 M KCl) as the reference electrode, and a platinum
RESULTS AND DISCUSSION
In this study, we prepared a new biosensor based hydrogen peroxide for the quantification of hypoxanthine. Immobilization of xanthine oxidase and uricase upon of PPy-DBS film was carried out. The electrochemical responses of the biosensor electrode towards hypoxanthine were investigated. All parameters were examined. Besides, we determined pH, temperature, operational and storage stabilities.

CONCLUSIONS
An amperometric biosensor for the determination of hypoxanthine was fabricated by the immobilization of xanthine oxidase and uricase in a polypyrrole- sodium dodecylbenzene sulphonate film by electrochemical polymerization. Operational stability and long term storage stability are good. In addition, PPy–DBS can provide a biocompatible and electrochemical microenvironment for immobilization of enzyme, making this material a good candidate for the fabrication of highly sensitive and selective xanthine biosensors.

ACKNOWLEDGMENTS
We acknowledge the support of this project by Gazi University Research Fund (FEF 05/2013-04).

REFERENCES

P-163: PREPARATION OF BIOSENSOR FOR CHOLESTEROL DETERMINATION IN BLOOD
S. Cete1, A. Alii Dada2

Gazi University, Faculty of Science
1Department of Chemistry, 2Institute of Science
Ankara, TURKEY

INTRODUCTION
In recent years various types of biosensors have been developed, some of which are already in practical use. Such sensors have been used for different applications, including health care, food and environmental monitoring. The determination of cholesterol levels is of importance in clinical diagnosis of diseases such as coronary heart disease, myocardial infarction and arteriosclerosis. Cholesterol is a sterol found in eggs, meats, yellow cheese, and derivatives. Biosensors for cholesterol have been used in biochemical analysis owing to their good selectivity, fast response, low cost, small size and long term stability [1,2]. Most of the literature on cholesterol biosensors has focused on diagnosing disorders.

MATERIALS AND METHODS
Instrumentation and Reagents: All electrochemical experiments carried out using an Epsilon EC electrochemical analyzer. A conventional three-electrode system was equipped with a Pt plate (0.5 cm2) as the working electrode, an Ag/AgCl electrode (3 M KCl) as the reference electrode, and a platinum wire (diameter and length, 1 mm, 4 cm respectively) for the counter electrode.

Preparation of Pt/PPy–PSS Film Electrode: The surface of the Pt plate electrode was cleaned [3] and covered with PPy-PSS(poly(sodium-4-styrene sulphonate)) film by electropolymerization of pyrrole in an anionic dopant containing. The electrode was immersed in a 10 mL solution of 50 mM pyrrole and 25 mM PSS (poly(sodium-4-styrene sulphonate)). The solution was purged with nitrogen in order to remove the oxygen. The electropolymerization pyrrole upon the electrode surface was performed by the cyclic voltammetric scans between -0.8 and -0.8 V at a scan rate 20 mV/s. The electrode was washed with buffer solution after the coating process.

RESULTS AND DISCUSSION
In this study, a PPy-PSS (polypyrrole-poly(sodium-4-styrene sulphonate)) electrode sensitive to hydrogen peroxide was prepared for the use as a biosensor. The optimum conditions for the enzyme electrode were found to be the pyrrole concentration, 50 mM; the PSS concentration, 25 mM; the working potential, 0.3 V; the number of cycles, 10. On account of the increased pore size of the PSS-doped PPY film which is suitable for the immobilization of enzymes. It is shown that enzyme electrode was very sensitive against to
cholesterol. This enzyme electrode can be used for determination of cholesterol in blood and urine. This film electrode can be used for preparing of enzyme electrodes to determine of the hydrogen peroxide.

CONCLUSIONS
In this work, cholesterol oxidase was successfully immobilized on a poly(pyrrrole)-PSS(poly-styrene sulphonate) (PPy–PSS) composite film. The experimental results showed clearly that the biosensor exhibited good performance in the determination of cholesterol. The cholesterol biosensor has high sensitivity and good selectivity. Operational stability and long term storage stability are good. In addition, PPy–PSS can provide a biocompatible and electrochemical microenvironment for immobilization of enzyme, making this material a good candidate for the fabrication of highly sensitive and selective cholesterol biosensors.

ACKNOWLEDGMENTS
We acknowledge the support of this project by Gazi University Research Fund (FEF 05/2011-57).

REFERENCES


P-164: ANTIOXIDANT-ANTIMICROBIAL ACTIVITY AND CHARACTERISATION OF PHENOLIC COMPOUNDS OF *TEUCRIUM ORIENTALE VAR. GLABRESCENS*

R. Aliyazicioglu,¹* O.E. Eyüpoğlu², S.O. Sener,¹ M. Badem,¹ U. Özgen³, S. Yıldırım⁴

¹Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey
²Department of Chemistry, Faculty of Arts, Karadeniz Technical University, 61080 Trabzon, Turkey
³Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

INTRODUCTION
Medicinal plants represent the most important natural source of drugs for traditional and folk medical systems, modern medicines, chemical constituents of synthetic drugs, and food supplements. The purpose of this study was to determine the fenolic composition, antioxidant, and antimicrobial activity of *Teucrium orientale var. glabrescens*.

MATERIALS AND METHODS
*Teucrium orientale var. glabrescens* were collected from Artvin, Turkey and identified by Prof. Dr. Ufuk Özgen. Aquous and methanolic extracts were prepared. Sixteen different phenolic compounds were determined by reverse phase-high performance liquid chromatography (RP-HPLC). Total phenolic compounds, ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), and 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity were utilized for antioxidant capacity analysis. The antimicrobial activity was studied using agar diffusion method.

RESULTS AND DISCUSSION
Both methanolic and aqueous extracts showed significantly antioxidant and antimicrobial activity and included large amounts of antioxidant compounds. Rosmarinic acid, p-coumaric acid, and p-OH benzoic acid in a great percentage and gallic acid, protocatechuic acid, *proto*-catechuic aldehyde, gentisic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, vanillin, syring aldehyde, ferulic acid, sinapic acid, benzoic acid in minor percentage were determined in methanolic extract of *Teucrium orientale var. glabrescens*. Extracts showed strong antimicrobial activity against *M. Smegmatis* ATCC®607. Previous studies showed that *Teucrium* genus have antifungal [1], antimicrobial [2], and antioxidant activity [3].

CONCLUSIONS
The *Teucrium orientale var. glabrescens* extracts have significantly antioxidants, antimicrobial agents, the plant might protect health and fight against several diseases.

ACKNOWLEDGMENTS
This study was supported by KTU Research Fund (Project No: 9731).

REFERENCES

P-165: THE INVESTIGATION OF DNA CLEAVAGE PROPERTIES AND ANTIOXIDANT CAPACITIES OF NEW HYDRAZIDE SUBSTITUTED BIMETALLIC COMPLEXES

S. Akkaya1, A. Ozel1, K. Karaoglu2, K. Serbest2
1Karadeniz Technical University, Faculty of Pharmacy, Department of Biochemistry, Trabzon, Türkiye, 2Recep Tayyip Erdoğan University, Faculty of Science, Department of Chemistry, Rize, Türkiye

INTRODUCTION
The interaction of transition metal complexes with DNA has received much attention due to their possible applications in cancer therapy and molecular biology [1]. Bimetallic complexes have enhanced the nuclease activity according to recent studies. This work focused on a study of the nuclease activities of new hydrazide-substituted bimetallic complexes.

MATERIALS AND METHODS
The DNA cleavage activity of ligands (1-3) and their bimetallic complexes (1a-3a) were evaluated by their ability to catalyze the conversion of the supercoiled pBR322 DNA (form I) to nicked circular DNA (form II) and linear DNA (form III) using agarose gel electrophoresis. Total volume of reaction mixture was 10 µl and contained TrisHCl buffer (pH 5.0-9.0), supercoiled plasmid pBR322 DNA (250 ng) and 1-3 and 1a-3a (100 µM). After 1 hour incubation at 37°C, the reaction was stopped by adding 5 µl of loading buffer, samples were then loaded on 0.8% agarose gel electrophoresis. Total volume of reaction mixture was 10 µl and contained TrisHCl buffer (pH 5.0-9.0), supercoiled plasmid pBR322 DNA (250 ng) and 1-3 and 1a-3a (100 µM). After 1 hour incubation at 37°C, the reaction was stopped by adding 5 µl of loading buffer, samples were then loaded on 0.8% agarose gel containing EB (1 mg/ml in Tris-acetate-EDTA), electrophoresis was carried out at 100 V for 1 h and resulting image was photographed.

RESULT AND DISCUSSION
Gel electrophoresis experiments were performed using pBR322 DNA with ligands and complexes in pH ranging from 5.0 to 9.0 and in the presence of H2O2, ME, AA. As a result of these experiments, it was determined that optimum pH was 7.0. The DNA cleavage activity was evaluated in the presence of different activators, viz. H2O2, ME and AA. The efficacy of 1-3 and 1a-3a followed the order as ME>H2O2≥AA. In this study, antioxidant activities of these ligands and complexes were determined. The 1a and 3a complexes have exhibited good free radical scavenging activity, 2a complexes showed less activity compared with BHA. Consequently, these complexes can be evaluated as potential drug.

CONCLUSIONS
The gel electrophoresis results showed that ligands and their bimetallic complexes cleave supercoiled plasmid pBR322 DNA at pH 7 and in presence oxidative agents. These studies suggested that a synergistic combination of ligands and metal ions were important in the design of a potential chemotherapeutic drug targeting DNA, in addition to their antioxidant activities which was helpful to understand biological properties of drugs.

REFERENCES

P-166: SERUM TGF-β AND E-CADHERIN CONCENTRATIONS IN PANCREATIC AND COLORECTAL CANCER PATIENTS

T. Turan1, A. Soyagir2, A. Gonenç3, A.O. Tarhan3, D.M. Kaya4, N. Erk5
1Department of Biochemistry, Gazi University Faculty of Pharmacy, Ankara, Turkey
2Department of Family Medicine, Ankara University Faculty of Medicine, Ankara, Turkey
3Ankara University Faculty of Pharmacy, Ankara University Faculty of Medicine, Ankara, Turkey
4Department of Medical Oncology, Ankara University Faculty of Medicine, Ankara, Turkey
5Department of Analytical Chemistry, Ankara University Faculty of Pharmacy, Ankara, Turkey

INTRODUCTION
Colorectal and pancreatic cancers are located in the third and fourth most common cause of cancer related deaths in the world, respectively [1,2]. Recently, researches focused on effects of chemical mediators on the pathogenesis of cancer. Transforming growth factor beta (TGF-β) promotes tumor growth, invasion and metastasis. Expression of TGF-β prevents inhibition of growth in normal cells but in cancer expression of TGF-β increases invasion of cells. It is known that cancer cell dispersion and metastases are strongly related with the loss of cell-cell adhesion. E-cadherin is one of the epithelial adhesion molecules and has a pivotal role in cell proliferation, tissue specificity and recognition. Therefore regulation TGF-β and E-Cadherin expression is significant and may be used for protective purposes in pancreatic and colorectal cancers. In this study, we aimed to
investigate the serum TGF-β and E-Cadherin levels in these cancers.

MATERIALS AND METHODS
Fifty three total patients (N=53) and twenty nine healthy controls (N=29) were involved in this study. Twenty three of them (N=23) were pancreatic cancer and the rest of the patients were colorectal cancer (N=30). In the present study, the serum levels of TGF-β and E-Cadherin from the patients with pancreatic and colorectal cancers and healthy controls were measured by ELISA method.

RESULTS AND DISCUSSION
In total patient group, a significant difference was found in TGF-β levels compared to controls (p<0.01, p<0.05, Table 1). TGF-β levels were increased significantly in both colorectal and pancreatic cancers as compared to controls (p<0.01, p<0.05).

Table 1. TGF-β and E-Cadherin levels in study group.

<table>
<thead>
<tr>
<th></th>
<th>TGF-β (pg/mL)</th>
<th>E-Cadherin(ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Patients (N=53)</td>
<td>635.68±187.88a</td>
<td>34.27±1.74b</td>
</tr>
<tr>
<td>Pancreatic Cancer (N=23)</td>
<td>616.15±287.68b</td>
<td>34.23±3.37</td>
</tr>
<tr>
<td>Colorectal Cancer (N=30)</td>
<td>650.65±252.32a</td>
<td>34.29±1.72b</td>
</tr>
<tr>
<td>Healthy Controls (N=29)</td>
<td>123.16±22.04</td>
<td>28.32±1.42</td>
</tr>
</tbody>
</table>

Significant difference from controls; a (p<0.01), b (p<0.05).

E-Cadherin levels were increased significantly in colorectal cancer as compared to controls (p<0.05). Increased TGF-β levels were found in various cancers. Naef et al. shows that patients with gastric carcinoma had increased TGF-β mRNA and/or protein levels compared to normal gastric mucosa [3]. There are conflicting results about E-Cadherin in cancer. Bonaldi et al. suggest that E-cadherin was lower in patients with prostate cancer compared to controls [4], while Liang et al. revealed that the serum E-cadherin in breast cancer patients were significantly higher than controls [5].

CONCLUSIONS
In conclusion, our study revealed that serum TGF-β expression was importantly enhanced in both colorectal and pancreatic cancer, but E-Cadherin was only in colorectal cancer. These results indicate that carcinogenesis is associated with TGF-β signalling pathway.

ACKNOWLEDGEMENTS
This project was founded by University of Ankara Scientific Research Project with the number of 12Ö3336002.

REFERENCES
4. Bonaldi, C.; Azzalis, LA.; Junqueira, VB.; de Oliveira, CG.; Vilas Boas, VA.; Gáson, TM.; Gehrke, FS.; Kuniyoshi, RK.; Alves, BC.; Fonseca, FL., Plasma levels of E-cadherin and MMP-13 in prostate cancer patients: correlation with PSA, testosterone and pathological parameters, Tumori., 2015, doi: 10.5301/tj.5000237.

P-167: STUDY OF CHOLINESTERASES UNDER THE INFLAMMATORY CONDITIONS

T. Cavojsky1, L. Paskova1, F. Bilka1, L. Slovak 2, K.Bauerova2, I. Paulikova1

Comenius University, Faculty of Pharmacy
1Department of Cell and Molecular Biology of Drugs
2Institute of Experimental Pharmacology and Toxicology, SAS, Bratislava, SLOVAKIA

Inflammation plays an important role in the pathophysiology of many common diseases such as Alzheimer and atherosclerosis. The group of cholinesterases responds to the pathological changes by increasing or reducing activity, with serious consequences for the function of the cholinergic system mediated by acetylcholinesterase (AChE) and biotransformation activity of butyrylcholinesterase (BuChE). Determination of cholinesterase activity has clinical significance in diagnosis of various diseases. Activity of BuChE varies depending on pathological condition of the organism, thus affecting the biotransformation of ester type drugs often used in pharmacotherapy.

INTRODUCTION
Inflammation is a protective response whose goal is to eliminate the injury-inducing agent, prevent tissue damage and initiate the repair process. BuChE is involved in the several functions: in lipoprotein
metabolism, hydrolysis of acetylcholine and non-choline esters [1]. Important function is suggested for BuChE also in the cholinergic anti-inflammatory pathway mediated by the neurotransmitter acetylcholine (Ach) which exerts and directs inhibition of the pro-inflammatory cytokines production [2]. Increasing of BuChE activity may lead to the greater hydrolytic destruction, this resulted in a reduction of Ach concentration, what could trigger and perpetuate systemic inflammation. Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease, manifested by inflammation of the joints also in other organs especially the lungs, blood vessels and heart. The reason of RA is determined by autoimmune processes which leads to the deficit of T-suppressor function of lymphocytes [3]. Characteristic for the RA is increased levels of proinflammatory cytokines, mostly prominent are considered IL-1β and TNFα.

MATERIALS AND METHODS
For simulation of RA conditions we used the model of experimental arthritis. Experimental arthritis was induced by intradermal injection of a suspension of Mycobacterium butyricum . The injection contained 100 mg of heat-killed mycobacteria suspended in incomplete Freund's adjuvant (0.1 ml). The experiment lasted 28 days, involving 3 groups of the experimental rat animals: the group of RA, the group of treated RA with methotrexate and the control group; each contained 10 animals. We determined BuChE activity by Ellman method and its biotransformation activity using BCH2 as a substrate. We also determined the mRNA transcriptional level of BuChE.

RESULTS AND DISCUSSION
The model of RA was confirmed by arthritic score, body weight change and reduced expression of CRP and IL-1β protein level in plasma. Under the inflammatory conditions, we observed a significant decrease of BuChE biotransformation activity, which accounted for 50% of the original activity in the rat lungs and even 30% in the liver comparing to the control group. The kinetics of the enzyme hydrolysis of BuChE was also altered significantly. In both organs of the inflammatory model we observed a significant increase of the KM value. The expression of BuChE gene has shown interesting finding by increased transcription level of BuChE in the liver (p<0,05), and by contrast the significant reduction in the lungs (p<0,0001) and spleen (p<0,001).

CONCLUSIONS
Decreased biotransformation activity of BuChE in both tissues may eventually result in an increased concentration of the drug in the cell with serious consequences for the treatment.

ACKNOWLEDGMENTS
This work was supported by grants: APVV 0052-10, UK/296/2014 and VEGA 2/0045/11, 2/0044/15. This contribution is the result of the project implementation: Comenius University in Bratislava Science Park supported by the research and development operational programme funded by the ERDF. Grant number: ITMS 26240220086.

REFERENCES

P-168: BIOACTIVITY OF PROTOIURUS KRAEPELINI (SCORPIONE: IURIDAE) VENOM

T. Somay Doğan1,2, A. Biber3, S. Gerekçi3, H. Hüsnügil3, A. İzbirak2, C. Özen1,3,4
1Central Laboratory, Middle East Technical University (METU),
2Hacettepe University, Faculty of Science, Department of Biology, Ankara, TURKEY
3Graduate School of Biotechnology, METU
4Center of Excellence in Biomaterials and Tissue Engineering, METU, Ankara 06800, Turkey

Aim of this study was to conduct bioactivity screening of Protoiurus kraepelini venom. Size exclusion and reverse phase fractionation of the venom was performed using high performance liquid chromatography. Antimicrobial, cytotoxic and K+ ion channel blocking activity of crude and fractionated venom was investigated.

INTRODUCTION
Animal venoms are a rich source of natural compounds that have evolved high affinity and selectivity for a diverse range of biological targets, especially membrane proteins such as ion channels, receptors, and transporters. Field of venomics has therefore emerged as an important addition to modern drug discovery efforts [1,2].

MATERIALS AND METHODS
Crude venom was injected to size exclusion column (SEC) and peptide fraction was separated. SEC peptide fraction was then further fractionated (1 through 6) on a C-18 reversed phase column. Antimicrobial Assay: Antimicrobial activity and antifungal activity of peptide fractions and crude venom were tested by determining the bacterial growth inhibition based on broth dilution method.
**RESULTS AND DISCUSSION**

- Crude venom showed highest growth inhibitory effect on *S. aureus*, *E. coli* and *C. albicans*. RP-HPLC fraction 6 showed potent antimicrobial effect on *E. coli*. Other fractions (2-5) had no observable antimicrobial effect.
- We could not observe a detectable K ion channel blocking activity for crude venom (1 mg/mL) or venom fractions (40-80 ug/mL).
- Crude venom showed no cytotoxic effect but SEC peptide fraction showed dose-dependent effect on Jurkat cells.

**CONCLUSIONS**

Our studies showed that *P. kraepelini* venom contains peptides with antimicrobial and cytotoxic activity. Sequence and structure of these peptides will be determined in future studies. The high potency and specificity of many venom-derived peptides, their ease of chemical synthesis and/or recombinant production, and the resistance of many disulfide-rich peptides to proteolytic degradation, make them attractive drug leads [3].

**ACKNOWLEDGMENTS**

We thank K.B. Kunt for help in venom milking.

**REFERENCES**

CONCLUSIONS

In the current work, new thiazole derivatives were synthesized and investigated for their cytotoxicity against MCF-7 and NIH/3T3 cell lines. MTT assay indicated that compound 3 can be identified as the most promising anticancer agent against MCF-7 cells due to its inhibitory effect with an IC₅₀ value of 20.6 μg/mL when compared with cisplatin (IC₅₀= 35.31 μg/mL) and low toxicity against NIH/3T3 cells.

REFERENCES


**P-170: SYNTHESIS AND ANTITUMOR ACTIVITY OF 2-((1Z,4E/Z)-1,5-DIARILPENTA-1,4-DIENE-2-YL)BENZO[d]THIAZOLES**

A. Şahin Yağlıoğlu¹, M. Ceylan², B. Şahin²

¹Çankırı Karatekin University, Faculty of Science, Department of Chemistry, Çankırı-TURKEY
²Gaziosmanpaşa University, Faculty of Science and Arts, Department of Chemistry, Tokat-TURKEY

INTRODUCTION

Benzothiazole derivatives are an attractive class of biologically active molecules. Among them, 2-phenyl substituted benzothiazoles are of particular interest since many of them have been reported to possess antitumor [1-3] and antimicrobial [4] activities. Halogen-containing derivatives of 2-(4-aminophenyl) benzothiazole were shown to be useful as probes for detecting β-amyloid plaques in Alzheimer’s disease [5]. Aim of this work is to synthesis and screening of anti-tumor activity against HeLa and C6 cell lines.

MATERIALS AND METHODS

2-Alkylbenzothiazol derivatives were synthesized starting from cyclobutanone. Condensation of benzaldehyde derivatives with cyclobutanone in basic medium gave the double addition product 1. Reaction of 2-aminobenzotiyol with 1 in EtOH in reflux conditions afforded 2-Alkylbenzothiazol derivatives. The crude products were purified on silica gel column chromatography and submitted to anti-tumour activity tests.

![Scheme 1. Synthesis of target compounds](image)

The antitumor activities of 3a and 3b were investigated using BrdU Cell Proliferation ELISA kit against HeLa and C6 cell lines. 5-fluorouracil (5-FU) was used as positive control. Measurements were measured in ELISA reader at 450 nm. The inhibition of cell proliferation was calculated as follows: (1-A treatments /A vehicle control) x100.

RESULTS AND DISCUSSION

The antitumor activities of 3a and 3b were determined against HeLa and C6 cell lines compared with 5-FU (Table1 and Table 2).

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>3a IC₅₀ (μM)</th>
<th>3b IC₅₀ (μM)</th>
<th>5-FU IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>66.81±0.86</td>
<td>74.21±0.10</td>
<td>90.71±0.45</td>
</tr>
<tr>
<td>75</td>
<td>25.77±1.21</td>
<td>17.30±0.18</td>
<td>88.75±0.78</td>
</tr>
<tr>
<td>50</td>
<td>14.69±0.93</td>
<td>7.60±0.08</td>
<td>87.22±1.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>3a IC₅₀ (μM)</th>
<th>3b IC₅₀ (μM)</th>
<th>5-FU IC₅₀ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>77.37±0.37</td>
<td>79.60±0.37</td>
<td>86.11±0.01</td>
</tr>
<tr>
<td>75</td>
<td>54.37±1.28</td>
<td>69.63±1.18</td>
<td>85.22±1.99</td>
</tr>
<tr>
<td>50</td>
<td>41.86±0.18</td>
<td>35.00±0.22</td>
<td>81.94±0.00</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Compounds (3a and 3b) showed moderate activity than the standard against both cell lines. And, they did not show activity at low concentrations (below 50 μg/mL).
ACKNOWLEDGMENTS
The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No; 111T111) for financial supports.

REFERENCES

P-171: SYNTHESIS AND ANTITUMOR ACTIVITY OF (7E)-7-(4-BROMO BENZYLIDENE)-5-[(4-BROMOPHENYL)(HYDROXY) METHYL] BICYCLO[3.2.0]HEPTAN-6-ONE DERIVATIVES
A. Şahin Yağcıoğlu¹, M. Ceylan², N. Akdoğan²

¹Çankırı Karatekin University, Faculty of Science, Department of Chemistry, Çankırı-TURKEY
²Gaziosmanpaşa University, Faculty of Science and Arts, Department of Chemistry, Tokat-TURKEY

INTRODUCTION
Chalcones are natural and synthetic compounds and are well known intermediates for the synthesis of various organic heterocycles. The compounds with the backbone of chalcone have been reported to possess various biological and medicinal activities such as antimicrobial, anticancer, antioxidant, antimalarial, antifeedant, antitumor, antimalarial, anti-inflammatory, antitubercular and anti-HIV activities [1-4]. In this study, chalcone like compounds (3a-d) containing bicyclo[3.2.0]heptan-6-one unit were synthesized and screened their anti-tumor activity against HeLa and C6 lines.

MATERIALS AND METHODS
Addition of dichloroketene to cyclopentene and followed reduction of adduct with Zn gave the bicycle ketone 1. Reaction of compound 1 with benzaldehyde derivatives in EtOH-NaOH afforded the double addition products 3a-d as well as mono addition products. The crude products were purified on silica gel column chromatography and submitted to antitumour activity tests.

RESULTS AND DISCUSSION
The antitumor activities of 3a-d were investigated using BrdU Cell Proliferation ELISA kit against HeLa and C6 cell lines. 5-fluorouracil (5-FU) was used as positive control.

CONCLUSIONS
Compounds 3a-d except 3b showed higher activity than the standard against C6 cell lines. While compounds 3c and 3d showed high activity, the other compounds 3a and 3b were inactive against HeLa cell lines.

ACKNOWLEDGMENTS
The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No; 111T111) for financial supports.
REFERENCES

P-172: SYNTHESIS AND ANTITUMOR ACTIVITY OF 2-(2-STIRYLCYCLOPENT-3-ENIL)BENZO[d]-THIAZOL DERIVATIVES

Ayşê Şahin Yağlıoğlu1, Mustafa Ceylan2, Nuray Akdoğan2

1Çankırı Karatekin University, Faculty of Science, Department of Chemistry, Çankırı-TURKEY
2Gaziosmanpaşa University, Faculty of Science and Arts, Department of Chemistry, Tokat-TURKEY

INTRODUCTION
Thiazole derivatives have been isolated and synthesized in view of their versatile pharmacological activities. Some thiazole analogues are used as fungicidal, cardiotonic, bactericidal, anti-inflammatory, antiviral, anti-arrhythmic, and antitumor agents. Thiazoles are used as drugs for the treatment of hypertension, HIV infections, and pain [1-4]. In this research, 2-stirylcyclopentyl benzothiazol derivatives were synthesized (3a-g) and screened their anti-tumor activity against HeLa and C6 lines.

MATERIALS AND METHOD
Addition of benzaldehyde derivative to bicyclo[3.2.0]hept-2-en-1-one in basic medium gave the unsaturated bicyclic ketone 1. Reaction of 2-aminobenzotiyol with 1 in EtOH in reflux conditions afforded the novel 2-stirylcyclopentyl benzothiazol derivatives. The crude products were purified on silica gel column chromatography and submitted to anti-tumour activity tests.

RESULTS AND DISCUSSION
The antitumor activities of 3a-g were investigated using BrdU Cell Proliferation ELISA kit against HeLa and C6 cell lines. 5-fluorouracil (5-FU) was used as positive control. Measurements were measured in ELISA reader at 450 nm. The inhibition of cell proliferation was calculated as follows: (1- Atreatments /Avehicle control) x100.

CONCLUSIONS
All compounds showed moderate to high activity against both cell lines. The most active compound was 3g containing dichloride atoms.
ACKNOWLEDGMENTS
The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No; 111T111) for financial supports.

REFERENCES

P-173: SYNTHESIS AND ANTITUMOR AND CYTOTOXIC ACTIVITIES OF 2-{(1S,2S)-2-{[(E)-2-ARYLVINYL]CYCLOPENTYL}-1,3-BENZOTHIAZOL DERIVATIVES

A. Şahin Yağlıoğlu1, M. Ceylan2, N. Akdoğan2, M. Üreşiç2

1Çankırı Karatekin University, Faculty of Science, Department of Chemistry, Çankırı-TURKEY
2Gaziosmanpaşa University, Faculty of Science and Arts, Department of Chemistry, Tokat-TURKEY

INTRODUCTION
Thiazole derivatives have attracted a great deal of interest owing to their anticancer [1], antibacterial, antifungal, anti-inflammatory [2], cardiotonic and antidegenerative activity on cartilage [3] etc. Thiazoles are known to be allosteric enhancer of A1 adenosine receptors whereas other analogs are known to be inhibitors of protein phosphatases [4]. In this research, 2-stirylcyclopentyl benzothiazol derivatives were synthesized (3a-f) and screened their anti-tumor activity against HeLa and C6 lines.

MATERIALS AND METHODS
Firstly, addition of dichloroketene to cyclopentene and following reduction of ketene adduct with Zn afforded bicyclo[3.2.0]hept-6-one. Unsaturated bicyclic ketone (1a-f) was obtained by addition of benzaldehyde derivatives to bicyclo[3.2.0]hept-6-one in basic medium. Reaction of 2-aminobenzotiyol with 1 in EtOH in reflux conditions afforded the 2-arylvinylcyclopentyl-1,3-benzothiazol (3a-f). The crude products were purified on silica gel column chromatography and submitted to anti-tumor activity tests.

RESULTS AND DISCUSSION

Table 1. The cytotoxicity values (%) of 3a-3f against C6.

<table>
<thead>
<tr>
<th>Comp.name</th>
<th>Cytotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>13</td>
</tr>
<tr>
<td>3b</td>
<td>15</td>
</tr>
<tr>
<td>3c</td>
<td>19</td>
</tr>
<tr>
<td>3d</td>
<td>20</td>
</tr>
<tr>
<td>3e</td>
<td>27</td>
</tr>
<tr>
<td>3f</td>
<td>29</td>
</tr>
<tr>
<td>5-FU</td>
<td>24</td>
</tr>
</tbody>
</table>

The antitumor activities of 3a-f were investigated using BrdU Cell Proliferation ELISA kit against HeLa and C6 cell lines. 5-fluorouracil (5-FU) was used as positive control. The cytotoxic activity of 3a-f were carried out using LDH cytotoxicity detection kit.

CONCLUSIONS
While all compounds (3a-f) showed higher activity than the standard against C6 cell lines, except compound 3e the other compounds showed lower activity than standard against HeLa cell lines.

ACKNOWLEDGMENTS
The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No; 111T111) for financial supports.
REFERENCES

P-174: SYNTHESIS AND ANTITUMOR ACTIVITY OF (E)-2-(2-STIRYLCYCLOOCTYL)BENZO[d]THIAZOL DERIVATIVES
Ayse Sahin YAĞLIÇOĞLU1, Mustafa CEYLAN2, Muhammed ÜREMIŞ3
1Çankırı Karatekin University, Faculty of Science, Department of Chemistry, Çankırı-TURKEY
2Gaziosmanpaşa University, Faculty of Science and Arts, Department of Chemistry, Tokat-TURKEY

INTRODUCTION
Benzothiazole derivatives are an attractive class of biologically active molecules. Among them, 2-phenyl substituted benzothiazoles are of particular interest since many of them have been reported to possess antitumor [1-3] and antimicrobial [4] activities. Halogen-containing derivatives of 2-(4-aminophenyl) benzothiazole were shown to be useful as probes for detecting β-amyloid plaques in Alzheimer’s disease [5]. For this, the numerous researchs are reported about 2-(4-aminophenyl)benzothiazoles in literature. In this research, 2-(2-stirlycyclooctyl)benzothiazol derivatives were prepared and tested their anti-tumor activity against HeLa and C6 line.

MATERIALS AND METHODS
Firstly, addition of dichloroketene to cyclooctene and following reduction of ketene adduct with Zn afforded bicyclo[6.2.0]dect-9-one. Unsaturated bicyclic ketone (1a-c) was obtained by addition of benzaldehyde derivatives to bicyclo[6.2.0]dect-9-one in basic medium. Reaction of 2-aminobenzotiyol with 1 in EtOH in reflux conditions afforded the 2-(2-stirlycyclooctyl) benzothiazol derivatives (3a-c). The crude products were purified on silica gel column chromatography and submitted to anti-tumour activity tests.

RESULTS AND DISCUSSION
The antitumor activities of 3a-3c were invetigated using BrdU Cell Proliferation ELISA kit against HeLa and C6 cell lines. 5-fluorouracil (5-FU) was used as positive control.

CONCLUSIONS
While all compounds (3a-c) showed higher activity than the standard against C6 cell lines, except compound 3b the other compounds showed lower activity than standard against HeLa cell lines. Compounds have very high activity and very low cytotoxicity.
ACKNOWLEDGMENTS
The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBITAK) (Project No; 111T111) for financial supports.

REFERENCES

P-175: THE SYNTHESIS OF (+)-DUOCARMYCN ANTIBIOTIC ANALOG: ITS EFFECT ON DNA

B. Çeken Toptancı, M. Kızıl
Dicle University, Faculty of Science, Chemistry Department, Diyarbakir, TURKEY

INTRODUCTION
The interaction of small molecules with DNA plays an essential role in many biological processes. DNA is often the target for majority of anticancer and antibiotic drugs, therefore, study related the interaction of drug with DNA has a key role in pharmacology. Moreover, understanding the interactions of small molecules with DNA is significance in the rational design of more powerful and selective anticancer agents [1]. CC-1065 and Duocarmycin antitumor antibiotics are specifically bind to double strand DNA. Duocarmycin have similar structure to the CC-1065. However it is not very toxic.

MATERIALS AND METHODS
In this study, water-soluble DNA-binding subunits, 5-[2-(Dimethylamino)acetylamino]-1H-indole-2-carboxylic Acid Hydrochloride (1) was synthesised [2]. DNA cleavage activity of this ligand (10-1000 μM) were checked, in the absence and presence of Cu (II) ions, on pBluescript M13+ plazmid DNA (3.2 kb) and Calf Thymus DNA (8-15 kb) [3].

RESULTS AND DISCUSSION
The ligand showed concentration and time dependent DNA cleavage activity. To verify if reactive oxygen species (ROS) involved in the mechanism of DNA cleavage, the experiments were carried out in the presence of histidine, thiourea, TEMPO and DMSO. It has been found that DNA cleavage was inhibited in the presence of these radical scavengers. This finding indicated that ROS plays a key role in the DNA cleavage.

CONCLUSIONS
In this study the results revealed that 5-[2-(Dimethylamino) acetylamino]-1H-indole-2-carboxylic Acid Hydrochloride (1) has DNA cleavage activity. Therefore it could be used as a chemical nucleases and chemotherapeutic agents.

ACKNOWLEDGMENTS
The authors would like to give thanks to TÜBITAK “The Scientific and Technical Research Council of Turkey” for financial support under Project Number 109T788.

REFERENCES
INTRODUCTION
Chalcones [(1,3-diaril)-2-propen-1-on], a member of flavonoid family, have been known to display interesting pharmacological activities such as anticancer, anti-inflammatory, antibacterial, antifungal, antimicrobial, antimalarial and anti-HIV activities. There have been many researchers done because they have safe effect profile, can be used orally and can be synthesized easily. It was proven that synthetically and naturally formed chalcones are effective on each level of carcinogenesis and offer activity against cancer cells (1). The aim of this research was to synthesize fluorinated chalcone derivatives containing morpholine ring and to investigate their anticancer activity.

MATERIALS AND METHODS
All chemicals and solvents were in analytical grade and purchased from Sigma-Aldrich, Merck and Roche. All chemical reactions were monitored with thin layer chromatography (TLC) using Merck silica gel 60 F254 plates. Melting points were determined by EZ-Melt melting point apparatus and were uncorrected. Electronic spectra were recorded in DMF on a PG Instruments T80+ UV-visible Spectrophotometer. IR spectra were determined with a Perkin Elmer Spectrum 100 FT-IR spectrophotometer. Elemental analyses (CHNS) were performed on a Vario MICRO elemental analyzer. 1H, 13C, 19F, DEPT 90, DEPT 135 DEPT, COSY, HETCOR, HMBC NMR spectra were recorded on a Agilent Technologies 600 MHz spectrometer. General Synthesis Method: Equimolar quantities of substituted benzaldehyde and morpholinooacetophenone were dissolved in methanol, three equivalents of NaOH was added to the mixture and stirred at the room temperature. The resulting solution was stirred for 2 days and kept in refrigerator overnight. The reaction mixture was extracted with dichloromethane. Organic phase was dried with anhydrous MgSO4. The solvent was evaporated and the crude product recrystallized from n-hexane /DCM.

Anticancer Activity Method
Fluorinated chalcones derivatives were synthesized and evaluated for their in vitro antiproliferative activities against HeLa and C6 cells using the BrdU ELISA assay (2,3). 5-fluourouracil (5-FU) and cisplatin were used as positive controls. Measurements were measured in ELISA reader at 450 nm. The inhibition of cell proliferation was calculated as follows: (1- Atreatments /Avehicle control) x100.

RESULTS AND DISCUSSION
The structure of synthesized compounds were proved by FTIR, 1H-NMR, 19F-NMR, 13C-NMR, DEPT 90, DEPT 135 DEPT, COSY, HETCOR, HMBC, mass spectra and elemental analysis. The FTIR spectra of the compounds exhibited characteristic bands at 1651-1663 cm-1 (C=O), 1214-1221 cm-1 (C-F). In the 1H-NMR spectrum, protons at α, β unsaturated carbonyl system of 1-7 resonated doublet at 7.47-7.73 ppm and the other doublet at 7.74-7.98 ppm which confirmed the presence of chalcone moiety. The coupling constants of the vinylic system ( j=14.4-16.2Hz) confirmed the trans configuration of the chalcones. 19F-NMR spectra indicated that the CF3 and F signal were at (-58)-(-63.04) and (-111.57)-(-115.79) ppm. Antiproliferative effects of the fluorinated chalcones were investigated on HeLa and C6 cell lines using proliferation with BrdU ELISA assay.

CONCLUSIONS
In our previous research, we found that (2E)-1-[4-(morpholin-4-yl)phenyl]-3-[4-(trifluoromethyl) phenyl] prop-2-en-1-one for HeLa cell line was more effective compounds than cisplatin with comparison of IC50 (4). Therefore novel chalcone derivatives having fluor atom and morpholine ring were synthesized in this study and the anticancer activity have been investigated.

ACKNOWLEDGMENTS
This work was supported by Gaziantep University Scientic Research Projects Governing Unit (BAPYB) (Grant no: FEF.14.01, Gaziantep, Turkey).

REFERENCES
P-177: NOVEL 6-SUBSTITUTED-3-(2H)-PYRIDAZINONE-2-ACETYL-2-(2,4-DISUBSTITUTEDBENZAL) HYDRAZONE DERIVATIVES: SYNTHESIS AND STRUCTURE ELUCIDATION

A.B. Özçelik1, S. Utku2, Ö. Güler1,3, M. Uysal1

1Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY
2Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Mersin, TURKEY
3Dicle University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Diyarbakır, TURKEY

INTRODUCTION

In recent years, a great deal of work have been directed to the synthesis of 3(2H)-pyridazinones derivatives. These nitrogen heterocyclic compounds are biological importance and therefore, design and strategy for their synthesis is important in medicinal chemistry [1]. Pyridazinone derivatives have been reported to exhibited wide range of pharmacological activities such as antidepressant, antihypertensive, antithrombotic, anticonvulsant, cardiotonic, antibacterial, diuretic, anti-HIV, aldose reductase inhibitors, anti-inflammatory, anticancer [2]. Here we report a convenient and versatile synthetic approach to novel 6-Substituted-3-(2H)-pyridazinone-2-acetyl-2-(2,4-disubstitutedbenzal) hydrazone derivatives.

MATERIALS AND METHODS

The fine chemicals and all solvents used in this study were purchased from Merck and Aldrich Chemical Co. Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus and the values given are uncorrected. FTIR spectra were recorded on an ATR apparatus on a Perkin Elmer Spectrum 100 Fourier Transform spectrophotometer. Elemental analyses were performed with Costech Combustion System CHNS-O analyzer and 1H NMR spectra were recorded in DMSO-d6 on a Bruker AV400 MHz FT NMR spectrometer.

The synthesis of I, II, III has already been reported in the literature [3]. Ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate IV derivatives were obtained by the reaction of III with ethyl bromoacetate in the presence of K2CO3 in acetone. 6-Substituted-3(2H)-pyridazinone-2-yl acetohydrazide derivatives V were synthesized by the condensation reaction of III derivatives with hydrazine hydrate (99%). Synthesis of title compounds VI were performed reaction of 6-substituted-3(2H)-pyridazinone-2-yl acetohydrazide derivatives V with 2,4-disubstituted benzaldehydes.

RESULTS AND DISCUSSION

Seventeen new final compounds IV, V and VIa-VIo were synthesized according to the procedures depicted in Figure. The elemental analysis data for each compound were in good agreement with the empirical formula proposed. In the IR spectra of newly synthesized compounds Va-o exhibited characteristic \( v (C=O) \) bands at 1703-1707 and 1662-1668 cm\(^{-1}\) for acetyl side chain and pyridazinone ring respectively. The \( v (N=H) \) stretching bands centered at 3212-3218 cm\(^{-1}\).

The \(^1\)H NMR spectra of all complexes were consistent with their corresponding protons as chemical shift values and the number of hydrogen. Synthesized compounds IV, V and VIa-VIo derivatives were reported first time in this study.

CONCLUSIONS

We report here in the synthesis of a series of novel IV, V, VIa-VIo derivatives. All synthesized compounds have been structurally elucidated and the basis of spectroscopic means.
spectrometer in CDCl 3 solutions using TMS as the internal standard. Mass spectra (ESI-TOF-MS) were recorded on Waters Synapt MS System mass spectrometer.

**RESULTS AND DISCUSSION**

The reaction of acetylsalicylic acid chloride with each aminoacid methyl ester, in the presence of non-nucleophilic base triethylamine, gave the corresponding aminoacid methyl esters.[3]: Thionyl chloride (1.8 mL, 25 mmol) was added dropwise to methanol (20 mL) at 0°C. Amino acid (10 mmol) was added and the mixture was stirred at room temperature for 100 h. The residue was evaporated *in vacuo*, dissolved in 20 mL of methanol and evaporated *in vacuo*. This was repeated for three times. Then the residue was dissolved in 20 mL of ether and evaporated *in vacuo*. This was repeated for three times. The residue was crystallized in ether to provide the title compounds as colourless powder.

**P-178: SYNTHESIS OF AMINO ACID ESTER DERIVATIVES OF ACETYSALICYLIC ACID**

B. Eymur, G. Taskor, N. Saygılı

Hacettepe University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Ankara, TURKEY

Synthesis of new acetyl salicylic acid derivatives is significant due to their activities in biological systems with reduced side effects. Acetylsalicylic acid chloride was reacted with different aminocids to afford aspirin derivatives. The new acetylsalicylic acid derivatives were characterized by $^1$H NMR, $^{13}$C NMR and MS.

**INTRODUCTION**

Aspirin (1), 2-acetoxybenzoic acid, is the most widely used safe non-steroidal anti-inflammatory drug (NSAID). It has the ability to decrease pain (an analgesic) and to reduce fever (an antipyretic), to reduce swelling and soreness (an anti-inflammatory agent) [1]. It may be used to prevent stroke, heart attack, and cancer [2].

Long term use of aspirin is a concern because of its side effects. Some of the undesirable side effects of aspirin result from acidity to induce gastric or intestinal ulceration [2]. It can be avoided by decreasing the acidity. In the present study, it was planned to synthesize derivatives of aspirin in order to reduce its side effects and to make it more biocompatible as a drug.

**MATERIALS AND METHODS**

All reagents were of commercial quality and reagent quality solvents were used without further purification. The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker DPX-400MHz FT-NMR spectrometer in CDC$_3$ solutions using TMS as the internal standard. The purity of the compounds was assessed by TLC on silica gel 60 F$_{254}$. Column chromatography was conducted on silica gel 60 (mesh size 0.063–0.200mm). Mass spectra (ESI-TOF-MS) were recorded on Waters Synapt MS System mass spectrometer.

**REFERENCES**


**PROCEDURE FOR THE PREPARATION OF AMINO ACID METHYL ESTERS**

1. Thionyl chloride (1.8 mL, 25 mmol) was added dropwise to methanol (20 mL) at 0°C. Amino acid (10 mmol) was added and the mixture was stirred at room temperature for 100 h. The residue was evaporated *in vacuo*, dissolved in 20 mL of methanol and evaporated *in vacuo*. This was repeated for three times. Then the residue was dissolved in 20 mL of ether and evaporated *in vacuo*. This was repeated for three times. The residue was crystallized in ether to provide the title compounds as colourless powder.

2. Acetylsalicylic acid chloride (2.0 mmol) in CH$_2$Cl$_2$ (5 mL) was added and treated with Et$_3$N (4.4 mmol). Acetylsalicylic acid chloride (2.0 mmol) in CH$_2$Cl$_2$ (5 mL) was added and the mixture was stirred at room temperature for ~1 week. The crude product purified using column chromatography.

**RESULTS AND DISCUSSION**

The reaction of acetylsalicylic acid chloride with each aminoacid methyl ester, in the presence of non-nucleophilic base triethylamine, gave the corresponding aminoacid ester derivatives of aspirin. The products formed were confirmed by $^1$H NMR, $^{13}$C NMR and MS.

**Synthesis of Dimethyl 2-(o-acetoxybenzamido) pentanedioate:** Acetylsalicylic acid chloride (2 mmol, 397 mg), dimethyl 2-aminopentanedioate (2.2 mmol, 385 mg) Et$_3$N (0.6 mL, 4.4 mmol) in CH$_2$Cl$_2$; eluent EtOAc–hexane 1:1 (v/v) gave compound 3 as a yellow viscous liquid (485 mg, 72 % yield). $^1$H NMR (CDCl$_3$) $\delta$ 7.80 (d, 1H, J = 8.0 Hz, ArH$_a$), 7.41 (t, 1H, J = 8.0 Hz, ArH$_b$), 7.23 (t, 1H, J = 8.0 Hz, ArH$_c$), 7.06 (d, 1H, J = 8.0 Hz, ArH$_d$), 6.56 (s, 1H, NH), 4.77 (m, 1H, 2-H), 3.70 (s, 3H, OCH$_3$), 3.55 (s, 3H, OCH$_3$), 2.38 (t, 2H, J = 8.0 Hz, 4-H), 2.33 (s, 3H, CH$_3$), 2.24 (m, 2H, 3-H); $^{13}$C-NMR (CDCl$_3$) δ 172.16, 168.67, 164.98, 148.17, 132.18, 130.34, 126.85, 126.24, 123.37, 52.64, 51.82, 29.68, 27.63, 21.07. HRMS (EI) calcd for C$_{16}$H$_{19}$NO$_7$ (M$^+$) 338.1246, found 338.1240.
properties. These molecules may help to prevent gastric irritation and bleeding. Further studies mainly including analgesic activity tests will be carried out in order to define these new compounds.

Scheme 1. Reagents: (i) thionyl chloride and CH₂Cl₂; (ii) triethyl amine and CH₂Cl₂.

REFERENCES

P-179: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SOME NEW 2,5-DISUBSTITUTED OXADIAZOLE DERIVATIVES

B. Kaya1, L. Yurttaş1, G. Akalin-Çiftçi2, Z.A. Kaplancıklı1
Anadolu University, Faculty of Pharmacy
1Department of Pharmaceutical Botany, 2Department of Biochemistry, Eskişehir, TURKEY

INTRODUCTION
Of the various human diseases, cancer has proven to be one of the most intractable diseases to which humans are subjected, and as yet no practical and generally effective drugs or methods of control are available. Therefore, identification of novel potent, selective, and less toxic anticancer agents remains one of the most pressing health problems [1]. Oxadiazole nucleus has shown quite good response as an anticancer agent, hence this nucleus has become an interest in the field of research. Among different types of oxadiazole nucleus containing molecules, 2,5-disubstituted 1,3,4-oxadiazoles have attracted a great deal of interest due to their anticancer activity [2]. Also there are some studies that shows some moieties including pyrimidine possess antiproliferative activity against different cancer cell lines [3]. Based on the advantages of 2,5-disubstituted 1,3,4-oxadiazoles moieties, we are interested in synthesizes new compounds and test their antiproliferative activity against A549 lung cancer cells. The antiproliferative activity of the new compounds was evaluated by MTT method. The cytotoxic potency of compounds 4a–4g was studied in comparison with the known anticancer drug cisplatin.

MATERIALS AND METHODS
2-(2-aryl-2-oxoethylthio) 5-(pyrimidin-2-ylthio) propyl -1,3,4-oxadiazole derivatives (4a–g) were synthesized according to our previous study [2]. For antiproliferative activity determination, conventional MTT method [4] was used against A549 cell line (human non-small lung cancer cell) using cisplatin as standard drug.

RESULTS AND DISCUSSION
Target molecules (4a–g) were synthesized in four steps. In the first step, ethyl 4-(pyrimidin-2-yl)thiobutanoate (1) was synthesized. Pyrimidine-2-thiol and ethyl 4-chlorobutanoate were refluxed in acetone with potassium carbonate to obtain an irritant intermediate product (2). In the second step, 4-(pyrimidin-2-ylthio)butanoylhydrazide was obtained from ethyl 4-(pyrimidin-2-ylthio)butanoate with excess of hydrazine hydrate in ethanol. 5-(3-(Pyrimidin-2-ylthio)propyl)-1,3,4-oxadiazole-2-thiol (3) was synthesized by the ring closure reaction of 4-(pyrimidin-2-ylthio)butanoylhydrazide with carbon disulfide in ethanolic KOH. Finally, 5-(3-(Pyrimidin-2-ylthio)propyl)-1,3,4-oxadiazole-2-thiol was reacted with appropriate α-bromoacetophenone derivatives to give the intended 2-(2-aryl-2-oxoethylthio)5-(pyrimidin-2-ylthio)propyl-1,3,4-oxadiazole derivatives (4a–g) which have shown in Scheme 1.
The antiproliferative activity of the compounds were determined against A549 cell lines. The results are presented in Table 1. However compound 4a including phenyl substituent was the most active compound against the A549 cell line. For compound 4g, IC50 value could not be calculated at tested concentrations. Phenyl and 4-methoxy phenyl including derivatives have been identified as the most effective compounds for anticancer activity. 4-Floro including derivative possesses moderate activity. 4-Nitro including derive In this study, it was seen once again that 2-(2-aryl-2-oxoethylthio)-5-(pyrimidin-2-ylthio)propyl)-1,3,4-oxadiazole derivatives were anticancer compounds.

CONCLUSIONS
The synthesis and cytotoxic activity of seven 2-(2-aryl-2-oxoethylthio)-5-(pyrimidin-2-ylthio)propyl)-1,3,4-oxadiazole derivatives (4a-g) have been reported in this work. It was determined that some of synthesized compounds had considerable anticancer activity against A549 cell lines. Compound 4a with phenyl moiety was measured as the most active compound.

REFERENCES

P-180: DESIGN, SYNTHESIS AND BIOLOGICAL EVOLUTION OF SOME 2-(5-SUBSTITUTED-BENZOTHIAZOL-2-YLSULFANYL)-N-(SUBSTITUTEDBENZYL)-N-(4-SUBSTITUTEDPHENYL)ACETAMIDE DERIVATIVES AS MONOAMINE OXIDASE INHIBITORS

B. Kaya1, Y. Ozkay2, Z.A. Kaplancikli1

Anadolu University, Faculty of Pharmacy, 1Department of Pharmaceutical Chemistry, Eskişehir-Turkey

INTRODUCTION
Monoamine oxidase (MAO) is a beneficial target in the management of neurodegenerative diseases and depressive conditions. There are two types of this enzyme as MAO-A and MAO-B, known to play critical roles in disease progression, and as such, the identification of new potent and selective inhibitors is a significant research aim [1]. Benzothiazole derivatives have shown fairly good response as MAO inhibitor agents, so this nucleus has turn out to be an important subject in the field of research [2]. Furthermore, there are some studies that confirm some moieties including benzylamine possess monoamine oxidase inhibitory [3]. In this study, new benzothiazole derivatives having benzylamine functional moiety were synthesized and their MAO-A and MAO-B inhibitory activities were investigated.

MATERIALS AND METHODS
Preparation of N-(3 or 4-nitrobenzylidene)-4-substituted anilines (1a-d): Corresponding nitrobenzaldehyde derivative (30 mmol) appropriate 4-substituted aniline (30 mmol) and catalytic amount of glacial acetic acid (0.5 mL) were refluxed in ethanol (100 mL) for 2h. After completion of reaction the mixture was cooled, precipitated product was filtered and recrystallized from ethanol.

Preparation of N-(3 or 4-nitrobenzyl)-4-substituted anilines (2a-d): The compounds 1a-d (30 mmol), were dissolved in methanol (100 mL). Sodium borohydride (60 mmol, 2.4 g) was divided to 4 poriton (4 x 0.6 g) and added to the methanolic solution 15 min intervals. After addition of last portion reaction mixture was allowed to stir for 2h at room temperature. The excess of solvent was evaporated under reduced pressure, crude product was washed with water, dried and recrystallized from ethanol.

Preparation of 2-Chloro-N-(3 or 4-nitrobenzylidene)-N-(4-substitutedphenyl)acetamides (3a-d): The compounds 2a-d (30 mmol) were dissolved in tetrahydrofuran (100 mL) and triethylamine (41 mmol, 3.483 g) was added. The mixture was cooled in an ice bath and chloroacetyl chloride (41 mmol, 3.88 g) was added dropwise with stirring. After addition of chloroacetyl chloride completed the reaction mixture was stirred for additional 1h at room temperature. The solvent was evaporated under reduced pressure, product was washed with water, dried and recrystallized from ethanol.

Preparation of N-(3 or 4-nitrobenzyl)-N-(4-substitutedphenyl)-2-((5-substitutedbenz[d]thiazol-2-yl)thio)acetamides (4a-l): The compounds 3a-d (1.5 mmol), appropriate 5-substituted benzothiazole derivative (1.5 mmol) and potassium carbonate (1.5 mmol) in acetone (40 mL) was refluxed for 2 days.
After TLC screening, the solvent was evaporated under reduced pressure. The product washed with water, dried, and recrystallized from ethanol.

In vitro MAO-A and MAO-B activity: The prepared compounds were investigated for their potential MAO-A and MAO-B inhibitory activities by an in vitro fluorimetric method. The fundamental of the activity measure is based on the ability of both MAO-A and MAO-B enzymes to metabolize non-fluorescent kynuramine, which is a suitable substrate for both isozymes, to the fluorescent product 4-hydroxyquinoline. Both enzymes were preincubated with test compounds and the MAO-inhibitory effect of the test compounds was correlated to the amount of 4-hydroxyquinoline formed. The read of the activity was performed by using proper excitation/emission wavelength pair [4].

RESULTS AND DISCUSSION

The chemical structures of the compounds (4a-l) were confirmed by IR, 1H NMR, MS spectral data and elemental analyses. Enzymatic activity test revealed that 2-(5-Chlorobenzothiazol-2-ylsulfanyl)-N-(3-nitrobenzyl)-N-(4-fluorophenyl)acetamide (4h) possesses significant inhibitory against both MAO-A and MAO-B enzymes.

CONCLUSIONS

The monoamine oxidase inhibitory effects of the compounds (4a-l) on MAO A and MAO B were determined by a fluorimetric method. Some of the test compounds showed promising inhibitory activity against MAO enzymes.

REFERENCES

1. Delogu G.L; Serra S.; Quezada E.; Uriarte E.; Vilar S.; Tatonetti N.P. and Vila D., Monoamine Oxidase (MAO) Inhibitory Activity: 3-Phenylcoumarins versus 4-Hydroxy-3-phenylcoumarins. ChemMedChem 2014, 9, 1672 – 1676

P-181: CYTOTOXIC EFFECTS OF FOUR TRITERPENOIDS ON BREAST CANCER CELL LINES

B. Çulhaöglu1,2, L. Türker Şener3, S. Damla Hatipoğlu1, T. Kuşman2, H. Birman4, I. Albeniz3, G. Topçu2

1Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry, 2Istanbul, Istanbul
2Bezmialem Vakıf University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34093, Fatih, Istanbul
3Istanbul University, Faculty of Medicine, Department of Biophysics, 34390, Çapa, Istanbul

Terpenoids are one of the important secondary metabolites of the plants. Triterpenoids have skeleton with 30 carbons, consisting of different structures and their structures are elucidated by spectroscopic measurements. Because of the diversity of their chemical structures they have interesting biological activities.

INTRODUCTION

Oleanolic acid and ursolic acid are pentacyclic triterpenes have been found in almost all Salvia species exhibit antimicrobial, anti-inflammatory, antihyperlipidemic, antiulcer, hepatoprotective, hypoglycemic, antifertility, anticarcinogenic, antiangiogenic, cytotoxic and protection against cyclophosphamide-induced toxicity activities [1-4]. In this study we prepared the semi-synthetic acetyl derivatives of oleanolic acid and ursolic acid: 3-acetyl oleanolic acid and 3-acetyl ursolic acid which were also isolated from several Salvia species previously by our group. Their (4 triterpenes) cytotoxic activity were investigated against MDAMB-231 breast cancer and HUVEC (human umbilical vein endothelial cell) cell lines in vitro.

MATERIALS AND METHODS

Cell Culture:

- MDA-MB-231 breast cancer cells were grown in DMEM (Life Technologies) supplemented with 2 mM L-glutamine, %10 FBS, %1 (v/v) antibiotics media.
- Human umbilical vein endothelial cells (HUVECs) are cells derived from the endothelium of veins from the umbilical cord.
- Cytotoxic Activity Measurement were done with xCELLigence System

MDA-MB-231 breast cancer cells were reproduced in a suitable medium and incubator. Antiproliferative effect of oleanolic acid (1), ursolic acid (2), 3-acetyl-oleanolic acid (3) and 3-acetyl ursolic acid (4) were measured with using a real-time cell analysis system (xCELLingence System). Antiproliferative effect had been evaluated for 72 hours after determining the number of cells. IC50 values of compounds were measured with a special quantitative value software.
RESULTS AND DISCUSSION
The tested triterpenoids, oleanolic acid (1), ursolic acid (2) and 3-acetyl ursolic acid (4) didn’t show any cytotoxic effect at lower doses, however they showed at higher doses in MDA-MB-231 breast cancer cell lines.
Oleanolic acid (1) and 3-acetyl ursolic acid (4) have no cytotoxic effect on HUVECs.
HUVEC test assays are still continuing for other two triterpenoids.

CONCLUSIONS
In conclusion, cytotoxic activity of four triterpenoids were investigated against MDA-MB-231 breast cancer and HUVECs in vitro. There is no cytotoxic effect on HUVECs, so they can be a potential drug in breast cancer treatment. Our studies are still going on other natural steroids, flavonoids and their semi-synthetic derivatives.

REFERENCES

P-182: BIOLOGICAL EVALUATION OF NEW TRICYCLIC DIHYDROPYRIDINE BASED DERIVATIVES ON POTASSIUM CHANNELS

C. Şafak1, M. G. Gündüz2, Y. Kaya2, R. Şimşek4, I. Erdemli2
Hacettepe University, Faculty of Pharmacy
1Department of Pharmaceutical Chemistry, 2Department of Pharmacology
Ankara, TURKEY

In this study a microwave-assisted method was applied for the synthesis of novel 8-(disubstituted phenyl)-2,3,4,5,6,8-hexahyrdithieno[3,2-b:2',3'-e]pyridine-1,1,7,7-tetra-oxide and 7,7-dimethyl-9-(disubstituted phenyl)-2,3,5,6,7,9-hexahydrothieno[3,2-b]quinolin-8(4H)-one 1,1-dioxide derivatives. The structures of the compounds were confirmed by spectral methods and elemental analysis. The Emax and pD2 values of the compounds and pinacidil were determined on rat superior mesenteric artery and urinary bladder strips.

INTRODUCTION
Potassium channels selectively conduct potassium ions across the cell membrane along its electrochemical gradient. This diverse and ubiquitous channel family plays important role in cellular signaling processes, neuronal excitability, neurotransmitter release, smooth muscle contraction, heart rate and cell volume regulation. Tricyclic dihydropyridine-based analogues, comprising a variety of heterocyclic rings fused to the dihydropyridine nucleus, were also found to be active as potassium channel openers [1].

MATERIALS AND METHODS
The mixture of tetrahydrothiophene-3-one-1,1-dioxide (2 mmol) / tetrahydrothiophene-3-one-1,1-dioxide (1 mmol) and 4,4-dimethyl-1,3-cyclohexanedione, disubstituted benzaldehyde and ammonium acetate was heated under microwave irradiation. After the reaction was completed, the reaction mixture was poured into ice-water, the obtained precipitate was filtered and crystallized from appropriate solvents or the solvent (methanol) was removed via a rotary evaporator and the crude product was then purified by column chromatography. The effects of the compounds on potassium channels were evaluated on rat superior mesenteric artery and urinary bladder strips.

RESULTS AND DISCUSSION
The test compounds (10-8-10-4 M) and pinacidil elicited concentration-dependent relaxation response in precontracted mesenteric arteries. The relaxation responses of the synthesized compounds were evaluated in the presence of ATP dependent potassium channel (KATP) antagonist glibenclamide and TEA (the antagonist of calcium activated potassium channels). It was determined that some compounds possessed their relaxant activities via ATP dependent potassium channel.

Fig. 1. The structures of the synthesized compounds
CONCLUSIONS

We reported herein an easy method for the preparation of tricyclic 1,4-DHPs. The obtained pharmacological results showed that several tricyclic DHP derivatives have relaxant activity on rat superior mesenteric artery and urinary bladder strips.

Glibenclamide changed the relaxation responses of some effective compounds. These results point out that there is a contribution of ATP sensitive potassium channels to relaxant effects of the compounds.

REFERENCES


P-183: SYNTHESIS AND SPECTRAL ANALYSIS OF NOVEL OXAZOLIDINONES

C. Kus, E. Ugurlu

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100Tandogan, Ankara-Turkey

INTRODUCTION

A variety of 4-(substituted benzylidene)-2-(substituted phenyl) oxazole-5(4H)-on derivatives (E1-E12) was prepared from hippuric acid derivatives. Our aim is to synthesized novel oxazolidinon derivatives. Oxazolone ring is a noteworthy scaffold in the area of drug discovery. Oxazolone based derivatives have shown diverse biological and pharmacological applications such as anticancer [1], and antibacterial [2].

MATERIALS AND METHODS

Uncorrected melting points were measured on an Electrothermal 9100 capillary melting point apparatus. ¹H-NMR and ¹³C-NMR spectra were recorded on a Varian Mercury 400 MHz and 100 MHz FT spectrometer, chemical shifts (δ) are in ppm relative to TMS, and coupling constants (J) are reported in Hertz. Mass spectra were taken on a Waters Micromass ZQ using the ESI(+)-method. Microanalyses were performed by Leco CHNS-932. All chemicals and solvents were purchased from commercial sources and used without further purification.

RESULTS AND DISCUSSION

Our final compounds showed parallel results at ¹H NMR, ¹³C NMR spectra and Electrosprey Ionization Mass Spectrometer (ESI-MS) as other research (Pāsha et al., 2007), (Younesi et al., 2009), (Kawai et al., 2003).

CONCLUSIONS

As expected, in the mass spectra of E2 and E12, both mono and two chlorine isotopes of this atom were seen. Compound E2 has two chlorine atoms, so there are M⁺, M+2 and M+4 (9:6:1) signals. On the other hand, Compound E12 has only one chlorine atom, so there are M⁺ and M+2 (3:1) signals.

REFERENCES


P-184: DESIGN, SYNTHESIS AND CYTOTOXICITIES OF HALOGEN BEARING NEW PHENOLIC BIS-MANNICH BASES

C. Yamali¹, H. I. Gul¹, H. Sakagami²

¹Ataturk University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Erzurum, Turkey.
²Meikai University, Division of Pharmacology, School of Dentistry, Sakado, Saitama, Japan

INTRODUCTION

The classical Mannich reaction, a three-component condensation between a compound containing at least one active hydrogen atom, secondary amine and formaldehyde. Mannich reactions are also known as aminomalkylation reactions. Under normal reaction conditions, substitution of a compound with a single aminomethyl groups results in mono-Mannich bases, but two aminomethyl groups may also be grafted onto a compound containing more than one active hydrogen atom, leading to bis-Mannich bases.[1]

Bioactivity of Mannich bases has been proposed due to alkylating potential of α,β-unsaturated ketones towards cellular nucleophiles which are liberated in situ following deamination.[2]
Mannich bases display varied activities, such as anticancer, antibacterial, antifungal, antimalarial, antiviral, anticonvulsant, anti-inflammatory, analgesic, antioxidant, blood pressure regulation, antulcer, inhibitors of cholinesterases.[1] In addition, Mannich bases of chalcones derived from either phenolic aldehydes or ketones have been examined as cytotoxic agents.[3]

In this study, we synthesized bis-Mannich derivatives of 4-hydroxychalcone to evaluate their cytotoxic activities.

MATERIALS AND METHODS

4-hydroxychalcone derivatives (1-3) were synthesized as described in literature.[4]

For the synthesis of bis-Mannich bases, 1a-c, 2a-c and 3a-c (Fig. 1.): amine (12.4 mmol) was treated with paraformaldehyde (12.4 mmol) in acetonitrile (20 ml) for 5 minutes at 80 °C, 150 Watt, and 13 barr). 4-Hydroxychalcone (21 mmol) was added into the reaction mixture and heated for 40 minutes (at 120 °C, 200 Watt, and 13 barr). Acetonitrile was removed under vacuum and residue was crystallized from methanol-diisopropylether for 2-3 times.

![Synthesis pathway of compounds](image)

Fig. 1. Synthesis pathway of compounds

RESULTS AND DISCUSSION

Chemical structures of the compounds were confirmed by 1H NMR, 13C NMR and HRMS spectra. For compound 3a, 1H NMR δ 7.79 (s, 2H, H-2), 7.69 (d, J=15.7 Hz, 1H, H-9), 7.58-7.49 (m, 5H, B-ring and H-8), 3.65 (s, 4H, H-5), 2.49 (s, 8H, H-6a, 1.64-1.58 (m, 8H, H-7, 1.47 (s, 4H, H-8); 13C NMR δ 188.7, 162.4, 142.1, 134.4, 132.3, 130.0, 129.9, 128.8, 124.5, 123.3, 122.9, 59.6, 54.4, 26.0, 24.3; HRMS (ESI-MS) Calc. for C27H34N2O2Br [MH]+ 497.1806; found: 497.1804.

As an example for NMRs, the compound 3b’s data were presented here, the morpholine protons which are H-7,7” were observed as multiplet at δ 3.77-3.74 and H-6,6” protons were observed as a singlet at 2.57 ppm. Whereas the benzylic methylene protons (H-5) appeared at δ 3.71 ppm as singlet. Chemical shift of C-5 carbon was observed at δ 67.00 ppm while carbonyl group was at δ 188.5 ppm.

![Table 1](image)

Table 1. Cytotoxic evaluation of the some compounds

<table>
<thead>
<tr>
<th>Tumor cell lines</th>
<th>HSC-2</th>
<th>HSC-3</th>
<th>HSC-4</th>
<th>Ave1</th>
<th>Ave2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod e</td>
<td>CC50</td>
<td>SI</td>
<td>CC50</td>
<td>SI</td>
<td>CC50</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
<td>11.2</td>
<td>6.8</td>
<td>5.9</td>
<td>6.6</td>
</tr>
<tr>
<td>3a</td>
<td>2.8</td>
<td>7.4</td>
<td>&lt; 1.6</td>
<td>12.9</td>
<td>2.5</td>
</tr>
<tr>
<td>3b</td>
<td>&lt; 1.6</td>
<td>12.3</td>
<td>&lt; 1.6</td>
<td>12.3</td>
<td>2.0</td>
</tr>
<tr>
<td>3c</td>
<td>&lt; 1.6</td>
<td>5.4</td>
<td>&lt; 1.6</td>
<td>5.4</td>
<td>2.7</td>
</tr>
<tr>
<td>5-FU</td>
<td>15.2</td>
<td>65.8</td>
<td>41.7</td>
<td>24.0</td>
<td>133</td>
</tr>
<tr>
<td>Ave3</td>
<td>63.4</td>
<td>32.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The CC50 value refers to the concentration of compounds in micromoles which kill 50% of the cells. The SI value indicate the selective index, i.e., the quotient of the averaged CC50 value for non-malignant cells divided by CC50 value for a specific malignant cell line. The PSE value indicates the potency selectivity expression which is the product of the averaged CC50 value and the averaged SI value for all four malignant cell lines.

Cytotoxicities of the compounds were compared with the reference compound 5-Fluorouracil (5-FU). CC50 values of compounds are in the low micromolar range towards HSC-2, HSC-3 and HSC-4 (oral squamous cell carcinoma) cell lines. In case of Mannich bases which were derived from bromine bearing chalcone...
analogue 3b and 3c, cytotoxities increased 1.3-2.6 times comparing with starting compound chalcone 3. The average selectivity index (SI) revealed that morpholine containing Mannich base, 3b, had the highest average SI value.

CONCLUSIONS

Lead compounds should have cytotoxic potencies and selective toxicity for tumours. The potency selectivity expression (PSE) values increases in Mannich bases comparing with reference compound 5-FU. The increases were as follows, 8 times for compound 3a, 13 times for compound 3b and 4.6 times for compound 3c.

The compound 3b may serve as a lead molecule for further studies.

ACKNOWLEDGMENTS

This work was financially supported by Research Foundation of Ataturk University, Turkey. (Project Number: 2012/74, 2012/75)

REFERENCES


P-185: THE SYNTHESIS OF THIOUREA DERIVED FROM HOMOCHIRAL AMINES AND THEIR BIOLOGICAL ACTIVITIES

Demet Begüm KORKMAZ¹, Eyüp BAŞARAN¹, Ayşegül KARAKÜÇÜK-İDOĞAN¹, Yusuf SİCAK², Emine Elçin ORUÇ-EMRE¹, Mehmet ÖZTÜRK²

¹Gaziantep University, Faculty of Arts and Sciences, Department of Chemistry, Gaziantep, Turkey
²Muğla Sıtkı Koçman University, Faculty of Sciences, Department of Chemistry, Muğla, Turkey

INTRODUCTION

In the last decade, as a crucial functional group, thiourea derivatives which exists in the substance of many active compounds and is in the structure of the many compounds of medicine active compound candidate, can demonstrate important pharmacological activities such as antioxidant, anticancer, antibacterial and other activities in accordance with the substitutes differences [1-2].

MATERIALS AND METHODS

In this study, all chemicals and solvents purchased from Aldrich, Fluka and Riedel de Haen. Melting points were determined in a EZ-Melt MPA 120 Automated Melting Point apparatus and were uncorrected. IR spectra were recorded on a Perkin Elmer 1620 model FT-IR Spectrophotometer Universal with universal ATR Sampling Accessory. ¹H NMR spectra were recorded with a Bruker AVANCE- DPX NMR Spectrometer in DMSO-d6 using TMS as a internal standard at 400 MHz and 100 MHz respectively. Elemental analysis were performed by Thermo Scientific Flash 2000 Organic Elemental Analyzer instrument. The purity of the compounds was confirmed by thin layer chromatography on silica gel F254 (Merck). The synthesis of target chiral thioureas was accomplished using the reaction sequence in Scheme. The homochiral amines were reacted with 4-substitute phenyl isothiocyanates in dichloromethane at room temperature for 24h as described in previously method [3]. The antioxidant activities of all chiral thioureas were tested by β-carotene-linoleic acid, DPPH free radical scavenging, ABTS cation radical scavenging and Cupric reducing antioxidant capacity assay [4]. The acetylcholinestarase and butyryl cholinesterase inhibitory activity of all chiral thioureas was evaluated by spectophotometric method [5].

RESULTS AND DISCUSSION

In present work, a series of new chiral thioureas from homochiral amines in both enantiomeric forms were synthesized in good yields and high enantiopurity. They were evaluated for antioxidant capacity and anticholinasteraerase activity. The chiral thioureas were prepared by treatment of (R)-(+)-1-(2-naphtyl) ethylamine and (S)-(−)-1-(2-naphtyl) ethylamine with 4-substitute phenyl isothiocyanates in CH2Cl2. Therefore, the chiral center from homochiral amines was retained. The chemical structure of all chiral thioureas were characterized by combined use of IR, ¹H NMR, element analysis (CHNS) and physical properties.
CONCLUSIONS

Herein, we have synthesized twenty chiral thioureas derived from enantiopure amines in both enantiomeric forms and tested their antioxidant and anticholinesterase activity.

REFERENCES


P-186: SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF NEW SCHIFF BASES OF 1,3,4-THIADIAZOL-2-AMINE

D. Cirdakli1, K. Ozadali-Sari1, D. Kart2, M. Sagiroglu2 and F. Ozkanli2

Hacettepe University, Faculty of Pharmacy
1Department of Pharmaceutical Chemistry, 2Department of Pharmaceutical Microbiology
Ankara, TURKEY

INTRODUCTION

The derivatives of 1,3,4-thiadiazole was known to posses various pharmacological activities like anti-inflammatory, analgesic, antimicrobial, anti-tubercular, anticonvulsant activities. Schiff bases have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [1]. The development of a potent, safe and selective antifungal agent is of prime importance for medicinal chemist in the quest for effective chemotherapeutic treatment for fungal diseases. At present antifungal treatment with existing drugs proved to be less effective, due to drug toxicity and drug resistance against a wide variety of fungal species [2]. Among the several classes of antifungal agents, azoles are the most widely used antifungal agents with specified mechanism of action and targeting. On the basis of these findings, this study aimed to synthesize and investigate antimicrobial activities of some new N-(4-substitutedbenzylidene)-5-phenyl-1,3,4-thiadiazol-2-amine containingazole rings.

MATERIALS AND METHODS

5-phenyl-1,3,4-thiadiazol-2-amine (I) was prepared by cyclization of benzoic acid with thiosemicarbazide in the presence of phosphorus oxychloride [3]. The target compounds 2a-e were obtained by condensation of 5-phenyl-1,3,4-thiadiazol-2-amine (I) with the appropriate azole substituted benzaldehydes (Scheme).

Scheme: Synthetic pathway of the compounds.

The antimicrobial activities of compounds against to Gram-positive and Gram-negative bacteria and yeast-like fungi were screened according to the microdilution broth method reported by National Committee for Clinical Laboratory Standards [4, 5]. Minimum inhibitory concentration (MICs) values which are the lowest concentrations of the substances that had no visible turbidity were used for determination of the antimicrobial activity.

RESULTS AND DISCUSSION

The structures of synthesized compounds were proved by spectroscopic (IR, NMR and Mass) and elemental analyses. The antimicrobial activities of the target compounds were less than the reference compounds.
CONCLUSIONS
In this study, new Schiff bases of 5-phenyl-1,3,4-thiadiazol-2-amine were synthesized. IR, MASS, NMR and elemental analysis data were used to characterize synthesized compounds. The antimicrobial activities of the target compounds were less than the reference compounds.

REFERENCES

P-187: SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF CO(III) COMPLEXES OF N-BENZOYL-4,6-DIOXO-HEXAHYDROPYRROLO[3,4-C]PYRROLE-2(1H)-CARBOTHIOAMIDE DERIVATIVES
Y. Nural1, M. Gemili1, D. Ersen1, M. Ulger2, S. Belveren1, S. Poyraz1, M.S. Serin2, HA. Dondas1

Faculty of Pharmacy, Mersin University
1Department of Analytical Chemistry, 2Department of Pharmaceutical Microbiology
Mersin, TURKEY

INTRODUCTION
Cobalt complexes, containing monobasic bidentat (O, S) ligands, play an important role in drug research studies and it has been reported that some of Co(III) complexes show antimycobacterial, antimicrobial, antiviral and other biological activities [1]. Likewise, N-aryl/acyl thiourea derivatives have been intensively studied in many chemistry disciplines due to their significant complexation and biological properties [2]. One of the other important classes of drug research studies is heterocyclic compounds containing nitrogen atom as pyrrolidine and its derivatives [3].

MATERIALS AND METHODS
The N-benzoyl-4,6-dioxo-hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carbothioamide derivative ligands were synthesized according to literature method [4]. Co(III) complexes were synthesized from reaction of these ligands with Co(Ac)2•4H2O dissolved in methanol. Structure of the Co(III) complexes were characterized by various analitical methods. Antimycobacterial activity studies of the Co(III) complexes were performed against M. tuberculosis H37Rv strain by using Microplate Alamar Blue assay.

RESULTS AND DISCUSSION
In our previous studies, we reported some novel aminocarbo-N-thiol pyrrolidine derivatives and their complexes with transitional metals such as Ni(II), Pd(II) and Cu(II) [4]. In this study, synthesized novel Co(III) complexes of N-benzoyl-4,6-dioxo-hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carbothioamide derivatives, having pyrrolidine and pyrrolidinedione rings as monobasic bidentat (O, S) ligands, were characterized by various analitical methods such as IR, UV-vis, magnetic susceptibility measurements.

CONCLUSIONS
Novel Co(III) complexes of N-benzoyl-4,6-dioxo-hexahydropyrrolo[3,4-c]pyrrole-2(1H)-carbothioamide derivatives, having substituted pyrrolidine and pyrrolidinedione rings, were synthesized and the Co(III) complexes showed moderate antimycobacterial activity against M. tuberculosis H37Rv strain

REFERENCES
INTRODUCTION

Alzheimer’s Disease (AD) is a chronic neurodegenerative disease which characterized by memory loss, difficulty in speaking, problems with communication and reasoning [1]. There are many causes about the emergence of this disease, like genetic factors, autoimmune reactions, protein plaques and tangles [2,3]. One of these reasons is cholinergic hypothesis that AD is caused by reduced synthesis of the neurotransmitter acetylcholine (ACh). AChE inhibitors inhibit the hydrolysis reaction of ACh, so low ACh level at AD patients is raised [3,4]. Many inhibitors such as tacrine, donepezil, physostigmine are used as drugs for AD treatment [4]. In this study, we report synthesis, characterization and AChE inhibition properties of new Schiff base derivatives of tacrine (9-amino-1,2,3,4-tetrahydroacridine).

MATERIALS AND METHODS

Schiff base derivatives of tacrine (9-amino-1,2,3,4-tetrahydroacridine) were synthesized with different aldehydes (salicylaldehyde and its derivatives). Inhibitors were characterized by elemental analysis, TGA, FT-IR, 1H-NMR, 13C-NMR spectroscopy. AChE activity of the synthesized compounds were investigated by spectrophotometric Ellman method [5]. Inhibitors’ inhibition types, IC50, Kx, Km, Vmax values were determined.

RESULTS AND DISCUSSION

Synthesized inhibitors’ elemental analysis, TGA, FT-IR, 1H-NMR, 13C-NMR spectroscopy results were observed at the expected value. As a result of spectrophotometric studies, it was seen that all compounds had reversible AChE inhibitor property. It was concluded that synthesized compounds showed a greater AChE inhibition property than tacrine. Compounds’ inhibition types were determined as mix, noncompetitive and uncompetitive type. When the inhibitory potency of compounds (IC50) was compared with respect to K values, same affinity was observed.

CONCLUSIONS

In summary, a range of Schiff base derivatives of tacrine were prepared for preliminary screening as inhibitors against AChE. The structural characterizations of synthesized compounds were made by using the elemental analyses and different spectroscopic methods. All synthesized compounds behaved as inhibitors against AChE.

REFERENCES


**P-189: STUDIES ON SOME ARYLOXYMETHYL THIOSEMICARBAZIDE, 1,3,4-THIADIAZOLE AND 1,2,4-TRIAZOLE-5-Thione Derivatives**

M.M. Shirzad1, K. Özalı̇ Sarı2, O. Ünsal Tan1, E. Palaska1

1Hacettepe University, Faculty of Pharmacy Department of Pharmaceutical Chemistry, Ankara, TURKEY

INTRODUCTION

It is well known that, 1,4-disubstituted thiosemicarbazides and related 2-substituted amino-1,3,4-thiadiazole and 4-substituted-1,2,4-triazole-5-thiones have analgesic, anti-inflammatory [1], antimicrobial [2], antiviral [3], anticonvulsant [4] and anticancer [5] activities. In this study, twelve 1-(7-methoxy-2-naphthoxyacetyl)-4-substituted-3-
thiosemicarbazide (1a-d), 5-(7-methoxy-2-naphthoxy)methyl)-2-substituted amino-1,3,4-thiadiazole (2a-d) and 3-(7-methoxy-2-naphthoxy)methyl)-4-substituted-1,2,4-triazole-5-thione (3a-d) derivatives have been synthesized and evaluated for their inhibitory effects on COX-2 and COX-1 enzymes. The interaction between the 2b and the COX-2 enzyme was interpreted by molecular modeling studies.

MATERIALS AND METHODS
1,3,4-thiadiazole and 1,2,4-triazole-5-thione derivatives were synthesized by cyclization of the corresponding thiosemicarbazides. Chemical structures of the compounds were elucidated by FT-IR, 1H-NMR, 13C-NMR, MS spectra and elemental analysis. The target compounds were screened for their ability to inhibit COX-2 and COX-1 enzymatic activity using a COX inhibitor screening assay kit. Molecular modeling studies were performed by using Molecular Operating Environment (MOE).

RESULTS AND DISCUSSION
All spectral data were in accordance with the assumed structures. The resulting data of COX inhibitor screening experiment showed that 2-(7-methoxy-2-naphthoxy)methyl)-5-ethylamino-1,3,4-thiadiazole (2b) is more selective against COX-2 than COX-1 (COX-2 IC₅₀: 150.2 μM, COX-1 IC₅₀>250 μM). On the other hand, 3-(7-methoxy-2-naphthoxy)methyl)-4-ethyl-1,2,4-triazole-5-thione (3b) exhibited remarkable selective activity against COX-1 (COX-1 IC₅₀: 45.6 μM; COX-2 IC₅₀: 176.5 μM). As a result of the docking studies on COX-2 enzyme, it was observed that 2b is fitted and interacted with the hydrophobic cavity formed Val349, Tyr355, Leu359 and Leu531 in the active pocket of COX-2.

CONCLUSIONS
Among the synthesized compounds, 2b was found as most selective derivative on COX-2 enzyme. Also, 3b and 3d showed selective inhibitory effect on COX-1 enzyme. Based on the inhibitory activity and docking studies, ethyl substitution to 2-amino group increased the inhibitory activity and selectivity of COX-2 in the 1,3,4-thiadiazole derivatives.

ACKNOWLEDGMENTS
This study was supported by Hacettepe University, Scientific Research Fund (Project no: 012D12301).

REFERENCES
2. Palaska, E.; Şahin, G.; Kelicen, P.; Durlu, T.N.; Altinok, G. Synthesis and Anti-inflammatory Activity of 1-Acylthiosemicarbazides, 1,3,4-Oxadiazoles, 1,3,4-Thiadiazoles and 1,2,4-Triazole-3-thiones. *Il Farmaco*, 2002, 57, 101-107.

P-190: DECIPHERING BIOMACROMOLECULAR TARGETS FOR DESIGNING NOVEL ANTITUMOUR METALLODRUGS

E. Süleymanoğlu

Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

The development of metal complexes with therapeutic activity has had an enormous impact on cancer chemotherapy. Thus, coordination complexes, either as drugs or prodrugs, become very attractive moieties as potential anticancer agents. Due to a dramatic increase especially of oncopathologic cases, the direction of research is towards characterizing various metals and other metal-containing complexes as potential novel anticancer drugs. Other interests in this field address concerns for uptake, toxicity, and resistance to metallo drugs.

INTRODUCTION
The current work emphasizes the characterization of selected metals that have gained considerable interest in both the development and the treatment of cancer. The characterization of yet undiscovered cellular targets of Boron, Gallium, Germanium, Rhodium and Gold, is emphasized as the major goal. Studying such
major cellular macromolecular targets would pave the way of designing novel bioorganometallic therapeutic strategies to target molecular pathways as anticancer agents.

MATERIALS AND METHODS
Affinities of metals to cellular phospholipids, serum and intracellular proteins and nucleic acids (both DNA and RNA) are followed by spectroscopic measurements (CD, Fluorescence, FTIR, Raman Scattering). Biomolecular structural transitions of various cellular macromolecular assemblages are investigated by thermodynamic measurements (DSC, ITC and UV Melting Curves analyses), by Langmuir-Blodgett lipid monolayers and microscopic studies (AFM and Fluorescence Microscopy).

RESULTS AND DISCUSSION
A novel hypothesis concerning the possible mechanisms of action of these inorganic drugs at the molecular level is proposed (Fig. 1). This hypothesis is based on our newly developed theoretical model considering cellular membrane phospholipids, serum and intracellular proteins and cellular nucleic acids as targets of these metals.

CONCLUSIONS
Such biophysical approach gives important clues for designing novel therapeutic strategies towards biomacromolecule directed drug design with further therapeutic implications in cellular oncology research.

ACKNOWLEDGMENTS
This work is undertaken in the frame of EU CMST COST Action CM1105 Functional metal complexes that bind to biomolecules (TÜBİTAK project No: 112S047) and was also partially supported by The National Boron Research Institute (BOREN).

REFERENCES

P-191: SUPRAMOLECULAR SELF-ASSEMBLING NUCLEIC ACID-LIPID SURFACE STRUCTURES DESIGNED AS PHARMACEUTICAL NON-VIRAL GENE DELIVERY NANOMEDICINES

F. F. Demirsoy Kaya1, N. Eruygur2 and E. Süleymanoğlu3
Ankara University, 1The Central Laboratory of The Institute of Biotechnology, Gazi University, Faculty of Pharmacy 2Department of Pharmacognosy and 3Department of Pharmaceutical Chemistry Ankara, TURKEY

Despite the significant efforts towards improving efficacies of nonviral vectors, little is achieved in terms of developing suitable nanocarriers based on polyelectrolyte properties of cell surfaces, poly- or lipoplex uptake and endosomal escape. Particular emphasis is put on the bioelectrochemical behaviour of lipoplexes and their surface parameters that influences both the uptake of non-viral gene carriers and the endocytic routes of interacting cells, and explores the molecular links that may trigger these interactions.

INTRODUCTION
The present study is a continuation of our previous work on the use of zwitterionic lipid vesicles in a relevant polyelectrolyte lipoplex design. The problematic high positive charge of the cytotoxic cationic lipids is substituted by small charge of Mg2+, which brings about complex formation of negatively charged plasmid DNA and also negatively charged cell surfaces to be transfected.

MATERIALS AND METHODS
Lipid-nucleic acid self-assembly formations are approached by applying lipid monolayer, bilayer and multilamellar systems, as described [1].

RESULTS AND DISCUSSION
Interfacial studies depict the high affinity of formation of DNA binary complexes with neutral lipids and their ternary complexes with Mg2+ at the air-water interface, as well as on bilayers and multilayers, as seen from their K0 profiles (Fig. 1).
Fig. 1. Kinetics of ternary complex formation between unilamellar DPPC vesicles and bacteriophage T7 DNA, due to Mg$^{2+}$ complexation, as determined by isothermal titration calorimetry (ITC).

CONCLUSIONS
The induced phase separations, free energy releases and kinetic rearrangements at the nanoscale surfaces allows the development of predictive links between structure and activity of lipoplexes with further implications in gene transfections.

ACKNOWLEDGMENTS
This work was supported by EU BMBS COST Action TD1104: European network for development of electroporation-based technologies and treatments (EP4Bio2Med) (TÜBİTAK project No: 111S495).

REFERENCES

P-192: STEREOCHEMICAL THERMODYNAMICS of Pt DRUGS-DNA INTERACTIONS
D. Lando$^1$ and E. Süleymanoğlu$^2$

Institute of Bioorganic Chemistry, $^1$National Academy of Sciences of Belarus, 220141, Minsk, BELARUS, Gazi University, Faculty of Pharmacy $^2$Department of Pharmaceutical Chemistry, Ankara, TURKEY

As nucleic acid damaging agents Pt compounds have been highly successful antitumour agents in a wide use against various human malignancies. Better understanding of their antineoplastic cellular effects would be employed for further rational design and development of novel and improved chemotherapeutics.

INTRODUCTION
Cellular nucleic acids are targets of numerous anticancer drugs, which upon helix binding affect gene expressions and further act in cell cycle arrests and programmed cell dead [1]. Currently, the pharmacologic efficacy of these agents is limited due to the occurrence of detrimental side effects and drug resistant tumour cells. One of the interesting issues to be studied remains the role of drug stereochemistry in DNA and RNA recognitions and their effects on chemotherapeutic trials. In this work, we studied thermodynamic control of Pt drug isomers on the fine structure of the thermal melting curves of calf thymus DNA and supercoiled plasmid DNA.

MATERIALS AND METHODS
Thermal and thermodynamic properties of complexes of cisplatin and transplatin with short oligonucleotide duplexes are studied by using highly sensitive Privalov’s type differential adiabatic scanning microcalorimetry and mathematical modeling.

RESULTS AND DISCUSSION
For cisplatin monofunctional adducts are intermediate, but their fraction is higher 30% of final product formed in DNA by its ineffective isomer transplatin (Fig. 1). These chemical modifications strongly destroy DNA structure at sites of their location.

Fig. 1. Proposed effects of cisplatin and transplatin on cellular nucleic acids.

CONCLUSIONS
Applying such thermodynamic measurements valuable structural data possibly relevant to drug cytotoxicities could be accumulated. However, to relate this to gene expression profiles, more elaborate experiments are needed employing reconstituted human chromosome fractions, for instance.

ACKNOWLEDGMENTS
This work is undertaken in the frame of Turkey-Belarus dual agreement for scientific cooperation
REFERENCES


P-193: A DFT BASED QSAR STUDY OF SCHIFF BASE DERIVATIVES OF BENZENESULFONAMIDE WITH INHIBITORY ACTIVITY AGAINST CARBONIC ANHYDRASE ISOFORMS I

N. Yorulmaz¹, M. Durgun², E. Erglu³

¹Harran University, Faculty of Sciences and Arts, Department of Physics, Şanlıurfa, TURKEY
²Harran University, Faculty of Sciences and Arts, Department of Chemistry, Şanlıurfa, TURKEY
³Akdeniz University, Faculty of Education, Department of Science Education, Antalya, TURKEY

Here, we present a validated QSAR model that demonstrates the correlations between the inhibition constant (Ki) data of the 37 benzene sulfonamide against carbonic Anhydrase I Isozyme (CA I) and the descriptors of the molecules. In order to obtain interpretable QSAR models, the descriptors which have physical meaning have been preferable chosen from the quantum mechanical calculations on the molecules using DFT/B3LYP level of the theory together with 6-31G(d) basis set.

INTRODUCTION

Carbonic anhydrases (EC 4.2.1.1) that belong to the lyase family are ubiquitous zinc enzymes present in prokaryotes and eukaryotes, all over the phylogenetic tree. Many of the CA isozymes involved in some physiological processes, are important therapeutic targets with the potential to be inhibited to treat a range of disorders including edema, glaucoma, obesity, cancer, epilepsy and osteoporosis [1]. Quantitative structure-activity relationships (QSAR) studies are tools for predicting endpoints of interest in organic molecules acting as drugs. In this study, a validated DFT-based QSAR model has been developed between the inhibition constant (Ki) data of the 37 benzene sulphonamides against carbonic Anhydrase I Isozyme (CA I) and the descriptors of the molecules. We believe that obtained QSAR model may be used to design new benzene sulphonamides with high inhibitory activity.

MATERIALS AND METHODS

For all the compounds investigated, 3D modelling and calculations were performed using the Gaussian 03 (G03) Quantum Chemistry package. CodessaPro (Comprehensive Descriptors for Structural and Statistical Analysis), Version 2.7.2 has been used for descriptors calculation, feature selection and statistical analysis. Duplex algorithm has been applied to whole molecules for training and test sets separation. Multi linear regression algorithm has been used for model construction. Application domain of the model has been determined using the leverage approach.

RESULTS AND DISCUSSION

To construct QSAR models by combining quantum chemical calculation based descriptors together with partial charged molecular surface area ones are expected to be mechanistically interpretable. During the model construction, an attempt was made to introduce more meaningful descriptors into the models by replacing some of the selected descriptors with other correlated (and physically meaningful) descriptors from the reduced descriptor pool. By take in account above issues, the best model obtained is as below:

\[
CA \ (K_i) = 489.28\ (36.15) + 1282.9\ (138.31)Q_{\text{N11}} - 72.36\ (20.42)\text{PPSA3} - 67\ (21.27)E_{\text{HOMO-1}}
\]

\[
N_{\text{training}} = 31 \quad \text{and} \quad N_{\text{test}} = 6,
\]

\[
R^2 = 0.79, \quad R^2_{\text{adj}} = 0.73, \quad F = 35.38
\]

\[
s^2 = 9233, \quad R^2_{\text{ext}} = 0.95, \quad \text{RMSD} = 77.72
\]

where \(Q(\text{N11})\) is the Millikan charge on N atom that is located in between benzene ring and substituted functional groups for all the molecules, \(\text{PPSA3}\) is the Zefirov’s atomic charge weighted partial positively charged surface area and \(E_{\text{HOMO-1}}\) is the second highest occupied molecular orbital of the molecules.

CONCLUSIONS

Results presented in this study shows that Millikan charge on bridging Nitrogen atom is the most dominant factor to determine inhibitory activity of the Schiff base derivatives of benzenesulfonamide. Energy of the second highest occupied molecular orbital of the molecules and atomic charge weighted partial positively charged surface area also give a similar contribution to determine the activity.

REFERENCES


(TÜBİTAK project No: 113Z494) and by the Belarusian Republican Foundation for Fundamental Research (X13-068).
P-194: REGIO AND DIASTEREOSELECTIVE SYNTHESIS OF AMINOCHROMENE SUBSTITUTED FULVENES

G. Koz, N. Coskun

Uludag University, Faculty of Arts and Sciences, 1Department of Chemistry, Bursa, TURKEY

INTRODUCTION

Chromene frameworks are commonly found in natural products and biologically active molecules [1]. Especially, 2-amino-3-nitrile chromenes have been indicated as common structural motifs in a diverse set of biologically important molecules, such as protein kinase inhibitors, tumor antagonists and anticancer drugs [2]. The development of catalytic approaches toward the efficient synthesis of 2-amino-3-nitrile chromenes is of significant interest [3]. Herein we report a one pot three component method for the synthesis of cyclopentadiene (Cp) substituted chromenes and the regio and diastereoselective synthesis of their fulvene analogues.

MATERIALS AND METHODS

An organocatalytic one pot method was developed for the synthesis of Cp substituted chromenes. The Cp Michael adducts were obtained as a mixture of isomers using 2° and 3° amine bases such as piperidine and DBU. These isomeric mixtures were subsequently converted to their fulvene analogues regio- and diastereoselectively under catalytic conditions. All reactions were performed in aqueous medium and the precipitated products were isolated easily by filtration.

RESULTS AND DISCUSSION

One of the direct methods for the synthesis of 2-amino-4H-chromene derivatives is through a Michael addition of common nucleophiles such as indole, nitromethane or ethylcyanoacetate. We used cyclopentadiene as a nucleophile for the first time in this manner and obtained an isomeric mixture of Cp ring in the Michael addition step (Fig. 1). We tried a variety of bases to optimize the reaction conditions.

In the second step we obtained 1,3-substituted (E)-fulvenes selectively as a result of catalytic conditions and aromatic aldehydes (R’CHO) we used.

CONCLUSIONS

In summary, we have developed a new efficient conjugate addition of Cp to in situ formed Knovenagel adducts mediated by a 2° and 3° amine organocatalyst. The method generates 2-amino-3-nitrile-chromenes in good to excellent yields (80-95%) as an isomeric mixture. Under our catalytic conditions using pyrrolidine, we obtained 1,3-substituted (E) fulvene derivatives of the aminochromenes as the only product in moderate to high yields (62-94%).

ACKNOWLEDGMENTS

G. K. acknowledges a BIDEB 2218-Postdoctoral Fellowship from the Turkish Scientific and Technological Research Council (TUBITAK).

REFERENCES


P-195: SYNTHESIS AND ANTICANCER ACTIVITY OF THIOSEMICARBAZIDES DERIVED FROM TOLMETIN

Y. Dadaş, G.P. Coşkun, Ö. Bingöl-Akpınar, D. Özsavc, Ş. Güniz Küçükgüzel
INTRODUCTION
Thiosemicarbazides, which are intermediate products of the synthesis for bioactive heterocyclic compounds, have taken attention of the researchers because of their clinical use and diverse biological activities [1,2]. Tolmetin inhibits prostaglandine synthesis and is a non steroidal anti-inflammatory drug. It was also reported that, tolmetin prevented the growth of colon cancer cells [3]. The ten compounds bearing thiosemicarbazides as the main structure had been synthesised from tolmetin in our study.

MATERIALS AND METHODS
Tolmetin [1] was synthesized by the hydrolysis of tolmetin sodium dihydrate in acidic conditions. Tolmetin ester [2] was prepared from tolmetin in the presence of methanol and concentrated sulphuric acid. Tolmetin hydrazide [3] was prepared from [2] in the presence of hydrazine-hydrate and methanol [4,5]. Compound [3] and substituted isothiocyanates were refluxed in anhydrous ethanol and the reaction led us the synthesis of substituted Tolmetin thiosemicarbazides [4a-j].

RESULTS AND DISCUSSION
The synthesized compounds were identified by FT-IR, 1H-NMR, 13C-NMR and 2D-NMR and their purity were proven by elemental analysis, and TLC. Tolmetin [1] and tolmetin fluoro substituted thiosemicarbazide [4d] were screened for anticancer activities in Marmara University, Faculty of Pharmacy, Department of Biochemistry.

CONCLUSION
The structures of Tolmetin thiosemicarbazides were elucidated after their synthesis. Tolmetin [1] and tolmetin fluoro substituted thiosemicarbazide [SGK-524; 4d] were evaluated for anticancer activity against androgen independent cancer cell line PC-3 (ATCC, CRL-1435), human colon cancer cell lines HCT-116 (ATCC, CCL-247), HT-29 (ATCC, HTB-38) using MTT assay and showed anticancer activity against PC-3 cancer cell line.

ACKNOWLEDGMENTS
Authors are grateful to Santa-Pharma Pharmaceuticals for providing Tolmetin sodium dihydrate. This work was supported by Research Fund of the Marmara University; Project number: SAG-C-YLP-100914-0318.

REFERENCES

P-196: SYNTHESIS AND ACETYLCHOLINESTERASE INHIBITOR ACTIVITY STUDIES ON N-BENZYL-PIPERIDINE AND N-BENZYL-MORPHOLINE DERIVATIVES

G. Sayar, S. Parlar, V. Alptüzün, E. Erciyas
Ege University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Izmir, TURKEY

INTRODUCTION
Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive memory deficit and reduced levels of acetylcholine (ACh) neurotransmitter. Cholinergic hypothesis is one of the major therapeutic strategies in AD. It is considered that the decrease of acetylcholine as neurotransmitter may play an important role in cognitive deficiency and memory loss. Acetylcholinesterase (AChE) inhibitors can increase the level of ACh in the synaptic cleft. To date, AChE inhibitors are the most successful approach for the treatment of AD. Tacrine [1], donepezil [2], rivastigmine [3], and galantamine [4] are AChE inhibitors approved by FDA for the symptomatic treatment of AD. Among these drugs donepezil is recognized by AChE by interactions in the middle gorge of the active site of the enzyme, mainly by three subunits: the benzyl moiety, the nitrogen atom at the piperidine ring and the dimethoxy-indanone group of donepezil. It is well known that N-benzylpiperidine pharmacophoric moiety has a key role in the AChE inhibitor activity. Thus, in this study we synthesized ‘N-benzylpiperidine’ and ‘N-benzylmorpholine’ derivatives and tested their inhibitor activity against AChE.
MATERIALS AND METHODS

The compounds (Fig 1) were synthesized as described in Ref [5, 6]. The enzyme inhibitor activity of synthesized compounds was measured using colorimetric Ellman’s method [7].

RESULTS AND DISCUSSION

In this study, some ‘N-benzylpiperidine’ and ‘N-benzylmorpholine’ derivatives were synthesized and evaluated for AChE inhibitor activity. The structures of title compounds were confirmed by spectroscopic methods (IR, 1H NMR, MS) and their AChE enzyme activities have been examined by using Ellman’s method [7]. The final compounds exhibited AChE inhibitor activity with different ratios.

Fig. 1. The structures of synthesized compounds

REFERENCES


P-197: NOVEL REVERSIBLE AND SELECTIVE PYRAZOLINE BASED hMAO-A INHIBITORS: SYNTHESIS, DOCKING STUDIES AND BIOLOGICAL EVALUATION


a Analysis and Control Laboratories of General Directorate of Pharmaceuticals and Pharmacy, Ministry of Health of Turkey, 06100 Sihhiye, Ankara, Turkey
bDepartment of Biochemistry, Faculty of Pharmacy, Hacettepe University, 06100 Sihhiye, Ankara, Turkey
cDepartment of Bioinformatics and Genetics, Faculty of Engineering and Natural Sciences, Cibali Campus, Kadir Has University, 34083 Fatih, Istanbul, Turkey

INTRODUCTION

Monoamine oxidases (MAOs) which are responsible for the oxidative deamination of neurotransmitters and exogenous amines, exist in two isoforms named as MAO-A and -B [1]. MAO-A inhibitors are used as antidepressant and anxiolytic drugs while MAO-B inhibitors are used in the treatment of Parkinson’s and Alzheimer’s diseases [2]. On the basis of the previous reports indicating that 2-pyrazoline derivatives have MAO inhibitor activities [3], and our earlier studies [4], we recently synthesized seven 2-pyrazoline analogues and investigated their human MAO (hMAO) inhibitory activities.

MATERIALS AND METHODS

A series of 2-pyrazoline derivatives was synthesized according to the previous methods [4]. Their structures were elucidated by IR, 1H NMR, Mass spectral data and elementary analysis. Synthesized compounds were docked computationally to the active site of the hMAO-A and -B isoenzymes. The AutoDock 4.2 program was employed to perform automated molecular docking. MAO inhibitory activity and reversibility tests were performed according to the previous methods [4].

RESULTS AND DISCUSSION

All tested compounds inhibited hMAO-A selectively, reversible and competitively. Among them, compound 5c, which is carrying 3-hydroxyphenyl group at the position 3, and 4-methoxyphenyl group at position 5 and 4-pyridyl carbonyl group at the first position of 2-pyrazoline ring appeared as the most potent compound with the Ki value of 0.009±0.4x10-3. Compound 5c was found more selective than moclobemide, the well known selective MAO-A inhibitor, with the selectivity index of 9.57x10-4. The computationally obtained results were in good agreement with the corresponding experimental values.
CONCLUSIONS

In the present study, new 2-pyrazoline derivatives were successfully identified and presented as potent, reversible and selective inhibitors of hMAO-A. Data gave important insights for designing more selective and reversible MAO inhibitors in future.

REFERENCES


P-198: SYNTHESIS OF AMINO ACID ESTER DERIVATIVES OF IBUPROFEN

G. Taskor, B. Eymur, N. Saygili

Hacettepe University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Ankara, TURKEY

New derivatives of ibuprofen have been synthesized by nucleophilic addition–elimination reaction of acyl chloride of ibuprofen with various amino acid methyl esters. Ibuprofen derivatives have been characterized by 1H NMR and 13C NMR.

INTRODUCTION

Ibuprofen (IBU), 2-(4-Isobutylphenyl)propionic acid, is a Non-steroidal anti inflammatory drug (NSAID) that inhibits the cyclooxygenase system. It is generally used as analgesic and antipyretic for a variety of inflammatory pathologies [1]. Despite being better tolerated in comparison to other NSAIDs [1], it has demonstrated NSAIDs-related gastric toxicity, including gastric irritation and bleeding, abdominal pain and ulcers [2].

IBU causes bleeding in the stomach due to its acidic property. In order to prevent this side effect, it was planned to reduce its acidic property by derivatization of the drug molecule. In order to do this, the synthesis of amino acid ester derivatives of ibuprofen was designed in this work.

RESULTS AND DISCUSSION

Synthesis of Methyl 2-(2-(4-isobutylphenyl)propanamido)-3-methylbutanoate (1). IBU (449.5 mg, 2 mmol), (S)-methyl 2-amino-3-methylbutanoate (288.6 mg, 2.2 mmol), Et3N (0.6 mL, 4.4 mmol) in CH2Cl2; eluent EtOAe–hexane 1:2 (v/v) gave compound 1 as a light yellow liquid (215 mg, 66% yield). 1H NMR (CDCl3) δ 7.13 (d, 2H, J = 8.0 Hz, ArH), 7.03 (d, 2H, J = 8.0 Hz, ArH), 5.96 (s, 1H, NH), 4.43 (m, 1H, 2-H), 3.57 (s, 3H, OC), 1.41 (d, 3H, J = 8.0 Hz, CH3PH), 1.35 (m, 1H, CH(CH3)2), 1.14 (d, 3H, J = 8.0 Hz, 3’-H), 0.80 (d, 12H, J = 8.0 Hz, 4-H, 3-CH3, & CH(CH3)2); 13C-NMR (CDCl3) δ 172.45, 140.52, 137.85, 129.43, 56.88, 51.93, 46.52, 44.95, 31.12, 30.18, 22.26, 18.82, 17.32.

In the present study, the amino acids were converted into their corresponding methyl ester by reacting with methanol in the presence of thionyl chloride [3]. These
amino acid esters were then reacted with acid chloride of ibuprofen [2-(4-Isopropyl-phenyl)propionyl chloride] to afford the desired products. The structures of synthesized molecules were confirmed by $^1$H NMR and $^{13}$C NMR.

CONCLUSIONS
As is known, the ulcerogenic property of NSAID is a factor limiting their use in clinics. These new ibuprofen derivatives may prevent the accumulation of the drug in gastric mucosa results in decrease of gastrointestinal irritant without loss of pharmacological response. For this purpose, further extensive studies on anti-inflammatory effect and analgesic activity of amino acid ester derivatives of ibuprofen should be done.

Scheme 1. Reagents: (i) thionyl chloride and DCM; (ii) triethyl amine and DCM.

REFERENCES

P-199: INTERACTIONS OF COUMARINS WITH TWO CYTOSOLIC CARBONIC ANHYDRASES MEASURED WITH STEERED MOLECULAR DYNAMICS SIMULATIONS

H. Unan¹, Z. E. Boyluğ², M. Tekpınar¹, E. Eroğlu³

¹Yuzuncu Yil University, Faculty of Science, Department of Physics, Van, TURKEY
²Aksaray University, Faculty of Science and Arts, Department of Physics, Aksaray, TURKEY
³Akdeniz University, Faculty of Education, Department of Science Education, Antalya, TURKEY
RESULTS AND DISCUSSION

Fig. 1. Force-Time curves for CA I (top panel) and CA II (bottom panel) with 1000 pN/Å spring force constant show how pulling forces change when two coumarin derivatives are used.

Fig. 2. Force-Time curves for CA I and CA II show that a higher force barrier has to be overcome when Coumarin2 5Z is pulled out of CA I (1hcb) binding pocket.

CONCLUSIONS
We obtained three important results from this study: Ring opening is very important for interactions of Coumarins with carbonic anhydrase forms. As observed in X-ray crystallography studies, our simulations also verify that open ring ligand Coumarin2 5Z interacts more with the enzymes. We observed that force-extension profiles differ for various forms of carbonic anhydrases when interacting with Coumarin2 5Z. Our results are in qualitative agreement previous with experimental molar affinity studies (3).
Finally, our results are robust and reproducible even if a different spring force constant is used.

ACKNOWLEDGMENTS
This project has been supported financially by TUBITAK under project number 114Z045.

REFERENCES
P-200: SYNTHESIS AND IDENTIFICATION OF IMINE-STRUCTURED BORONIC COMPOUNDS AS PROSTATE CANCER THERAPEUTIC

H.Temel¹, S. Paşa¹, A. Doğan², S.Demirci², F. Şahin²

¹Dicle University, Faculty of Pharmacy Department of Pharmaceutical Chemistry, Diyarbakır, TURKEY
²Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, TURKEY

Boron derivative compounds such 3-((4-acetyl phenyl imino)methyl) phenyl boronic acid (B1), 3-((2,6-dimethoxy phenyl imino)methyl)-4-fluoro phenyl boronic acid (B2) were synthesized and identified. Prostate cancer treatment was investigated employing these boronic structures. Therapeutic effect of boron as anti-cancer treatment was tried to enlighten that the prostate cancer cells decreased while the most of healthy cells are still viable. Cell viability has tested three times to prove the efficiency of boronic molecules.

INTRODUCTION
Boron and derivative compounds have been behaving remarkable features in hormone and mineral metabolism, enzyme reactions, and cell membrane functions even being classified as trace elements for human life. However, previous studies have also shown that the deficiency of boron caused decreasing the plant photosynthetic capacity [3-5]. The insufficiency of nutritional boron has an impact on brain electrophysiology and brain behaviors. Furthermore, boron compounds have been commonly employed in cancer treatment that is called as Boron Neutron Capture Therapy [7]. Various boronic structures have been exerted in tumor and cancer therapy with limited thriving outputs.

MATERIALS AND METHODS
Boron containing imine structures were evaluated for their anticancer activity by using PC-3 (Human Prostate Cancer Cells-ATCC® CRL-1435®) and L929 cells (Mouse Fibroblast Cells-ATCC® CCL-1™) as healthy cell line. Four separate concentrations (0.5-1-2-5µM) of the boronic compounds were applied to these cells.

Prepared compounds were added to the cultured cells, incubated for 24, 48, and 72 hours. Cell viability was measured by the 3-(4,5-di-methyl-thiazol-2-yl)-5-(3-carboxy-methoxy-phenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium(MTS)-assay (Cell Titer 96 Aqueous One Solution; Promega, Southampton, UK) according to the manufacturer’s instructions. Cells were incubated with the MTS solution for 2 hours at 37 °C and absorbance was measured at 490 nm by using an ELISA plate reader (Biotek, Winooski, VT).

RESULTS AND DISCUSSION
Boron containing imine (C=N) structures were synthesized by the condensation between amine and aldehyde group bearing phenyl boronic acids. They were characterized with various spectroscopic methods such ¹H and ¹³C NMR, LC-MS, FTIR, UV-Vis, Elemental Analysis, SEM. These boronic compounds were investigated against prostate cancer cells. The cell viability was depicted in Figure 1 and Figure 2.

The analysis revealed that although B1 and B2 were cytotoxic for PC-3 cells, there were no significant toxic effects on L929 cells. B1 exerted a time and dose dependent cytotoxicity on cells. Although 5µM B1 decreased cell viability to 33 % (Fig. 1) at day 3 while the healthy cells are preserved approximately 71 %.

B2 demonstrated similar results as B1 and exerted a time and dose dependent cytotoxicity for cells. 5µM B2 decreased prostate cancer cell viability to 44 % while healthy cells are maintained 95 % alive (Fig. 2).
Fig. 2 Cell viability ratio of B2 against PC-3 cells

CONCLUSIONS
Results showed that boron containing imine compounds specifically exhibited cytotoxicity on prostate cancer cells. These boronic compounds have acceptable toxicity on fibroblast cells which were used as healthy cell line in the study. The results of the current study clearly show that boron containing imine compounds could be promising candidates for drug design in cancer research.

ACKNOWLEDGMENTS
Support for analysis and research by the Dicle University Science and Technology Research Center (DUBTAM) and Yeditepe University are gratefully acknowledged.

REFERENCES

P-201: A STUDY ON SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 4-ACYL-PYRAZOLE-3-CARBOXYLIC ACIDS AND THEIR DERIVATIVES

İ. Bildirici1, A. Çetin2

1 Yüzüncü Yıl University, Faculty of Pharmacy Department of Pharmaceutical Chemistry, Van, TURKEY
2 Muş Alparslan University, Faculty of Education, Department of Sciences, Muş, TURKEY

INTRODUCTION
The pyrazole ring is a prominent heterocyclic scaffold in lots of bioactive molecules. They are important substances and have gained widespread attention in agrochemical, pharmaceutical and chemical industries. For example, they possess a wide range of bioactivities, including antimicrobial, antiviral, antitumor, anti-inflammatory, antihistaminic, pesticidal, antifungal, antibacterial, anti-rheumatoid-arthritis, anticonvulsant, antidepressant and antipyretic agents. In recent years, several drugs including patented ones are developed from the pyrazole core containing compounds. For instance, celecoxib demonstrates anti-inflammation effect and inhibits COX-2; rimonabant functions as cannabinoid receptor and is utilized in obesity treatment; fomepizole inhibits alcohol dehydrogenase; antiviral agents (Pyrazomycin); and sildenafil inhibits phosphodiesterase.

MATERIALS AND METHODS
All reagents and solvents were obtained from commercial suppliers and were of reagent grade quality. Melting points were determined on an Electrothermal Gallenkamp apparatus. FT-IR spectra were recorded on a Matson 1000 FT-IR spectrophotometer.1H- and 13C-NMR spectra were obtained on Bruker Instrument Avance Series-Spectrospin DPX-400 Ultra Shield. Elemental analyses were performed on a Thermo Scientific Flash 2000. Follow up of the reactions and checking the purity of the compounds was made by thin layer chromatography (TLC). Compounds were visualized by Camag TLC devices UV.

RESULTS AND DISCUSSION
4-Acyl-Pyrazole-3-Carboxylic Acids were synthesized via the reaction of furandiones with hydrazine by heating in solventless media, and its acid chlorides were obtained easily. Then these novel derivatives could be easily converted into the corresponding derivatives such as the ester, ureid and the amide via reactions of acid chloride with various O- and N-nucleophiles. In addition, the other pyrazole derivatives that studied antibacterial activity were obtained with various reaction condition. The in-vitro
antibacterial activity of new synthesized compounds were carried out against Bacillus subtilissus and Staphylococcus aureus as examples gram-positive bacteria, Eschericha coli, Pseudomonas aeroginosa and Klebsiella pneumonia as examples of gram-negative bacteria by well diffusion method (zone inhibition).2 Erythromycin (15 mg), Rifampicin (5 mg) and Amikacin (10 mg) antibiotics were used as reference drugs. Dimethylsulfoxide was used as control and no visible inhibition zone were observed on control groups.

**CONCLUSIONS**

It may be suggested that these new synthesized compounds have much potent of antibacterial activity owing to containing of both pyrazole and pyridazine groups. Besides the among this series, some compounds showed similar activities comparing to reference drugs those were containing chloro, fluoro groups at different position at the phenyl ring. The compounds containing electron withdrawing groups at different position such as chloro and fluoro showed good activity against P. aeroginisa and S. Aureus. Our results have showed obviously that substituents containing different groups such as chloro, fluoro, methyl and NO₂ have increased the activity of compounds. In conclusion, the substituted pyrazoles showed a wide variety of biological and pharmacological activities and have wide application both pharmaceuticals and in the agricultural industry. For this reason, the methods developed for the synthesis of these compounds are becoming more importance.

**ACKNOWLEDGMENTS**

The authors wish to express their appreciation and gratitude to the Scientific Research Projects Chairmanship of Yuzuncu Yil University for its financial support of this study. Project Number: 2011-FBE-D006.

**REFERENCES**


---

**P-202: POLYVINYLPYRROLIDONE (PVP) / POLY (E-CAPROLACTONE) (PCL) BLEND NANOFOBRES FOR THE RELEASE OF DOXORUBICINE**

K. Cicek1, F.O. Gokmen2, N. Pekel Bayramgil1

1Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, TURKEY
2Bilecik Seyh Edebali University, Central Research Laboratory, Bilecik, TURKEY

In this study, we prepared biocompatible Polyvinylpyrrolidone (PVP) and biodegradable Poly (ε-Caprolactone) (PCL) blend nanofibers by electrospinning and searched the usability for controlled drug delivery systems trying doxorubicine as a model drug for oncological treatment. pH and conseration effects on drug release were studied by using UV-vis. spectroscopy.

**INTRODUCTION**

Controlled drug delivery systems, after when the drug into the body to provide the continuity of reception of the drug therapeutic amounts, it is reducing harmful side effects due to the positioning of the target are, it can reduce the amount of dosage, it provides different drug administration for short or long half-life medicaments. Controlled drug release systems, because of the innovative and their healing aspects were introduced in the world of medicine fastly and in short time it has been priority system used in cardiology, endocrinology, immunology, ophthalmology and oncology[1,2]. Fibers with a size of nanometer have high surface area (high length / weight), because of high porosity, stiffness and such as flexibility properties to offer the opportunity in a wide use like electrical and optical applications, defense and aerospace applications, filtration, agricultural, biomedical, composites and many other fields [3,4]. Electrostatic spinning is considered today as the most promising method to produce fiber in a wide range. Electrostatic spinning is can be summarized first charged on polymers very high voltage then polymer solidifies when it flows through from a catapult to grounded plate and becomes fibres. The nanofibers obtained by electrostatic spinning, is the subject of research in intensively medicine, pharmacy, bioengineering. As a spinning by an electrostatically and suitable for bioengineering natural polymers are proteins (collagen, gelatin, silk), polysaccharides (cellulose acetate, hyaluronic acid), and polyhydroxyalkanoates, among synthetic...
polymers is polyvinylpyrrolidone, polyvinyl alcohol, polyethylene glycol etc.

**MATERIALS AND METHODS**

PVP (MW: 25000, Sigma-Aldrich), PCL (Sigma-Aldrich), Dichloro methane (Merck), N,N-Dimethyl Formamide (Sigma)

**RESULTS AND DISCUSSION**

Fig. 1. SEM picture of Polyvinylpyrrolidone (PVP) nanofibers

Fig. 2. FT-IR Spectra of Polyvinylpyrrolidone (PVP) / Poly (ε-Caprolactone) (PCL) Blend Nanofibres and their composites fibers.

**REFERENCES**


**P-203: SYNTHESIS AND STUDY OF PHYSICO-CHEMICAL PROPERTY OF DITHIOL-ONE’S DERIVATIVES FROM DITHIOL-THIONE.**

Laifa E.A1, B. Khaled2 and Zouaoui Nassima3

1,2,3 Département of Chemistry, Faculty of Science, University of Constantine1, Algeria

1,2- dithiole- 3-ones and 1,2-Dithiole-3-thiones are an important class of oxygen and sulfur containing heterocycles with antioxidant, chemopreventive and radioprotective activities, the biological action of which has been attributed to their redox reactions[1]. One of the main metabolites of the cancer chemopreventive agent, 4-methyl-5-(2-pyrazinyl)-3 H-1,2-dithiole-3-thione (Oltipraz)

![Fig.1: 4-methyl-5-(2-pyrazinyl)-3 H-1,2-dithiole-3-thione (Oltipraz)](image)

is a result of a reductive methylation process. This metabolite was synthesized also by chemical reduction of Oltipraz with sodium sulfide followed by alkylation with methyl iodide [2]. Therefore, the reduction of 1,2-dithiole-3-thiones was intensively studied and has been shown that both biochemical [3], electrochemical [4] and reduction with sodium sulfide affords a dianionic intermediate, which can be alkylated to give dithioester derivatives or undergoes an intramolecular cyclization as in case of Oltipraz this product inhibits HIV-1 (AIDS) [5]. An other group described the synthesis of 4-fluoro-5-polyfluoroalkyl-1,2-dithiole-3-thiones (R1=CF3, HCF2 CF2, R2= F) and studied their cycloaddition properties as dienophiles and as 1,3-dipoles. Also The natural product leinamycin

![Fig.2: leinamycin](image)

an antitumor, antibiotic isolated from Streptomyces sp, displays potent antitumor and cytotoxic activities and an interesting activity against Gram-positive bacteria.

We therefore undertook a study in order to develop a method to synthesize, identification and study biological and pharmaceutical interest of various derivatives of 1,2-dithiolan-3-one 1-oxides. The intramolecular cyclization of 2,2’dithiodibenoic acid and they derivatives offered 3H-1,2-benzodithiol-3-
thione which, by oxidation gave 3H-1,2-benzodihiol-3-thione 1-oxide. which we report in this communication.

REFERENCES

P-204: SYNTHESIS AND IN VITRO EVALUATION OF SOME THIAZOLE DERIVATIVES AS NEW ANTIMICROBIAL AGENTS

M.D. Altuntop1, Z. Cantürk2, Z.A. Kaplancıklı1

Anadolu University, Faculty of Pharmacy
1Department of Pharmaceutical Chemistry, 2Department of Pharmaceutical Microbiology
Ankara, TURKEY

INTRODUCTION
Search for antimicrobial agents is a never ending task, as pathogens are endowed with the ability to thwart therapeutic regimens by rapidly developing resistance. Pharmaceutical industry has focused on the discovery of potential antimicrobial agents which inhibit the growth of pathogens or kill them and have no or low toxicity to host cells [1,2].

In the continuation of our ongoing research in the field of design, synthesis and evaluation of new thiazole derivatives as antimicrobial agents [3], herein we synthesized a new series of thiazolyl hydrazone derivatives and investigated their antimicrobial activity.

MATERIALS AND METHODS
New thiazolyl hydrazone derivatives (Fig. 1) were obtained via the ring closure of 4'-[morpholino/piperidino]acetophenone thiosemicarbazone with phenacyl bromides. The in vitro effects of these derivatives were evaluated against S. aureus (ATCC 25923), E. faecalis (ATCC 29212), E. faecalis (ATCC 51922), L. monocytogenes (ATCC 1911), K. pneumoniae (ATCC 700603), P. aeruginosa (ATCC 27853), E. coli (ATCC 35218), E. coli (ATCC 25922), C. albicans (ATCC 90028), C. glabrata (ATCC 90030), C. krusei (ATCC 6258), C. parapsilosis (ATCC 22019) using CLSI broth microdilution method. Based on this assay, the minimum inhibitory concentrations (MICs) of the compounds were determined. Chloramphenicol and ketoconazole were used as reference drugs for antibacterial and antifungal activity, respectively.

RESULTS AND DISCUSSION
All compounds showed remarkable antibacterial activity against E. faecalis (ATCC 51922), P. aeruginosa, and E. coli (ATCC 35218) with a MIC value of 200 μg/mL when compared with chloramphenicol (MIC= 200 μg/mL). The microbiological results demonstrated that the antibacterial effects of these compounds did not depend on the substituents.

Among these compounds, 2-[2-[1-(4-(piperidin-1-yl)phenyl)ethylidene]hydrazinyl]-4-(4-fluorophenyl)thiazole and ketoconazole exhibited the same level of antifungal activity against C. glabrata and C. parapsilosis.

CONCLUSIONS
In the current work, we described the synthesis of a new series of thiazolyl hydrazone derivatives and evaluated their antimicrobial effects against pathogenic bacteria and Candida species. Among these compounds, 2-[2-[1-(4-(piperidin-1-yl)phenyl)ethylidene]hydrazinyl]-4-(4-fluorophenyl)thiazole can be identified as the most promising antimicrobial agent due to its inhibitory effects on E. faecalis, P. aeruginosa, E. coli, C. glabrata and C. parapsilosis. Further research is needed to understand the mechanism of action for the antimicrobial activity of this compound.

REFERENCES
P-205: ANTIPROLIFERATIVE ACTIVITIES OF SOME 2-[4-(1H-BENZIMIDAZOL-1-YL)PHENYL]-1H-BENZIMIDAZOLE DERIVATIVES

T. Ozkan1, A. Sunguroglu1, M. Alp2

1Ankara University, Faculty of Medicine, Department of Medical Biology, Sihhiye, Ankara, Turkey
2Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Tandoğan, Ankara, Turkey

A series of 2-[4-(1H-benzimidazol-1-yl)phenyl]-1H-benzimidazoles (13-22) were evaluated for their in vitro antiproliferative activities against human chronic myeloid leukemia (CML) cell lines, K562s (imatinib sensitive) and K562r (imatinib resistance). Compound 20 and 21 exhibited remarkable antiproliferative activity against K562 cell lines.

INTRODUCTION
In our previous paper, we have reported antileukemic activities of some 2-(4-phenoxy phenyl)-1H-benzimidazoles against human CML cell line K562 [1]. These findings prompted us to investigate a series of some 2-substituted phenyl-1H-benzimidazoles to evaluate their anti-proliferative activity against CML cell lines.

MATERIALS AND METHODS
Compounds [2] were dissolved in DMSO in a stock solution at a concentration of 10 mM, stored at -20°C and protected from light. In each experiment DMSO concentration never exceeded 0.5% and this percentage did not interfere with cell growth.

MTT test was used to determine cell viability. Briefly, cells (4x10^4 cells/well) were seeded to 96-well plates. The cell proliferation assay was carried out using the Cell Proliferation Kit I [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoli-um bromide ;(MTT)] (Roche, Germany) as described by the manufacturer using cells treated at various concentrations (0.1, 1, 5, 10, 15, 20, 25, 30, 35, 40 μM) of the compounds for 48h. [3]

RESULTS AND DISCUSSION
Benzimidazole derivatives containing 5,6-dimethyl (20) and 5-chloro-6-methyl (21) exhibited antiproliferative activities against CML cell lines, K562s and K562r. Compounds 15, 16 and 17 bearing electronegative group at 5th position of benzimidazole ring, cyano, nitro and carboxy respectively, did not exhibit a significant activity.

CONCLUSIONS
In this study, some benzimidazole derivatives were evaluated for their in vitro antiproliferative activities against CML cell lines, K562s and K562r. Compounds 20 and 21 exhibited remarkable activity.

REFERENCES

P-206: COMPUTATIONAL DESIGN AND SYNTHESIS OF POTENTIAL PROTEIN KINASE INHIBITORS FOR CANCER TREATMENT

F. Cömert Önder1, M. Ay1, T. Taskin Tok2

1Çanakkale Onsekiz Mart University, Faculty of Sciences and Arts, Department of Chemistry, Natural Products and Drug Research Laboratory, Çanakkale, Turkey
2 Gaziantep University, Faculty of Science and Arts, Department of Chemistry, Gaziantep, Turkey

INTRODUCTION
Protein kinases play a crucial role in a wide range of cellular processes. Aberrant activation of kinase signaling pathways is involved in many types of disease, such as cancer. Protein kinases, therefore, have become one of the most intensively investigated target classes for therapeutic intervention [1a, b]. The purpose of the proposed study is to design using quantum chemistry calculations by the Functional of Density Theory method and synthesize the more effective new PKCα and/or eEF-2 kinase inhibitors for the treatment of breast and pancreatic cancers.

MATERIALS AND METHODS
To examine the effects of different substituents, we synthesized six compounds including 2H-1-benzopyran-2-one and 7-hydroxycoumarin core.
structures (Scheme 1). Various synthesis methods were used for all target compounds (1-6), 6 looks as a new compound, successfully [2a-c]. The structures were characterized by melting point, FT-IR, 1H-NMR, 13C-NMR spectral analysis. All the calculations were performed with the Gaussian 09 molecular package [3a]. Prior to any DFT [3b] calculations all structures were submitted to PM3 [3c] geometry conformational search. All PM3 geometries were fully optimized at the B3LYP/6-311++G** computational level [3d]. Frequency calculations at both levels were carried out to confirm that the obtained structures correspond to energy minima. The structure–activity relationship has been used to understand the role played by the different structural features of the six molecules according to the standard compounds [4]; and to obtain new compounds endowed with better PKCδ and/or eEF-2 Kinase inhibitors properties on the B3LYP/6-311++G** optimized geometries.

RESULTS AND DISCUSSION

The inhibitor activities of six different compounds were theoretically measured. The selection of these compounds was based on their chemical structure characteristics including various quantum mechanical parameters such as dipole moment, global hardness (η) chemical potential (μ) and electrophilicity index, (ω).

Scheme 1. The structures of potential protein kinase inhibitors for the treatment of breast and pancreatic cancers.

CONCLUSIONS

Our results manifest that electrophilicity index, (ω) values of the compounds (3.175-2.023) are potential PKCδ and/or eEF-2 kinase inhibitors for the treatment of breast and pancreatic cancers according to the ω values of standard compounds (3.122-1.192). Synthesized compounds (1-6) are going to be studying first time as protein kinase inhibitors.

REFERENCES


P-207: SYNTHESIS AND BIOLOGICAL EVALUATION OF FUMARAMIDES AS POTENTIAL CHE INHIBITORS

M. Koca1, E. Tosun1, K.O. Yerdelen1, Z. Kasap1, B. Anil2
Ataturk University

1Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Faculty of Science, Department of Organic Chemistry

A series of fumaramide derivatives was synthesized and evaluated as acetylcholinesterase and butyrylcholinesterase inhibitors against Alzheimer’s disease. Their inhibitory ability was compared to chosen reference compounds, (galantamine bromide, neostigmine bromide and ambenonium dichloride). Lineweaver-Burk plot studies showed that one of the most potent compounds 1o (IC50= 0.03 μM) could bind to both catalytic and peripheral active sites of acetylcholinesterase.

INTRODUCTION

Alzheimer’s disease (AD), the most common form of dementia, is a complex neurodegenerative disorder occurring in the central nervous system, characterized by progressive cognitive decline, memory loss and learning and behavioral disturbances [1]. The cholinergic hypothesis is one of the oldest and most popular hypothesis outlining the pathogenesis of AD. Studies have shown that AD is defined by the rapid loss of acetylcholinesterase (AChE) activity in the early stages of the disease, along with the increasing ratio of AChE as the disease progresses [2]. In this work, eighteen fumaramide derivatives were synthesized, evaluated and molecularly modeled. The pharmacological evaluations of these compounds included AChE and butyrylcholinesterase (BuChE) inhibition, the kinetics of enzyme inhibition and metal chelation.

MATERIALS AND METHODS

A general procedure for the synthesis of fumaramides (1a-s): A mixture of meta-substituted benzaniline (1.3 mmol), triethylamine (1.3 mmol) and dry ethylacetate was cooled with an ice bath to 0–5 °C and fumaryl chloride (0.65 mmol) in dry ethylacetate was added dropwise by syringe over 30 min. The target compounds were recrystallized from EtOH.

270
**Inhibition Studies on AChE and BuChE:** Inhibitory activities of cholinesterase enzymes (ChEs) of the test compounds were evaluated by colorimetric method [3] with some modifications. All test compounds were prepared at different concentrations such as 0.195, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 \(\mu\)g/ml. The absorbance of the reaction mixture was then measured three times at 412 nm every 45 s using a microplate reader. Results are presented as means ± standard errors of the experiment. The IC\(_{50}\) values of the compounds showing percentage inhibition, the measurements and calculations were evaluated by non-linear regression analysis using GraphPad Prism software.

**Kinetic study:** Compounds 1o and 1p were selected for kinetic measurements because they were found to have the highest inhibitory activity against AChE and BuChE, respectively. The test was carried out without the inhibitor and in 0.02, 0.03 and 0.04 \(\mu\)M concentrations of the inhibitors 1o and 1p. Substrate concentrations were varied from 0.1 to 1.5 mM. The obtained data were used to create substrate-velocity curves, which were transformed in GraphPad Prism to Lineweaver-Burk plots (Fig. 1).

**RESULTS AND DISCUSSION**

All fumaramide compounds 1a-s showed good inhibitory activity to both ChEs with \(\mu\)M concentrations. Among the target compounds, 1o (IC\(_{50}\)= 0.03 \(\mu\)M) showed the most potent inhibitory activity for AChE, being 2.33-, 35-, and 63-fold stronger than the reference compounds neostigmin, galantamine and ambenonium, respectively. 1p exhibited the strongest inhibition to BuChE with an IC\(_{50}\) value of 0.03 \(\mu\)M, which was 3-, 154-, and 441-fold more potent than those of neostigmine, ambenonium, and galantamine.

**CONCLUSIONS**

In summary, a series of fumaramide analogues was synthesized and evaluated as AChE and BuChE inhibitors. According to the inhibition data, the fumaramides generally showed moderate to high anticholinesterase activity in which compounds 1o and 1p were the most potent inhibitors acting in low micromolar concentrations towards AChE and BuChE, respectively.

**ACKNOWLEDGMENTS**

This research work was supported by Ataturk University Research Fund (Project No: 2014/167), Turkey.

**REFERENCES**


**P-208: STUDIES ON THE ANTICANCER ACTIVITIES OF SOME CHROMONYL 2,4-THIAZOLIDINE-DIONE / 2,4-IMIDAZOLIDINEDIONE / THIOHYDANTOINE COMPOUNDS**

M. Ceylan Ünlüsoy\(^1\), Ç. Özen\(^2\), M. Öztürk\(^2\), O. Bozdağ Dündar\(^1\)

\(^1\)Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, TURKEY
\(^2\)Izmir Biomedicine and Genome Center, iBG-Izmir, Dokuz Eylül University, Izmir, TURKEY

**INTRODUCTION**

Hepatocellular carcinoma (HCC) is a strongly resistant cancer type against conventional chemotherapy and radiotherapy. Therefore drug treatments in hepatocellular carcinoma fail at the end. More than 80 percent of primer liver cancers are HCC and every year more than 500,000 people are diagnosed with HCC [1]. The chromone and related compounds are widespread in the plant kingdom. Chromones are able to cause cytotoxic effect in various types of cells. They are widely known to have anticancer, antioxidant, antiproliferative, antiHIV, antiinflammatory, and many other activities. In recent years, numerous compounds containing 2,4-thiazolidinedione (TZD), imidazolidinedione, thiohydantoin have been recognized as new potential anticancer agents [2].

Previously we combined these two active structures to synthesize 18 new hybrid molecules, named chromonyl-2,4-TZDs/imidazolidinediones/thiohydantoines (C1-C18) (Formula) [3,4]. In this study, we tested their biological activity against hepatocellular carcinoma.
MATERIALS AND METHODS

3-formyl chromones was reacted with thiazolidine-2,4-dione/imidazolidine-2,4-dione/thiohydantoin by using Knoevenagel reaction. Methyl or ethyl substituted compounds [3,4] were obtained by reacting these non substituted compounds with methyl iodide or ethyl iodide.

Primer screening of 18 derivatives was done by sulforhodamine B (SRB) assay in two HCC cell lines, Huh7 and Plc/Prf/5 (PLC). Five different HCC cell lines (Huh7, PLC, Snu449, Hep3B, HepG2) and one breast cancer cell line (MCF7) were used to calculate 50% growth inhibitor concentration (IC50) values. All breast cancer cell line (MCF7) were used to calculate 50% growth inhibitor concentration (IC50) values. All cell lines were tested after 24 and 72 hrs drug treatments by PI staining and the data was obtained from flow cytometer.

RESULTS AND DISCUSSION

Primer screening of 18 derivatives was done by sulforhodamine B (SRB) assay in two HCC cell lines, Huh7 and Plc/Prf/5 (PLC). Five different HCC cell lines (Huh7, PLC, Snu449, Hep3B, HepG2) and one breast cancer cell line (MCF7) were used to calculate 50% growth inhibitor concentration (IC50) values. All cell lines were examined and photographed under inverted microscope, in term of morphological chances after 72 hrs treatment. Senescence associated beta-galactosidase assay was performed in order to find any increased galactosidase activity. We stained these non substituted compounds with methyl iodide to ethyl iodide.

CONCLUSIONS

Compound C9 was found more bioactive or very similar in very resistant liver carcinoma cell lines than some TZD derivatives in literature [5]. Furthermore, regarding to cell cycle arrest data, we suggest that C9 inhibits cancer cell proliferation in a different way from literature. In conclusion we believe the structure of C9 molecule may be served as a new kind of anticancer drug model.

ACKNOWLEDGMENTS

This work was supported by Research Organization of Ankara University, Turkey (No:09B3336003 and 12B3336003). One of the authors was supported by TUBA DSAP program.

REFERENCES


P-209: SYNTHESIS OF 4-NAPHTHYL-1,4-DIHYDROPYRIDINES CONTAINING DIFFERENT ESTER GROUPS AS POTENTIAL CALCIUM CHANNEL MODULATORS

M. G. Gündüz1, E. Albayrak1, F. İşli2, G. S. Öztürk Fincan3, Ş. Yıldırım4, R. Şimşek1, C. Safak1,Y. Sanoğlu3, S. Öztürk Yıldırım5, R. J. Butcher6

1 Hacettepe University, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara, TURKEY
2 Department of Rational Drug Use and Drug Supply Management, Turkish Medicines and Medical Devices Agency, Ankara, TURKEY
3Gazi University, Department of Pharmacology, Faculty of Medicine, Ankara, TURKEY
4Kirikkale University, Department of Pharmacology, Faculty of Medicine, Kirikkale, TURKEY
5Erciyes University, Department of Physics, Faculty of Sciences, Erciyes University, Kayseri 38039, Turkey

The present study reports the synthesis and myorelaxant activity evaluation of a series of 16 novel fused 4-naphthyl-1,4-dihydropyridine derivatives. The compounds were achieved by one-pot microwave-assisted method. The structures of the compounds were confirmed by various spectral methods including X-ray studies. To evaluate their myorelaxant
activities, the $E_{\text{max}}$ and $pD_2$ values of the compounds were determined on isolated rabbit gastric fundus smooth muscle strips.

**INTRODUCTION**

Calcium ions ($\text{Ca}^{2+}$) play a versatile role in a variety of biological functions and also in pathological processes such as muscle contraction and even cell death. 1,4-DHPs represent the most important group of calcium-channel modulating agents and are widely prescribed as treatments for cardiovascular diseases, particularly hypertension and angina pectoris [1]. In this study, we synthesized sixteen DHP derivatives and determined how different ester groups and the naphthyl moiety affect the myorelaxant activities of these compounds.

**MATERIALS AND METHODS**

The general procedure for the preparation of the compounds was as follows: The mixture of 4,4-dimethyl-1,3-cyclohexanedione, 1- or 2-naphthaldehyde, appropriate alkyl acetoacetate and ammonium acetate were heated under microwave irradiation in methanol.

The $E_{\text{max}}$ and $pD_2$ values of the compounds were determined on isolated strips of rabbit gastric fundus smooth muscle. A ligand-based pharmacophore model represented by color-coded pharmacophore features was generated for the compounds, which were found to be the most active ones in this series by LigandScout 3.1.

**RESULTS AND DISCUSSION**

The obtained results indicated that introduction of long chain alkyl groups such as 2-methoxyethyl or 2-(methacryloyloxy)ethyl moiety to the ester group led to the most active compounds. Ligand-based pharmacophore studies suggested two hydrogen bond acceptor groups should be required in the ester group for the calcium channel blocking effect.

**CONCLUSIONS**

We reported herein a convenient method for the preparation of condensed 1,4-DHPs under MW irradiation. The pharmacological results showed that all compounds have relaxing effects on isolated rabbit gastric fundus smooth muscle. The introduction of long chain alkyl groups to the ester group led to the most active compounds, suggesting that two hydrogen bond acceptor groups can be required for the calcium channel blocking activity.

**REFERENCES**


**P-210: SYNTHESIZED OF POLY-ELECTROLYTE HYDROGEL FOR DRUG DELIVERY SYSTEM**

F. Taktak¹, M. Yıldız², R. Şahin¹

¹Uşak University, Faculty of Engineering
²Department of Chemical Engineering, Uşak, TURKEY

**INTRODUCTION**

During the last couple of decades many different kind of polymeric systems are proposed as drug carrier systems. One of these systems is poly-electrolyte polymers which contain relatively ionizable groups at levels ranging from a few mols to 100% of the repeating units. Controllable volume changes in response to small variation in solution conditions such as temperature, pH, and electric signal which are also employed the solution variables in typical physiological, biological and chemical systems. The pH sensitivity also imparts additional advantages to these systems by causing an overall retardation in the release of drug as compared with non-electrolyte gels [1].

In this study, an attempt has been made to synthesis a pH responsive smart hydrogel by using copolymers made from 2-(dimethylamino) ethyl methacrylate (DMAEMA) and itaconic acid (IA).

**MATERIALS AND METHODS**

Materials: 2-(dimethylamino) ethyl methacrylate (DMAEMA) and itaconic acid (IA) monomers were purchased from Aldrich. Monomers were passed through basic alumina column before used. The cross-linker methylene bisacrylamide (MBAAm) and the initiator ammonium persulfate (APS) were obtained from Aldrich, and used without further purification. All other reagents are analytical grade or highest purity available. Deionized water was used throughout the experiments in the preparation of hydrogels, of buffer solutions and in the swelling experiments.
**Preparation of poly (DMAEMA-co-IA) hydrogels:**

Hydrogels were prepared through free radical copolymerization in aqueous solution. Different amounts of DMAEMA and IA monomers (DMAEMA/IA mole ratios, 70:30, 75:25, 80:20, and 85:15) and certain amount of MBAAm crosslinking agent (4% based on the monomers) were dissolved in deionized water for 20 min. APS initiator was transferred to the mixture via cannula. After stirring 10 min, the mixture was carried in cylindrical PVC straws and allowed to the complete of the copolymerization at room temperature for 24 h. When the copolymerization was completed, hydrogel samples were cut into disk with diameter of 0.5 cm and thickness of 0.5 cm. Then, the disk samples washed with distilled water and immersed in 100 ml of distilled waters for 2 days in order to remove the unreacted chemicals. During this treatment, distilled water was refreshed every 6 h. Finally, pure hydrogels were dried in ambient temperature for 4 days. Polymers were characterized by using scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FT-IR).

**Drug loading and in vitro drug release studies:**

The polymer disks (30 mg) were immersed in 20 ml of ethanol/water solution (1:1, v/v) containing 1 mg/mL of Naproxen Sodium for 24 h to reach equilibrium at room temperature. After that, the gels were taken out carefully from the solution and washed with ethanol/water mixture to remove the free Naproxen Sodium on the surface. Then, the drug-loaded hydrogels were dried completely in vacuum. In vitro release profiles of Naproxen Sodium-loaded gels were performed in phosphate buffer pH 7.4 (PBS) at 37 °C. The amount of Naproxen sodium released was determined spectrometrically at 330 nm on a UV–vis spectrometer UV-1800 Shimadzu.

**RESULTS AND DISCUSSION**

DMAEMA-co-IA was synthesized by using free radical copolymerization in aqueous solution. For the characterization of structure of gels, FTIR spectra and SEM analysis were performed. Naproxen sodium was used as a model drug and the release profiles from the DMAEMA-co-IA as a potential carrier system were investigated.

**CONCLUSIONS**

In conclusion, we found that our novel DMAEMA-co-IA was ideal candidate for controlled release of Naproxen sodium.

**REFERENCES**


---

**P-211: A NOVEL HYDROGEL CONTAINING ZWITTERIONIC SULFOBETAINE FOR CONTROLLED RELEASE OF NAPROXEN**

F. Taktak, M. Yıldız, R. Şahin

Uşak University, Faculty of Engineering
1Department of Chemical Engineering, Uşak, TURKEY
2Uşak University Ulübey Vocational Higher School, Department Technical Science Uşak, TURKEY

**INTRODUCTION**

The zwitterionic functionality has attracted more interest in recent years. An ideal zwitterionic polymer is characterized by having ionic moieties containing both positive and negative charges in an exact balance. Consequently, although the surface density of charged moieties is high, the material has no net charge. Thanks to these properties, zwitterionic polymers are candidate for drug delivery system [1]. In this study, an attempt has been made to synthesis smart hydrogel by using copolymers made from 2-(dimethylamino) ethyl methacrylate (DMAEA) and 3-dimethyl (methacryloyloxyethyl) ammonium propanesulfonate (DMAPS).

**MATERIALS AND METHODS**

**Materials:** 2-(dimethylamino) ethyl methacrylate (DMAEA) and 3-dimethyl (methacryloyloxyethyl) ammonium propanesulfonate (DMAPS) monomers were purchased from Aldrich. Monomers were passed through basic alumina column before used. The cross-linker methylene bisacrylamide (MBAAm) and the initiator ammonium persulfate (APS) were obtained from Aldrich, and used without further purification. All other reagents are analytical grade or highest purity available. Deionized water was used throughout the experiments in the preparation of hydrogels, of buffer solutions and in the swelling experiments.

**Preparation of poly (DMAEMA-co-DMAPS) hydrogels:**

Hydrogels were prepared through free radical copolymerization in aqueous solution. Different amounts of DMAEMA and DMAPS monomers (DMAEMA/DMAPS mole ratios, 75:25, 50:50, and 25:75) and certain amount of MBAAm crosslinking agent (4% based on the monomers) were dissolved in deionized water for 20 min. APS initiator was transferred to the mixture via cannula. After stirring 10 min, the mixture was carried in cylindrical PVC straws and allowed to the complete of the copolymerization at room temperature for 24 h. When the copolymerization was completed, hydrogel samples were cut into disk with diameter of 0.5 cm and thickness of 0.5 cm. Then, the disk samples washed with distilled water and immersed in 100 ml of distilled waters for 2 days in order to remove the unreacted chemicals. During this treatment, distilled water was refreshed every 6 h. Finally, pure hydrogels were dried in ambient temperature for 4 days.
Polymers were characterized by using scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FT-IR).

Drug loading and in vitro drug release studies: The polymer disks (30 mg) were immersed in 20 mL of ethanol/water solution (1:1, v/v) containing 1 mg/mL of Naproxen Sodium for 24 h to reach equilibrium at room temperature. After that, the gels were taken out carefully from the solution and washed with ethanol/water mixture to remove the free Naproxen Sodium on the surface. Then, the drug-loaded hydrogels were dried completely in vacuum.

In vitro release profiles of Naproxen Sodium-loaded gels were performed in phosphate buffer pH 7.4 (PBS) at 37 °C. The amount of Naproxen sodium released was determined spectrometrically at 330 nm on a UV–vis spectrometer UV-1800 Shimadzu.

RESULTS AND DISCUSSION

DMAEMA-co-DMAPS were synthesized by using free radical copolymerization in aqueous solution. For the characterization of structure of gels, FTIR spectra and SEM analysis were performed. Naproxen sodium was used as a model drug and the release profiles from the DMAEMA-co-DMAPS as a potential carrier system were investigated. Within copolymer, if DMAEMA ratio was increased it was determined that controlled release increased.

CONCLUSIONS

In conclusion, we found that our novel DMAEMA-co-DMAPS were ideal candidate for controlled release of Naproxen sodium.

REFERENCES


P-212: SYNTHESIZED OF POLY-ELECTROLYTE HYDROGEL FOR DRUG DELIVERY SYSTEM

F. Taktak1, M. Yıldız2, R. Şahin3

Uşak University, Faculty of Engineering
1Department of Chemical Engineering, Uşak, TURKEY
2Uşak University Ulubey Vocational Higher School, Department Technical Science Uşak, TURKEY

INTRODUCTION

During the last couple of decades many different kind of polymeric systems are proposed as drug carrier systems. One of these systems is poly-electrolyte polymers which contain relatively ionizable groups at levels ranging from a few mols to 100% of the repeating units. Controllable volume changes in response to small variation in solution conditions such as temperature, pH, and electric signal which are also employed the solution variables in typical physiological, biological and chemical systems. The pH sensitivity also imparts additional advantages to these systems by causing an overall retardation in the release of drug as compared with non-electrolyte gels [1]. In this study, an attempt has been made to synthesis a pH responsive smart hydrogel by using copolymers made from 2-(dimethylamino) ethyl methacrylate (DMAEMA) and itaconic acid (IA).

MATERIALS AND METHODS

Materials: 2-(dimethylamino) ethyl methacrylate (DMAEMA) and itaconic acid (IA) monomers were purchased from Aldrich. Monomers were passed through basic alumina column before used. The cross-linker methylene bisacrylamide (MBAAm) and the initiator ammonium persulfate (APS) were obtained from Aldrich, and used without further purification. All other reagents are analytical grade or highest purity available. Deionized water was used throughout the experiments in the preparation of hydrogels, of buffer solutions and in the swelling experiments.

Preparation of poly (DMAEMA-co-IA) hydrogels: Hydrogels were prepared through free radical copolymerization in aqueous solution. Different amounts of DMAEMA and IA monomers (DMAEMA/IA mole ratios, 70:30, 75:25, 80:20, and 85:15) and certain amount of MBAAm crosslinking agent (4% based on the monomers) were dissolved in deionized water for 20 min. APS initiator was transferred to the mixture via cannula. After stirring 10 min, the mixture was carried in cylindrical PVC straws and allowed to the complete of the copolymerization at room temperature for 24 h. When the copolymerization was completed, hydrogel samples were cut into disk with diameter of 0.5 cm and thickness of 0.5 cm. Then, the disk samples washed with distilled water and immersed in 100 ml of distilled waters for 2 days in order to remove the unreacted chemicals. During this treatment, distilled water was refreshed every 6 h. Finally, pure hydrogels were dried in ambient temperature for 4 days. Polymers were characterized by using scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FT-IR).

Drug loading and in vitro drug release studies: The polymer disks (30 mg) were immersed in 20 mL of ethanol/water solution (1:1, v/v) containing 1 mg/mL of Naproxen Sodium for 24 h to reach equilibrium at room temperature. After that, the gels were taken out carefully from the solution and washed with ethanol/water mixture to remove the free Naproxen Sodium on the surface. Then, the drug-loaded hydrogels were dried completely in vacuum.
In vitro release profiles of Naproxen Sodium-loaded gels were performed in phosphate buffer pH 7.4 (PBS) at 37 °C. The amount of Naproxen sodium released was determined spectrometrically at 330 nm on a UV-vis spectrometer UV-1800 Shimadzu.

RESULTS AND DISCUSSION
DMAEMA-co-IA was synthesized by using free radical copolymerization in aqueous solution. For the characterization of structure of gels, FTIR spectra and SEM analysis were performed. Naproxen sodium was used as a model drug and the release profiles from the DMAEMA-co-IA as a potential carrier system were investigated.

CONCLUSIONS
In conclusion, we found that our novel DMAEMA-co-IA was ideal candidate for controlled release of Naproxen sodium.

REFERENCES

P-213: KOJIC ACID DERIVATIVES AS ANTIDERMATOPHYTIC AND ANTIMYCOBACTERIAL AGENTS WITH CYTOTOXICITY EVALUATION

M. D. Aytemir1, G. Karakaya1, B. Özçelik2

1Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY
2 Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, TURKEY

INTRODUCTION
Tuberculosis caused by Mycobacterium tuberculosis is the leading bacterial cause of death with approximately two million people dying from M. tuberculosis infections every year [1]. The increasing numbers of drug resistance and the lack of sufficient chemical diversity to existing azole antifungal represent a serious challenge for tuberculosis control. Also, the introduction of new oral therapies has improved cure rates of cutaneous mycoses however approximately 20% of the patients with oncomycoses are still failing on antifungal therapy [2]. So, new antifungal and antitubercular drugs that are active against latent tuberculosis and dermatophytes are urgently needed. Previously, Mannich bases of hydroxyprone derivatives were synthesized in our laboratory and examined for their anticonvulsant, antibacterial, antifungal and antitubercular activities [3]. Hence, herein, we present the results of a preliminary evaluation of cytotoxicity, antidermatophytic and antimycobacterial activities of fourteen Mannich bases of kojic acid.

MATERIALS AND METHODS
Mannich bases were prepared by the reaction of appropriate substituted piperazine/piperidine derivatives with kojic acid and formaline at room temperature with quite significant yield (Fig.1). The basic structures compounds were confirmed by IR, 1H-NMR and ESI-MS.

In vitro antidermatophytic activity of the derivatives against Microsporum gypseum, Trichophyton mentagrophytes var. erinacei and Epidermophyton floccosum were screened as broth microdilution method. Terbinafine, itraconazole, ketoconazole, fluconazole, and griseofulvin were used as the control agents. Cytotoxicity was evaluated by the maximum non-toxic concentrations (MNTCs) of each sample, which was determined based on cellular morphologic alteration. As for antimycobacterial activity the breakpoint concentrations (µg mL⁻¹) of the compounds were determined against standard strains of M. tuberculosis H37Rv and M. avium (ATCC 15769) clinical isolated strains by using the colorimetric resazurin microtiter assay (REMA). Isoniazid and ethambutol were used as control agents [3].

RESULTS AND DISCUSSION
All of the synthesized compounds exhibited antidermatophytic activity with the range of 2-32 µg mL⁻¹. Also, these compounds showed antimycobacterial activity with the MIC values ranging 16-64 µg mL⁻¹ and 2-32 µg mL⁻¹ against M. tuberculosis and M. avium, respectively.

CONCLUSIONS
As a result, compounds bearing piperidine structure showed highest activity against dermatophytes. Besides this, compound having piperonylpiperazine structure was the most active against M. avium. These compounds seem good candidates for novel antidermatophytic and antitubercular agents and further studies will be carried out.

ACKNOWLEDGMENTS
This study is supported by TÜBİTAK. Project no: SBAG-1135527.

REFERENCES

P-214: SYNTHESIS, ANTIPROLIFERATIVE AND APOPTOSIS-INDUCING ACTIVITY OF SOME NOVEL N-(5-ARYLOXYMETHYL-4-BENZYL-4H-1,2,4-TRIAZOLE-3-YLTHIO)ACETANILIDES

Necla Kulaba1, Esra Tatar1, Özlem Bingöl Özakpinar2, Derya Özsavcı2, Ilkay Küçükgüzel1

1 Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Haydarpaşa 34668 Istanbul, Turkey
2 Marmara University, Faculty of Pharmacy, Department of Biochemistry, Haydarpaşa 34668 Istanbul, Turkey

INTRODUCTION
Cancer is the second cause of death after cardiovascular diseases worldwide. Although chemotherapy has been widely employed for various cancer treatments, severe toxicity and resistance to traditional chemotherapeutic agents makes it a necessity to develop novel and safer drugs for cancer therapy. 1,2,4-Triazole derivatives represent an interesting class of five-membered heterocycles, possessing many biological properties such as antimicrobial [1], anti-tuberculosis [2], anti-inflammatory [3], analgesic [3] and anticancer activities [4].

MATERIALS AND METHODS
In this study, we synthesized several new 1,2,4-triazoles starting from phenolic compounds such as 2-naphtol, paracetamol and thymol. These 1,2,4-triazoles containing 3-thioxo moiety were reacted with 2-chloroacetanilides in DMF to give 2-{[4-benzyl-5-(aryloxymethyl)-4H-1,2,4-triazol-3-yl]thio}-N-phenylacetamides 18–32. Structures and purity of the target compounds were confirmed by the use of their IR, 1H-NMR and HR-mass spectral data; besides TLC, HPLC-UV/DAD and elemental analysis. Among the synthesized compounds, three representatives 18, 19 and 25 were selected and evaluated by the National Cancer Institute (NCI) against the full panel of 60 human cancer cell lines derived from nine different cancer types. The compounds which exhibited good inhibition in the primary screen were then evaluated for cell viability in the second stage by using the mouse embryonic fibroblast cell line NIH-3T3. Anticancer activity of these compounds were determined against prostate cancer (PC-3), lung cancer (A549/ATCC) and leukemia (K-562) cell lines at different concentrations. After the cell viability assay, caspase activation and Bcl-2 activity of the selected compounds were measured and the loss of mitochondrial membrane potential (MMP) was detected. The data were reported as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey’s multiple comparison tests using GraphPad Prism 5. Differences between means at p<0.05 level were considered significant.

RESULTS AND DISCUSSION
Antiproliferative effects of the selected compounds were demonstrated in human tumor cell lines K-562, A549 and PC-3. These compounds inhibited cell growth assessed by MTT assay. Compound 18, 19 and 25 exhibited anti-cancer activity with IC50 values of 5.96 μM (PC-3 cells), 7.9 μM (A549/ATCC cells) and 7.71 μM (K-562 cells), respectively. Compounds 18, 19 and 25 showed a significant increase in caspase 3 activity in a dose dependent manner. The same situation was not observed for compound 18 and 25 while caspase 8 activity of compound 19 was significantly elevated only at the dose of 50 μM. In addition, all of three compounds significantly decreased the mitochondrial membrane potential and expression of Bcl-2.

CONCLUSIONS
These results demonstrated that compound 19 triggers apoptosis by using intrinsic and extrinsic pathways, while compounds 18 and 25 induce apoptotic cell death by triggering intrinsic pathway.

ACKNOWLEDGMENTS
We thank the Division of Cancer Research, National Cancer Institue (NCI), Bethesda, MD, for the anticancer activity screening. The authors are grateful to Dr. Jürgen Gross from the Institute of Organic Chemistry, University of Heidelberg, for his generous help on obtaining ESI mass spectra of the compounds.

REFERENCES

277
**P-215: SYNTHESIS AND CHARACTERIZATION OF NEW IMIDAZO[2,1-B]THIAZOLE DERIVATIVES**

N. Ulusoy Güzeldemirci¹, S. Cimok¹

Istanbul University, Faculty of Pharmacy, ¹Department of Pharmaceutical Chemistry, Istanbul, TURKEY

**INTRODUCTION**

Among fused five-membered heterocyclic rings containing bridgehead nitrogen atom, the levamisole (Fig. 1) is the most popular commercial derivative. This compound, apart from its anthelmintic properties, belongs to a general class of agents called biologic response modifiers, has immune-modulating and immuno-stimulating properties and it is also used in cancer adjuvant therapy. We report here, the synthesis and structural determination of a new imidazo[2,1-b]thiazole series.

![Scheme 1. Synthesized compounds](image)

**MATERIALS AND METHODS**

Melting points were determined on a Büchi B-540 melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses were done on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Shimadzu IR Affinity-1 FT-IR spectrophotometer. ¹H-NMR spectra were measured on a Varian UNITY INOVA (500 MHz) spectrometer using DMSO-d₆. Mass spectra were acquired on a Thermo Finnigan LCQ Advantage Max instrument.

**RESULTS AND DISCUSSION**

Hydrazide and alkyl/aryl isothiocyanates were heated in ethanol to yield 4-alkyl/aryl-1-[(6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl)acetyl]-3-thiosemicarbazides [1]. The thiosemicarbazides were then reacted with ethyl α-bromoacetate/ethyl 2-bromopropionate in the presence of anhydrous sodium acetate in absolute ethanol to yield 3-alkyl/aryl-2-[(6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl)acetyl] hydrazono]-5-nonsubstituted/methyl-4-thiazolidinones (Scheme 1).

**CONCLUSIONS**

A series of new 1,4-disubstituted thiosemicarbazides and 4-thiazolidinones bearing imidazo[2,1-b]thiazoles moieties were synthesized. The new compounds were characterized by spectral data (IR, ¹H-NMR and mass spectra) and elemental analyses. Structures proposed have been confirmed by spectral data.

**ACKNOWLEDGMENTS**

This work was supported by Istanbul University Scientific Research Projects. Project Number: 40810.

**REFERENCES**


---

**P-216: SYNTHESIS, CHARACTERIZATION AND ANTIVIRAL ACTIVITY EVALUATION OF NEW THIAZOLIDINONES BEARING IMIDAZO[2,1-B]THIAZOLE MOIETY**

N. Ulusoy Güzeldemirci¹, S. Cimok¹

Istanbul University, Faculty of Pharmacy, ¹Department of Pharmaceutical Chemistry, Istanbul, TURKEY

**INTRODUCTION**

Diverse antiviral drugs are now available for the treatment of infections by HIV, herpes-, influenza, hepatitis B or hepatitis C viruses. Except for the broad antiviral agent ribavirin, there is no approved therapy for diverse emerging RNA viruses. In addition, new antiviral molecules are required to tackle the problems of drug toxicity and rapid development of drug resistance, which is particularly problematic for mutation-prone RNA viruses. For this reasons, we here report the synthesis, structural determination and antiviral evaluation of 3-alkyl/aryl-2-[(6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl)acetyl]hydrazono]-5-methyl-4-thiazolidinones.
MATERIALS AND METHODS
Melting points were determined by using a Büchi B-540 melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Shimadzu IR Affinity-1 FT-IR spectrophotometer. 1H-NMR, 13C-NMR (APT), 13C-NMR (DEPT) and HSQC (1H-13C) spectra were measured on a Varian UNITY INOVA (500 MHz) spectrometer using DMSO-d6. Mass spectra were recorded on a Thermo Finnigan LCQ Advantage Max instrument. The starting materials were either commercially available or synthesized according to the references cited.

RESULTS AND DISCUSSION
The target compounds were prepared from 2-[6-(4-bromophenyl)imidazo[2,1-b]thiazol-3-yl]acetohydrazides [1] by a five step synthesis. In the IR spectra, some significant stretching bands due to N-H and C=O were observed at 3182-3115 cm⁻¹ and 1666-1635 cm⁻¹, respectively. New strong bands at 1753-1716 cm⁻¹ and 1716-1689 cm⁻¹ in the spectra of compounds provided firm support for ring closure [2]. 1H-NMR and 13C-NMR data were also in agreement with the formation of 4-thiazolidinone ring.

CONCLUSIONS
In conclusion, we synthesized a series of imidazo[2,1-b]thiazoles bearing 4-thiazolidinone moieties. The new compounds were characterized by IR, NMR spectra, mass spectra as well as elemental analyses. Antiviral activity testing revealed that some of the compounds exhibited antiviral activity against diverse RNA viruses. In order to improve the antiviral potency, chemical synthesis of additional 4-thiazolidinone fused imidazo[2,1-b]thiazoles is warranted.

ACKNOWLEDGMENTS
We thank Prof. Lieve Naesens from the Rega Institute for Medical Research, KU, Leuven, Belgium for evaluation of anviral activity. This work was supported by Istanbul University Scientific Research Projects. Project Number: 24304.

REFERENCES

P-217: STUDIES ON THE SYNTHESIS OF SOME NEW TETRAZOLO THIAZOLYL-METHYL 4-{(2-SUBSTITUTEDPHENYL)CARBAMOYL}BENZYL CARBAMATES AS ANTICANCER AGENTS AND HDAC INHIBITORS
O. Bozdag-Dundar, M. Albayrak, D. Konyar
Ankara University, Faculty of Pharmacy Department of Pharmaceutical Chemistry Ankara, TURKEY

INTRODUCTION
Histone deacetylases (HDACs) are a class of enzymes with a predominant role in regulating gene expression through a chemical modification to DNA-associated proteins known as histones [1]. With the realization that HDACs are often overexpressed in cancer and that inhibition of HDACs arrests cell cycling and often drives cancer cells, but not normal cells, into cell death pathways, there has been an explosion of interest in testing and synthesizing novel HDAC inhibitors. There are many HDAC inhibitors in clinical trials either alone or in combination with chemotherapy agents [2]. A great number of HDAC inhibitors under development have a common general pharmacophore, which consisting of a ‘capping region’ linked to a ‘zinc binding group (ZBG)’ via a lipophilic linker (3). In this study, in view of the anticancer property of the HDACi pharmacophore, a new series of tetrazolo thiazolyl-methyl4-{(2-substitutedphenyl)-carbamoyl}benzyl carbamates have been synthesized.

MATERIALS AND METHODS
To a suspension of 1,1’-CDI in THF was added (4-chloro-2-(1-methyl-1H-tetrazol-5-ylthio)-thiazol-5-yl)methanol in THF at 10°C and the mixture stirred for 2 h at room temperature. The resulting solution was added to a suspension of 4-(aminomethyl)benzoic acid, DBU and TEA in THF. After stirring for 5h at room temperature, the mixture was evaporated to remove THF and then dissolved in water. The solution was acidified with HCl (pH=5) to precipitate a white solid which was collected by filtration, washed with water and methanol, respectively, and dried to give carboxylic acid. To a suspension of carboxylic acid compound in toluene was added DMF and oxalyl chloride.
chloride and the mixture stirred for 4 h at room
temperature. The precipitate was collected by
filtration, washed with diethylether and dried. The
precipitate was dissolved in THF and was added
imidazole in THF. The mixture stirred for 1 h at room
temperature. The precipitate was filtrated. The filtrate
was added o-substitutedphenylamine and
trifluoroacetic acid. The mixture was stirred for
several hrs at room temperature. The crude product
was purified by column chromatography.

RESULTS AND DISCUSSION
In this study, in view of the anticancer property of
benzamid pharmacophores, in order to increase the
activity of compounds, a new series tetrazolothiazole
derivatives containing benzamide structure have been
synthesized as seen in below Formula. The structural
evaluation of the synthesized compounds was based
on the 1H NMR, Mass and elementary analysis data.
We are expecting that compounds can bind the
enzymes active site and make the inhibition of HDAC.

CONCLUSIONS
Their docking studies are shown that they have
enough interactions for the enzyme inhibition. The
synthesized compounds are going to be investigated
for their anticancer and HDAC inhibitory activities.

ACKNOWLEDGMENTS
This work was supported by The Scientific and
Technological Council of Turkey (TUBITAK),
Turkey (Project No: 213S097).

REFERENCES
1-Kalyaanamoorthy, S.; Chen, Y.P. P., Biochimica et
2-Delcuve, G.P.; Khan D.H.; Davie, J.R., Expert Opin.
Ther. Targets 2013, 17(1), 29-41.
3-Manzo, F.; Tambaro, F. P.; Mai, A.; Altucci, L. Expert
Opin. Ther. Pat., 2009, 19, 761-774.

P-218: THE STUDIES ON NOVEL
BENZIMIDAZOLE DERIVATIVES AS
POTENTIAL MULTIFUNCTIONAL AGENTS
FOR THE TREATMENT OF ALZHEIMER’S
DISEASE

O. Unsal-Tan1, K. Ozadali-Sari1, B. Ayazgok2, T. Tüylü
Küçükkülünkç2, A. Balkan1

Hacettepe University, Faculty of Pharmacy
1Department of Pharmaceutical Chemistry, 2Department of
Biochemistry
06100 Ankara, TURKEY

INTRODUCTION
Alzheimer’s disease (AD) is a progressive
neurodegenerative brain disorder. It is known that
there are some factors that seem to play a significant
role in the pathology of AD, such as β-amyloid (Aβ)
aggregation, tau protein aggregation, oxidative stress
and low levels of acetylcholine (ACh) [1]. However
there is no drug that can definitively cure AD, the main
therapeutic strategy today is the use of
acetylcholinesterase (AChE) inhibitors such as
tacrine, donepezil, rivastigmine ve galantamine.
Recently, it has been demonstrated that
butyrylcholinesterase (BuChE) play a significant role
in the cholinergic system function and can be an
important target in the treatment of AD [2].
Considering this strategy we aim to study on new
compounds which can be used to treat Alzheimer’s
disease by inhibiting both AChE and BuChE enzymes.
For this purpose, with the help of molecular modeling
studies, some new benzimidazole derivatives expected
to show AChE/BuChE inhibitory activities were
designed, synthesized and evaluated for their
activities.

MATERIALS AND METHODS
The molecular modeling studies were performed with
the MOE software program. The target compounds
were synthesized via the route outlined in Scheme [3].
Chemical structures of the compounds were elucidated
by FT-IR, 1H-NMR, mass spectra and elemental
analysis.

Scheme: Synthetic route of the target compounds

The AChE and BuChE inhibitory activities were
determined by Ellman’s assay [4]. IC50 values were
determined by the plots of residual activity percentage,
calculated in relation to a sample of the enzyme treated
under the same conditions without inhibitor, versus inhibitor concentration [I]. Dose-response curves were obtained by Graphpad Prism software.

RESULTS AND DISCUSSION
In the light of molecular modeling studies, the designed compounds with reasonable binding modes were selected for synthesis (Figure). The structures of the compounds were confirmed by spectral data and elemental analysis. It was found that the target compounds showed remarkable AChE/BuChE inhibitory activity.

CONCLUSIONS
The benzimidazol derivatives possessed good inhibitor activity to AChE/BuChE. These results showed that our docking method can be used to predict potent AChE/BuChE inhibitors.

ACKNOWLEDGMENTS
The authors gratefully acknowledge the financial support of the Turkish Scientific Research Institution (TUBITAK, 114S374).

REFERENCES

P-219: SYNTHESIS OF SOME BIOLOGICALLY EFFECTIVE 2,5-DISUBSTITUTED-BENZOXAZOLES
Özlem Temiz-Arpaci, Meryem Taşçı

a Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100, Tandogan, Ankara, Turkey.
b Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Kayseri, Turkey

INTRODUCTION
Recent observations suggested that benzoxazoles and related fused heterocycles indicated potential antitumor, antiviral and antibiotic activities as the new topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors with lower toxicities in the chemotherapeutic approach. [1-4].

MATERIALS AND METHODS
In the present investigation, a new series of 2-(p-tert-butylphenyl)-5-[2-{4-[(p-substitutedphenyl)]-1-piperazynyl- / 1-piperidinynl}acetamido]-benzoxazoles was synthesized using a three step procedure and the synthetic pathways for preparation of the target compounds are shown in Scheme 1 with the hope of discovering new effective antimicrobial agents.

RESULTS AND DISCUSSION
Firstly, 5-Amino-2-(p-tert-butylphenyl)-benzoxazole (1) was synthesized by heating 2,4-diaminophenol with p-tert-butyl benzoic acid in polyphosphoric acid (PPA). Then compound 2 was obtained by treating a solution of 2-chloroacetyl chloride with 5-amino-2-(p-tert-butyl phenyl)-benzoxazole. Finally, compound 2 was treated by some 4-(p-substituted piperazine / piperidinyl)acetamides. All the results compounds (3-8) were prepared as original products. The structures of them were supported by spectral data. The 1H-NMR, 13C NMR and mass spectra and elemental analysis results agree with those of the proposed structures.
ACKNOWLEDGMENTS
This work was supported by Ankara University Research Fund (Grant No 12L 3336001). The Central Laboratory of the Faculty of Pharmacy, Ankara University, Turkey supported the acquisition of the NMR and mass spectra and elemental analyses in this work.

REFERENCES

P-220: SYNTHESIS AND ANTIMICROBIAL EVALUATION OF 2-(P-SUBSTITUTED PHENYL)-5-[(4-SUBSTITUTEDPHENYL) SULFONYLAMIDO]-BENZOXAZOLES

Ozlem Temiz-Arpacib, Fatma Doğançih, Duygu Saçi, Elmas Sarib, Fatma Kaynak-Onurdagb, and Suzan Oktenb

a Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100, Tandogan, Ankara, Turkey. b Trakya University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 22030, Edirne, Turkey

INTRODUCTION
The treatment of infectious diseases still remained as an important and challenging problem due to emerging infectious diseases and increasing number of multidrug resistant microbial pathogens [1]. In spite of the large number antibiotics and chemotherapeutics the emergence of old and new antibiotic resistant bacterial strains constitutes a substantial need for the new class of potent antimicrobial agents [2]. Benzoxazoles, structural isosteres of natural nucleotides that can interact with biopolymers, constitute an important class of heterocyclic compounds with antimicrobial and antifungal activity [3,4].

MATERIALS AND METHODS
The desired benzoxazole derivatives were synthesized using a two-step procedure in Scheme 1. Firstly, 5-Amino-2-(p-substitutedphenyl)-benzoxazoles (1a-1c) were synthesized by heating 2,4-diaminophenol with p-substituted benzoic acid in polyphosphoric acid (PPA). Then compounds 2-19 were obtained by treating a solution of p-substituted-benzenesulfonyl chlorides with 5-amino-2-(p-substituted phenyl)-benzoxazoles.

Their structures were elucidated with Mass and 1H NMR spectroscopy, 13C NMR for compounds 4,13,19, their purity was analyzed through elemental analysis that was within ±0.4% of theoretical values.

All the newly synthesized benzoxazoles (2-19) were evaluated for their antimicrobial activity with microdilution method, against some Gram-positive, Gram-negative bacteria and fungus Candida albicans and their drug-resistant isolates in comparison with standard drugs.

RESULTS AND DISCUSSION
In this study, A series of new 2-(p-substituted phenyl)-5-[(4-substitutedphenyl) sulfonyl amido]-benzoxazoles were synthesized and tested for their antimicrobial activities. The structures of the new derivatives were elucidated by spectral techniques. The minimum inhibitory concentrations (MIC) of the new benzoxazoles, were determined against standard bacterial and fungal strains and drug-resistant isolates and compared to those of several reference drugs.

CONCLUSIONS
In this study, we aimed to develop new effective antimicrobial agents possessing benzoxazole nuclei in their structure.
Benzoxazole derivatives 2-19 as a new class of synthetic antimicrobial agents along with their \textit{in vitro} antimicrobial activity tested against some Gram-negative, Gram-positive bacteria, and its isolate and \textit{C. albicans} and its isolate. So that we put a para-substituted-phenyl sulfonylamido moiety on fifth position and different p-substitutedphenyl groups on second position of benzoxazole ring for increasing the antimicrobial activity. Nevertheless the benzoxazole derivatives were found to possess a broad spectrum of antimicrobial activity with MIC values of 32 - 1024\,\mu g/mL and the standard drugs were more active against the tested pathogens.

\section*{REFERENCES}

\section*{P-221: SYNTHESIS OF SOME ESTERS AND HYDRAZIDES OF 3(2H)-PYRIDAZINONE}
\begin{flushleft}
\textsuperscript{1}Güler\textsuperscript{1,3}, \textsuperscript{2}A.B. Özçelik\textsuperscript{1}, \textsuperscript{3}S. Utku\textsuperscript{2}, \textsuperscript{2}M. Uysal\textsuperscript{1}, \textsuperscript{3}H. Temel\textsuperscript{1}
\end{flushleft}
\begin{itemize}
\item \textsuperscript{1}Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY
\item \textsuperscript{2}Mersin University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Mersin, TURKEY
\item \textsuperscript{3}Dicle University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Diyarbakır, TURKEY
\end{itemize}

\section*{INTRODUCTION}
The pyridazinone nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs. Some of the present day drugs such as emor fazone (analgesic) \cite{1}, pimobendan (positive inotropic, vasodilator) \cite{2}, levosimendan (calcium sensitizer) \cite{3}, imazodan (cardiotonic) \cite{4} and zardaverin (cardiotonic) \cite{5} are the best examples for potent molecules possessing pyridazinone nucleus. Due to favorable presence a pyridazinone moiety in known active structures, these derivatives provoked a special interest in the search for new potent agents. In view of the above mentioned findings and as continuation of our effort to identify new candidates that may be of value in designing new, potent, selective and less toxic anticancer compounds, we report herein the synthesis of some new ester and hydrazide derivatives of 6-substituted-3(2\textit{H})-pyridazinones.

\section*{MATERIAL AND METHODS}
The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected. IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal. Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in ESI method, also coupled to an AQUITY Ultra Performance Liquid Chromatography system. The \textsuperscript{1}H-NMR of the compounds spectra were recorded on a Bruker Avance 300 MHz UltrashieldTM NMR Spectrometer using tetramethylsilane as an internal standard.

\section*{RESULTS AND DISCUSSIONS}
New ester and hydrazide derivatives of 6-substituted-3(2\textit{H})-pyridazinones were synthesized according to Figure 1. Initially, reaction of glyoxalic acid monohydrate with substituted acetophenones and then hydrazine hydrate afforded 6-substituted phenyl-3(2\textit{H})-pyridazinones \textbf{I}. Ethyl 6-substituted-3(2\textit{H})-pyridazinone-2-ylacetate \textbf{II} derivatives were obtained by the reaction of \textbf{I} with ethyl 2-bromoacetate in the presence of \textit{K}_2\textit{CO}_3 in acetone. 6-Substituted-3(2\textit{H})-pyridazinone-2-yl acetohydrazide derivatives \textbf{III} were synthesized by the condensation reaction of ethyl 6-substituted-3(2\textit{H})-pyridazinone-2-ylacetate \textbf{II} derivatives with hydrazine hydrate (99\%).

The elemental analysis data for each compound were in good agreement with the empirical formula proposed. The \textsuperscript{1}H NMR spectra of all complexes were
consistent with their corresponding protons as chemical shift values and the number of hydrogen. IR spectra of newly synthesized compounds II and III derivatives exhibited characteristic ν (C=O) bands at 1713-1715 and 1668-1674 cm⁻¹ for acetyl side chain and pyridazinone ring respectively.

Fig. 1. Synthesis pathway of ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate II and 6-substituted-3(2H)-pyridazinone-2-yl acetoxydiode derivatives III

CONCLUSIONS
All of the II and III derivatives were synthesized for the first time in this study. Chemical structures of synthesized compounds were confirmed by elemental analysis, IR, ¹H-NMR and Mass spectral data. Detailed anticancer activity of synthesized compound will be investigated in our next study.

REFERENCES

INTRODUCTION
In recent years, a great deal of work have been directed to the organic synthesis of 3(2H)-pyridazinones derivatives. Pyridazinone derivatives have established a variety of pharmacological activities most of them are related to cardiovascular effects. In this field a number of compounds such as zardaverine, bemoradan, indolindan, pimobendan are few examples of pyridazinones that are active as cardio tonic agents/platelet [1,2]. During our literature research, we observed that compounds synthesized in the recent studies possessed notable antidepressant, antihypertensive, antithrombotic, antifungal, antibacterial, analgesic anti-inflammatory, antiplatelet, anticancer, anti-HIV, vasodilator and antiasthmatic effects on cardiovascular system [3,4]. This study was an attempt together the different developments for synthesis and anticancer activities of pyridazinone derivatives.

MATERIALS AND METHODS
The fine chemicals and all solvents used in this study were purchased locally from E. Merck (Darmstadt, F. R. Germany) and Aldrich Chemical Co. (Steinheim, Germany). Melting points of the compounds were determined on Electrothermal 9200 melting points apparatus (Southent, Great Britain) and the values given are uncorrected. IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal. Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in ESI method, also coupled to an AQUITY Ultra Performance Liquid Chromatography system. The ¹H-NMR of the compounds spectra were recorded on a Bruker Avance 300 MHz UltrasoundTM NMR Spectrometer using tetramethylsilane as an internal standard.

New 6-substituted-3(2H)-pyridazinone-2-acetyl-3-(p-substituted/nonsubstituted benzal)hydrazone IV derivatives were synthesized according to Figure 1. Ethyl 6-substituted-3(2H)-pyridazinone-2-ylacetate II derivatives were obtained by the reaction of I with ethylbromacetate in the presence of K₂CO₃ in acetone. 6-Substituted-3(2H)-pyridazinone-2-yl acetoxydiode derivatives III were synthesized by the condensation reaction of II derivatives with hydrazine hydrate. Synthesis of title compounds IV were performed reaction of 6-substituted-3(2H)-pyridazinone-2-yl acetoxydiode derivatives III with substituted/nonsubstituted benzaldehydes.

RESULTS AND DISCUSSION
We describe here the synthesis of new 6-substituted-3(2H)-pyridazinone-2-acetyl-3-(p-
substituted/nonsubstituted benzal)hydrazone IV derivatives. The elemental analysis data for each compound were in good agreement with the empirical formula proposed. Chemical structures of synthesized compounds were confirmed by elemental analysis, IR, 1H-NMR and Mass spectral data.

CONCLUSIONS
All of the IV derivatives were synthesized for the first time in this study. Our aim was to establish more detailed structure-activity relationship in this series and evaluate the modification of the pharmacological profile induced by the substituents in the 6-position of the 3(2H)-pyridazinone derivatives. Detailed anticancer activity of synthesized compound will be investigated in our next study.

REFERENCES

P-223: MICROWAVE-ASSISTED SYNTHESIS OF CONDENSED 1,4-DIHYDROPYRIDINE DERIVATIVES AS POTENTIAL CALCIUM CHANNEL MODULATORS

R. Şimsek1, E. K. Özer2, M. G. Gündüz1, A, El-Khouly1, Y. Sara3, A. B. Iskit3, C. Şafak3

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey
2Department of Pharmacology, Faculty of Medicine, Selçuk University, Konya, Turkey
3Department of Pharmacology, Faculty of Medicine, Hacettepe University, 06100, Ankara, Turkey

This study reports the synthesis and calcium channel modulatory activity evaluation of 14 novel fused 1,4-dihydropyridine derivatives. The compounds were achieved by one-pot microwave-assisted reaction according to a modified Hantzsch synthesis. The structures of the compounds were confirmed by spectral methods and elemental analysis. To evaluate their relaxant activities, the maximum relaxant response (Emax) and pD2 values of the compounds and nifedipine were determined on isolated rat aorta rings. The Emax values (a measure of efficacy) of five compounds have been found higher than that of nifedipine.

INTRODUCTION
Calcium channel blockers are the class of drugs that inhibit selectively the calcium influx through cell membranes. L-type channels are highly sensitive to 1,4-dihydropyridines (DHPs), which represent a well-known class of calcium antagonists. DHPs are clinically used as treatments for cardiovascular diseases particularly hypertension and angina [1]. Here, we described an efficient method based on microwave irradiation for fourteen novel DHP derivatives in which substituted cyclohexane rings are fused to the DHP ring, and we determined how different ester groups affect calcium channel block.

MATERIALS AND METHODS
The general procedure for the preparation the compounds was as follows: The mixture of 4,4-dimethyl-1,3-cyclohexanedione, chloro-substituted-salicylaldehyde, appropriate alkyl acetoacetate and ammonium acetate were heated under microwave irradiation in methanol. The Emax and pD2 values of the compounds were determined on isolated rat aorta rings.

RESULTS AND DISCUSSION
The obtained pharmacological results showed that all synthesized compounds are potent relaxing agents on isolated rat aorta smooth muscle due to blockade of calcium channels, similar to that of nifedipine. Given that the main difference between these compounds is
their ester groups; this suggests that ester moiety plays a key role in the ability of these compounds to block calcium current. The introduction of the second chlorine atom on the phenyl ring did not mediate a significant change in blocking activity.

\[
\begin{array}{c}
\text{NH} \\
\text{CH}_3 \\
\text{H}_3\text{C} \\
\begin{array}{c}
\text{OH} \\
\text{H}_3\text{C} \\
\end{array} \\
\text{COOR}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{Cl} \\
\end{array}
\]

\[R_1: \text{H, Cl} \]

\[R_2: \text{CH}_3, \text{C}_2\text{H}_5, \text{CH}_2\text{(CH}_3)_2, \text{C(CH}_3)_3, \text{CH}_2\text{CH}_2\text{OCH}_3, \text{CH}_2\text{C}_6\text{H}_5\]

### Compound 1-14

**Fig. 1.** The synthesized compounds

**CONCLUSIONS**

We reported herein a convenient method for the preparation of condensed 1,4-DHPs under MW irradiation. The pharmacological results showed that all compounds have relaxing effects on isolated rat aorta rings.

**REFERENCES**


**P-224: EVALUATION OF TRIPLE RESPONSIVE COPOLYMERIC GEL AS DRUG DELIVERY DEVICE**

F. Taktak¹, M. Yildiz², R. Şahin³

Uşak University, Faculty of Engineering

¹Department of Chemical Engineering, Uşak, TURKEY

²Uşak University Ulubey Vocational Higher School, Department of Technical Science Uşak, TURKEY

**INTRODUCTION**

Superabsorbent hydrogels are three-dimensional cross-linked hydrophilic, linear or branched polymers with the ability to absorb large quantities of water, saline or physiological solutions compared with general absorbing materials. Because of their excellent hydrophilic properties, high swelling ratio, and biocompatibility, hydrogels have been widely used in biomedical area as antibacterial materials [1] and drug delivery [2]. Usually, most hydrogels were prepared from synthetic polymers by radical copolymerization.

Due to pH, temperature and salt sensitive properties of DMAEMA-co-MEMA [3] was used as superabsorbent.

**MATERIALS AND METHODS**

**Materials:** 2-(dimethylamino) ethyl methacrylate (DMAEMA) and 2-(N-morpholino) ethyl methacrylate (MEMA) monomers were purchased from Aldrich. Monomers were passed through basic alumina column before used. The cross-linker methylene bisacrylamide (MBAAm) and the initiator ammonium persulfate (APS) were obtained from Aldrich, and used without further purification. All other reagents are analytical grade or highest purity available. Deionized water was used throughout the experiments in the preparation of hydrogels, of buffer solutions and in the swelling experiments.

**Preparation of DMAEMA-co-MEMA Polymers:** Hydrogels were prepared through free radical copolymerization in aqueous solution. Different amounts of DMAEMA and MEMA monomers (DMAEMA/MEMA mole ratios, 80:20, 50:50, and 20:80) and certain amount of MBAAm crosslinking agent (2% based on the monomers) were dissolved in deionized water for 20 min. APS initiator was transferred to the mixture via cannula. After stirring 10 min, the mixture was carried in cylindrical PVC straws and allowed to the complete of the copolymerization at room temperature for 24 h. When the copolymerization was completed, hydrogel samples were cut into disk with diameter of 0.5 cm and thickness of 0.5 cm. Then, the disk samples washed with distilled water and immersed in 100 ml of distilled waters for 2 days in order to remove the unreacted chemicals. During this treatment, distilled water was refreshed every 6 h. Finally, pure hydrogels were dried in ambient temperature for 4 days. Polymers were characterized by using scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FT-IR).

**Drug loading and in vitro drug release studies:** The polymer disks (30 mg) were immersed in 20 mL of ethanol/water solution (1:1, v/v) containing 1 mg/mL of IBU for 24 h to reach equilibrium at room temperature. After that, the gels were taken out carefully from the solution and washed with ethanol/water mixture to remove the free IBU on the surface. Then, the drug-loaded hydrogels were dried completely in vacuum.

In vitro release profiles of IBU-loaded gels were performed in phosphate buffer pH 7.4 (PBS) at 37 °C. The amount of IBU released was determined spectrometrically at 223 nm on a UV–vis spectrometer UV-1800 Shimadzu.
RESULTS AND DISCUSSION

DMAEMA-co-MEMA were synthesized by using free radical copolymerization in aqueous solution. For the characterization of structure of gels, FTIR spectra and SEM analysis were performed.

IBU was used as a model drug and the release profiles from the DMAEMA-co-MEMA as a potential carrier system were investigated. Within copolymer, if DMAEMA ratio was increased it was determined that controlled release increased.

CONCLUSIONS

In conclusion, we found that our novel DMAEMA-co-MEMA was ideal candidate for controlled release of IBU.

REFERENCES


RESULTS AND DISCUSSION

Here, we prepared novel type cryogel by using pH- and salt- sensitive 2- (N morpholino ethyl methacrylate) as a functional monomer through freeze-thawing process.

MATERIALS AND METHODS

Materials: 2-(N-morpholino) ethyl methacrylate (MEMA) methylene bisacrylamide (MBAAm), ammonium persulfate (APS) were purchased from Sigma All other chemicals were of analytical grade.

Preparation of PMEMA Cryogels: PMEMA cryogels were prepared by repeated freeze-thaw method. An appropriate amount of MEMA monomer and MBAAm crosslinking agent (2% based on the monomer) were dissolved in 5 ml of deionized water. Then, APS initiator was added to the reaction mixture. After stirring 10 min, this mixture was transferred to PVC straws and allowed to freeze and then allowed to thaw for completion of the polymerization. Such freezing–thawing cycles were repeated at three times.

After completion of the polymerization period, gels were cut into disk with diameter of 0.5 cm and thickness of 0.5 cm. Then, the disk samples washed with distilled water and immersed in 500 ml of distilled waters for 2 days in order to remove the unreacted chemicals. During this treatment, distilled water was refreshed every 6 h. Finally, cryogel disks were dried in ambient temperature for 4 days. Cryogel characterization was performed by using scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FT-IR).

Drug loading and in vitro drug release studies: The cryogel disks (60 mg) were immersed in 20 mL of ethanol/water solution (1:1, v/v) containing 1 mg/mL of IBU for 24 h to reach equilibrium at room temperature. After that, the gels were taken out carefully from the solution and washed with ethanol/water mixture to remove the free IBU on the surface. Then, the drug-loaded hydrogels were dried completely in vacuum.

In vitro release profiles of IBU-loaded gels were performed in phosphate buffer pH 7.4 (PBS) at 37 °C. The amount of IBU released was determined spectrophotometrically at 223 nm on a UV–vis spectrometer UV-1800 Shimadzu.

RESULTS AND DISCUSSION

PMEMA cryogels were synthesized by using free radical chemistry in cryogenic conditions. For the characterization of structure of gels, FTIR spectra and SEM analysis were performed. As shown in Fig. 1, the freeze-dried gels had highly porous structure.

IBU was used as a model drug and the release profiles from the PMEMA cryogels as a potential carrier system were investigated. The drugs were released gradually from gel matrix with the increase of time and no significant burst release was observed.
Fig. 1. SEM images of PMEMA cryogel

CONCLUSIONS
In conclusion, we found that our novel PMEMA cryogels were ideal candidate for controlled release of IBU.

REFERENCES

P-226: SYNTHESIS OF NOVEL AMINOCARBO-\(N\)-THIOL PYRROLIDINE DERIVATIVES AND THEIR ANTITUBERCULOSIS ACTIVITIES

S. Poyraz1, Y. Nural1, M. Ülger1, S. Belveren1, M. Gemili2, D. Erşen1, M.S. Serin2, H.A. Döndaş1

Mersin University, Faculty of Pharmacy
1Department of Analytical Chemistry, 2Department of Pharmaceutical Microbiology
Mersin, TURKEY

INTRODUCTION
Pyrrolidine and aminocarbothiol pyrrolidine derivatives have been extensively studied during the last decade due to possessing biological and pharmacological properties such as antifungal, antibacterial and antimalarial activity [1-3]. Pyrrolidine ring also have important place in drug research and this structure is present in many alkaloids and drug molecules [1].

MATERIALS AND METHODS
As a part of our ongoing research work, some novel pyrrolidine and aminocarbo-\(N\)-thiol pyrrolidine derivatives were prepared by modification of literature method [4].

Antimycobacterial activity of these novel compounds were tested and performed by using Microplate Alamar Blue assay [5] against *M. tuberculosis* H37Rv strain.

RESULTS AND DISCUSSION
In this study, the novel pyrrolidine and aminocarbo-\(N\)-thiol pyrrolidine compounds were prepared and their structures were characterized by NMR, IR, MS and microanalysis.

The antimycobacterial activity of these novel compounds were performed by using Microplate Alamar Blue assay against *M. tuberculosis* H37Rv strain. In antimycobacterial activity studies, isoniazid and ethambutol drugs were used as standard drugs. The antimycobacterial activity were found in the range of 62.5-125 μg/mL. Isoniazid and ethambutol showed antimycobacterial activity with 1 μg/ml and 10 μg/ml respectively.

CONCLUSIONS
The synthesized novel compounds showed moderate activity against *M. tuberculosis* H37Rv strain.

ACKNOWLEDGMENTS
The authors thank Mersin University (Project Grant BAP-SBE TEB (SP) 2014-4 YL) for the financial support.

REFERENCES
P-227: SYNTHESIS OF PYRIMIDINE BASED DERIVATIVES FROM PHENOLIC CHALCONE USING MICROWAVE METHOD, AND ANALYSIS OF BIOLOGICAL ACTIVITIES

N. Yayl, S. Fandakli, N. Kahriman, S. Ulker, A. Bozdevci

1Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, Trabzon-TURKEY
2Department of Chemistry, Faculty of Science, Karadeniz Technical University, Trabzon-TURKEY
3Department of Biology, Faculty of Arts and Science, Recep Tayyip Erdoğan University, Rize-TURKEY

INTRODUCTION

Pyrimidine and derivatives; both synthetic and natural origin with various groups in their structure, electron-rich heterocycles containing nitrogen atom are compounds.1 Being a building unit of RNA and DNA, pyrimidine derivatives were found to be related to a variety of biological effects including antifungal, antiviral, antimicrobial, antitubercular, antimalarial and antitumor.2,3

METARIAL AND METHODS

In this study, we synthesized three new 2,4,6-trisubstituted pyrimidine with microwave method compounds starting from hydroxy and methoxy

RESULT AND DISCUSSION

In this study, we synthesized three new pyrimidine compounds by the reaction which proceeds either by 1,2-addition and/or 1,4-addition of urea to the chalcone, followed by cyclization to give the corresponding 2-hydroxy-4,6-disubstituted pyrimidine derivatives (4-6) and biological activities of the compounds were investigated.

### Table 1: IC_{50} Values of Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Residual Activity %</th>
<th>IC_{50} (µM) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>88.59 ± 2.88</td>
<td>36.51 ± 0.94</td>
</tr>
<tr>
<td>5</td>
<td>48.84 ± 0.31</td>
<td>15.04 ± 2.18</td>
</tr>
<tr>
<td>6</td>
<td>37.41 ± 3.4</td>
<td>9.56 ± 1.33</td>
</tr>
<tr>
<td>Acarbose</td>
<td>63.44 ± 9.61</td>
<td>13.34 ± 1.26</td>
</tr>
</tbody>
</table>

Fig 2. Result of α-glucosidase enzyme inhibition of compounds (4-6)

### Table 2: Lipase IC_{50} Values of Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Residual Activity %</th>
<th>IC_{50} (µM) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9.59 ± 3.00</td>
<td>0.85 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>26.31 ± 2.60</td>
<td>2.97 ± 0.52</td>
</tr>
<tr>
<td>6</td>
<td>18.21 ± 1.97</td>
<td>4.47 ± 0.74</td>
</tr>
<tr>
<td>Orlistat</td>
<td>0.7 ± 0.02</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Fig 3. Result of lipase enzyme inhibition of compounds (4-6)

REFERENCES


P-228: ANTICANCER EVALUATION OF SOME 5-(4-METHYLPIPERAZIN-1-YL)-2-PHENYL-1H-BENZIMIDAZOLE DERIVATIVES

M. Alp, A.S. Gurkan-Alp, T. Ozkan, A. Sunguroglu

1Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY
2Ankara University, Faculty of Medicine, Department of Medical Biology, Ankara, TURKEY

A series of some 5-(4-methylpiperazin-1-yl)-2-substituted phenyl-1H-benzimidazoles (5-14) were evaluated for their in vitro anticancer activities against human leukemia cell line, HL-60. Compounds 5-7, 10-12 exhibited potent anticancer activities against HL-60 cell line. The quantitative analysis of apoptosis by flow-cytometry demonstrated that the percentages of apoptotic HL-60 cells treated with compounds 5, 10-12 were significantly higher than the control.

INTRODUCTION

It is known that Hoechst 33342 and Hoechst 33258 (Fig. 1) are adenine-thymine-specific dyes which stain DNA by binding to its minor groove. It was reported that Hoechst 33342 bearing an ethoxy group induced apoptosis and led to cell death in HL-60 cells [1]. In our previous paper, we have reported antileukemic activities
of some 2-(4-phenoxyphenyl)-1H-benzimidazoles against human CML cell line K562 [2]. These findings prompted us to investigate a series of 5-(4-methylpiperazin-1-yl)-2-substituted phenyl-1H-benzimidazoles 5-14 (Fig. 2) to evaluate their anticancer activity against AML cell line HL-60.

Fig. 1. Chemical structures of Hoechst 33342 (R= C2H5) and Hoechst 33258 (R= H)

Fig. 2. General structure of 5-(4-methylpiperazin-1-yl)-2-phenyl-1H-benzimidazoles

MATERIALS AND METHODS
MTT test was used to determine cell viability. Cells were seeded to 96-well plates. The cell proliferation assay was carried out using the Cell Proliferation Kit I as described by the manufacturer using cells treated at various concentrations of compounds 5-14 for 48 h [3]. Number of viable cells was calculated from untreated cells, and the data were expressed as percent cell viability. Cisplatin was used as a positive control in all viability experiments. The quantitative analysis of apoptosis was demonstrated by flow cytometry.

RESULTS AND DISCUSSION
This work indicated that compounds 5, 10-12 exhibited potent anticanter activity, anti-proliferative and apoptosis-inducing effect on HL-60 cells. The percentage of early apoptotic HL60 cells, treated with compounds 5, 10-12 containing benzyloxy group were significantly higher than the control cells. The percentage of late apoptotic / death HL60 cells, treated with compounds 5, 10-12 were significantly higher than the control cells at 48 h.

CONCLUSIONS
According to the results some of the 5-(4-methylpiperazin-1-yl)-2-substituted phenyl-1H-benzimidazoles have cytotoxic activity in HL-60 cell line. More active derivatives against AML can be obtained by chemical modification of these compounds.

REFERENCES

P-229: SYNTHESIS OF SOME NOVEL 1-(4-CHLOROPHENYL)-2-(1H-TRIAZOLE-1-YL)ETHANOL ESTER DERIVATIVE COMPOUNDS AS POTENTIAL BIOLOGICAL ACTIVE COMPOUNDS
I.S. Dogan1, G.Tosun2, E.Sellitepe1, S.Sarac Tarhan3
1Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Trabzon-TURKEY, 2Karadeniz Technical University, Faculty of Arts and Sciences, Trabzon-TURKEY,
3Hacettepe Faculty of Pharmacy, Department of Pharmaceutical Chemistry Ankara- TURKEY

INTRODUCTION
Nafimidone [1-(2-naphthyl)-2-(imidazol-1-yl)ethanone] and its derivatives have attracted great interest due to their promising biological effects, firstly anticonvulsant and also antibacterial, antiviral, activities [1]. Nafimidone and its major metabolite nafimidone alcohol found active as (arylalkyl)azole anticonvulsant compounds and modifications are made. In our previous study, we have reported some carboxylic acid esters of nafimidone alcohol and aliphatic carboxylic acid ester derivatives of 1-phenyl/1-(4-chlorophenyl)-2-(1H-imidazol-1-yl)ethanol carrying phenyl ring as a lipophilic aromatic group instead of naphthalene have been described [2-4]. In this study, parallel to our previous work [3], we aimed to synthesize eight 1-(4-chlorophenyl)-2-(1H-triazole-1-yl)ethyl esters (IIIa-h), three of which were reported in the literature [5].

MATERIALS AND METHODS
Compounds a-h were prepared by the reaction of 1-(4-chlorophenyl)-2-(1H-triazole-1-yl)ethanol with various carboxylic acids under Steglich esterification reaction conditions [6]. We tried different acids to establish the relationships between the type and the size of the alkyl chain of the ester group and the activity.
RESULTS AND DISCUSSION
The structures of those synthesized and purified compounds (IIIa-h) confirmed through IR, 1H-NMR, 13C NMR, mass spectral data. Figures 1 shows example of spectral data for 1H-NMR spectrum of compound IIIh.

CONCLUSIONS
In this study synthesis of some new 1-(4-chlorophenyl)-2-(1H-triazole-1-yl)ethanol esters (IIIa-h) having “phenyl ring” instead of “naphthalene ring” in nafimidone, which were designed as prodrugs of nafimidone alcohol were realized. The anticonvulsant and antimicrobial activities of the compounds are in progress. The relationship between the structure and the biological activity of the compounds will be concluded after their biological activity screening tests.

ACKNOWLEDGMENTS
This study was supported by TÜBİTAK Fund (3001Project-114S862)

REFERENCES

P-230: SYNTHESIS AND CHOLINESTERASE INHIBITORY POTENTIAL OF NEW 1-[4-(4-SUBSTITUTED Piperazine-1-YL) PHENYL]-3-PHENYLPROP-2-EN-1-ONE DERIVATIVES

B. N. Saglık1, U. Acar2, Y. Özkay3

1AnadoluUniversity, Faculty of Pharmacy, Department of PharmaceuticalChemistry, Eskişehir-Turkey

INTRODUCTION
Alzheimer’s disease (AD) is the most common reason of progressive dementia in the old population. In recent years, many studies have been alleged to find out the complex pathophysiology of AD, but its etiology remains obscure [1]. Acetycholinesterase (AChE) is the main enzyme responsible for the hydrolysis of the ACh at the cholinergic synapses, while butrylycholinesterase (BuChE) acts as a co-regulator of the activity of AChE. Present therapies for AD mainly focus on the use of FDA accepted acetylcholinesterase inhibitors (AChEIs), i.e. donepezil, rivastigmine, galantamine, tacrine. These medications are counted as solely symptomatic. Thus there is a need to find more efficient agents to stop the disease progression [2]. Piperazine ring possesses two tertiary nitrogen elements that act as proton acceptor. Thus, nitrogen elements convert to quaternary form and can interact with anionic site of AChE by electrostatic attraction. Due to this property of piperazine it is usually sited into chemical structure of new inhibitor candidates of AChE [3]. In consideration of above referred observations we synthesized a new series of piperazine derivatives and investigated their BuChE and AChEinhibitory activitiy in order to acquire new biologically active compounds.

Materials and Methods
Preparation of 4-[4-[2-(dimethylamino)ethyl]-piperazine-1-y1] acetophenone(1)
4-Fluoroacetophenone (10 mmol, 1.214 mL), K2CO3 (10 mmol, 1.38 g), 1-[2-(dimethylamino)ethyl]piperazine(20 mmol), and DMF (5 mL) were added into a vial (30 mL) of
microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 200°C and 10 bars for 15 min. After the control of reaction by TLC, the mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

**General Synthesis procedure for 1-[4-{[4-[2-(dimethylamino)ethyl]-piperazine-1-yl][phenyl]-3-phenylprop-2-en-1-one derivatives (2a-l)**

The compounds 1a(10mmol), appropriate benzaldehydedervative (10 mmol) and potassium hydroxide (10 mmol) in methanol (10 mL) was stirred at room temperature for about 12 h. After TLC screening, the resulting solid was filtered, washed with water, dried, and recrystallized from ethanol.

**AChE and BuChE inhibitory activity**

All compounds were subjected to a slightly modified method of Ellman’s test [4,5] in order to evaluate their potency to inhibit the AChE and BuChE. Enzyme solutions were prepared in gelatine solution (1%), at a concentration of 2.5 units/mL.AchE/BuChE and compound solution (50 μL) which is prepared in 2% DMSO at 0.1 and 1 mM concentrations were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB (50 μL) and ATC (10 μL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 μL 2% DMSO, 50 μL DTNB and 10 μL substrate. All processes were assayed in triplicate.

**RESULTS AND DISCUSSION**

Structure elucidations of the final compounds (2a-l) were performed with IR, 1H-NMR, and ES-MS spectroscopic methods and elemental analysis. Among the tested compounds, 2b was determined as the most active derivative.

**CONCLUSIONS**

The anticholinesterase effects of the compounds (2a-l) on AChE and BuChE were determined by a modification of Ellman’s spectrophotometric method. Some of the compounds showed enzyme inhibitory potency to different extents and will be evaluated in further detailed studies.

**REFERENCES**


**P-231: SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF NOVEL IMIDAZOLE DERIVATIVES**

**S. Levent1, B.N. Saglik1, U. Acar1, Y. Ozkay1**

Anadolu University, Faculty of Pharmacy, 1Department of Pharmaceutical Chemistry, Eskişehir-Turkey

**INTRODUCTION**

A dysfunction of acetylcholine (ACh) containing neurons in some areas of the brain such as cortex and hippocampus is related to the deficits in memory and cognitive function. This observation led to the development of therapeutic agents that inhibit acetylcholinesterase (AChE) enzyme, which reduces the level of ACh in central nervous system. Acetylcholinesterase inhibitors (AChEI) prolong the duration of action of acetylcholine (ACh) and render symptomatic relief in AD. The use of AChEI as anti-Alzheimer drugs has beneficial effects on cognitive, functional and behavioral symptoms of the disease [1,2].

In the present study due to the physiological importance of imidazole some new imidazole derivatives were synthesized for their inhibitory potency against cholinesterases enzymes.

**MATERIALS AND METHODS**

Microwave assisted synthesis of 4,5-Bis(4-substituted phenyl)-2-(4-substitutedphenyl)-1H-imidazoles (1a-h): Corresponding benzaldehydedervative (10,5 mmol) appropriate 4-substituted benzil (10 mmol) and ammonium acetate (80 mmol, 6 gr) were dissolved in glacial acetic acid (10 mL) into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 220°C and 10 bars for 15 min. After the control of reaction by TLC, the mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

Synthesis of 2-[4,5-Bis(4-substituted phenyl)-2-(4-substitutedphenyl)-1H-imidazole-1-yl]-1-(4-substitutedphenyl) ethaneone (2a-p): The compounds 1a-h (1 mmol), appropriate phenacyl bromide (1 mmol) and K₂CO₃ (1 mmol, 138 mg) were stirred in acetone (30 ml) for 24h at room temperature. After the control of reaction by TLC, the excess of solvent was evaporated under reduced pressure, crude product was washed with water, dried and recrystallized from ethanol.

**AChE and BuChE inhibitory activity:** All compounds were subjected to a slightly modified method of Ellman’s test [3,4] in order to evaluate their potency to inhibit the AChE and BuChE. Enzyme solutions were prepared in gelatine solution (1%), at a concentration of 2.5 units/mL.AchE/BuChE and compound solution (50 μL) which is prepared in 2% DMSO at 0.1 and 1 mM concentrations were added to 3.0 mL phosphate buffer.
buffer (pH 8±0.1) and incubated at 25 oC for 5 min. The reaction was started by adding DTNB (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 µL 2% DMSO, 50 µL DTNB and 10 µL substrate. All processes were assayed in triplicate.

RESULTS AND DISCUSSION

The chemical structures of the compounds (4a-l) were confirmed by IR, 1H NMR, MS spectral data and elemental analyses. Enzymatic activity test indicated that the compound 2d possess the highest inhibitory potency against AChE among the tested compounds.

CONCLUSIONS

The anticholinesterase effects of the compounds (2a-p) were determined by Elman’s method. Enzymatic studies showed that some modifications on the chemical structure of the compounds are required to enhance biological activity.

REFERENCES


CONCLUSIONS

In the present study, seven novel molecules are synthesized as potential anticancer drug candidates, and their structures are enlightened. After the tests, it is stated that the synthesized molecules don’t have effects, such as anticancer, antifungal, antibacterial, anti-inflammatory and HIV-protease inhibitory activities, depending on the substituents contained in this structure.

INTRODUCTION

In this research, we aimed to synthesis a new series of sulphanylamine substituted ureas (1a-g) as possible anticancer agents, by using sulfonamide derivatives, which are also known as antibacterial, diuretic and antitumor agents.

MATERIALS AND METHODS

In the present study, new urea derivatives were synthesized by reacting the sulfonamide derivatives with substituted aryl isocyanates. All new compounds were characterized by elemental analysis and various spectroscopic methods (IR, 1H-NMR, MS). The newly synthesized compounds have been tested for their possible cytotoxic activity by using MTT test L929 (cytotoxicity) and different cancer cell line (antiproliferative).

RESULTS AND DISCUSSION

P-232: SYNTHESIS, CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITY OF SULPHANYLAMIDE SUBSTITUTED UREA DERIVATIVES

S. Türk1, S. Karakuş1, S. Özbaş-Turan2, J. Akbuğa2

Marmara University, Faculty of Pharmacy
1Department of Pharmaceutical Chemistry, 2Department of Pharmaceutical Biotechnology
İstanbul, TURKEY

Nowadays, urea structure is located in many compounds as candidate for drug active molecules. It is suggested that it has different pharmacological

CONCLUSIONS

In the present study, seven novel molecules are synthesized as potential anticancer drug candidates and their structures are enlightened. After the tests, it is stated that the synthesized molecules don’t have
cytotoxic activity (MTT). And also, the antiproliferative activity studies are going on the following cell lines: lung (ATCC, CC-185), breast (ATCC, HTB-26) and cervix (ATCC, CCL-2).

REFERENCES

P-233: QUORUM SENSING INHIBITOR (QSI) ACTIVITY AND BINDING MODE ON LASR OF CELECOXIB DERIVATIVES

Seyhan Ulusoy1, Sevil Senkardeş2, İnci Coşkun2, Gülgün Boşgelmez-Tınaz2, Laurent Soulere4, Yves Queneau4, Ş. Güniz Küçükgüzel2

1 Süleyman Demirel University, Faculty of Fen-Edebiyat, Department of Biology, Çiçinür Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2 Department of Basic Pharmaceutical Sciences, Haydarpaşa 34668 Istanbul, Turkey.
3 Université de Lyon, INSa- Lyon, ICBMS, Bât Jule Verne, 20 avenue Albert Einstein 69621 Villeurbanne Cedex, France

INTRODUCTION
The inappropriate and indiscriminate application of antibiotics in pharmacotherapy has led to the development of widespread bacterial resistance to these agents. Today there is an urgent need for the discovery of novel antibacterial drug targets and treatment strategies. One such new target is quorum sensing (QS). QS is a cell-to-cell communication system employed by a variety of bacteria to coordinate group behaviors as function of cell-density. QS depends on the production of N-acylated-L-homoserine lactone (AHL) signal molecules. AHLs diffuse from bacterial cells and accumulate in the medium. The accumulated AHLs interact with a transcriptional activator protein then induce the expression of QS-regulated genes. (1). The discovery that many pathogenic bacteria employ QS to regulate their pathogenicity and virulence factor production makes the QS system an attractive target for antimicrobial therapy (2). Some homoserine lactone-derived sulfonylureas have been reported as inhibitors of Vibrio fischeri QS system (3). The sulfonylthiourea and 4-thiazolidinone groups have emerged as the most favourable pharmacophore. Therefore, in this study anti-QS capacities and binding mode of celecoxib sulfonylthiourea and 4-thiazolidinone on LasR protein are presented.

MATERIALS AND METHODS
A series of novel N-(3-substituted aryl/alkyl-4-oxo-1,3-thiazolidin-2-ylidene)-4-[5-(4-methylphenyl)-3-( trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamides [SGK 328, 329, 330, 334, 337] were synthesized by the addition of ethyl α-bromoaacetate and anhydrous sodium acetate in dry ethanol to N-(substituted aryl/alkylcarbamothioyl)-4- [5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamides [SGK 318, 319, 320, 324, 327], which were synthesized by the reaction of alkyl/aryl isothiocyanates with celecoxib (4). Anti-QS potential of these compounds was evaluated using quorum sensing inhibitor selector 1 (QSIS1) assay. A molecular modeling study of the binding mode was achieved using these compounds and the protein LasR (pdb 2UV0) (5).

RESULTS AND DISCUSSION
Compounds SGK 324, 327 and 330 were found to have anti-QS activities against biosensor bacteria (QSIS1) at 0.1 M concentration. Additionally, compounds SGK 324 and 327 showed antibacterial activity at this concentration. QS inhibitory compounds that do not kill or inhibit microbial growth are less likely to impose a selective pressure for the development of resistant bacteria. Therefore, to eliminate antibacterial effect, QSIS1 strain was grown in the presence of different concentrations of these molecules. Concentrations up to 4 mM had no effect on QSIS1 growth. QSIS1 assay was carried out at 2mM concentration and found that these compounds showed strong QS inhibitory activity without growth inhibition. These compounds were found to significantly affect QS regulated elastase production in P. aeruginosa PA01 strain. The binding mode study via molecular docking suggests that compounds SGK 324 and SGK 327 could not interact within LasR. In contrary, compound SGK 330 seems to fit within the binding site of LasR via hydrophobic/hydrophilic interactions with hydrogen bonds.

CONCLUSION
These results suggest that celecoxib derivatives may provide a starting point for the design and development of new QS inhibitory drugs that restrict virulence of clinically important human pathogens. In
addition, these molecules could be used in combination with conventional antibiotics to increase the efficiency of current antimicrobials by reducing the minimal inhibitory concentration. Additionally, inhibition of bacterial QS, rather than bactericidal or bacteriostatic strategies, offers a promising way to overcome the bacterial resistance problem. It is assumed that targeting the pathogenesis instead of killing the organism will apply a gentler selective pressure for the development of bacterial resistance.

ACKNOWLEDGMENTS
This work (synthesis and elucidation of celecoxib derivatives) was supported by Research Fund of the Marmara University. Project number: SAG.A.310510 / 0175.

REFERENCES

P-234: SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME FURAN-BASED THIOSEMICARBAZONE DERIVATIVES
S. Gökbülüt1, M.D. Altıntop1, A. Özdemir2, F. Demirci2, Z.A. Kaplançikl2
Anadolu University, 1Faculty of Pharmacy, Department of Pharmaceutical Chemistry,
2Faculty of Health Sciences, Eskişehir, TURKEY

INTRODUCTION
The spread of microbial resistance of pathogens to currently available antimicrobial agents poses a serious threat to public health. Consequently, intensive efforts are underway worldwide to develop new antimicrobial agents [1]. To identify compounds with a potent antimicrobial profile, we designed and synthesized some furan-based thiosemicarbazone derivatives as new antimicrobial agents.

MATERIALS AND METHODS
Initially, 4-(2,4-difluorophenyl)-3-thiosemicarbazide was synthesized via the reaction of 2,4-difluorophenyl isothiocyanate with hydrazine hydrate. The reaction of 4-(2,4-difluorophenyl)thiosemicarbazide with 5-arylfulural derivatives afforded new thiosemicarbazone derivatives. The chemical structures of the compounds were elucidated by elemental analyses, IR, 1H-NMR and mass spectral data.

The in vitro effects of these derivatives were evaluated against Candida parapsilosis (ATCC 22019), Candida albicans (ATCC 24433), Staphylococcus aureus (ATCC BAA 1026), methicillin-resistant Staphylococcus aureus (MRSA) (ATCC 700699), Pseudomonas aeruginosa (ATCC 13388) using CLSI broth microdilution method [2,3]. Ciprofloxacin and amphotericin B were used as reference drugs for antibacterial and antifungal activity, respectively.

RESULTS AND DISCUSSION
Among the newly synthesized compounds, 4-(2,4-difluorophenyl)-1-[(5-(2-nitrophenyl)furan-2-yl)methylene]thiosemicarbazide, 4-(2,4-difluorophenyl)-1-[(5-(3-nitrophenyl)furan-2-yl)methylene]thiosemicarbazide and 4-(2,4-difluorophenyl)-1-[(5-(4-chlorophenyl)furan-2-yl)methylene]thiosemicarbazide exhibited significant antimicrobial activity against C. parapsilosis, C. albicans, S. aureus, MRSA, P. aeruginosa.

CONCLUSIONS
In the current work, we described the synthesis of a new series of furan-based thiosemicarbazone derivatives and evaluated their antimicrobial effects against pathogenic bacteria and Candida species. Among these compounds, 4-(2,4-difluorophenyl)-1-[(5-(2-nitrophenyl)furan-2-yl)methylene]thiosemicarbazide, 4-(2,4-difluorophenyl)-1-[(5-(3-nitrophenyl)furan-2-yl)methylene]thiosemicarbazide and 4-(2,4-difluorophenyl)-1-[(5-(4-chlorophenyl)furan-2-yl)methylene]thiosemicarbazide can be identified as the most promising antimicrobial agents due to their inhibitory effects on C. parapsilosis, C. albicans, S. aureus, MRSA, P. aeruginosa. Further research is needed to understand the mechanism of action for the antimicrobial activity of these compounds.

REFERENCES

2. EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts.


**P-235: SYNTHESIS AND BIOLOGICAL ACTIVITY STUDIES ON ACETOPHENONE-HYDRAZONE DERIVATIVES AS ACETYLCOLINESTERASE INHIBITORS**

S. Parlar, G. Bayraktar, V. Alptüzün, E. Erciyas

Ege University, Faculty of Pharmacy Department of Pharmaceutical Chemistry, İzmir, TURKEY

**INTRODUCTION**

Acetylcholinesterase enzyme (AChE) catalyzes the hydrolysis of neurotransmitter acetylcholine (ACh) and is present in the most prominent constituents of central cholinergic pathways. AChE have a vital role for both central and peripheral nervous systems. Terminating the impulse transmission at cholinergic synapses, rapid hydrolysis by AChE into acetylcholine is the crucial function of AChE and it is a target of drug development to combat the neuromuscular disorders such as myasthenia gravis, glaucoma and Alzheimer’s disease (AD) [1].

In our previous study, a group of 1,4-dihydropyridine-benzaldehydehydrazone derivatives were synthesized and evaluated for their inhibitor activity against AChE enzyme [2]. Most of the compounds were exhibited good inhibition. In this study, we aimed to synthesize a series of compounds consisting acetophenone moiety instead of benzaldehyde which are linked to the hydrazone. Aromatic and aliphatic moiety were connected to the pyridine nitrogen with alkyl linkers with different lengths.

**MATERIALS AND METHODS**

The target compounds were prepared by three-step synthesis. In the first step, 4-chloropyridine and hydrazinium hydroxide were reacted to obtain 4-hydrazinylpyridine and 4-hydrazinylpyridine was condensed with nonsubstituted and 4-chlorosubstituted-acetophenones. Then the compounds were furnished by the quaternization of the pyridine nitrogen with various alkyl halides. The final compounds’ AChE inhibitor activities were evaluated in vitro by using Ellman’s method [3].

**RESULTS AND DISCUSSION**

In this study, a group of acetophenone-hydrazone compounds (Fig 1) were synthesized and their structures were identified by spectroscopic methods (IR, 1H NMR, MS). Their AChE enzyme activities have been examined by using Ellman’s method [3]. Some of the final compounds exhibited promising inhibitor activities to the AChE enzyme compared to the standard drug.

**REFERENCES**


**P-236: SYNTHESIS OF NEW N,N’-DIPHENYL UREA DERIVATIVES AND EVALUATION OF ANTIMICROBIAL ACTIVITIES**

S. Dilem Doğan1, Esra Köngül2, Perihan Gürbüz2, Nilay Ildiz2

Erçiyes University, Faculty of Pharmacy

1Department of Pharmaceutical Basic Sciences,
2Department of Pharmacognosy, 3Department of Pharmaceutical Microbiology

Kayseri, TURKEY

**INTRODUCTION**

The multi-drug resistance to most of all antibiotics has become a serious medical problem therefore development of new antibiotic compounds in place of traditional antibiotics is very important. Urea
derivatives are found in many biologically active compounds. Urea is class of compounds constituted carbonyl group attached to two amino groups. Urea derivatives are formed by replacement of hydrogen with different substituent and formula is \(-\text{N-CO-N}^+\).

\[
\text{diphenyl urea}
\]

It is a well fact that some substituted urea derivatives have been the subject of considerable attention due to their applications, for example cellulose fibers, antioxidants in gasoline for automobiles, additives in detergents to prevent carbon deposits, inhibit corrosion, dyeing of hair and intermediate for the production of the carbamates[1].

Diphenyl urea derivatives (1) have been reported to display a wide range of biological activities, such as the HIV protease inhibitors, selective NK1 antagonists, Chk1 inhibitors, anti Alzheimer’s disease, antimicrobialidal agents, antifungal agents[2,3].

MATERIALS AND METHODS

Chemistry: Diphenyl urea derivatives were synthesized according to the reaction between different isocyanates and amines. Carboxylic acid was treated with ethylchloroformate in the presence of triethylamine followed by the addition of solution of NaN\(_3\) in water gave acyl azide [4]. To obtain the corresponding isocyanate derivatives, acyl azide was refluxed in benzene to transformation to the corresponding isocyanate which can be generated by the Curtius rearrangement. To obtain the urea derivatives, acyl azide was directly treated with different isocyanates and amines. Carboxylic acid was synthesized according to the reaction between acyl azide and amin derivatives and tested their antibacterial and antifungal activity.

RESULTS AND DISCUSSION

New diphenyl urea derivatives (1-18) were synthesized and characterized using the \(^1\)H-NMR, \(^{13}\)C-NMR, IR and mass spectroscopy. Compounds 1-18 were screened in vitro for their anti-microbial and antifungal activity against *Pseudomonas aeruginosa* AACC 27853, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* NJ-1, *Basilus subtilis* ATCC 6633, *Candida albicans* 10231 and *Candida glabrata* ATCC 90030. Compounds 5 (1-(2-mercaptoethyl)-3-(3,4,5-trimethoxyphenyl)urea) and 11 (1-(3,4-dimethoxyphenyl)-3-(2-mercaptoethyl)urea) were effective against *Basilus subtilis* ATCC 6633 and 14 (1-(2-mercaptoethyl)-3-(4-methoxyphenyl)urea) and 17 (methyl (4-methoxyphenyl)carbamate) were effective against *Staphylococcus aureus* ATCC 25923. Antifungal activity was not effective against Gr negative standard strains.

CONCLUSIONS

In this study, several new diphenyl urea derivatives were synthesized by the reaction between acyl azide and amin derivatives and tested their antibacterial and anti fungal activity. Synthetic compounds 5 and 11 showed potent *Basilus subtilis* inhibitory activities and compounds 14 and 17 showed *Staphylococcus aureus* inhibitory activities.

ACKNOWLEDGMENTS

The authors are indebted to the Faculty of Pharmacy at Erciyes University and Research Foundation of Erciyes University (Project Number: TCD-2015-5602) for their financial support of this work.

REFERENCES

**P-237: SYNTHESIS AND CYTOTOXIC ACTIVITY OF SOME HETEROCYCLIC COMPOUNDS**

Şirin Uysal1, Murat Çizmecioğlu1, Zeynep Soyer1, Yalçın Erzurumlu2, Petek Ballar Kirmızibayrak2

Ege University, Faculty of Pharmacy
1 Department of Pharmaceutical Chemistry, 2 Department of Biochemistry
İzmir, TURKEY

**INTRODUCTION**

Heterocyclic compounds play major role in pharmaceutical processes and exhibit a broad spectrum of biological activities. It is well known that many drugs containing heterocycle skeletons are used as core structures. Among these heterocycles, quinoline and benzoazole rings are the privileged structures in drug discovery. Derivatives of this scaffold have been reported to exhibit various biological activities such as anticancer, antiinflammatory, antimicrobial, anticonvulsant, antimalarial and antimycobacterial [1, 2]. In this preliminary study, some compounds bearing benzoazole and quinoline rings were synthesized and evaluated for their cytotoxic activities against 293T, HeLa and U20S cell lines.

**MATERIALS AND METHODS**

The final compounds (Figure 1) were synthesized according to the procedures described earlier [3, 4]. The cytotoxic activities of the title compounds were determined against three cancer cell lines (293T, HeLa and U20S), using WST-1 cell proliferation assay.

**RESULTS AND DISCUSSION**

In this present study, 3-substituted-5-chloro-2-oxo-benzoazole and 4-substituted-2-phenyl-quinoline derivatives have been synthesized to explore their antiproliferative activities. Structures of the synthesized compounds was confirmed by IR, $^1$H NMR, and ESI-MS spectral data. Antiproliferative activity of the prepared compounds were evaluated by performing in vitro assays of the inhibition ratios of these compounds to the proliferation of human cancer cells. The compounds possessed different antiproliferative activity profile depending on the changes in their chemical structures.

Fig. 1. The structure of the synthesized compounds

**REFERENCES**


---

**P-238: SYNTHESIS OF 4-N-SUBSTITUTED PIPERAZINE DERIVATIVES CONTAINING INDOLE-2-CARBOXYLIC ACID**

T.G. Altuntas1, A.Baydar1, Z. Kilic Kurt1

1 Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06100 Tandogan, Ankara-Turkey
Ankara, TURKEY

**INTRODUCTION**

Human immunodeficiency virus type-1(HIV-1) contains an enzyme, reverse transcriptase (RT), which catalyzes the conversion of the genomic viral RNA into the proviral DNA. Reverse transcriptase inhibitors (RTIs) such as 3’azido-3’deoxythymidine (AZT) and 2’, 3’-dideoxyinosine (ddi) are nucleoside drugs. Unfortunately, administration of these compounds to patients often causes serious toxic side effects (1).
order to maximize chances of discovering an inhibitor, non-nucleoside RT inhibitors were discovered. Bis(heteroaryl)piperazines (BHAP) were new class of RT inhibitors. These compounds were dramatically improved anti HIV-1 activity (2,3). We initially became interested in preparing various indole containing heteroaryl piperazines.

MATERIALS AND METHODS

General Procedure: Coupling of monosubstituted piperazines with carboxylic acids utilizing CDI: Indole-2-carboxylic acid or 5-substituted indole-2-carboxylic acid was added to a solution of 1,1'-carbonyldiimidazole in THF at room temperature. After 1 h of stirring the reaction was cooled to 0°C and various 4-N-substituted piperazine dissolved in THF was added via cannula. Then the reaction was slowly warmed to room temperature and stirred a further 18 h. Basic workup (CH2C12, NaHCO3, Na2SO4) was carried out. The products were afforded by concentration in vacuo, and purification by flash column chromatography (n-hekzan-EtOAc).

RESULTS AND DISCUSSION

Coupling of indole-2-carboxylic acid or 5-substituted indole-2-carboxylic acid with 4-N-substituted carbonyldiimidazole). Chemical structures of synthesized compounds were confirmed by elemental analysis, IR, 1H-NMR and Mass spectral data. Results were in good agreement with their chemical structures. Their RT inhibitor activity will be tested.

CONCLUSIONS

A series of indole containing piperazine derivatives were successfully synthesized as RT inhibitors.

ACKNOWLEDGMENTS

We thank to Prof. Dr. Hakan Goker (Central Lab., Faculty of Pharmacy, Ankara University) for providing the NMR, Mass spectrometer, and elemental analyzer results.

REFERENCES

P-239: SYNTHESIS AND ANTIOXIDANT ACTIVITY OF INDOLE DERIVATIVES CONTAINING 4-SUBSTITUTED PIPERAZINE MOIETIES

T.G. Altuntas¹, N. Yılmaz¹, T. Çoban², Z. Kılıç Kurt¹, S. Öłgen¹

¹Department of Pharmaceutical Chemistry and ²Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Ankara University 06100 Tandogan, Ankara, Turkey

INTRODUCTION

Free radicals, which are generated in many bioorganic redox process, play a role in the pathogenesis of several diseases including cancer, arthritis, hemorrhagic shock, inflammatory, autoimmune, cardiovascular, neurodegenerative diseases and age-related degenerative brain diseases (1). Exposures of normal cell to free radical damages several structures; oxidizes nucleic acids, proteins, lipids or DNA. As pharmacophores of medical interest, piperazine derivatives promotes various types of biological activities such as antifungal, antibacterial, antimalarial, antipsychotic and anti-HIV protease. calcium antagonists, antimalarial agents, antifungal agents and antimicrobial agents. In recent study, N-aryl and N-alkyl piperazine derivatives are reported as a novel class of anti-inflammatory and antioxidant agents with sturdy cell viability (2). Many publications have covered the antioxidant role of several indole derivatives. Indoles are featured in a wide variety of biologically and pharmacologically active compounds (3). In connection with these studies, we focused on the development of new designed compounds with the combination of these both structure, hoping that these compounds might possess certain antioxidant properties.

MATERIALS AND METHODS

Coupling of monosubstituted piperazines with indole-2-carboxylic acid utilizing CDI: Indole-2-carboxylic acid (3.1 mmol) was dissolved in THF (5 ml). A solution of 1,1'-carbonyldiimidazole (CDI) in THF (3 ml, 3.7 mmol) was added at room temperature. After 1 h of stirring the reaction under N2, it was cooled to 0°C and N-substituted piperazine derivatives (3.7 mmol) dissolved in THF was added. Then the reaction was slowly warmed to room temperature and stirred a further 17-18 h. Basic workup (CHCl3, NaHCO3(saturated), Na2SO4), concentration in vacuo, and recrystallization from ethyl acetate : n-hexane provided the desired compounds. In vitro antioxidant activity studies: Superoxide radical scavenging activity (Cytochrome c assay), DPPH free radical scavenging activity and Lipid peroxidation (LP) were carried out according to general literature methods.
RESULTS AND DISCUSSION
All the compounds of the series (1-15) were synthesized with a view to structural elucidation, first time in vitro antioxidant screening, and evaluation of the structure activity relationship. The prepared compounds structures were in good agreement with their 1H-NMR, MS and IR characteristics. Antioxidant capacity of the synthesized indole piperazines in three different in vitro assays; superoxide anion formation, DPPH stable radical scavenging activity and LP were investigated. All the compounds were evaluated against vitamin E ( α-tocopherol) which was chosen as a reference compound because of its high antioxidant capacity.

\[ \text{The results indicated that compounds 6 (75%), 7 (66%), 8 (75%), 11 (88%), and 12 (69%) showed strong inhibitory effects on superoxide radical formation compare to vitamin E (62%). Compound 10 (38%) has similar activity to vitamin E in lipid peroxidation inhibition assay. Compounds did not showed good inhibition on DPPH except compound 10, which has 20% inhibition.} \]

CONCLUSIONS
Compounds 3, 6, 8, 10, and 11 scavenged superoxide and DPPH radical and inhibited lipid peroxidation at 1mM concentration. The remainder of the compounds showed different patterns of effect on these parameters. The inhibitory effects of compounds were noted at the level of superoxide radical but neither on DPPH radical nor LP inhibition. The observation of different effects of synthetic compounds on superoxide anion, DPPH radical scavenger and LP inhibition activity was not surprising since the mechanism of production of oxidative stress by these methods were different.

REFERENCES

P-240: STUDIES ON THE SYNTHESIS OF SOME NEW (3,4-DIHYDRO-2H-PYRANO [2,3-B]PYRIDIN-6-YLMETHYL4-(2-SUBSTITUTEDPHENYLCARBAMOYL) BENZYL CARBAMATES AS ANTICANCER AGENTS AND HDACIS

T.Z. Yesiloglu, O. Bozdag-Dundar
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

INTRODUCTION
Histone deacetylases (HDACs) are enzymes that are commonly deregulated in a variety of tumors [1] because of their importance in the regulation of gene expression through modification of histone and nonhistone substrates [2].

HDAC inhibitors (HDACIs) are under development as anticancer drugs. In cell-based studies, HDACIs have been shown to induce cell cycle arrest, cell differentiation, and apoptosis. Furthermore, HDACIs were reported to intensify the host immune response and decrease angiogenesis [3]. For these reasons, several HDACIs are currently at various stages of clinical trials, individually or in combination with radiotherapy and/or chemotherapy for cancer treatment in patients with hematological and solid malignancies [4]. HDACIs currently in clinical trials belong to four different chemical classes: hydroxamic acids, cyclic peptides, benzamides, and shortchain fatty acids.

In this study, in view of the anticancer property of the HDACi pharmacophore, a new series of (3,4-dihydro-2h-pyran-2,3-bipyridin-6-yl)methyl4-(2substitutedphenyl carbamoyl)benzyl carbamates have been synthesized.

MATERIALS AND METHODS
To a suspension of 1,1′-CDI in THF was added pyrano pyridin methanol in THF at 10°C and the mixture stirred for 2 h at room temperature. The resulting solution was added to a suspension of 4- (aminomethyl)benzoic acid, DBU and TEA in THF. After stirring for 5h at room temperature, the mixture was evaporated to remove THF and then dissolved in water. The solution was acidified with HCl (pH=5) to precipitate a white solid which was collected by filtration, washed with water and methanol, respectively, and dried to give pyrano pyridin carboxylic acid. To a suspension of pyrano pyridin carboxylic acid in toluene was added DMF and oxalyl chloride and the mixture stirred for 5 h at room temperature. The precipitate was collected by filtration, washed with diethylether and dried. The precipitate was dissolved in THF and was added imidazole in THF. The mixture stirred for 1 h at room temperature. The precipitate was filtrated. The filtrate
was added o-substituted phenylamine and trifluoroacetic acid. The mixture was stirred for 15 h at room temperature. The crude product was purified by column chromatography.

RESULTS AND DISCUSSION
In this study, in view of the anticancer property of these pharmacophores, a new series pyrano pyridinyl carbamate derivatives containing benzamide structure have been synthesized as seen in below Formula. The structural evaluation of the synthesized compounds was based on the $^1$H NMR, Mass and elementary analysis data. We are expecting that compounds can bind the enzymes active site and make the inhibition of HDAC.

CONCLUSIONS
Their docking studies are shown that they have enough interactions for the enzyme inhibition. The synthesized compounds are going to be investigated for their anticancer and HDAC inhibitory activities.

ACKNOWLEDGMENTS
This work was supported by The Scientific and Technological Council of Turkey (TÜBİTAK), Turkey (Project No: 213S097).

REFERENCES

P-241: INVESTIGATION OF ANTICANCER ACTIVITES OF THIAZOLOCOUMARIN–BASED FLUORESCENCE SCHIFF BASE AND ITS Pt(II) COMPLEX ON VARIOUS CANCER CELLS

Ü. ÖZDEMİR ÖZMENa, Ö. ŞAHİNA, Z. KARAGÖZ GENÇb, B. AYDINERa, Z. SEFEROĞLUa, S. TEKİNc

aDepartment of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey
bDepartment of Metallurgy and Materials Engineering, Faculty of Engineering, Adıyaman University Adıyaman, Turkey
cDepartment of Physiology, Faculty of Medicine, İnönü University, Malatya, Turkey

INTRODUCTION
Thiazolocoumarin derivatives are well known biological active compounds. Coumarin derivatives are a class of compounds with biological activity, such as analgesics, anticoagulant, human leukocyte elastase, diuretics, anticancer [1], inhibitor of HIV-1 protease and antibacterial agents. The thiazole nucleus also appears frequently in the structure of various natural products and biologically active compounds, notably thiamine (vitamin-B), antibiotics such as penicillin, micrococcin [2], and many metabolic products of fungi and primitive marine animals.

MATERIALS AND METHODS
Thiazolocoumarin-based fluorescence Schiff base (Fig.1) and its Pt(II) complex (Fig.2) have been synthesized and characterized by using elemental analysis, spectrophotometric methods ($^1$H- $^{13}$C NMR, FT-IR, LCMS). The cytotoxic effects on cancer cells (breast, colon and prostate cancers) of synthesized Schiff base and its Pt(II) complex have been determined through experimental work using MTT [3-(4,5-dimethylthiazol-2-il)difeniltetrazoli umbromid] assay method, that is widely used as an enzymatic test method.

Fig.1. Structure of fluorescence Schiff base
RESULTS AND DISCUSSION

Anti-cancer activity of synthesized fluorescence Schiff base and its Pt complex were evaluated in vitro mode using MTT assay on the human cancer lines MCF-7 (human breast adenocarcinoma), LS174T (human colon carcinoma) and LNCAP (human prostate adenocarcinoma) with 1, 5, 25, 50 μM doses. According to the obtained results, our studies show that Schiff base and Pt(II) complex induced loss of cell proliferation in concentration and time-dependent manner for different cell lines. Schiff base owns greater antitumor activity against MCF-7 and LNCAP whereas Pt(II) complex illustrated more less antitumor activity against LNCAP and LS174T.

REFERENCES


P-242: STUDIES ON THE SYNTHESIS OF COUMARINE-BASED FLUORESCENCE SCHIFF BASES AS ANTICANCER AGENTS AGAINST VARIOUS CANCER CELLS

Ü. ÖZDEMİR ÖZMEN, E. BAKAN, Ö. ŞAHİN, Z. KARAGOZ GENÇ, B. AYDINER, Z. SEFEROGLU, S. TEKIN

Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey
Department of Metallurgy and Materials Engineering, Faculty of Engineering, Adiyaman University, Adiyaman, Turkey
Department of Physiology, Faculty of Medicine, Inonu University, Malatya, Turkey

INTRODUCTION

Coumarin derivatives constitute an important class of heterocyclic compounds with anticoagulant (e.g., warfarin, acenocoumarol) [1] and antibacterial (e.g., novobiocin, clorobiocin) pharmacological properties. On the other hand, the thiophene derivatives were found to possess analgesic and antiinflammatory activities. The cytotoxic activities of coumarin and its known metabolite 7-hydroxycoumarin were tested in several human tumor cell lines. Both compounds inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line, a hepatoma-derived cell line, and a lymphoblastic cell line [2].

In this study the authors report the synthesis, structural determination, and in vitro antitumor activity of the new coumarine-based fluorescence Schiff bases incorporated thiazole moiety.

MATERIALS AND METHODS

Coumarin-based fluorescence Schiff bases (Fig.1 and Fig.2), have been synthesized and characterized by using elemental analysis, spectrophotometric methods (1H, 13C NMR, FT-IR, LCMS). The cytotoxic effects on cancer cells (breast, colon and prostate cancers) of synthesized Schiff bases have been determined through experimental work using MTT [3-(4,5-dimetiltiazol-2-il)difeniltetrazolium bromid] assay method, that is widely used as an enzymatic test method.

RESULTS AND DISCUSSION

In this work, anticancer activities on various cancer cells of (E)-3-(2-((2-hydroxy-4-methoxybenzylidene)amino)thiazol)-4-yl)-2H-chromen-2-one (CTS-1) and (E)-3-(2-((4-diethylamino)-2-hydroxybenzylidene)thiazol)-4-yl)-2H-chromen-2-one (CTS-2) have been investigated for the first time. In vitro anticancer activity of the synthesized compounds (CTS-1 and CTS-2) were evaluated against the human cancer lines MCF-7 (human breast adenocarcinoma), LS174T (human colon carcinoma) and LNCAP (human prostate adenocarcinoma) using MTT assay with 1, 5, 25, 50 μM doses. According to the results, it was observed that Schiff base CTS-1 illustrated temperate to anticancer activity against MCF-7 cell lines, whereas Schiff base CTS-2 showed broad spectrum anticancer activity all of the cancer types that used in this work.
REFERENCES
This work is supported by TUBITAK 214Z152

P-243: CARBONIC ANHYDRASE I (HCA I) INHIBITOR: DETERMINATION OF INHIBITION EFFECT OF BIS(FURAN-2-ALDEHYDEMETHANESULFONYLHYDRAZONE) COPPER(II) CHLORIDE BY SPECTROPHOTOMETRIC METHOD

A. Balaban Gündüzalp1, G. Parlakgümüş1, Ü. Özdemir Özmen1, N. Özbek2
1 Gazi Üniversitesi Fen Fakültesi Kimya Bölümü, ANKARA,
2 Ahievran Üniversitesi Eğitim Fakültesi Fen Bilgisi Öğretmenliği ABD, KİRŞEHİR

INTRODUCTION
The carbonic anhydrases (CAs) are the metalloenzymes containing zinc center which classically participate in the maintenance of pH homeostasis. CAs catalyze the reversible hydration of carbon dioxide in a two-step reaction to yield bicarbonate and protons. Inhibitors of the carbonic anhydrase (CA) enzymes have clinical usage of major diseases such as glaucoma, epilepsy, gastroduodenal ulcers, acid-base disequilibria and neurological disorders [1]. The synthesis of CA inhibitors from various classes of sulfonamides (such as sulfonylhydrazone, sulfonylhydrazone, sulfonylurea etc.) have importance of specificity towards isoenzymes and affect biologic systems by interacting with enzyme active sites [2]. The inhibition effect of bis(furan-2-aldehyde methanesulfonylhydrazone)copper(II) chloride against hCA I was investigated by spectrophotometric method. The activity parameters (Km, IC50 and Ki) were calculated by Lineweaver-Burk graph, activity % - [inhibitor] graph and Cheng-Prusoff equation.

RESULTS AND DISCUSSION
The inhibition effect of bis(furan-2-aldehydemethanesulfonylhydrazone)copper (II) chloride against HCA I was investigated by spectrophotometric method and its inhibition parameter (IC50) was found as 7.91×10⁻⁵ M. It shows that Cu(II) complex (Fig.1) has good inhibition activity against hCA I isoenzyme. This is probably due to the further effect of the electronegative groups (such as furan, NH, SO₂ etc.) that may increase the interaction of sulfonylhydrazone Cu(II) complex with enzyme active sites.

Fig.1.Structure of Cu(II) complex

REFERENCES
anhydrase species. Methanesulfonamides \((\text{CH}_3\text{SO}_2\text{NH}_2)\) are also used in drug industry because of their biological activity on a large scale. In our previous studies, we reported the antibacterial and cytotoxic effect of methanesulfonic acid hydrazide \((\text{H}_2\text{N}-\text{NH}-\text{SO}_2-\text{CH}_3)\) and its hydrazone derivatives, as well as its metal carbonyl complexes. Furthermore, propane sulfonic acid hydrazide \((\text{H}_2\text{N}-\text{NH}-\text{SO}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3)\) and its aromatic/heteroaromatic propanesulfonylhydrazine derivatives were investigated for inhibitory effects on carbonic anhydrase II (CA II) enzyme [2].

**MATERIALS AND METHODS**

In this study, two aromatic butane sulfonylhydrazine derivatives; 5-chlorosalicylaldehydebutanesulfonylhydrazone (Compound 1) and 2-hydroxy-5-chloroacetophenonebutanesulfonylhydrazon (Compound 2), have been synthesized and characterized by using elemental analysis, FT-IR, \(^1\)H-NMR, \(^{13}\)C-NMR, LC/MS techniques.

**RESULTS AND DISCUSSION**

The structure of 2-hydroxy-5-chloroacetophenonebutanesulfonylhydrazon (Comp. 2) was supported by X-ray crystal diffraction studies (Figure. 1). The crystal structure contains intramolecular hydrogen bonds which play an important role in stabilization.

![Fig.1. The molecular structure of Compound 2](image)

In \(^1\)H-NMR spectra of compound 1 and 2; NH protons are observed at 10.40 and 10.69 ppm. Azomethine (HC=N) proton of compound 1 exhibits at 8.18 ppm and CH\(_2\)C=N protons of compound 2 exhibit at 2.28 ppm. In FT-IR spectra of synthesized compounds, bands in the region of 3153 ± 3230 cm\(^{-1}\) may be due to \((\text{NH})\) stretching vibration. The most characteristic absorptions at 1605, 1618 cm\(^{-1}\) are assigned to the stretching vibration of the azomethine (C=N) group for compounds 1, 2.

**REFERENCES**


mmol) in acetone for 7 h. After TLC screening, the mixture was filtered and the obtained crude product was washed with water, then crystallized from ethanol. **ACHE and BuChE inhibitory activity:** All compounds were subjected to a slightly modified method of Ellman's test [3,4] in order to evaluate their potency to inhibit the AChE and BuChE. Enzyme solutions were prepared in gelatine solution (1%), at a concentration of 2.5 units/mL. AChE/BuChE and compound solution (50 μL) which is prepared in 2% DMSO at 0.1 and 1 mM concentrations were added to 3.0 mL phosphate buffer (pH 8.0) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB (50 μL) and ATC (10 μL) to the enzyme-inhibitor mixture. The production of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading was performed with an identical solution of the enzyme without the inhibitor. The inhibition of cell proliferation was calculated as follows: (1 - treatment / vehicle control) x 100.

**RESULTS AND DISCUSSION**

The chemical structures of the compounds (C1-C26) were confirmed by IR, 1H-NMR, MS spectral data and elemental analyses. In the IR spectra significant stretching bands were observed at expected regions. In the 1H-NMR spectra, all of the aromatic and aliphatic protons were observed at estimated areas. M+1 peaks in Ms spectra agreed well with the calculated molecular weight of the compounds. All compounds gave satisfactory elemental analysis results. The anticholinesterase effects of the compounds (C1-C26) on AChE and BuChE were determined by a modification of Ellman's spectrophotometric method. Among the tested compounds C13 can be identified as the most promising anticholinesterase agent due to its inhibitory effect on AChE.

**CONCLUSIONS**

Consequently synthesis and anticholinesterase activity determination of some novel piperazinedithiocarbamate derivatives were reported in this study. Activity screening results point out that tested compounds have inhibitory potency against cholinesterase enzymes.

**REFERENCES**


# P-246: ANTICANCER ACTIVITIES OF PINE AND OREGANO HONEYS

**Y. Sıçak**, M. Öztürk, A. Çatak, A. Şahin-Yağlıkolu

1. Department of Chemistry, Faculty of Sciences, Muğla Sıtka Koçman University, Muğla, Turkey
2. Department of Biology, Faculty of Sciences, Muğla Sıtka Koçman University, Muğla, Turkey
3. Department of Chemistry, Faculty of Sciences, Çankırı Karatekin University, Çankırı, Turkey

**INTRODUCTION**

After the cardiovascular diseases, cancer has the second death rate in the world. Cervical cancer is one of the leading causes of cancer death in women worldwide (1) is a largely preventable disease. In our country, 38% of the people die from cardiovascular disease and 29% from cancer diseases. In developed countries, one of the most common types of cancer is gynecological cancer. Another most common cancer among the cancer derivatives is the female reproductive system cancer. About 500 thousand women contract to the cervix (cervical) cancer, every year and almost half of them die according to WHO reports. Recently, anticancer drugs have used in the treatment, and they are very effective against certain tumors. But, the usage of some of them is restricted due to acquired resistance of cancer cells. Therefore, more effective new anticancer drugs are necessary to be developed. Honey has been used to treat some diseases since ancient times. Recent studies indicate that it has many pharmacological properties (2, 3, 4). We aimed to study the cytotoxic activity of the pine and oregano honeys and their fractions.

**MATERIALS AND METHODS**

All chemicals and solvents were in analytical grade and purchased from Sigma-Aldrich, Merck and Roche. **Honey Samples:** Honey Samples which were produced for us were obtained from MAY-BIR. **Anticancer Activity Assay:** Honey samples were evaluated in vitro antiproliferative activities against HeLa and C6 cells using the BrdU ELISA assay (5, 6). 5-fluorouracil (5-FU) and cisplatin were used as positive controls. Measurements were measured in ELISA reader at 450 nm. The inhibition of cell proliferation was calculated as follows: (1 - treatments /vehicle control) x100.
RESULTS AND DISCUSSION
Anti-proliferative effects of pine and oregano honeys were investigated on HeLa and C6 cell lines using proliferation with BrdU ELISA assay. The activity was dose dependents manner. The IC50 values were found between 50 and 100 μg/mL.

CONCLUSIONS
The results indicated that the oregano honey possessed more cytotoxic activity than that of pine honey. The n-butanol extracts have more potential on Hela cell lines. None was comparable with that of anticancer drugs. However, honey is a food and non-toxic to fibroblast cancer lines.

REFERENCES

P-247: MOLECULAR DOCKING STUDIES OF NOVEL INDO LIN-2-ONE DERIVATIVES AS VEGFR-2 KINASE INHIBITORS

Z. Kılıç-Kurt1, S. Öngen2

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY
İstanbul Kemerburgaz University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, TURKEY

INTRODUCTION
Vascular endothelial growth factor receptor-2 (VEGFR-2), is a major target in suppressing tumor growth and metastasis because of its crucial role in the regulation of angiogenesis [1]. The inhibition of VEGFR-2 signaling by small molecule inhibitors has been shown to reduce angiogenesis and suppress tumor growth. Therefore, inhibition of the VEGFR-2 has become an attractive strategy in the treatment of cancers [2]. Among the VEGFR-2 inhibitors, indolin-2-one scaffold has been extensively studied and the structure-activity relationships (SAR) are described. In the present work, to develop VEGFR-2 kinase inhibitors novel indolin-2-one derivatives (Figure 1) were designed and the binding modes of compounds into the active site of VEGFR-2 kinase were explored by docking studies.

Fig. 1. General structure representing series of designed indolin-2-one derivatives.

MATERIALS AND METHODS
AutoDock Vina and AutoDock Tools (ADT) package version 1.5.6rc3 were used for docking calculations. From the crystal structure of VEGFR-2 tyrosine kinase in complex with Sunitinib (PDB entry 4AGD), the ligand and all the crystallization waters molecules were removed and polar hydrogens were added to the protein saving the file in pdbqt extension. Grid points of 20x20x20 with a spacing of 1 Å were created. 2D structures of designed compounds were established by using ChemBioDrawUltra 11.0, then they were energetically minimized with ChemBioDrawUltra 11.0 and saved in mol2 format. The rigid root and rotatable bonds of compounds were defined and saved in pdbqt extension. The docking poses for each compound were analyzed by examining their binding free energy score and hydrogen bonding interaction.

RESULTS AND DISCUSSION
In this study, binding affinities of 45 novel designed indolin-2-one derivatives into the active site of VEGFR-2 kinase were evaluated by the docking studies. The validation of the scoring function implemented in Autodock vina was done by docking the native ligand (Sunitinib) into its binding site. Figure 2 illustrates that the docked Sunitinib is superimposed almost exactly on the native ligand with obtained binding free energy (AGb : -9.6 kcal/mol). The RMSD of docked ligand (Sunitinib) was found as 0.585Å. The docked Sunitinib exhibited one hydrogen bond between carbonyl of indolin-2-one moiety and backbone nitrogen of Cys919, whereas the native ligand exhibited two hydrogen bonds with Cys919 and Glu917 (Figure 2).
Docking studies of designed compounds showed that they exhibited favorable binding interactions with active site of VEGFR-2. The indolin-2-one group of compounds formed one or two hydrogen bond interaction with the key residues, Cys919 and Gly917 in the ATP-binding pocket of VEGFR-2 kinase. In addition, all molecules in the test series located in similar position to the Sunitinib with overlapping the side chains at C-3 position of indole ring of compounds and Sunitinib (Figure 3).

CONCLUSIONS
In conclusion, the molecular docking simulations demonstrated that all of compounds located into the active site of VEGFR-2 and showed binding modes like Sunitinib by forming same H-bond interactions with backbone amino acid residues of VEGFR-2. In further, these compounds will be synthesized and tested inhibitory activity against VEGFR-2 kinase. Results of biological activity will support us to evaluate the relationship between the VEGFR-2 inhibitory activity of designed compounds and the binding affinities predicted by Autodock vina.

REFERENCES

P-248: SYNTHESIS OF THIOSEMICARBAZONE DERIVATIVES AS NEW ANTICANCER AGENTS AGAINST A549 HUMAN LUNG ADENOCARCINOMA CELLS

Z.A. Kaplancıklı¹, Özlem Atlı², M.D. Altıntop¹, Sinem Ilgın²

Anadolu University, Faculty of Pharmacy,
¹Department of Pharmaceutical Chemistry, ²Department of Pharmaceutical Toxicology
Eskişehir, TURKEY

INTRODUCTION
In recent years, the development of resistance to anticancer drugs has emerged as a major obstacle in the fight against cancer and therefore medicinal chemists have carried out considerable research for the development of new effective anticancer agents with enhanced activity and limited toxicity [1,2].

In an effort to develop potent anticancer agents, herein we described the synthesis of a new series of thiosemicarbazone derivatives and evaluated their cytotoxic effects against A549 human lung adenocarcinoma and NIH/3T3 mouse embryonic fibroblast cell line.

MATERIALS AND METHODS
Initially, 4-(naphthalen-1-yl)thiosemicarbazide was synthesized via the reaction of 1-naphthyl isothiocyanate with hydrazine hydrate. The reaction of 4-(naphthalen-1-yl)thiosemicarbazide with fluoro-substituted aromatic aldehydes afforded new thiosemicarbazone derivatives. The chemical structures of the compounds were elucidated by ¹H-NMR, ¹³C-NMR and mass spectral data and elemental analyses.

XTT assay, which measures mitochondrial activity, was performed to determine the IC₅₀ values of the compounds against A549 cell lines. An ideal anticancer agent should kill cancer cells selectively without causing any toxicity on healthy cells [3]. For this purpose, the cytotoxic effects of the compounds on NIH/3T3 cell lines were also investigated.

RESULTS AND DISCUSSION
According to XTT assay, compounds 2, 8, and 10 exhibited the highest anticancer activity against A549 cell line without showing any cytotoxicity against healthy cells (Table 1). Particularly, 2,4-difluoro substituted compound 2 was found to be the most effective and selective anticancer agent with the IC₅₀ value of 31.25 µg/mL, which is very similar to the IC₅₀ of cisplatin, the positive control.
Table 1. IC₅₀ values of the compounds against cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µg/mL)</th>
<th>NIH/3T3 cell line</th>
<th>A549 cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt; 500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt; 500</td>
<td>31.25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt; 500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.25</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&gt; 500</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>&gt; 500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>500</td>
<td>51.30</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&gt; 500</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&gt; 500</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>288.96</td>
<td>16.28</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS
In the present study, compound 2 was found to be the most promising anticancer drug candidate. Further studies may be carried out to evaluate the anticancer activity of compound 2 in animal models.

ACKNOWLEDGMENTS
This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1210S165.

REFERENCES

P-249: SYNTHESIS AND INVESTIGATION OF AROMATASE INHIBITION/ ANTICANCER EFFECTS OF NEWLY SYNTHESIZED THIAZOLEs

Z. Sahin1, M. Ertas1, B. Berk1, L. Yurttas2, S. Demirayak1

1Istanbul Medipol University, School of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, TURKEY
2Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Eskisehir, TURKEY

INTRODUCTION
Now a days studies over breast cancer treatment involve non-steroidal inhibiton of aromatase enzyme instead of steroidal therapy. The reason for that drawback is prolonged effects of steroidal agents since the enzyme is inactivated even after the drug is cleared from the circulation. Resumption of oestrogen production depends on the synthesis of new aromatase molecules [1]. After the release of “Crystal structure of human placental aromatase cytochrome P450 in complex with androstenedione” (3EQM), it became a possibility to develop selective drugs towards the cytochrome P450 in aromatase, permitting more specific inhibition. Moreover, supporting data presented that adding an extra central 1,3-thiazole ring structure to 3,5,4'-trihydroxy-trans-stilbene structure enhances 6000 times aromatase inhibitory activity [2]. Therefore, we planned to synthesise 4-phenol-2-pyridinylthiazole derivatives for inhibiting aromatase cytochrome P450 active site and examine interactions by using molecular modelling techniques. Furthermore the compounds aromatase inhibition levels and IC₅₀ values were determined by fluorescence based techniques and XTT experiments on different cancer lines.

MATERIALS AND METHODS
Molecular Modelling: Subsequently protein, ligand preparations and dockings were carried out by using Schrodinger Maestro Suite’ protocols based on the crystal structure of 3EQM. Compound poses with the most negative e-model scores, which consider hydrophobic parameters extensively, were selected, analyzed visually and evaluated.

Synthesis: The general synthesis of 4-phenol-2-pyridinylthiazole derivatives are shown in Scheme 1. Purifications of the compounds were succeed with crystallization from ethanol and chromatographic techniques. The structures of the final compounds were evaluated by IR, ¹H/¹³C NMR and Mass spectroscopy techniques.
Scheme 1. General synthesis of the proposed compounds.

**Biological Evaluation:** To evaluate the inhibitory activities of the compounds, the fluorescent intensity which was formed by fluoresceine molecule hydrolyzed by aromatase enzyme were measured. Briefly, the test samples of 100-10 μg/mL concentrations were pre-incubated with a NADPH regenerating system for 10 min at 37 °C. Then the enzyme and substrate mixture were added to incubate for an additional 2 hours at 37 °C. Finally, the fluorescence of reaction solvent was measured at 485 and 530 nm. According to dose-reply curve IC50 values were calculated [3].

The XTT assay were performed by multiple parameter with appropriate concentrations and non-steroidal inhibitors as standards. IC50 values were calculated using non-linear regression.

**RESULTS AND DISCUSSION**

IR, 1H/13C-NMR data of the derivatives are clear and consisted with expectations and mass data showed M+ peaks convenient for all compounds. Hydroxyl bands for phenol structures were plainly seen between 3600-3200 cm⁻¹ at IR spectra where the 1H-NMR shifts of these groups differ between 9.55-11.4 ppm according to their substitution positions. The only H of the central thiazole were found as singlet between 8.0-8.75 ppm. The other aromatic H’s of both phenol and pyridine rings were stated between 6.9-8.9 ppm. 13C NMR shifts of phenol ring, thiazole and pyridine ring system were existed at 116-129, 162-191 and 150-155 ppm respectively.

Biological evaluation of the compounds showed promising results

**CONCLUSIONS**

Clearly studies on evaluating non-steroidal agents instead of steroidal therapy are more beneficial in clinical perspective which in this study we stated that 4-phenol-2-pyridinylthiazole derivatives are options. The interactions between the biological active molecules and the docking site we obtained from that study will sure contribute to both our future and other researchers studies.

**REFERENCES**

for inhibition of cell viability by this class of compounds.

Scheme 1. Novel indole-benzimidazole derivatives

CONCLUSIONS
In summary, a new series of indole-benzimidazoles have been successfully synthesized starting from o-phenylenediamines and substitute indole-3-carboxaldehydes. The in vitro anticancer activity analyses of the compounds 6 and 9 at higher concentrations are in progress.

ACKNOWLEDGMENTS
Central Lab. of Pharmacy Faculty of Ankara University provided support for acquisition of the NMR, Mass spectrometer, and elemental analyzer used in this work.

REFERENCES
1. Ates-Alagoz, Z.; Kus, C.; Coban, T. Synthesis and antioxidant properties of some novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments, Journal of Enzym Inhibition and Medicinal Chemistry, 2005, 20(4); 325-331.

RESULTS AND DISCUSSION
Nine different compounds were synthesized and tested for their effects on cell viability using MTT assays on MCF7 cells grown in 96 well plates (5000 cells/well) at different drug concentrations (0.25-40μM). Camptothecin was used as a positive control (0.25 and 2μM) while there was a DMSO group as the negative control for each concentration. BIO-TEK/μQuant – Universal Microplate Spectrophotometer and BIO-TEK/KC junior software (v.1.418) were used to perform the MTT assay. GraphPad Prism (v. 6.05) was used to analyze and plot the viability data. Our results showed that at least two of the compounds exhibited significant reductions on cell viability in MCF7 cells. These two compounds were then exposed to a wider range of drug concentrations in MCF7 as well as MDA-MB-231 cell lines. The cytotoxic effects of these compounds were thus validated. Furthermore, our findings suggested that there could be cell-dependent differences in the anticancer activity of these compounds.
CONCLUSIONS
Anticancer activity testing revealed that at least two of the compounds synthesized exhibited significant anticancer activity against MDA-MB-231 and MCF-7 breast cancer cell lines. In order to improve the anticancer potency, chemical synthesis of additional indole-benzimidazole derivatives are warranted.

ACKNOWLEDGMENTS
This work was supported by TUBİTAK-SBAG Research Project: 213S037

REFERENCES
2. Ates-Alagoz, Z.; Kus, C.; Coban, T. Synthesis and antioxidant properties of some novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments, *Journal of Enzym Inhibition and Medicinal Chemistry*, **2005**, 20(4); 325-331.

P-252: SYNTHESIS, STRUCTURE IDENTIFICATION, AND BIOLOGICAL EVALUATION OF NOVEL INDOLE-ALKYL SULFONYL BENZIMIDAZOLES

F. Zengin, Z. Ates-Alagoz
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara-Turkey

INTRODUCTION
Treatment of cancers has been a major endeavor; thus discovering novel agents that can selectively kill tumor cells or inhibit their proliferation without general toxicity remains a current research focus. In this study, novel indole alkyl sulfonyl benzimidazole derivatives were designed and synthesized as anticancer agents.

MATERIALS AND METHODS
Syntheses of the compounds were carried out starting from commercially available 1-Chloro-4-methanesulfonyl-benzene. This was followed by reaction with conc. H₂SO₄ and potassium nitrate to give nitro intermediates (1). Nucleophilic displacement of the chloro group with several amines in N,N-dimethylformamide, and their reduction with hydrogen gas by using palladium carbon and condensation of these derivatives with appropriate indole carboxaldehydes gave the targeted indole-benzimidazoles (2) (scheme 1). Purity control and structural elucidation were controlled by using elemental analyser and ¹H, ¹³C-NMR, Mass spectrometers, respectively.

RESULTS AND DISCUSSION
Several indole alkyl sulfonyl benzimidazoles were designed and synthesized as anticancer agents. Syntheses of the compounds were carried out starting from commercially available aryl sulfones followed by a series of steps including alkylation, nucleophilic displacement, reduction by hydrogen gas and condensation of the derivatives with appropriate indole carboxaldehydes to obtain the targeted indole-benzimidazoles.

Scheme 1. Novel indole-benzimidazole derivatives

CONCLUSIONS
Future studies will include testing anticancer activities of the synthesized compounds using transcriptome analysis in vitro and zebrafish embryonic toxicity and cell proliferation/apoptosis assays in vivo. In order to improve the anticancer potency, chemical synthesis of additional indole-benzimidazole derivatives are warranted.

ACKNOWLEDGMENTS
This work was supported by TUBİTAK-SBAG Research Project: 213S037

REFERENCES
2. Ates-Alagoz, Z.; Kus, C.; Coban, T. Synthesis and antioxidant properties of some novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments, *Journal of Enzym Inhibition and Medicinal Chemistry*, **2005**, 20(4); 325-331.

P-253: BIOLOGICAL ACTIVITY OF NEW CHOLINESTERASE INHIBITORS FOR ALZHEIMER’S DISEASE

Z. Kasap¹, K.O. Yerdelen¹, M. Koca¹, E. Karabiber¹, B.Anıl²
Ataturk University
¹Faculty of Pharmacy, Department of Pharmaceutical Chemistry, ²Faculty of Science, Department of Organic Chemistry, Erzurum, TURKEY
Several new compounds have been designed, synthesized and evaluated as the acetyl- and butyrylcholinesterase inhibitors for Alzheimer’s disease. The enzyme inhibitory activity of the synthesized compounds was measured using Ellman’s method. To better understand the enzyme-inhibitor interaction of the most active compounds towards cholinesterase enzymes, molecular modelling studies were carried out on high-resolution crystallographic structures.

**INTRODUCTION**

Alzheimer’s disease (AD), is a neurodegenerative disorder that attacks the brain’s nerve cells, and can cause symptoms like loss of memory, learning and language skills [1]. The progression of the AD are still mostly unknown, but the most favorite hypothesis has been put forward on the basis of the various causative factors such as cholinergic, amyloid, tau and metal hypothesis [2].

In this study we aimed the development of more active and selective dual cholinesterase inhibitors which are capable of interacting with the active sites of both ChEs. A new series of oxalamide derivatives was synthesized and evaluated their abilities of cholinesterase enzyme inhibition.

**MATERIALS AND METHODS**

*Synthesis of oxalamide compounds (1-15):* A mixture of the para-substituted benzaniline (1.3 mmol), triethylamine (1.3 mmol) and oxalyl chloride (0.66 mmol) in tetrahydrofuran (5 ml) was stirred at room temperature for 12h. The crude product was purified by column chromatography on silica gel with hexane:ethyl acetate (7:3) mobile phase. The obtained solid was recrystallized from EtOH.

*Cholinesterase inhibitory activity:* The inhibitory activity of the compounds 1-15 against AChE and BuChE was measured according to the colorimetric assay of Ellman [3]. Neostigmine and ambenonium were used as the reference compounds.

*Molecular modeling:* Molecular modeling studies were performed to investigate possible interactions between the most active compounds 11 with AChE, and 13 with BuChE. The molecular modeling was performed with the docking program Sybyl X 2.0. The structures were minimized using the Steepest descent conjugated gradient method until the gradient was 0.001 kcal/mol, max iterations: 1000 with the Tripos force field with the Gasteiger Huckel charge. The simulation system was built on the crystal structures of 1ACJ and 1P0I which were obtained from the Protein Data Bank.

**RESULTS AND DISCUSSION**

Oxalamide derivatives (1-15) were synthesized with moderate to good yields (26–70%). Compounds 10-15 showed good inhibitory activity against BuChE with micromolar concentrations (IC$_{50}$= 1.19-9.33 µM). Among the oxalamide compounds, compound 13 (IC$_{50}$= 1.19 µM) showed the most potent inhibitory activity for BuChE, being 3.66-, and 3.87- fold stronger than the reference compounds neostigmine (IC$_{50}$= 4.36 µM) and ambenonium (IC$_{50}$= 4.61 µM), respectively. On the other hand, compounds 6-15 were found to have a moderate inhibitory activity on AChE, with IC$_{50}$ values of 8.31-87.09 µM. Compound 11 showed the strongest inhibition against AChE with IC$_{50}$ value of 8.31 µM (Fig. 1).

**CONCLUSIONS**

Fifteen new compounds have been synthesized and evaluated their cholinesterase inhibitory activity. The obtained inhibition data revealed that the oxalamide compounds generally show a moderate to high anticholinesterase activity.

**ACKNOWLEDGMENTS**

This research work was supported by Ataturk University Research Fund (Project No: 2014/167), Turkey.

**REFERENCES**


**P-254: SYNTHESIS AND CYTOTOXIC ACTIVITY OF SOME N-SUBSTITUTED-5-CHLORO-2(3H)-BENZOXAZOLONE DERIVATIVES**

Zeynep Soyer1, Şirin Uysal2, Yalçın Erzurumlu2, Petek Ballar Kırımzıbayrak2
INTRODUCTION
Cancer is presently a major health problem and an important cause of mortality and morbidity worldwide. Cancer is a disease characterized by uncontrolled cell growth and invasion of tissues and organs. Various chemotherapeutic drugs have been developed to treat cancer, but most of them have systemic toxicity and many side effects. Thus, there is a need for development of new safer and effective anticancer drugs. 2\((3H)\)-benzoxazolone, as one of the most important heterocyclic rings, exhibits a wide range of biological activities such as anticancer, anti-HIV, analgesic, anti-inflammatory, antinociceptive, antimicrobial, anticonvulsant, antimalarial and human leukocyte MPO chlorinating inhibitor activity [1-3]. In this study, a series of N-substituted-5-chloro-2\((3H)\)-benzoxazolone derivatives were synthesized and evaluated for cytotoxic activity against 293T, HeLa and U20S cell lines.

MATERIALS AND METHODS
The target compounds (Figure 1) were synthesized by Mannich reaction of 5-chloro-2\((3H)\)-benzoxazolone with the appropriated amines. The cytotoxic activities of the title compounds were determined by WST-1 cell proliferation assay.

RESULTS AND DISCUSSION
In this study, eight N-substituted-5-chloro-2\((3H)\)-benzoxazolone derivatives have been synthesized to evaluate cytotoxic activity. The structures of the synthesized compounds were confirmed by spectral IR, \(^1\)H NMR, and APCI-MS analysis. The preliminary screening results indicated that majority of the compounds 5-chloro-2\((3H)\)-benzoxazolone scaffold demonstrated cytotoxic effects depending on the structural differences.

REFERENCES

P-255: CURRENT TURKISH PHARMACY EDUCATION WITHIN BOLOGNA PROCESS
B. Sözen Şahnel, E. Uluta, Z. Çalışg, S. Yeşenoglölu

INTRODUCTION
For globalizing the workforce and education, various changes are made in many areas in Turkey as in all over the world. Within this scope some changes are being made in higher education. Bologna process is one of them. This Process, starting within the scope of the European Union and having 47 members including Turkey, in which updating are carried out in the training program in order to provide international roaming. Pharmacy faculties in Turkey are trying to optimize their training program according to this Process.

Within the scope of this study, the websites of pharmacy faculties in Turkey are examined and will be attempt to demonstrate the current situation in the Bologna Process is demonstrated.

MATERIALS AND METHODS
The websites of pharmacy faculties providing education in Turkey are examined in terms of the data towards training on site, notably the Course Catalog/Information Package and Academic Program of those pharmacy faculties.

RESULTS AND DISCUSSION
There are 24 pharmacy faculties, 18 of which are state university and 6 of which are private university, providing undergraduate education by the year 2014 in Turkey. All of these faculties have website. There are contact information in mentioned every website. When these sites are examined in terms of availability of Course Catalog/Information Package and Academic Program due to the Bologna Process, different
information is accessed. Only 10 of the 24 faculties of the web site have details of the Course Catalog/Information Package. These information of some faculties can be accessed through the website of their universities. This is noteworthy that these packages have less accessibility on the website of faculties because they were originally prepared in order to provide international roaming. The learning outcomes such as the objectives, lecture contents and loads of program desired the scope of the Bologna Process are required being available in the lecture of the package contents which can be reached. It is seen that the information of the lecture’s title and credit is available but these information such as learning outcomes and the programs of lecture aren’t included in examined packages and all lecture programs. It is thought that when users reviewing Website access entire information, they achieve accurate information about the topic.

CONCLUSIONS

Nowadays, when the accessibility of information gets increasingly easier, accessing information by especially education has great importance. The information provided by educational institutions through the Internet is in a separate place. In this context, we propose that the pharmacy faculties must prepare carefully and diligently the content of their web site.

P-256: ACADEMIC PHARMACY RELATED EMPLOYMENT PROBLEMS

B. Sözen Şahne1, S. Şar2, Z. Çalışan1, M. Arslan2, S. Yeğenoğlu3

1Hacettepe University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara, TURKEY
2Ankara University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION

The imbalances in the distribution of workforce bring many problems despite the growing working area around the world. It’s known that similar problems exist in our country. The employment problems are increasing in the field of academic pharmacy too. In this study, the current employment situation of Turkish Pharmacy Faculties is examined and the reasons of undesired employment level in faculties will be discussed.

MATERIALS AND METHODS

A literature survey was made for examining similar studies and researching pharmacist academic workforce. In the light of the literature, an evaluation was made in order to put forward the current situation.

RESULTS AND DISCUSSION

Pharmacy is a popular profession in our country as well as around the world. There are approximately 27,000 pharmacist at different areas in Turkey. In 2003-2004, 11 pharmacy faculties provided education for training pharmacists but in 2014-2015 the number of the faculties increased to 24. In this context, the number of pharmacy students and graduates between the years of 2007-2013 increased (1). However, there are huge differences in the sufficient number of academic staff among the pharmacy faculties. These differences are remarkable on the departments. In accordance with this information, the ratio of students per academic staff increased from 12 in 2003 to 14.7 in 2013 (2).

Despite the increasing number of students, the number of academic staff didn’t change and the workload of these staff gets heavier day by day. There are several reasons for the low rates of academic staff. One of this is the limitation of the academic member to open postgraduate programs. Moreover low work satisfaction levels, unsatisfactory wages and high work stress are some of the other reasons.

CONCLUSIONS

Increasing the quality of pharmacy services is closely related with the quality of pharmacy education. Therefore, supporting the academic staff has a great importance. For improving the current situation and increasing the number of academic staff, bringing innovations, provide employment is necessary to raise the work satisfaction. Besides, the advantages of being an academic member must be introduce to graduate students. Thus, the need of faculties of qualified persons will be provided.

REFERENCES


P-257: OVERVIEW OF BUSINESSES ISSUES OF PHARMACIES THROUGH BORNOVA DISTRICT CASE

B. Kran1, E.G. Taşkaran2, S. Şar3

1,2Ege University, Faculty of Pharmacy, Department of Pharmacy Management, İzmir TURKEY
3Ankara University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara TURKEY
INTRODUCTION
Current health policies, applications of Social Security Institution (SSI), changing practices of stakeholders in pharmaceutical manufacturers and distribution channels directly affect work conditions and practices of pharmacists. This study was planned to be determined working conditions of pharmacists, business problems they faced, reflection of health policies and practices to pharmacy in Bornova district in Izmir.

MATERIALS AND METHODS
It is a cross-sectional, descriptive study. Population includes pharmacists in Bornova district in Izmir. A questionnaire containing questions about socio-demographic characteristics, working conditions and occupational problems of pharmacists was applied on a voluntary basis to all pharmacists in Bornova district between 5 April to 30 June 2013, without selecting the sample. The questionnaire was answered by 75 pharmacists with self-report. In this context, survey's comprehensiveness of pharmacists in Bornova district in Izmir was 52.8%. Data is assessed by frequency and percentage distributions using SPSS18 software package.

RESULTS AND DISCUSSION
Of pharmacies participating in survey, 50.6% were neighborhood pharmacies, 73.4% were female and 26.6% were male pharmacists. 57.4% of pharmacists said that they were not satisfied with working hours of pharmacies, and this time was too long. In a study conducted by the Turkish Pharmacists' Association, it was determined that majority of pharmacists work 11-13 hours a day. It is noted that it is 1/3 more than 9 hours a day adopted as a legal working hours in our country (1). 94.7% of pharmacists participating in the study reported that they had economic difficulties because of not being met by the companies the damage caused by decrease in drug prices, decrease in profit rates of pharmacists, and increased costs unlike the income. In a study conducted in 2011, it has been determined that 75.2% of pharmacists had experienced financing problems for their pharmacies (2). In a study conducted in 2004 in Ankara, 61% of pharmacists stated that economic issues are the most important issue of profession (3). 60% of pharmacists thought that decline in profit margins and drug prices and so on, adverse developments push pharmacists the behaviors that leads to unfair competition. In a study conducted in Ankara, professional reputation, professional ethics and deontological problems are in second place among all issues related to profession stated by pharmacists (3). 54.7% of pharmacists participating in the study stated that Pharmacy Act No. 6308 amending the Law No. 6643 did not provide solutions to current problems of profession, 85.3% of pharmacists said that changing of general communique on healthcare applications too often caused problems in professional practice, 89.4% of pharmacists said that there are too much price difference in prescriptions and the collection of these fees are caused problems with patients. In a study conducted in Turkey wide in 2011, it was determined that changing of health policy and applications frequently is the most common problem in pharmacy applications (2).

CONCLUSIONS
It was observed that more than half of pharmacist in Bornova district in Izmir were not satisfied with the working hours, and approximately 9 of 10 had problems in pharmacy financing and professional practice as a result of health policy and practice. In this context, it is thought that effective participation of pharmacy professional organizations as stakeholders to the regulatory processes affecting pharmaceutical applications will be beneficial.

REFERENCES

P-258: STOCK CONTROL MANAGEMENT IN PUBLIC HOSPITAL PHARMACIES
F.Tarkan, G. Gülpinar, M.B. Uzun, G. Özçelikay
Ankara University Faculty of Pharmacy, Pharmacy Management Department, Ankara, Turkey

INTRODUCTION
Hospital pharmacy defined as the department which provide drug, information and counseling to inpatients and outpatients, , health professionals and other health care employees in hospital. At the same time Hospital pharmacy manage the procurement, storage, preservation, packaging, sterilization, compounding, preparation, dispensing or distribution of medicine in the hospital [1]. Unlike other service providers, health institutions have to carry all equipment and supplies for medical treatment as it is ready to use at any time. Hospital pharmacies usually stock a larger range and amount of medications, including more specialized medications, than would be feasible in the community setting [2]. The smallest error that will occur in drug storage process can cause complications which can result even
to death. Therefore, stock control management in hospital pharmacies is an important issue that needs to be addressed well [3]. The purpose of this study is to determine stock control methods used in the public hospital pharmacies in Ankara, Turkey.

MATERIALS AND METHODS
Materials of this study consists of the questionnaires which are applied to responsible pharmacist of the public hospitals pharmacies. Survey forms have been prepared considering the literature and pharmaceutical applications. The questionnaire was applied to 11 chief pharmacists of public hospitals in Ankara with face to face interviews. The population of the study constitutes all of the pharmacies of public hospitals in Ankara. The obtained data evaluated with SPSS version 16.0.

RESULTS AND DISCUSSION
90.9% of surveyed pharmacists uses spreadsheets method as a stock control method in the hospital. When determining the stock policies, surveyed pharmacists are mostly affected by the cost of stock keeping as 90.9. 63.6% of surveyed pharmacists working with high stock amount for preventing the workflow interruption. 54.5% of the pharmacists surveyed believed they stored the material in pharmacy appropriate and sufficient conditions. 54.5% of the pharmacists surveyed believed they have an efficient stock in policy their pharmacy.

CONCLUSIONS
Pharmacists have a right to say about purchase drugs and medical supplies, inventory control methods and management in the hospital pharmacy. Duties and responsibilities of authorized pharmacist should be clearly defined. For patient safety medicines and medical supplies should be stored in physically fit and sufficient warehouses.

ACKNOWLEDGMENTS
All participated pharmacists were greatly appreciated.

REFERENCES

P-259: PHARMACY DISTRIBUTION IN TURKEY: POTENTIAL RISKS FOR TURKISH PHARMACISTS
E.Aydin, G.Ozcelikay
Ankara University, Faculty of Pharmacy, Department of History of Pharmacy & Management, Ankara, TURKEY

INTRODUCTION
With the pharmaceuticals and pharmacy services they offer, pharmacists are one of the important components of today’s healthcare system. Pharmacists are the most accessible healthcare workers that people can consult at any time and get free advice service. Pharmacies are seen as the right places for the enhancement of public health because of the respectable location of the pharmacist in the community and large number of people benefit from the services of pharmacists [1].

In our country, pharmacies are licensed according to the Regulation About Pharmacists and Pharmacies prepared pursuant to the Law No. 6197. Law No. 6197 was amended by Law No. 6308 and a restriction rule for new pharmacies was introduced. According to this amendment only one pharmacy would have been permitted for every three thousand five hundred people in a county.

In this study, distribution of pharmacies by regions in Turkey will be discussed based on the potential risks for pharmacists.

MATERIALS AND METHODS
Pharmacy numbers in the counties published by Turkish Ministry of Health and Turkish Pharmacists’ Association are used in this study.

RESULTS AND DISCUSSION
Pharmacies are the most common recruitment area for pharmacists with 92.35% proportion [2]. There are 24550 pharmacies in our country. 31% of them are located in Marmara Region, 18% in Central Anatolia Region, 16% in the Aegean Region, 14% in the Mediterranean Region, 10% in the Black Sea Region, 7% in Southeastern Anatolia Region, 4% Eastern Anatolia Region. According to the restriction in the regulation, it was determined 2337 quota [3,4]. According to 2014 OSYM data there are 27 faculties of pharmacy, including 3 in TRNC, and they accept 1931 students in total [5]. Taking into account of the quota which will come from new faculties that haven’t begun admission of students yet, it is apparent that pharmacist number will increase in the coming years.

CONCLUSIONS
Without taking into account of population, distribution of pharmacies restricts people to access them in some
areas. This also raises the additional costs and difficulties with it. By regulating the distribution and the number of pharmacies all segments of society can benefit equally from healthcare services. On the other hand restriction that limits the number of newly opened pharmacies and the increasing number of graduated pharmacists raise the recruitment problem in the pharmacy profession. Therefore, various employment opportunities should be created for the Turkish Pharmacists, new pharmacy faculties shouldn’t be open and pharmacy student quotas should be reduced.

REFERENCES
2. Türkiye’de Sağlık Eğitimi ve Sağlıklı İnsan Gücü Durumu Raporu, 2014 [www.yok.gov.tr/saglik/3ee8eafe-9fbe-4eb6-bc05-152f62a6ec747]
4. Nüfusa göre eczane sayıları [http://www.teb.org.tr/news/6438/N%C3%BCfusa-G%C3%B6re-Eczane-Say%C4%B1%2c-B"%Hak%C4%B1nd%C4%B1%2c- disponíveis em http://www.titck.gov.tr/UnitDetails.aspx?DetailId=2f50v7RD/A=]

P-260: OCCUPATIONAL PROBLEMS OF PUBLIC HOSPITAL PHARMACISTS

E.G. Taşkıran, B. Kiran

Ege University, Faculty of Pharmacy,
Department of Pharmacy Management, Izmir TURKEY

INTRODUCTION

Despite advances in science and technology today, pharmacists working in hospitals continue their classic roles consisting of supply, storage and distribution in our country. Legislative regulations for clinical pharmacy practice that allow roles of pharmacists working in hospitals in more effective, safe, economic and rational use of drugs come into prominence, are not yet complete. With this research, it is aimed to determine that socio-demographic characteristics, working environment and perceptions of workload, job satisfaction and expectations from trade bodies of pharmacists who are working in hospitals making official inpatient treatment in central districts of Izmir.

MATERIALS AND METHODS

It is a cross-sectional, descriptive study. Population includes pharmacists (n = 80) who are working in hospitals making official inpatient treatment in central districts of Izmir in 2012. 32-item questionnaire for socio-demographic characteristics, work environment, job satisfaction and occupational problems of pharmacists was applied on a voluntary basis to all pharmacists, without selecting the sample. The questionnaire was answered by 63 pharmacists. In this context, survey’s comprehensiveness of pharmacists working in hospitals in central districts of Izmir was 78.8%. Data are evaluated in 95% statistical confidence interval by frequency, percentage, chi-square tests using SPSS18 software package.

RESULTS AND DISCUSSION

Of pharmacies participating in survey, it was assessed that 42.9% “Choose working in hospital for guaranteed job and fixed income”, 61.9% works average 8 hours daily and mainly deal with routine business such as drug distribution (76.2%), inventory control (77.8%). In a study conducted in 2005 in Ankara, it was determined that pharmacists mainly perform tasks such as drug distribution and inventory control and do not professional practice in clinics (1). More than 3/4 of pharmacists participating in survey (76.2%) stated that they learn their duties, powers and responsibilities with own experiences, 85.7% felt that there is missing information in their exercise of the profession, and more than half (57.1%) cannot find benefits of theoretical training received at university during application. In a study conducted in Ankara on pharmacists working in hospitals, more than half of pharmacists (63.9%) stated that they found insufficient themselves in one or more subject in hospital pharmacy practice (2). 28.6% of pharmacists mentioned that they are not satisfied their profession, 57.1% of them are partially satisfied, the majority of them described their job as routine (60.3%) and exhausting (55.5%). According the research; job satisfaction in between gender of the pharmacists are statistically significant. Through the determination male pharmacists has more job satisfaction than female pharmacists. In a study conducted in the United States, it was determined that 78% of pharmacists working in hospital are satisfied their profession (3). 93.6% of pharmacists thought that trade bodies do not show sufficient interest to problems of pharmacists working in hospital, 77.7% of them are expected realization of legislative regulations to increase the pharmacist employment in hospitals.

CONCLUSIONS

More than half of pharmacists working in hospital spent 8 hours for work per day and a large part of their time spent roles such as drug distribution, inventory control as they describe routine and exhausting. In addition, more than 3/4 feel that there is missing information in their exercise of the profession and more than half cannot find benefits of theoretical
training at university during application. It is thought that undergraduate education for clinical pharmacy practice that allow roles of pharmacists working in hospitals in more effective, safe, economic and rational use of drugs come into prominence and to increase their job satisfaction and making legislative regulations immediately on working life will be beneficial.

REFERENCES

P-261: DRUG LABELING SYSTEMS IN PUBLIC HOSPITALS

B. Süzen⁴, M.B. Uzun², G. Gülpınar³, G. Özçelikay⁴
Ankara University, Faculty of Pharmacy, ¹Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION
There aren’t any process or technology that prevents misuse of drugs. Because of this health professionals should provide patient safety by some developments in technology. Color using in drug labeling is seen as important for patient safety. When using colors convenient, distribution of drugs can be much easy and process will get better. Studies about benefits of labeling are less but according to these studies, researchers, pharmacists and nurses reported the advantages of color use in labeling. The purpose of this study is determining the drug labelling systems used in public hospital pharmacies in Turkey and comparing with the applicable other systems.

MATERIALS AND METHODS
In the study, the questionnaire prepared considering Public Hospitals Authority recommends about the use of colors in labeling and was applied to responsible pharmacists of public hospitals which are located in the center of Ankara. Questionnaire form includes questions about labels, label usage and usage of labeled drugs hospital pharmacies.
11 questionnaires were evaluated in the scope of the research. Survey results are evaluated with SPSS 16.0v and given in the table.

RESULTS AND DISCUSSION
According to the survey results drug labeling is carried out %90,9 of hospital pharmacies and %70 of hospital pharmacies use uniform (red) label. 70% of hospitals participating our study reported they are using 120 and more labeled drugs daily, although 20% of hospitals use 0-30 labeled drugs. Whil 80% of pharmacists participating our study label drugs, 40% of technicians and employees label drugs in their hospitals. Although system about drug labeling is become a must by Public Hospitals Authority, no directive is sent to hospitals about labeling criteria. Only a list about drugs need to be labeled is prepared. Hospital pharmacists label drugs according to that list. Pharmacies in the hospitals related to “Institution of Public Hospitals” do not use standard labelling system. Pharmacies in the hospitals related to “Institution of Public Hospitals” do not use standart labelling system. Because the fact that lack of staff, drug labeling can be ignored and all drugs cannot be labeled.

CONCLUSIONS
Consequently, drug labeling in the hospitals is important but because of the lack of the staff and the high amounts of drugs, labeling can be done limited. Making the labelling system which is important for safety of patient and drug management by producer firms in the procedure of packaging may decrease the risks.

ACKNOWLEDGMENTS
Hospital pharmacists who made contribution to this study were greatly appreciated.

REFERENCES
3. Dursunbey Devlet Hastanesi Baştabıipliği (2013). Eczane İşleisys Prosedürü (S.2-4)

P-262: THE COMPARISION OF TWO METHODS USED IN ETHICS COURSES IN ANKARA UNIVERSITY FACULTY OF PHARMACY

G. Gülpınar⁴, M.B. Uzun³, G. Özçelikay¹
Ankara University, Faculty of Pharmacy, ¹Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION
Ethics education is known as important for gaining information about ethical problems and creating a different viewpoint about ethical cases. Because of the fact that the necessity of ethics education for pharmacists who can encounter several ethical cases is
indisputable. In addition, ethics and ethical dilemma become one of the inseparable part of pharmacy education in US and UK. The goals of ethics education was discussed in most of studies. They can be counted as; solving the ethical dilemmas much easier, gaining information about professional ethics, increasing the ability of ethical decision making and professional awareness.

Reaching these goals has been tried with the course of “General Ethics- Pharmacy Ethics and Pharmacist-Patient Communication” given in Ankara University Faculty of Pharmacy. Ethical cases, pharmacists can encounter, were taught to students within this course by using two different teaching methods. First method was making presentation and second one was role-play. The aim of this study is to provide students to evaluate these two methods in terms of effectiveness and efficiency.

MATERIALS AND METHODS
The students who are taking this course have been included to the study. Some ethical issues have been identified for applying the presentation method. Students were asked to choose the ethical case that they are interested in, search and present the issue in class to other students. Similarly, some ethical cases that are common in pharmacy practice have been identified for role-play method. These ethical cases were chosen by students in groups of threes fours. Students have written a script of these cases and played on camera. Videos have been showed in class and ethical dilemmas and problems were discussed in videos by all students.

A survey prepared for applying students after course constitutes the material of this study. Participating to survey is voluntary basis. The survey consists of two parts. First part is related to demographics and the second part includes some statements for obtaining RSLQLRQVRIVWXGHQWVRIWKHFRXUVH¶VHIIHFWLYHQHVVDQG efficiency.

RESULTS AND DISCUSSION
While nearly 78% of the students thought that they learn ethical issues better with presentation method, almost 95% of students impressed role-play is much more beneficial than presentation. The rate of students thinking that role-play is providing a comprehension of other groups’ issues is 95%. The same ratio for presentation method is almost 50%. Most of the students (90%) mentioned that they comprehended ethical problems related to pharmacy easily with role-play method.

Additionally, students expressed that they utilized from both of the two methods separately. They believed that they added some skills themselves owing to the two methods. Some of these can be counted as; being more comfortable in front of the community, increasing the interest to ethics, developing the ability of research, improving time management and ability of communication.

Student centered method providing an active participation to a course begins to take the place of teacher-centered lecture which was considered as a classical teaching method. Together with interactive education, ethics education is started to convert to student-centered learning too. Owing to becoming more active during the course, it is shown up that students can communicate with patients and other health professionals effectively, they can solve ethical problems easily, they may adopt a solution based questioning perspective and they can coordinate a team-work well.

There are some researches showing students enjoy the course with role-play method and their willingness to learn is increased.

CONCLUSIONS
Students are pleased with the teaching methods of the course. In addition role play method that can ease learnability of course are found more effective.

ACKNOWLEDGMENTS
Students who made contribution to this survey were greatly appreciated.

REFERENCES

P-263: PATIENT SAFETY AND DRUG MANAGEMENT

D. Akalgan1, G. Özcelikay2

1Ankara University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION
A number of definitions have been applied to medical errors and patient safety. InTo Err is Human, the IOM adopted the following definition: An error is defined as the failure of a planned action to be completed as intended or the use of a wrong plan to achieve an aim.
Errors can include problems in practice, products, procedures, and systems. The enhancement of patient safety encompasses three complementary activities: preventing errors, making errors visible, and mitigating the effects of errors. Medical errors may occur in hospitals, clinics, physician’s offices, pharmacies, in nursing homes and in patients’ homes. The term “patient safety” as used applies to initiatives designed to prevent adverse outcomes from medical errors. An adverse event is defined broadly as an injury that was caused by medical management and that resulted in measurable disability. Some adverse events, termed “unpreventable adverse events,” result from a complication that cannot be prevented given the current state of knowledge. Many drugs, even when used appropriately, have a chance of side effects, such as nausea from an antibiotic. The occurrence of nausea would be an adverse event, but it would not be considered a medical error to have given the antibiotic if the patient had an infection that was expected to respond to the chosen antibiotic. 34-56% of medical errors are preventable mistakes. Some of the factors associated with medication errors include the following:

- Medications with similar names or similar packaging
- Medications that are not commonly used or prescribed
- Commonly used medications to which many patients are allergic (e.g., antibiotics, opiates, and nonsteroidal anti-inflammatory drugs)
- Medications that require testing to ensure proper (i.e., nontoxic) therapeutic levels are maintained (e.g., lithium, warfarin, theophylline, and digoxin)

Look-alike/sound-alike medication names can result in medication errors. Misreading medication names that look similar is a common mistake. These look-alike medication names may also sound alike and can lead to errors associated with verbal prescriptions.


**MATERIALS AND METHODS**

Complying of a variety of sources on the subject.

**RESULTS AND DISCUSSION**

Medication errors; may occur as a result of malpractice, product defects, the procedure errors and system failures. Lack of education, other factors such as time pressure also contributes to the formation of medication errors. Negligence medication errors, improper dosages, errors arising from the unauthorized use of drugs are the most common medication errors. Current approaches in patient safety primarily focuses on medication errors. Medication errors are the errors which can be avoided. Drug Safety Applications are a mandatory procedure for not only patients but also health professionals.

**REFERENCES**

4. Prof. Dr. H. Erdal Akalın, Fako, FIDSA, Sağlıkta Kalite ve iyileştirme Derneği Yayınları, 2009.

**P-264: EVIDENCE BASED PHARMACY**

D. Akalgan¹, G. Öçelikay²

¹Ankara University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara, TURKEY

**INTRODUCTION**

In medicine, psychology, and numerous other disciplines, randomized controlled trials have become the gold standard for evaluating an intervention’s effectiveness. In medicine, early 1990’s EBM is “the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence-based medicine means integrating individual clinical experience with the best available external clinical evidence from systematic research (Sackett, 1996). EBP in many fields now strongly promoted. Evidence-based psychology, Evidence-based design, Evidence Based Library and Information Practice, Evidence-based management, Evidence-based medicine, Evidence-based nursing Evidence-based pharmacy, Evidence-based education . “Those fields that have displayed unprecedented development over the last century, such as medicine, technology, transportation, and agriculture have been those embracing research as the prime determinant of practice” (Shavelson & Towne, 2002).

"Evidence-Based Practice requires that decisions about health care are based on the best available, current, valid and relevant evidence. These decisions should be made by those receiving care, informed by the tacit and explicit knowledge of those providing care, within the context of available resources.” The integration of best evidence from current research, patient preferences and values, and clinical expertise to clinical questions. (Sackett, 2000)

There are 5 Step Process in EBP: 1. ASK: Formulate an answerable clinical question. 2. ACCESS: Track...
down the best Evidence. 3. APPRAISE: Appraise the
evidence for its validity and usefulness. 4. APPLY:
Integrate the results with your clinical expertise and
your patient values/local conditions. 5. ASSESS:
Evaluate the effectiveness of the process.
Extravagant prescribing: Expensive drugs are used
when less expensive equivalents are available.
Over-prescribing: Prescribed drugs are not needed, or
the dosage is too high. Incorrect prescribing: The
wrong drugs are selected for the patient’s condition.
Under-prescribing: Needed medications are not
prescribed, or the dosage is inadequate.
Multiple prescribing: Many drugs are used when fewer
would have the same effect.
Evidence-Based Prescribing

MATERIALS AND METHODS
Compiling of a variety of sources on the subject.
Aşağıda belirtilen arama motorlarında “Kanita Dayalı
Eczacılık” ile ilgili kaynaklar araştırılarak, yapılan
çalışmalar sınıflandırılmıştır.
The following search engines "Evidence Based
Medicine" and related resources are investigated,
studies were classified.
- Informa Health Care
- International Pharmaceutical Abstracts
- ProQuest
- Sage
- Science Direct – Elsevier
- Scopus
- Springer Link
- Taylor & Francis
- Wiley Interscience

RESULTS AND DISCUSSION
The current enthusiasm for evidence-based
prescribing should lead to safer and more effective use
of medicines. But it also poses some real problems for
prescribers: Reliable evidence to guide everyday
prescribing decisions at the point of prescription is
hard to find, Evidence is often inconclusive,
inconsistent with other reports, or irrelevant to clinical
realities; Even when there is good evidence, different
experts synthesize it to produce a variety of
conclusions about optimal prescribing; When several
are found, the prescriber has to decide which one of
them is the most reliable, accurate, and representative
of true evidence. These shortcomings should not
lessen prescribers’ appetite for sound, evidence-based
recommendations for rational prescribing (Maxwell,
2005).

REFERENCES
1. Sicily statement on evidence-based practice. BMC
Medical Education, 2005 Jan 5;5(1):1

P-265: THE EFFECTS OF SSI
IMPLEMENTATIONS ON COMMUNITY
PHARMACISTS’ JOB SATISFACTION: A
QUALITATIVE STUDY

G. Gülşnăr1, M.B. Uzun1, N.Y. Yalın2
Ankara University Faculty of Pharmacy, Pharmacy
Management Department1
Ankara University Faculty of Medicine, Medical History and
Ethics Department2; Ankara, Turkey

INTRODUCTION
Satisfaction can be defined as is to meet people's
expectations, requirements, demands and desires. In
other words, Satisfaction is an individual assessment
of his/her life positively according to his/her own
stated criteria. One of the key elements that needed
for people to be happy at work is providing satisfaction
from their work. According to Locke's definition, job
satisfaction “is feeling satisfactory or positive
emotional state of mind when the person evaluate
his/her job experience or job.” Job satisfaction also
includes the awards provided by the job along with the
expectations from job.

Although many studies have been carried out about the
job satisfaction of health care workers in Turkey,
noteworthy not yet a qualitative research has been
done.

The purpose of this study is to make a qualitative
research with in-depth interviews to understand how
pharmacists and their job satisfaction effected by
Social Security Institution (SSI) procedures while they
performing their job.

MATERIALS AND METHODS
Semi-structured face to face interview technique was
applied to 9 community pharmacists which were
added to study by the snowball method.
Pharmacists who participated in the study consists of
four groups: who has pharmacies near hospital, near
family health center, in the town or whose pharmacies
non-drug product sales are high. All interviews were
conducted face to face and over the last six months of
2014. The interviews lasted approximately 45
minutes.
Pilot study was carried out on questions prepared
before the start of semi-structured interviews.

RESULTS AND DISCUSSION
In this section themes derived from interviews with
pharmacists impacts of the SSI applications on
pharmacists' job satisfaction and pharmacists’
comments that covering these themes are given. The
resulting themes has been studied in 8 titles:
regulations related to pharmacy practice, vocational
training, professional reputation, relationships with colleagues, staff management, financial concerns, consulting services, patient behaviors and attitudes. SSI applications constitute large part of the daily workload of pharmacists. The important part of this applications consists of the procedures that have to perform in order to provide a prescriptions of the patients. Pharmacists think that they can not separating enough time for patient counseling.

CONCLUSIONS
Job satisfaction of pharmacists in our country are affected by the SSI applications. Our study brought up many new research questions and presenting a basic ground for asking this right questions in a proper discourse. Also this study shows many precautions needed to take for increasing the pharmacists’ job satisfaction.

ACKNOWLEDGMENTS
All participated pharmacists were greatly appreciated.

REFERENCES

P-266: FAMILY PHARMACY MODEL IN PHARMACY PRACTISE

Miray ARSLAN1, Bilge SÖZEN ŞAHNE2, Sevgi ŞAR1

1Ankara University, Faculty of Pharmacy, Pharmacy Management Department, 06100, Tandoğan-Ankara, TURKEY, 2Hacettepe University, Faculty of Pharmacy, Pharmacy Management Department, 06100, Şhhiye-Ankara, TURKEY

INTRODUCTION
Both pharmacy practice and the role of the pharmacist are changes with developments in the world as in all areas. The family pharmacy, one of these innovations as we face with, is being implemented in some countries in the world and is expected to improve the current services provided by pharmacists with this application. It is noteworthy that the application way of the offered services within the scope of the family pharmacy like being able to provide better patient follow-up in addition to providing easier access to pharmacy services are vary from one country to other. The aim of this study is explaining family pharmacy concept and related topics like as home care pharmacy and ambulatory pharmacy. After that their practice in the world will be investigated to determine if it is applicable in Turkey or not.

MATERIALS AND METHODS
In this study, by examining the concept of family pharmacy in which there are discussions about it can be applicable in Turkey or not, the application in the world will be given and issue will be discussed in several aspects by literature review and interviews with pharmacy professional organizations.

RESULTS AND DISCUSSION
It is observed that there are differences in many pharmaceutical applications such as family pharmacy worldwide. There are various specialization areas such as family pharmacy, family medicine pharmacy, home care pharmacy and ambulatory pharmacy in many different countries. These specialization areas are started to gain increasing importance with the development of pharmaceutical care.

In Turkey, one of the major changes made in recent years in the health sector is also family physician application. The concept of family pharmacy has also begun to be discussed again with the realization of family physician application in 2010 which’s pilot study started in 2005.

The concept of family pharmacy which is expected to be made more equitable distribution of the pharmacist-patient and allow the reduction of income inequality is started to be examined in more detail and establishment of Family Physician Integrated Family Pharmacy Model has been a priority targets at the Commission on Family Pharmacy founded within the pharmacy professional organizations especially in recent months.

As a result we can say that family pharmacy and related concepts are new in our country. The family pharmacy applications have started to come up more often in Turkey as well as all over the world with the increasing importance of pharmaceutical care and patient satisfaction. In this context, including family pharmacy practice, which help pharmacists get more active role in health care system, in the pharmacy education and the establishment of specialization areas on this subject will provide a major contribution to our profession.

REFERENCES
2. ASHP, Educational Outcomes, Goals, and Objectives for Postgraduate Year Two Ambulatory Care Pharmacy Residency Programs. http://www.ashp.org/DocLibrary/Accreditation/Regulations-Standards/RTP-PGY2-AmbCareProgram.pdf

P-267: PHARMACOECONOMIC MODELING METHODS AND FREQUENCY OF USE

M. Oral, G. Özcelikay

Ankara University, Faculty of Pharmacy, Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION

The pharmacoeconomics is a scientific discipline handling the comparison of the drugs used for the prevention, diagnosis and treatment of diseases within the scope of economy and efficiency with each other and with operational or non-operational alternative treatment options with incorporating the pharmacy services by using basically cost-benefit, cost-utility, cost-minimization and cost-effectiveness analysis. Long-term sustainability of health care services, equally transfer of these services and the flow management of the sources depend on a better understanding of the use of cost-effectiveness analysis[1]. As the health care costs are increasing, pharmacoeconomics is becoming increasingly important. But most of the clinicians have limited information about pharmacoeconomic methods. Clinical trials designed properly prove highly the efficacy of drugs, but in these studies cost-effectiveness is rarely examined. The effectiveness of drug is related with whether it really brings benefit or not while the cost-effectiveness is examining whether the effectiveness obtained from the drug is worth the cost or not [2]. For making comments according to the results being reached by using the findings obtained during the pharmacoeconomic evaluations, extrapolation of these findings are required. Pharmacoeconomic modeling methods have been developed according to this need. Pharmacoeconomic modeling methods are Sensitivity Analysis (One-way sensitivity analysis, Two or multi-way sensitivity analysis, Scenario analysis, Threshold analysis, Monte Carlo simulation method), Discounting, Decision Analysis methods (Decision trees, Markov modeling) [3]. In this study our aim is to give information about pharmacoeconomics, analysis methods and pharmacoeconomic modeling methods. In addition, the frequency of use of pharmacoeconomic modelling methods will be investigated.

MATERIALS AND METHODS

Literature search will be done to find out detailed information about pharmacoeconomic evaluations and the frequency of use of pharmacoeconomic modelling methods by using databases: Informa Health Care, International Pharmaceutical Abstracts, ProQuest, Sage, Science Direct–Elsevier, Scopus, Springer Link, Taylor & Francis, Wiley Interscience. The data for the last 10 years will be taken into account.

RESULTS AND DISCUSSION

The pharmacoeconomic evaluation methods will be analyzed in detail benefiting from the literature research. Also pharmacoeconomic modelling methods including Sensitivity Analysis, Discounting and Decision Analysis will be discussed in detail.

![Fig. 1. Simple hypothetical example of a decision analysis tree, capturing costs as outcomes](image)

Fig. 1. Simple hypothetical example of a decision analysis tree, capturing costs as outcomes [2].

![Fig. 2. Schematic overview of Markov Modeling](image)

Fig. 2. Schematic overview of Markov Modeling [2].

The frequency of use of pharmacoeconomic modelling methods obtained from the literature research via mentioned databases will be stated numerical and the distribution between last 10 years will be specified.

CONCLUSIONS

Countries' health expenditure is increasing worldwide. In these expenditures, drug expenditures are of high importance as well. In this manner, pharmacoeconomic evaluations are becoming increasingly important in recent years. Besides pharmacoeconomic analysis methods, the requirement of the extrapolation of the data obtained is giving rise to pharmacoeconomic modelling. In the lights of the findings of literature research, this study will specify detailed information about
pharmacoeconomic modelling methods and the frequency of use of these methods in the last 10 years. We believe that this study will be a guide for our colleagues aiming to do pharmacoeconomic studies in terms of pharmacoeconomic modelling.

REFERENCES
3. Yalçın-Başık, P.; Şahin, B., Sağlık Hizmetlerinde Maliyet Etkilik Analizi ve Karar Analizi (Cost Effectiveness Analysis and Decision Analysis in Health Services), Hacettepe Sağlık İdaresi Dergisi (Hacettepe Journal of Health Administration) 2013, 16 (2), 121-134.

RESULTS AND DISCUSSION
Most of the studies relating measuring expectations and perceptions of pharmacy customers in community pharmacies are handled through utilizing servqual technique. Those studies include a questionnaire related with five areas of servqual for pharmacy customers. Pharmacy customers answer what expect and what perceive from pharmacy services. Generally, in studies there are five questions for each dimensions of servqual. If the perceptions become less than the expectations, it is concluded that pharmacy service should ameliorate at the related issue. The sample group generally consists of pharmacy customers except pharmacy staff and doctors. The survey is commonly carried out in more than one pharmacy in a city and the questionnaire is prepared according to the five dimensions of servqual technique.

CONCLUSIONS
Pharmacy management is more important than ever before. Pharmacists confront with lots of challenges globally, such as increasing competition between pharmacies, decreasing drug prices and etc. In this regard, some pharmacists try to resist these challenges and sustain their operations throughout providing a quality service. The dual role of community pharmacies, as a retail business and as a health care provider prevents them to seek only profit. This causes

MATERIALS AND METHODS
Since 1988, a lot of researches about measuring service quality in different areas have been handled. As we make a research via using keywords “servqual, community pharmacy” on sciencedirect, we can reach limited number researches about servqual in community pharmacies. These published papers guide us about how to apply servqual technique in our country.

P-268: A REVIEW ON IMPLEMENTING SERVQUAL TECHNIQUE TO COMMUNITY PHARMACIES IN TURKEY

M. Kartal*, G. Ozcelikay*

*Ankara University, Faculty of Pharmacy
Department of Pharmacy Management and History
Ankara, Turkey

INTRODUCTION
Expectations and perceptions are the two factors that make up the quality. If perceptions are more than the expectations about a product or a service, we say that it is a quality product or service. Parasuraman and et al. improved a technique “SERVQUAL-Service Quality” in order to measure service quality in 1988 [1].

According to research of Parasuraman and et al., expectations cover five areas; tangibles, reliability, responsiveness, assurance and empathy. “Tangibles are the appearance of personnel, physical facilities; reliability is the ability to perform the desired service accurately; responsiveness is willing to helping customers; assurance is the knowledge, courtesy and ability to convey trust and confidence of employees and finally empathy is the provision of caring, individualized attention to customers” [1]. If an employee understands what customers need according to five areas of expectations and fulfills those needs in their services, they would satisfy the customers. At this paper we want to understand practicability and possible results of the servqual technique Turkish community pharmacies.
P-269: INCORPORATING ACTIVE LEARNING STRATEGIES IN A PHARMACOTHERAPY COURSE IN ISTANBUL KEMERBURGAZ UNIVERSITY

N. Abunahlah¹, J. Trujillo², A. Yeşilada³

¹Istanbul Kemerburgaz University, School of Pharmacy, Department of Clinical Pharmacy, Istanbul, Turkey
²University of Colorado, Department of Clinical Pharmacy, Colorado, USA
³Istanbul Kemerburgaz University, School of Pharmacy, Department of Basic Pharmaceutical Sciences, Istanbul, Turkey

INTRODUCTION
Active learning strategies in pharmacy education are commonly used in the United States¹ and may be more effective than traditional, didactic lectures². Istanbul Kemerburgaz University (IKU) and the University of Colorado (CU) collaborated to develop a progressive pharmacotherapy course that was patient-centered and used active learning strategies. The purpose of this study was to evaluate student acceptance and expectations of this course.

METHODS
Three CU faculty members developed the course content, structure, learning methods, assessments, and evaluations that were patient-centered and incorporate contemporary educational approaches that are not common in Turkey. The CU faculty traveled to Istanbul to teach the course in Spring 2015. Faculty at IKU participated in all course preparations, sessions, activities, and evaluations. The course was condensed into five weeks. The daily structure included 4 hours of knowledge acquisition and application including didactic lectures, case discussions, group assessments, and student-directed learning activities and 2 hours of communication-based activities. Evaluations include eleven quizzes, three assessments of communication skills, and one final examination. Students completed a ten-item questionnaire regarding their acceptance level for the course, learning strategies, and materials using a 5 point Likert scale (strongly agree to strongly disagree).

RESULTS
Twenty-nine students completed the course. All students agreed or strongly agreed that the active learning methods used in the course were more effective than traditional lectures and preferred active learning methods more than traditional lectures. All students agreed or strongly agreed that the course should be offered in the same format next year. All students agreed or strongly agreed that the course was made relevant to the practice of pharmacy and prepared them to practice pharmacy in a real practice environment. Students preferred more frequent quizzes instead of one midterm exam. Students believed that the condensed 5-week period was too intensive and should be extended.

CONCLUSION
This progressive international collaboration was designed to increase the knowledge, skills and abilities of graduating pharmacists in Turkey, consistent with IKU’s aim to graduate patient-centered pharmacists. Student responses indicate overall acceptance and preference for active learning methods compared to traditional lectures for this patient-centered pharmacotherapy course.

REFERENCES
P-270: THE ATTITUDE, BEHAVIOUR AND INTENTION OF PHARMACY PHARMACISTS TOWARDS PHARMACIST PARTNERSHIP

N. Tarhan¹, M. Arslan¹, S. Sar¹
Ankara University, Faculty of Pharmacy, ¹Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION

Different models of community pharmacies seem to be applied throughout the world depending on the ownerships of pharmacies. In some countries, a community pharmacy owner could be only one pharmacist but in some countries, community pharmacies could be opened with the participation of more than one pharmacist. The number of pharmacist partners, the distribution of shares and conditions of pharmacists partnership also change from a country to another. In our country according to the current legislation, a pharmacist could be only one pharmacy’s owner. Pharmacists partnership is not included in Turkey’s laws about the pharmacies. In this study, we investigated that pharmacists’ attitudes, intentions and behaviours towards pharmacist partnerships will provide contributions or not to the quality and efficiency of pharmacy services, pharmacists’ economic conditions. It will be the first investigation of community pharmacists’ approach to pharmacist partnerships and if it makes the difference according to the location of community pharmacies and working experience of pharmacists.

MATERIALS AND METHODS

A questionnaire study consisting, 8 demographic and 30 questions prepared by 5 point Likert scale, 38 questions totally will be asked to pharmacy pharmacists face to face in Ankara city center and districts which investigates pharmacists’ attitude, behaviour and intentions towards pharmacist partnership; the results will be analysed statistically.

RESULTS AND DISCUSSION

In Turkey, pharmacists are living economic difficulties and concerned about their future due to many reasons such as the decrement of drug prices frequently, the increase in operating costs, inflation, shortage of pharmacist profitability, taxes, repayment problems in the social security institutions etc. Pharmacists are trying to take individual measures such as reducing the number of employees and miscellaneous operating costs, tending to non-drug products to survive and manage the pharmacies well which are the closest and easily reachable health care institutions. Some authority on this subject suggests that the pharmacist partnership could be a solution to these problems. Primarily new graduates, lots of pharmacists will have troubles in finding location to open new pharmacies based on the population criterion with the law, the act no 6308, valid from 31 May 2012 Alteration of the Law Legislated on Pharmacists and Pharmacies, Supervision of the Narcotics. Will the pharmacists partnership be a solution to this problem? When the literatures were investigated no study searching of the pharmacy pharmacists’ intentions, behaviours and attitudes towards pharmacist partnership were observed. In this study, the partnership will be investigated with the pharmacist’s perspective.

REFERENCES


P-271: COMMUNITY PHARMACISTS’ KNOWLEDGE AND ATTITUDE ABOUT FIP CODE OF ETHICS

O.N. Erdogan¹, A.O. Araman², M.S. Erdogan³
İstanbul University ¹School of Pharmacy, Department of Pharmacy Management, ²School of Pharmacy, Department of Pharmaceutical Technology, ³School of Medicine, Department of Public Health, İstanbul, TURKEY

INTRODUCTION

Ethics (also moral philosophy) is the branch of philosophy that involves systematizing, defending, and recommending concepts of right and wrong conduct[1]. Community pharmacists most closely and directly impact on or interact with the final consumer of medicines [2]. It is known that the community pharmacy practice has been encountering many ethical conflicts because of the commercial nature of community pharmacy[2]. The FIP recommends that in every country, the appropriate association of pharmacists should produce a Code of Ethics for pharmacists setting out
their professional obligations and take steps to ensure that pharmacists comply with the provisions of that Code [3].

The aim of this study is to identify the community pharmacist knowledge and attitudes toward ethical issues at community pharmacy setting in Bahcelievler, Istanbul according to mainly FIP code of ethics.

MATERIALS AND METHODS

A cross-sectional, qualitative survey of community pharmacists was conducted and the survey was completed by a face-to face questionnaire. It was designed by using the literature [3,4,5]. Whole number of community pharmacies serving in Bahcelievler district of Istanbul were included into the study setting (n=206). Only103 pharmacists were accessed, 58 were accepted the participation, 45 was rejected the study, the rest was not found in the pharmacy.

The data were collected from 23rd of March to 6th of April 2015 using a structured questionnaire.

RESULTS AND DISCUSSION

53.4 % were woman, 46.6 % were man. 7 community pharmacists have postgraduate education: 28.6 % of those have masters degree from pharmacology, 28.6 % of have masters degree from pharmaceutical technology. It was stated that 37.9% of community pharmacists have received education on ethics. 72.7% of those who received ethics education referred those education as undergraduate curriculum, 18.2 % was provided by the Istanbul Chamber of Pharmacist.

Resources in the pharmacy are web (48,3%), books (12.1%).

ACKNOWLEDGEMENT

Special thanks to Ferit Yavuz who is 5th grade student of the School of Pharmacy, Istanbul University for applying the questionnaire to the community pharmacists.

REFERENCES

1. https://en.wikipedia.org/wiki/Ethics accessed 03.05.2015

P-272: ANTIMICROBIAL ACTIVITY AND CHARACTERISATION OF VOLATILE COMPOUNDS OF ECHIUM VULGARE BY SPME AND GC-FID/MS

R. Aliyazicioglu1, O. E. Eyüpoglu2, U. Özgen3, M. Badem1, S. O. Sener1, S. Yıldırım1

Karadeniz Technical University, Faculty of Pharmacy, Faculty of Sciences,
1Department of Biochemistry, 2Department of Chemistry, 3Department of Pharmacognosy, Trabzon, TURKEY

INTRODUCTION

The suitable planting distance and phosphorus, potassium fertilizer could reasonably improve photosynthetic characteristics and promote seedling growth and development of Echium vulgare [1]. Echium vulgare seed oils contain abundant polyphenols and flavonoids and Blueweed (Echium vulgare) seeds contain oil rich in γ-linolenic and stearidonic acids. Low-calorie structured lipids were produced from Echium seed oil by enzymic acidolysis reactions [2]. Honey from E. vulgare shows the best performance in inhibiting lipid peroxidation and scavenging superoxide anion radicals, hydroxyl radicals, and DPPH radicals [3]. In our study, solid phase microextraction (SPME) was used to analyze the ten volatile compounds in Echium vulgare and Antimicrobial Activity of the methanolic extracts of Echium vulgare was investigated against microorganisms.

MATERIALS AND METHODS

All chemical reagents and standards were used in gradient purities. For the SPME procedure, ~1.00 g of tree plant was placed in a 10 mL vial. The fiber coating was located on the head space for temperature and time values set according to the experimental protocol. Fibers with extracted aroma compounds were then injected into the GC injector (split mode). Finally, thermal desorption was performed at 250 °C for 4 min. GC analysis was performed using a Shimadzu 2010 Plus gas chromatograph, which was coupled to a Shimadzu QP2010 Ultra mass selective detector. Separation was performed using a Restek Rxi-5MS capillary column, 60 m length, 0.25 mm i.d. and a 0.25 µm phase thickness in split mode. Initial oven temperature was 60 °C for 2 min. This was then raised to 240 °C at 3 °C min⁻¹; 250 °C was maintained for 4 min. Helium (99.999%) was employed as carrier gas at a constant flow-rate of 1 mL min⁻¹. Electronic impact mode (EI) was used for detection after the ionization voltage had been stabilized at 70 eV. Mass acquisition was performed in scan mode (40-450 m/z) [4].

Antimicrobial activity test was practised by using Agar well diffusion method [5].
RESULTS AND DISCUSSION
95.5% of the oil composition of Echium vulgare were characterized using GC-FID/MS. The caffeic, salicylic, p-hydroxyphenylacetic, p-hydroxybenzoic, and 3,4-dihydroxybenzoic acids most abundant identified as common phenolic components by GC-MS were found in free, ester, and glycoside forms. Conclusion of antimicrobial activity test was that monitored effects of anti-tuberculosis.

CONCLUSIONS
This study is important in terms of
- analysis of volatile compounds of Echium vulgare
- investigated antimicrobial activity of Echium vulgare for producing of pharmaceutical active substance
- effects of anti-tuberculosis.

REFERENCES

P-273: ANTIOXIDANT ACTIVITY OF ECHIUM VULGARE
R. Aliyazicioglu1, O. E. Eyüpoglu2, U. Ozgen3, M. Badem1, S. O. Sener1, S. Yıldırım1
Karadeniz Technical University, Faculty of Pharmacy, Faculty of Sciences,
1Department of Biochemistry, 2Department of Chemistry, 3Department of Pharmacognosy, Trabzon, TURKEY

INTRODUCTION
Echium vulgare is a species of Echium native to most of Europe, and western and central Asia and also common in North America. Its pollen is blue but its filaments of the stamens remain red. It flowers between May and September[1]. This plant species are class of wild-growing medicinal plants and absorbs toxic some elements for human health [2]. Identified lipidic components from seeds of the plants especially Echium vulgare and similarly Cynoglossum officinale, and Lappula squarrosa of the family Boraginaceae are generally polyunsaturated fatty acids like linoleic, γ-linolenic, α-linolenic, and stearidonic acid [3]. Both easy availability with the proliferation of a wide area and positive effects to human health with rich sources of lipidic biomolecules are preoccupied that we must need search its antioxidative biological activity and in our study, antioxidant activities were measured using three different methods, total phenolic contents (TPC), ferric reducing / antioxidant power (FRAP), and 2,2-diphenyl - 1-picrylhydrazyl (DPPH) radical scavenging activity. Its phenolic acid composition responsible for antioxidant activity was identified by using RP-HPLC-DAD-UV.

MATERIALS AND METHODS
Used all chemical reagents were in gradient purities. The FRAP test was used to measure the total antioxidant capacity of Echium vulgare methanolic extract method is based on electron transfer and is regarded as accurate indicators of total antioxidant power, since total reducing power is defined as the sum of the reducing powers of the individual compounds contained in a particular sample [4]. The free radical scavenging activity was determined using the DPPH test by comparing to reference antioxidant like BHT for the sample of Echium vulgare methanolic extract. The phenolic compounds may thus have acted as free radical scavengers on the basis of their hydrogen donating ability [5].

Total phenolic content was determined in Echium vulgare methanolic extract by using the Folin-Ciocalteu method in comparison with standard gallic acid.

RP-HPLC analysis of phenolic compounds was conducted by using a reverse phase column (4.6 × 250 mm, 5 μm), on a gradient program with a two solvents

Fig. 1. A picture of GC combined system

SAMPLE VIAL
TRAP
GC
Headspace

328
system (A: 2% acetic acid in water; B: 0.5% acetic acid in acetonitrile: water [1:1]) at a constant solvent flow rate of 1.2 mL.min⁻¹. Injection volume was 20 μL. Signals were detected at 232, 246, 260, 272, 280, 290, 308, 328 nm by DAD and at 280 nm by UV detection. Column temperature was maintained at room temperature, 25°C.

RESULTS AND DISCUSSION
The total phenolic content of *Echium vulgare* methanolic extract consist of especially benzoic acid 15 times more than other phenolics when it was compared with the other phenolics which are from 15 phenolic compounds in searched with HPLC like gallic acid, *proto*-catechuic acid, *proto*-catechuic aldehyde, gentisic acid, chlorogenic acid, *p*-OH benzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, syring aldehyde, *p*-coumaric acid, ferulic acid, sinapic acid, rosmarinic acid. SC₅₀ radical scavenging-activity of *Echium vulgare* methanolic extract was very close to the standard BHT with 40-fold difference as in harmony with FRAP activity in terms of equivalent Trolox (TEAC).

CONCLUSIONS
This study is important in terms of
- Good antioxidant source
- High Phenolic acid content and dietary supplement
- Pharmaceutical active substance isolation in future

REFERENCES

P-274: USAGE OF NEUROMARKETING TECHNIQUES ON PHARMACY FIELD

S. N. Silkü¹, S. Şar²
Ankara University, Faculty of Pharmacy
Department of Pharmacy Management, Ankara, TURKEY

INTRODUCTION
Marketing is described as an interface function between suppliers and consumers. The aim of marketing is to submit proper goods, services and concepts to consumers in desired time, on desired place and also with right cost [1]. By technological developments, consumers can reach the market easily and product options are increased, so consumer and value based marketing types are started to get more attention. With increased competition, the important question of marketing is how consumers will give their decisions when buying products. Neuromarketing searches the effects of central nervous systems on consumers behaviors to improve marketing strategies. Besides consumers feelings, thoughts and expectations neuromarketing techniques give also information about buried opinions of them [2]. Neuromarketing utilizes some brain monitorize techniques like *electroencephalography* (EEG), *magnetoencephalography* (MEG) and *functional magnetic resonance* (fMRI) and also some physical techniques like *galvanic skin response* (GSR) and *eye tracking* [3].

This study is prepared to give information about neuromarketing and neuromarketing studies and also evaluating of applicability of neuromarketing studies in pharmacies and health services.
electroencephalography (EEG), magnetoencephalography (MEG) and functional magnetic resonance (fMRI) and also some physical techniques like galvanic skin response (GSR) and eye tracking [3].

This study is prepared to give information about neuromarketing and neuromarketing studies and also evaluating of applicability of neuromarketing studies in pharmacies and health services.

On the other hand, pharmaceutical regulations about marketing is different contry to country. In Turkey, according to Medicinal Products Promotion Legislation, drug advertisement is illegal. Just introduction about an health situation and after that leading to a health professional is permitted with some rules.

Pharmaceutical marketing is so critical and should be discussed about. Because the main purpose of the marketing action is to increase sales of that product. Therefore, drug advertisement may be ended with drug abuse and so some thinks that drug commercials should be just advertised to health professionals. On the other hand, some promotions can be used to encourage being healthy like vaccine commercials or quitting smoking promotions [3]. Marketing activities of non-medical products are considered according to Consumer Protection Law and these products can not claim about treatment.

By using neuromarketing techniques, perceptions about marketing activities of drugs or non-medical products on health professionals and/or consumers can be evaluated and also marketing actions are attractive or not, can be checked. Eye tracking technique can be useful about merchandising in pharmacies. Visuals and slogans of products which are used in pharmacies can be tested with EEG, MEG and/or IMRI to evaluate their impression on people. Besides, communication between patients and pharmacist can be observed by neuromarketing techniques and so weaknesses patient-pharmacist connection can be detected and handled. By using neuromarketing results, pharmacists can be educated to reach the right points of consumers’ brain to raise awareness and to sell. For example, while selling a product to quit smoking, first of all, pharmacist should bespeak to reptallian brain by mentioning the danger of losing wellbeing, also pharmacist should show visuals and concreate datas to point the recovery of the body and lungs after quitting. Furthermore, the speech about financial and physical benefits of quitting should be underlined at the beginnig and at the end of the conversation, so by these steps sales can be successful.

As a result, neuromarketing can be useful for marketing strategies of drugs and non-medical products by pharmaceutical industry and pharmacies. Regulations about neuromarketing should be revised by the evaluation of ethical issues and with the help of health professionals.
POSTER SESSION III
(Poster 275 - 424)

June 11, 2015
P-275: CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF MENTHA LONGIFOLIA SUBSP. TYPHOIDES VAR. TYPHOIDES

A. Ertas1, U. Kolak2

1Dicle University, Faculty of Pharmacy, Department of Pharmacognosy, Diyarbakır, TURKEY
2Istanbul University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul, TURKEY

The aim of the present study was to determine the chemical composition and antioxidant activity of essential oil of Mentha longifolia subsp. typhoides var. typhoides. Essential oil was obtained using a Clevenger apparatus by hydrodistillation from the aerial parts of M. longifolia subsp. typhoides var. typhoides. The essential oil composition was determined by GC/FID and GC/MS analysis. The major compounds of the oil were found to be cineole (32.0%) and p-cymen-8-ol (21.1%). To our knowledge, there is no study about M. longifolia subsp. typhoides var. typhoides regarding these main components of the essential oil. The essential oil of Mentha longifolia subsp. typhoides var. typhoides showed good antioxidant activity in four tested methods.

INTRODUCTION

The genus Mentha L. (Lamiaceae family) is widely distributed in Eurasia, Australia and South Africa [1]. The Mentha species which are the most important source of the essential oil production have been grown in damp or wet places. Some of them, especially M. piperita L. (peppermint oil), M. arvensis L. (cornmint oil) and M. spicata L. (spearmint oil), have been cultivated in the world for their essential oils, which have been used in the food, beverage, confectionary, cosmetic and pharmaceutical industries. Their essential oils have been also used externally as antipruritic, astrigent, antiseptic, antimicrobial, and rubefacient [2]. Since the mint essential oils have economic and medicinal values, their composition and biological activities have been investigated by many researchers [1]. The leaves, flowers and stems of the Mentha species have been used as carminative, antispasmodic, antiemetic, stimulant, analgesic, and emmenagogue in traditional medicine all around the world. Their leaves have been also consumed as herbal tea and spice [2].

MATERIALS AND METHODS

Plant material
M. longifolia subsp. typhoides var. typhoides L., which was collected by Dr. A. Ertaş from east Turkey (Mardin-Kızıltepe) in May 2014 and characterized by Dr. Y. Yeşil (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University). Voucher specimens have been stored in the Herbarium of Istanbul University, Faculty of Pharmacy (ISTE: 101084).

Preparation and GC/MS conditions for essential oil: Essential oil samples were obtained by a Clevenger apparatus from the whole parts of plants which were crumbled into small pieces and soaked in distilled water for 3 h. Then, these samples were dried over anhydrous Na2SO4 and stored at +4°C for a sufficient period of time. In the next step, the essential oil samples were diluted using CH2Cl2 (1:3 volume/volume) prior to GC/FID and GC/MS analyses. GC/FID and GC/MS analyses were performed using Thermo Electron Trace GC FID and Thermo Electron DSQ GC/MS detectors respectively.

Antioxidant activity: We used the β-carotene-linoleic acid test system, DPPH free radical and ABTS cation radical scavenging activities and cupric reducing antioxidant capacity (CUPRAC) methods to determine the antioxidant activity.

RESULTS AND DISCUSSION

The essential oil composition of M. longifolia subsp. typhoides var. typhoides was obtained by hydrodistillation and determined by GC/FID (Gas Chromatography / Flame Ionization Detector) and GC/MS analysis. 46 components were detected, constituting 100% of the essential oil composition of M. longifolia subsp. typhoides var. typhoides. The main components of the essential oil were identified as cineole (32.0%), p-cymen-8-ol (21.1%), menthone (8.0%) and β-pinene (5.9%). The essential oil of Mentha longifolia subsp. typhoides var. typhoides showed good antioxidant activity in four tested methods.

CONCLUSIONS

The species may help to protect the people against lipid peroxidation and free radical damage, and its extracts will probably be used for the development of safe food products and additives.

REFERENCES

P-276: A DETAILED STUDY ON THE CHEMICAL AND BIOLOGICAL PROFILES OF THREE HERBAL TEAS IN ANATOLIA

A. Ertas¹, M. Boga², M.A. Yilmaz³, M. Firtat⁴, H. Temel⁵

Dicle University, Faculty of Pharmacy, Diyarbakir, TURKEY
¹Department of Pharmacognosy, ²Department of Pharmaceutical Technology, ³Department of Pharmaceutical Chemistry
⁴Yüzüncü Yıl Üniversitesi, Eğitim Fakültesi Biyoloji Bölümü, Van

The aim of this study was to determine the chemical profile of three herbal teas in Anatolia, Melissa officinalis, Sideritis libanotica subsp. linearis and Stachys thirkei by LC-MS/MS and GC/MS. Additionally, the total phenolic-flavonoid content, antioxidant and anticholinesterase activities of three herbal tea extracts and their individual main compounds were determined. To determine the active principles responsible for the antioxidant and anticholinesterase activities, phenolic components, such as chlorogenic, rosmarinic, caffeic acids, rutin, quercetin, luteolin, naringenin etc. were quantified by LC-MS/MS in the methanol extracts. The results revealed that the antioxidant and anticholinesterase properties of the plants might be stemmed from the phenolic ingredients.

INTRODUCTION

Melissa officinalis L., Sideritis libanotica subsp. linearis and Stachys thirkei are included in the family Lamiaceae which is one of the most populated family of medicinal plants. Sideritis and Stachys are the largest genera of the family, the genus Sideritis represented by 46 species and the genus Stachys is represented by 89 species in Turkey [1, 2]. The genera Sideritis and Stachys are known as “mountain tea” or “plateau tea” in Turkey and decoctions and infusions of them are used as tea and for treatment to the common cold, flu, allergies, skin disorders, and being gastroprotective and digestive agents in Turkish folk medicine [3].

MATERIALS AND METHODS

Phenolic components in the methanol extracts were quantified by LC-MS/MS. Essential oil and fatty acid composition of three herbal teases were analyzed by GC/MS. β-Carotene method, ABTS cation radical decolorisation method, cupric reducing antioxidant capacity assays and DPPH free radical scavenging activity were carried out to indicate the antioxidant activity. The anticholinesterase potential of the extracts were indicated by Ellman method.

RESULTS AND DISCUSSION

Experimental results showed that the STM (Stachys thirkei methanol) extract was rich in terms of phenolic acids and some flavonoids. Chlorogenic (35372.8 μg/g extract) and quinic acids (26029.2 μg/g extract) were the most abundant compounds in the STM extract. In addition, malic (4213.9 μg/g extract) and rosmarinic acids (393.1 μg/g extract) were also abundant in the STM extract. In the case of MOM (Melissa officinalis methanol) extract, rosmarinic (65066.8 μg/g extract) and quinic acids (15955.9 μg/g extract) were the most abundant compounds and phenolic acids. Of all the studied species, the SLM (Sideritis libanotica subsp. linearis methanol) extract is the richest one in terms of quinic acid (30091.42 μg/g extract). Moreover, the SLM extract has considerable amounts of chlorogenic (5849.68 μg/g extract) and 4-OH benzoic acids (646.63 μg/g extract).

The major component of the essential oils was identified as valencene (23.2%) for M. officinalis, arachidic acid (17.9%) for S. libanotica subsp. linearis and germacrene-D (15.6%) for Stachys thirkei. The main constituent of the fatty acid obtained from the petroleum ether extracts was identified as linolenic acid (27.4%) for M. officinalis, palmitic acid (25.0%) for S. libanotica subsp. linearis and linoleic acid (50.1%) for Stachys thirkei. Especially, the methanol extracts of three herbal teas showed strong antioxidant activity in all methods. The acetone extract of M. officinalis indicated higher inhibitory effect against butyryl-cholinesterase enzyme (92.66%) than the reference compound, galanthamin.

CONCLUSIONS

The antioxidant capacity and anticholinesterase potential of MOM extract was higher than the other eleven extracts. The reason why MOM was the most active of all twelve extracts tested for four antioxidants methods used, could be related to its high total phenolic content or high amount of rosmarinic acid that have strong antioxidant activity.

As a result, rich total phenolic content and high antioxidant capacity of the MOM necessitates further studies in this field. The results of the present study showed that MOM extract can also be used as a food source due to its high phenolic acid content and strong antioxidant properties.

REFERENCES

P-277: PHENOLIC PROFILE, FATTY ACID AND ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT, ANTIALZHEIMER ACTIVITIES OF GUNDELLIA TOURNEFORTII (KENGER) EXTRACTS

A. Ertas, M. Boga, H. Temel, M.A. Yilmaz, Y. Yesil, Isil Aydin, I. Yener, Esra Yarış

Dicle University, Faculty of Pharmacy, Diyarbakır, TURKEY
1Department of Pharmacognosy, 2Department of Pharmaceutical Technology, 3Department of Pharmaceutical Chemistry, 4Department of Analytical Chemistry
5Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, TURKEY

In this research, chemical composition and biological activities of various extracts obtained from whole parts of Gundelia tournefortii were compared. The amounts of total phenolic and flavonoid components in crude extracts were determined by expressing as pyrocatechol and quercitin equivalents, respectively. Methanol and acetone extracts obtained from G. tournefortii showed good antioxidant activity in four tested methods. Furthermore, these extracts of G. tournefortii showed good inhibition activity against acetyl- and butyryl-cholinesterase enzymes. The main constituents of fatty acid and essential oil were identified as as linoleic (C18:2 omega-6) (40.3%) and arachidic acid (22.3%), respectively, by GC/MS. Finally, the chemical composition of the methanol extract was determined using LC-MS/MS for quantitative and qualitative purposes.

INTRODUCTION

G. tournefortii L. is an important food source and a well-known medicinal plant in Eastern Anatolia. It is recorded that the flowers, leaves, seeds and stems of G. tournefortii are used as food sources [1]. In the Middle East, the young and still undeveloped flower buds are sold in the local markets just like artichoke hearts; it is a highly sought item [2]. Dry seeds of G. tournefortii are also known to be effective for the treatment of vitiligo disease, in Eastern Anatolia folk medicine. Fresh seeds of G. tournefortii are used in pickles and also are effective diuretics [3].

MATERIALS AND METHODS

Plant material: G. tournefortii L., which was collected by Dr. A. Ertuş from east Turkey (Diyarbakır) in May 2012 and characterized by Dr. Y. Yeşil (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University). Voucher specimens have been stored in the Herbarium of Istanbul University, Faculty of Pharmacy (ISTE: 98068).

Methods: Phenolic components in the methanol extract was quantified by LC-MS/MS. Essential oil and fatty acid composition of G. tournefortii was analyzed by GC/MS. β-Carotene method, ABTS cation radical decolorisation method, cupric reducing antioxidant capacity assays and DPPH free radical scavenging activity were carried out to indicate the antioxidant activity. The anticholinesterase potential of the extracts were indicated by Ellman method.

RESULTS AND DISCUSSION

The fatty acid compositions of the petroleum ether extracts were determined by GC/MS analysis. As shown in Table 3, twelve components were identified, constituting 100.0% of the petroleum ether extract of G. tournefortii. The major constituents of the fatty acids obtained from the petroleum ether extract of G. tournefortii were determined as linoleic (C18:2 omega-6) (40.3%), oleic (C 18:1 omega-9) (24.7%) and palmitic (C16:0) (19.7%).

The essential oil composition of G. tournefortii was obtained by hydro-distillation and determined by GC/FID (Gas Chromatography / Flame Ionization Detector) and GC/MS analysis. 18 components were detected, constituting 96.1% of the essential oil composition of G. tournefortii. The main components of the essential oil were identified as arachidic acid (22.3%), α-pinene (14.2%) and α-terpineol (7.1%).

Experimental results showed that the G. tournefortii methanol extract was rich in terms of phenolic acids and some flavonoids. Chlorogenic (4247.4 μg/g extract) and Protocatechuic acids (4783.5 μg/g extract) were the most abundant phenolic acid compounds in the G. tournefortii methanol extract.

The methanol and acetone exhibited stronger ABTS cation radical scavenging activity than standard compounds, α-TOC and BHT. Additionally, these extracts of G. tournefortii showed good inhibition activity against acetyl- and butyryl-cholinesterase enzymes.

CONCLUSIONS

The methanol extract of G. tournefortii can be investigated in terms of both phytochemical and biological aspects to find natural active compounds. Also, results of the current study showed that methanol extract of G. tournefortii may also be used as food supplement.

REFERENCES

INTRODUCTION

The genus *Vinca*, which is a member of Apocynaceae family is used as diuretic, antipyretic and tonic for many diseases in Turkish traditional medicine [1]. *Vinca* species include indole alkaloids. For example, vinblastine and vincristine found in *Catharanthus roseus* (*Vinca rosea*) are commonly used in cancer treatment [2]. Also another alkaloid, serpentine, present in the *Catharanthus roseus* has potent acetylcholinesterase inhibitory activity [2]. Additionally, vincarine, herbamine, majdine and isomajdine possessing apoptotic, antioxidant, antiradical indole alkaloids were isolated from *Vinca herbacea* Waldst. [3]. In the present study, anticholinesterase activity of the aerial parts of *Vinca herbacea* Waldst. has been investigated.

MATERIALS AND METHODS

**Material:** The plant material was collected from Manisa in May 2010. The plant was identified by Volkan Eroglu (Ege University, Faculty of Science). Voucher specimen is deposited (No. 1491) in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

**Method:** Powdered plant material was extracted with methanol three times for 30 minutes in an ultrasonic bath at room temperature. Then, the acetylcholinesterase (AChE) and the butyrylcholinesterase (BuChE) enzyme inhibitory activities of the extract were tested spectrophotometrically by using a microplate assay based on in vitro Ellman’s method with 96-well microplate reader [4,5].

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>AChE (% inh.)</th>
<th>BuChE (% inh.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>43.95</td>
<td>99.0</td>
</tr>
<tr>
<td>100</td>
<td>14.21</td>
<td>67.54</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>8.07</td>
</tr>
</tbody>
</table>

The experiments were carried out in triplicate. The final concentrations of the extracts in the assay were determined to 250-2.5µg/mL. The enzyme inhibitory activities were calculated as a percentage compared to blank. Galanthamine was used as a positive control and galantamine showed an IC50 value of 0.15 µM.

CONCLUSIONS

As a result of this study, the AChE and BuChE inhibitory activities of *Vinca herbacea* Waldst. are determined. Significant BuChE inhibitory activity was observed for the plant extract 1000 and 100 µg/mL. Also, the BuChE inhibitory activity was found to be higher than the AChE inhibitory activity.

ACKNOWLEDGMENTS

This study was supported the Research fund of Ege University (Project No: 09/ECZ/021).

REFERENCES


P-279: ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITY OF TANACETUM ARGENTEUM SUBSP. ARGENTEUM

A Sen1, O.Bingol Ozakpinar2, S. BirteksozTan3, S. Kultur4, F. Uras2, L. Bitis1

Marmara University, Faculty of Pharmacy, 1Department of Pharmacognosy, 2Department of Biochemistry; Istanbul University, Faculty of Pharmacy, 3Department of Pharmaceutical Botany, 4Department of Pharmaceutical Microbiology, Istanbul, Turkey

INTRODUCTION

Some pathogens are resistant against firstly discovered effective antimicrobial drugs. New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants. Contrary to presently used antimicrobial drugs, antimicrobial compounds in plants might inhibit bacterial growth by different mechanisms and may be used as antibiotic against resistant microbial strains. Thus there is a need to find new bioactive compounds of plant origin which can be used in the treatment of resistant microbial strains [1]. New drugs exhibiting antitumor activity are needed worldwide due to escalating cancercases. This
requirement could be met either by producing new molecules or by isolating new substances from the plant. The great majority of the drugs used in cancer treatment are mainly obtained either from substances isolated from plants, from modification of natural molecules or from main structures of compounds of the natural origin. Therefore, it is so important to maintain research on the plants exhibiting anticancer activity in the discovery of new molecules [2]. In this study, we investigated antibacterial and antiproliferative activities of various extracts obtained from the aerial parts of Tanacetum argenteum (Lam.) Willd. subsp. argenteum against four cancer cell lines and eight microorganisms, respectively.

MATERIALS AND METHODS
Plant material and extraction: Plant samples were collected in the flowering periods from the Doğanşehir district of Malatya province of Turkey in 2012 and were identified by Dr. Sukran Kultur, a botanist of the Faculty of Pharmacy, University of Istanbul. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE No:98969). The preparation of extracts were performed as described in our previous study [3].

Antimicrobial and antiproliferative activity: Antimicrobial activity against Staphylococcus aureus ATCC 29213 (S.a), Staphylococcus epidermidis ATCC 12228 (S.e), Escherichia coli ATCC 25922 (E.c), Enterococcus faecalis ATCC 29212 (E.f), Klebsiella pneumoniae ATCC 4352 (K.p), Pseudomonas aeruginosa ATCC 27853 (P.a), Proteus mirabilis ATCC 14153 (P.m) and Candida albicans ATCC 10231 (C.a) were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations. Antiproliferative activity was measured against four human cancer cell lines (A549: lung adenocarcinoma, Hela; cervix adenocarcinoma, HT-29: colon adenocarcinoma, MCF-7; breast adenocarcinoma) using MTT assay.

RESULTS AND DISCUSSION
All extracts, except for TAAM, at the concentration of 100 μg/mL inhibited growth of four cancer cell lines in range of 63 to 93% in compared to a control. The criteria of cytotoxicity activity for the crude extracts, as established by the American National Cancer Institute (NCI) is an IC50<30 μg/ml in the preliminary assay[4]. TAH (MCF-7:74%; HT-29:75%) and TAC (A-549:75%; HeLa:62%; HT-29:89%; MCF-7:73%) extracts at the concentration of 30 μg/ml showed a strong antiproliferative activity against tested cancer cell lines (Figure 1).

Figure 1. Antiproliferative activity of T.argenteum subsp. argenteum extracts at the concentration of 30 μg/mL.

Table 1. The MIC values (μg/mL) of different extracts of T.argenteum subsp. argenteum against various microorganisms.

<table>
<thead>
<tr>
<th>Extracts / Standards</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.a</td>
</tr>
<tr>
<td>TAH</td>
<td>-</td>
</tr>
<tr>
<td>TAM</td>
<td>-</td>
</tr>
<tr>
<td>TAC</td>
<td>625</td>
</tr>
<tr>
<td>TAAM</td>
<td>1250</td>
</tr>
<tr>
<td>Levofloksasín</td>
<td>0.25</td>
</tr>
<tr>
<td>Flukonazol</td>
<td>-</td>
</tr>
</tbody>
</table>

* not active (>1250 μg/mL),

The antimicrobial activity of plant extracts considered to be significant (MIC<100 μg/mL), moderate (100<MIC ≤625 μg/mL) or weak (MIC>625 μg/mL) as described by Kuete [5]. TAC presented moderate activity against S.a (MIC: 625 μg/mL) and S.e (MIC: 625 μg/mL). TAM and TAAM showed weak antimicrobial activity against S.e and C.a with MIC values of 1250 μg/mL (Table 1).

CONCLUSIONS
The results show that TAH and TAC are good candidates for further activity-guided fractionation in the search for new active antimicrobial and antitumor compounds.

REFERENCES
3. Sen, A.; Turan, S.O.; Akbuga, J.; Bulut, G.; Bitis, L., Centaurea cuneifolia Sm. ve Centaurea kilaea Boiss. Türlerinin In Vitro Koşullarda Silotoksik ve Antiproliferatif
The total polyphenol content (TPC) of extracts was correlated to the TPC. In addition, the antioxidant activity of the extracts was determined spectrophotometrically by the Folin-Ciocalteau reagent. The antioxidant properties were examined in vitro by DPPH test, reducing power assay, and TBA assay. Further, the antimicrobial potential of Juniperus L. spp. leaves extracts against Microbial Infections: A Review. Planta Med. 2010, 76(14): 1479-1491.

P-280: COMPARATIVE STUDY OF BIOLOGICAL ACTIVITIES OF METHANOL AND AQUEOUS LEAVES EXTRACTS OF FIVE JUNIPERUS L. (CUPRESSACEAE) SPECIES GROWING IN TURKEY

M.F. Taviano1, A. Koroğlu2, A. Marino1, N. Miceli1

1University of Messina, Department of Scienze del Farmaco e Prodotti per la Salute, Messina, ITALY
2Ankara University, Department of Pharmaceutical Botany, Ankara, TURKEY

INTRODUCTION
Numerous Juniperus L. (Cupressaceae) species, particularly those under the Juniperus section, are frequently employed in Turkish folk medicine to treat several diseases. Since ancient times, Juniperus L. female cones and leaves have been utilized as an antihelmintic, diuretic, stimulant, wound-healing, and antiseptic [1].

In continuation of our studies on Juniperus L. species under Juniperus section growing in Turkey, this work was designed to define and compare the antioxidant and antimicrobial properties of methanol and aqueous extracts of five Juniperus species: J. communisL. var. communis (Jcc), J. communisL. var. sasatilisPall. (Js), J. drupaceaLabill. (Jd), J. oxycedrusL. ssp. oxycedrus (Joo), J. oxycedrusL. ssp. macrocarpa(Sibth. & Sm.) Ball.(Jom).

MATERIALS AND METHODS
The total polyphenol content (TPC) of Juniperus L. spp. leaves extracts was determined spectrophotometrically by the Folin-Ciocalteau reagent. The antioxidant properties were examined in vitro by DPPH test, reducing power assay, and TBA assay. In addition, the antioxidative activity of the extracts was correlated to the TPC. The Artemia salina Leach (brine shrimp) lethality bioassay was employed to predict the potential cytotoxic activity of the extracts. Further, the antimicrobial potential of Juniperus L. spp. extracts against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger ATCC strains was evaluated by standard methods [2].

RESULTS AND DISCUSSION
The TPC of Juniperus L. spp. leaves methanol extracts ranged from 71.76 ± 0.13 mgGAE/g (Jcc) to 133.28 ± 1.74 mgGAE/g (Joo); the TPC of water extracts ranged from 70.08 ± 2.51 mgGAE/g (Jcc) to 176.05 ± 1.15 mgGAE/g (Joo).

Both in the DPPH test and in the reducing power assay, Joo methanol extract was found to be the most active (IC50 = 0.092 ± 0.006 mg/mL and ASE/mL = 2.56 ± 0.06, respectively). A strong positive correlation between primary antioxidant activity and TPC was found. In the TBA assay, Jcs methanol extract exhibited the highest activity (IC50 = 4.39 ± 0.47 μg/mL). A positive correlation between TPC and anti-lipid peroxidation activity was highlighted for aqueous extracts, whilst no correlation with methanol extracts was found.

All the extracts resulted potentially non-toxic against Artemia salina (LC50> 1000 μg/mL). The MIC values obtained from the antimicrobial tests indicated that Juniperus L. spp. leaves extracts display bacteriostatic activity versus S. aureus, whereas they are not effective against the Gram-negative bacteria and the yeasts tested in the study.

CONCLUSIONS
The results of our investigation give a scientific basis to the ethnopharmacological use of these Turkish Juniperus L. species, also demonstrating the potential of their leaves as sources of natural antioxidant and antimicrobial compounds.

REFERENCES

P-281: ANTIMICROBIAL ACTIVITY OF SOME BIDENS SPECIES

Ü. G. İçöz1, M. Eryılmaz2, A.N. Yazgan3, B. Sever Yılmaz3, M.L. Altun3

1Republic of Turkey, Social Security Institution, Ankara, Turkey, 2Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 3Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION
The genus Bidens L., belongs to Asteraceae family and is distributed in many regions of the world with 280 species. The genus Bidens is mainly represented by four species in Turkey [1]. Phytochemical studies on Bidens species have shown the presence of
polycytenes, flavonoids, essential oils, coumarins, polysaccharides, carotenoids, amines, mineral elements and chalcones [2]. Several Bidens species are used in traditional medicine for the treatment of inflammation, rheumatism (arthritis), diabetes, enteritis, angina (pharyngitis), malaria, metabolic disorders, dermal diseases, seborrhea, psoriasis, wound and tumors [3,4].

The objective of this study was to evaluate the in vitro antimicrobial activities of aerial parts of B. cernua L. var. radiata DC., B. frondosa L. and B. tripartita L. against some bacteria and yeast.

MATERIALS AND METHODS

In vitro antibacterial and antifungal activities of Bidens Species: Aqueous and ethanolic aerial parts extracts of B. cernua var. radiata, B. frondosa and B. tripartita were screened for their potential in vitro antibacterial activities against S. aureus ATCC 25923, S. aureus ATCC 43300 (MRSA), B. subtilis ATCC 6633, E. coli ATCC 25922, P. aeruginosa ATCC 27853 and antifungal activities against C. albicans ATCC 10231. Prior to testing all extracts were filter-sterilized through a 0.45 μm membrane filter. Broth dilution assay was used for determination of the minimum inhibitory concentrations (MIC) [5].

RESULTS AND DISCUSSION

Ethanolic extracts of tested species exhibited better antimicrobial activity than aqueous extracts. Ethanolic extracts of tested species possessed activity having MIC values of 0.125-0.250 mg/ml against the tested microorganisms. No antibacterial activity was observed for all the aqueous extracts. Only aqueous extract of B. frondosa and B. tripartita showed antifungal activity having MIC values of 0.500-1.000 mg/ml against C. albicans, respectively.

CONCLUSIONS

In conclusion, this study provides significant information about antimicrobial activities of aqueous and ethanolic extracts of aerial parts of B. cernua var. radiata, B. frondosa and B. tripartita. It is conceivable that usage of Bidens species for treatment of various skin diseases and injuries, and also cold in folk medicine is because of its antimicrobial activity. To the best of our knowledge, this is the first research on the antimicrobial activity of Turkish Bidens species.

REFERENCES


P-282: FLAVONOIDS FROM SCORZONERA LATIFOLIA (FISCH. AND MEY.) DC.

B. Ergene Öz, Ö. Bahadir Aci karma, S. Özbilgin, G. Saltan

Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION

The genus Scorzonera L. which belongs to Asteraceae family, grows widespread from Central Europe to Central Asia, especially in Mediterranean region and has more than 175 species. In Turkey, this genus is represented by 49 species [1,2]. Scorzonera species are widely used as traditional medicine or consumed as vegetable in Europe as well as in Turkey. The latex obtained from the roots of Scorzonera latifolia (Fisch. and Mey.) DC. is traditionally used as analgesic and against infertility externally. The internal use of this mastic as anthelmintic is also reported [1,3]. According to the phytochemical studies, this species contains phenolic compounds such as scorzoveratin 4-O-β-glucoside, scorzoveratrin scorzoveratrizit and caffeoyl derivatives in roots [2,4].

In this study, two major compounds were isolated from the water soluble fraction of the methanol extract obtained from the aerial parts of S. latifolia.

MATERIALS AND METHODS

Plant material: S. latifolia was collected from Kars (AEF 23830) and identified by H. Duman.

Extraction: Dried and powdered aerial parts of the plant material were macerated in methanol at room temperature. Afterwards, the extract was filtered and evaporated until dryness under reduced pressure at 50ºC.

The crude extract was subjected to liquid partitioning yielding the fractions of petroleum ether, chloroform, ethylacetate and water. Water soluble fraction was applied to reverse phase column (C18). The extract was eluated with water:methanol mixture and two compounds were obtained. Chemical structures of the compounds were elucidated by 1H- and 13C-NMR as well as 2D-NMR (COSY, TOCSY, HSQC, HMBC) techniques.
RESULTS AND DISCUSSION
The chemical structures of the compounds were established as swertisin and 7-methyl isoorientin.

CONCLUSIONS
Flavonoid derivatives are the compounds which take attention with their various biological activities such as antioxidant, anti-inflammatory and antithrombotic activities [5]. In this respect, the compounds isolated from the aerial parts of S. latifolia are remarkable and this species may be suggested as a natural source of flavonoids. Therefore further studies either on chemical composition or on biological activity of this species are needed.

ACKNOWLEDGMENTS
This study is funded as a scientific research project by Ankara University.

REFERENCES

P-283: ANALYSIS OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF THREE ORNITHOGALUM L. SPECIES

G. Renda¹, A. Özel², B. Yaylı³, B. Barut², M. Çol Ayvaz³, E. Akyüz Turuntay³

Karadeniz Technical University, Faculty of Pharmacy
¹Department of Pharmacognosy, ²Department of Biochemistry Trabzon, TURKEY
³Ordu University, Faculty of Art and Sciences, Department of Chemistry, Ordu, TURKEY
⁴Recep Tayyip Erdogan University, Faculty of Art and Sciences, Department of Chemistry, Rize, TURKEY

INTRODUCTION
The genus Ornithogalum (Liliaceae) comprises about 34 species in Turkish flora [1]. The bulbs of the plant which has medical and economic value since the time of Dioscorides. O. sigmoideum bulbs are also consumed as food and sold in local markets [2]. Our aim is to compare the antioxidant capacity and investigate the phenolic compounds of both bulbs and aerial parts of three Ornithogalum species; O. sigmoideum Freyn & Sint (OS), O. orthophyllum Ten. (OO), O. oligophyllum E.D.Clarke (OL).

MATERIALS AND METHODS
Aerial parts and bulbs of plant species were collected from Ordu and Trabzon. Dried and powdered plant materials were separately extracted with methanol. Antioxidant activity of the crude methanol extracts of the aerial parts and the bulbs were determined with following methods; 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and superoxide radical scavenging, metal chelation, phosphomolibdenum-reducing antioxidant power (PRAP), ferric-reducing antioxidant power assay (FRAP) [3]. The total phenolic content was determined by Folin Ciocalteu method [4]. Crude methanol extracts of phenolic compounds from flowers and leaves of OS, OO and OL were evaporated and dissolved in water for liquid-liquid extraction using ethyl acetate and diethyl ether for better resolution of high performance liquid chromatography (HPLC). HPLC-UV analyses of phenolic compounds were carried out by the method validated on the standard phenolic compounds [5]. HPLC-UV analyses were performed on a reverse phase C18 column and compounds were detected with an UV-Vis detector. Benzoic acid derivatives (gallic, protocatechuic, p-hydroxybenzoic, vanillic, and syringic acid), flavanol (epicatechin) and cinnamic acid derivatives (chlorogenic, caffeic, p-coumaric and ferulic acid) were analyzed.

RESULTS AND DISCUSSION
Among the six extracts, methanol extract of the aerial parts of OO contains the highest (GAE, 11.00 mg/g extract) amount of phenolic compounds followed by the methanol extract of the bulbs of the OL. The SC₅₀ values of methanol extract of the aerial parts of OO were 0.39 mg/ml and 0.44 mg/ml which showed higher DPPH and superoxide radical scavenging activity, respectively, than the other extracts. Protocatechuic acid, p-hydroxybenzoic acid, vanillic acid and p-coumaric acid were among the most abundant compounds identified at all of the extracts. Diethyther phase of leaf part of OO extract has highest phenolic content according to HPLC-UV analysis whose total phenolic content and antioxidant potential were highest in the extracts as well.

CONCLUSIONS
The results showed that there was a high correlation between antioxidant activity potential and amount of phenolic compounds of the extracts.

ACKNOWLEDGMENTS
This research was supported by the Scientific Research Foundation of the Karadeniz Technical University (Project Number 8960). The authors are grateful to Professor Neriman Özhatay (Istanbul University, Faculty of Pharmacy, Department of...
Pharmaceutical Botany) for the help in the authentication of the species.

REFERENCES

RESULTS AND DISCUSSION
The experiments were carried out in triplicate. The enzyme inhibitory activities were calculated as a percentage compared to blank. Galanthamine was used as a positive control and the range of concentration was 300-0.003 μg/mL. According to the test results, the AChE inhibitory activity was higher than the BuChE activity. And the final concentrations of the extracts in the assay were 250, 25, 2.5μg/mL.

CONCLUSION
As a result, the AChE and BuChE inhibitory potentials of Glaucium leiocarpum BOISS. are determined. Significant anticholinesterase activity was observed for the plant extract prepared at 1000 μg/mL.

ACKNOWLEDGMENTS
This study was supported by the Research Fund of the Ege University. (Project number 09/ECZ/021)

REFERENCES
1. Baytop, T., Therapy with Medicinal Plants in Turkey (Past and Present), 1984, Publication no 3255, 189.

P-284: EVALUATION OF CHOLINESTERASE INHIBITORY PROPERTIES OF GLAUCIUM LEIOCARPUM BOISS.
C. Emir1, A. Emir1, B. Bozkurt1, G. I. Kaya1, M. A. Onur1, N. Unver Somer1

INTRODUCTION
The genus Glaucium consisting of about 25 species in Turkey belongs to the Papaveraceae family. Glaucium species are used as laxatives, hypnotic, antitussive in Turkey belongs to the Papaveraceae family. Therefore it can be used for the treatment of Alzheimer’s Disease (AD) [4]. In this study, the acetylcholinesterase (AChE) and butrylcholinesterase (BuChE) enzyme inhibitory activities of Glaucium leiocarpum BOISS. were investigated.

MATERIALS AND METHODS
Material: The plant material was collected from Manisa in May, 2010 and was identified by Volkan Eroglu (Ege University, Faculty of Science). The plant specimen with herbarium no 1481 is deposited in the Herbarium of Ege University, Faculty of Pharmacy, Department of Pharmacognosy.
Method: Dry-powdered plant material was extracted with methanol three times for 30 minutes in an ultrasonic bath at room temperature. Then, the AChE and BuChE inhibitory activities of the extract were determined spectrophotometrically by using a microplate assay based on in vitro Ellman’s method [5].

P-285: ANTIOXIDANT POTENTIAL AND FATTY ACID CONTENT OF ECHINOPS VISCOSUS DC. SUBSP. BITHYNICUS
A. Ertaş1, D. Dinçel2, T. Kuşman2, B. Çulhaoğlu2, Y. Yeşılı3, U. Kolak4, G. Topçu2

1Dicle University, Faculty of Pharmacy, Department of Pharmacognosy, Diyarbakır, Turkey
CONCLUSIONS
Determination of total phenolic and flavonoid contents and antioxidant activities (ABTS\(^+$\), CUPRAC, DPPH) of the obtained extracts will be carried out following our studies.

REFERENCES

P-286: IN VITRO CYTOTOXIC EFFECTS OF SOME TANNINS AND TANNIN PRECURSORS

S. Sabuncuoğlu\(^1\), D. Şöhretoğlu\(^2\)

Hacettepe University, Faculty of Pharmacy
\(^1\)Department of Pharmaceutical Toxicology, \(^2\)Department of Pharmacognosy Ankara, TURKEY

INTRODUCTION
Some Geranium species have been employed in folk medicine for their tonic, diuretic, antidiabetic, anti diarrheal, and anti hemorrhoidal properties. Moreover, they have been used in the treatment of fever, tonsillitis, cough, whooping cough, urticaria, dysentery, kidney pain, and gastrointestinal ailments. These traditional usages and the rich polyphenolic content of Geranium species make these plants interesting from the viewpoint of biological and toxicological research [1,2]. In this study, cytotoxic potential of f1,3,6-tri-O-galloyl-β-glucopyranoside (1), pusilagin (2), methyl gallate (3), 1,2,3,4,6-penta-O-galloyl-β-glucopyranoside (4) and gallic acid (5) isolated from G. psilostemon were evaluated against L1210, V79 and HeLa cell lines at different concentrations.

MATERIALS AND METHODS
Tested compounds were isolated from G. psilostemon in our previous study [3].

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay for the cytotoxicity of the compounds: Cell viability was evaluated by the reduction of MTT (Guan S 2006). Briefly, L1210, V79 or HeLa cells (5000 cells/well) were treated with 100...
μM or 1M H₂O₂ in the presence of different concentrations of the compounds (10 μg/ml, 20 μg/ml). After incubation with MTT, cells were lysed in DMSO and the MTT formazan was qualified by determining the absorbance. Cell viability was expressed as a percent of the control culture value. Statistical analysis: Student t-test was performed as a nonparametric test to evaluate the significance of the differences between groups. p < 0.05 was accepted as significant.

RESULTS AND DISCUSSION

Fig. 1. The effect of the compounds on cell viability against H₂O₂ induced cytotoxicity. Viability of the cells was determined by MTT assay. Data are shown as mean ± SD, *significantly different from negative control, # significantly different from 100 mM H₂O₂, & significantly different from 1 M H₂O₂.

According to our results, the compounds showed cytoprotective effect with MTT assay at 10 and 20 μg/ml against H₂O₂ induced cytotoxicity. On the other hand, IC₅₀ levels of the compounds were determined in different cell lines,

CONCLUSIONS

Oxidative stress in cardiomyocytes plays an important role in different pathologies. Several studies showed that phenolic compounds have antioxidant effect by scavenging the free radicals. In this study, it has been shown that the compounds, isolated from G. psilostemon, have cytoprotective effect due to their antioxidant potential. However, the extracts should be investigated in detail for its cytotoxic activity because of the possible prooxidant effect of the compounds in high concentrations. Finally, these results make the plant highly interesting to investigate the potential radical scavenging, protective and cytotoxic activities. Thus, further studies are necessary to determine the wide range for optimal cytotoxic or protective concentrations of the compounds from the plant which is used in folk medicine.

REFERENCES


P-287: ANTIMICROBIAL ACTIVITY AND FLAVONOIDS CONTENT OF IPSOLITHUS ARHIZUS MUSHROOM GROWN IN TURKEY

D. Onbasli¹, G. Yuvali Celik¹, B. Aslim²
¹Erciyes University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Kayseri, TURKEY
²Gazi University, Faculty of Science, Department of Biology, Ankara, TURKEY

ABSTRACT

The purpose of this study was to evaluate the antimicrobial effects and flavonoid content of Pisolithus arhizus. Antimicrobial activity of ethanol and methanol extracts was evaluated through the determination of the MIC by the microdilution method in culture broth. For this purpose, Salmonella typhimurium BAST01, Escherichia coli ATCC 35218, Enterobacter aerogenes BA-EA1 used as test microorganisms. Flavonoids analysis of the mushroom extracts has been carried out by high-performance liquid chromatography (HPLC). These results showed that the mushroom may be used in pharmaceutical applications because of its effective antimicrobial and antioxidant properties.

INTRODUCTION

Pisolithus arhizusus used in this study has a wide spread in our country (Antalya, Aydın, Balıkesir, Çanakkale, Denizli, İzmir, Kahramanmaraş, Kütahya, Malatya, Manisa, Mersin Muğla regions). The aim of the work presented here in were (i) to determine the antimicrobial activity of ethanol and methanol extracts of Pisolithus arhizus (ii) to characterise the flavonoid content in the mushroom extracts by HPLC.

MATERIALS AND METHODS

Extraction procedure

Extracts of the materials to be used in the study will be determined according to the method Barros et al (2007) [1].

Antimicrobial activity

Test bacteria: Salmonella typhimurium BAST01, Escherichia coli ATCC 35218 and Enterobacter aerogenes BA-EA1 (enteric pathogens) were used as test bacteria.

Determination of minimum inhibitory concentrations (MIC): Antimicrobial activity of the extracts was
evaluated through the determination of the MIC by the microdilution method in culture broth.  

**Determination of flavonoid contents:** Flavonoids analysis was carried out using an Agilent Technologies 1200 series High performance liquid chromatograph consisted of a Quart pump, an UV-detector and a ACE 5-C18 column (250x4.6 mm, 5μm particle size). The flavonoid content was calculated from the peak areas of HPLC chromatograms from the 3 replicate samples.

**RESULTS AND DISCUSSION**

**Determination of antimicrobial activity:** The results are given in Table 1. In the extracts prepared with different solvents was observed the concentration of the different inhibition. According to this results; The ethanol extracts of *P. arhizus*, against *S. typhimurium* and *E. coli* pathogens showed a greater effect than *E. aerogenes* pathogen. While the methanol and ethanol extracts of *P. arhizus* showed the same effecton *S. typhimurium* ve *E. aerogenes*, the ethanol extract of this mushroom showed less effect on *E. coli*.

**Table 1.** Minimum Inhibition Concentration (MIC) values (μg/ml) on test microorganisms of *Pisolithus arhizus*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Minimum Inhibition Concentration (MIC) (μg/ml)</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. typhimurium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EA1</td>
</tr>
<tr>
<td><em>P. arhizus</em></td>
<td>Ethanol ≥250</td>
<td>≥250</td>
</tr>
<tr>
<td></td>
<td>Methanol ≥250</td>
<td>≥500</td>
</tr>
</tbody>
</table>

**Determination of Flavonoid contents:** The results are given in Table 2. According to this results, kaempferol (14,32 mg/g) and biochanin A (0,81 mg/g) in the ethanol extracts of *P. arhizus* were higher than its methanol extracts. Also, we detected that the methanol extracts had higher luteolin (4,90 mg/g), rutin (40,12 mg/g), quercetin (mg/g) and catechin (2,57 mg/g) amount. Apigenin (3,34 mg/g) were found only in the ethanol extract.

**Table 2.** The results of High Performance liquid Chromatography (HPLC) analysis of Flavonoid content of *Pisolithus arhizus*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvents</th>
<th>Flavonoid Content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
</tr>
<tr>
<td><em>P. arhizus</em></td>
<td>Ethanol</td>
<td>3.56</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>4.90</td>
</tr>
</tbody>
</table>

ND: Not determined, L: Luteolin, R: Rutin, Q: Quercetin, K:Kaempferol, B: Biochanin A: Apigenin, C: Catechin

**CONCLUSIONS**

These results showed that *Pisolithus arhizus* may be used in pharmaceutical applications because of its effective antimicrobial and antioxidant properties. Further investigation on the underlying mechanism responsible for the biological activities should be attempted.

**ACKNOWLEDGMENTS**

The authors wish to thanks to University of Erciyes Scientific Research Projects Unit for financial supporting to the TSA-2014-5232 project and Dr. Hakan Alli, Department of Biology, Faculty of Science and Art, University of Mugla, for identification of the mushroom materials collected.

**REFERENCES**


**P-288: SECONDARY METABOLITES FROM ERICA MANIPULIFLORA SALISB.**

S. Avunduk

1Vocational School of Health Care, Mugla University, Marmaris, Mugla, 48187 Turkey

**INTRODUCTION**

The genus Erica L. (Ericaceae is represented by only four species of which one taxon (*Erica bocquetti* (Peşmen) P.F. Stevens) is endemic in Turkey[1]. Herbal teas are prepared from aerial parts of Erica arborea and Erica manipuliflora have been popularly used as diuretic, astringent and treatment of urinary infections in Turkey [2]. Erica species contain many active components such as flavonoids, anthocyanidins, coumarins, and triterpenoids [3], phenylpropanoid glycosides,diarylnonanoidglycosides. There is a limited number of reports on *Erica manipuliflora* Salisb.[4-5] The aim of our study is to make more detailed phytochemical investigation on *E. manipuliflora*Salisb., and isolation-characterization of new compounds.

**MATERIALS AND METHODS**

The aerial parts of *Erica manipuliflora* Salisb. was extracted with n-Hexane, CH₂Cl₂ and MeOH, respectively. MeOH extract was partitioned between n-BuOH and H₂O. All extracts were fractioned using various chromatographic techniques (Open-CC, VLC) on different adsorbents (NP-and RP-silica gel). 11 sub-fractions were obtained from n-Hexane extracts. 8, sub-fraction (EH8) has been achieved as pure compound. The CH₂Cl₂ extract were submitted on Open-CC using NP-silica gel. 9 sub-fractions were obtained. The n-BuOH and H₂O extracts were subjected to VLC on RP-silica gel. 5 and 8 sub-fractions were obtained, respectively.
The structure of pure compounds isolated from the extracts were elucidated by extensive 1D-and 2D-NMR, and mass spectroscopic techniques.

RESULTS AND DISCUSSION
EH8 which is the major compound of n-Hexane extract was isolated. Our phytochemical study on CH2Cl2, n-BuOH, and H2O extracts is still going on.

CONCLUSIONS
As a result of interpreting the 1H-NMR spectra, the structure of EH8 has been determined as free aglycone—contains no sugar molecule—and triterpene type aglycone.

Fig. 1. A picture of Erica manipuliflora Salisb.

REFERENCES

P-289: ISOLATION AND IDENTIFICATION OF FLAVONOID GLYCOSIDE FROM AERIAL PARTS OF ZOSIMA ABSINTHIFOLIA (VENT.) LINK

A. Atasoy, E. Kurtul, Ö. Bahadir Acikara

Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey

INTRODUCTION
Zosima absinthifolia (Vent.) Link belongs to Umbelliferae family, widely distributed in central and southwestern areas of Asia; Turkey, Iran, Afghanistan [1-2]. Aerial parts of the plant are consumed as a vegetable after cooked, and added to a traditional cheese made in East Anatolia [3]. Fruits of Z. absinthifolia are also used as a food spice in Turkey and Iran [4]. The fruit is ethnobotanically important in Turkish folk medicine for its digestive, carminative, and anti-inflammatory properties. Additionally, the aerial parts are used to relieve indigestion and stomach gas, to treat cough and bowel disorders. Flavonoids including 4′-ethoxy naringenin, acetylated chrysoeriol rhamnoside, 2′,3′,4′,6′- tetrahydroxy – 4 – O – angelylchalcon; comaraines such as (–)-deltoin, zosimin, bergapten, isobergapten, pimpinellin, isopimpinellin, imperatorin, sphonin and umbelliferone as well as alkaloids have been isolated from Z. absinthifolia [5]. In current study isolation and structure elucidation of a flavonoid glycoside from Z. absinthifolia aerial parts was aimed.

MATERIALS AND METHODS
Z. absinthifolia was collected from Bolu, Turkey. Identification of the plant sample was confirmed by Prof. Hayri Duman, a plant taxonomist from Gazi University. Voucher specimen was kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No: 23847). Aerial parts of the plant were dried and extracted with n-hexane, ethyl acetate and methanol respectively under reflux for 5 h. The methanolic extract was evaporated to dryness and subjected to column chromatography on silicagel column to obtain 72 fraction. Chloroform: methanol : water mixture was used for elution. Fr. 10-12 were applied to further purification procedure by using preparative TLC to obtain a flavonoid glycoside. The structure of the isolated compound was elucidated by 1H, 13C-NMR and 2D-NMR spectrometric techniques (1D and 2D) and measurements of mass spectrometry.

RESULTS AND DISCUSSION
A flavonoid glycoside isolated from aerial parts of Z. absinthifolia, was identified as isorhamnetin-3-O-rhamnoglucoside according to obtained data from spectroscopic analysis (1H, 13C-NMR and 2D-NMR, MS and UV).

CONCLUSIONS
A flavonoid glycoside, identified as isorhamnetin-3-O-rhamnoglucoside (narcissin) has been isolated from Z. absinthifolia aerial parts for first time.

ACKNOWLEDGMENTS
We are grateful to Prof. Hayri Duman for plant identification.

REFERENCES
INTRODUCTION

Epidemiological data have clearly indicated that regular consumption of wine in moderate amounts may have positive effects on health care. This health-promoting effect is generally attributed to their rich phenolic content and antioxidant effect [1,2]. Therefore interest in wines has been recently increased. There is a wide range of diversity of grape and other fruit wines and many of them are produced in Turkey [3]. However, the antioxidant potential of these fruit wines has not been explored scientifically so far. Eventually, this study was designed to investigate the antioxidant potential of sourcherry, blueberry and black mulberry wines. In addition, a Turkish red wine prepared from Papazkaras grapevines was also included in the study in order to compare their efficiency. An in vitro gastrointestinal digestion method was also applied to simulate the possible changes in the activity following the oral intake.

MATERIALS AND METHODS

Materials: Sourcherry, black mulberry, blueberry and Papazkaras wines were obtained from local markets in Turkey. After evaporating the alcohol, the remaining wines were lyophilized. The lyophilized extracts were dissolved in water at proper concentrations.

In Vitro Digestion Method: Based on the methodology described by McDougall et al. with a few modifications [4]. It consists of two major phases that are gastric and intestinal phases.

Determination of Total Phenolic Content: Total phenolic content of the samples were measured according to the method described previously [5].

Antioxidant Activity: The antioxidant activities was measured by various methods such as DPPH-radical and H2O2 -scavenging tests, total antioxidant capacity and cupric ion reducing capacity tests [5].

RESULTS AND DISCUSSION

According to in vitro digestion method, the digestion process directly affects the antioxidant activities of the samples. There are significantly differences antioxidant activity between digested and undigested extracts.

CONCLUSIONS

These results indicate that although antioxidant potentials of these fruit wines are close to each other, black mulberry wine shows higher antioxidant activity when compared to others.

REFERENCES


P-291: THE COMPARISON OF ANTIOXIDANT ACTIVITIES OF GINGER AND TURMERIC RHIZOMES BEFORE AND AFTER SUBMISSION TO DIGESTION SIMULATION

M. Charehsaz1, S. Akyüz2, E. Yeşilada1

Yeditepe University, Faculty of Pharmacy, 1Department of Pharmaceutical Toxicology, 2Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION

Various herbs and spices have been widely studied for their antioxidant effects. They are the most important targets in the search for natural antioxidants regarding safety issues. The rhizomes of ginger (Zingiber officinale Roscoe, Zingiberaceae) and turmeric (Curcuma longa L., Zingiberaceae) are one of the most popular spices containing natural antioxidants. There are numerous studies about their antioxidant activities in the scientific literature [1]. Although the assessment of antioxidant activity potential of plants are usually performed by in vitro
methods, the results may sometimes be conflicting with the results obtained from in vivo. [2]. In addition, a full-scale evaluation of bioavailability of phytochemicals requires information regarding their absorption, metabolism, tissue and organ distribution and excretion. In vitro digestion procedures mimic the biochemical and physicochemical conditions encountered in the upper GI tract [3]. In this study, we assess the effect of a two-step simulation of gastrointestinal digestion on total phenolic content, free radical scavenging activity and metal reducing activity of ginger and turmeric rhizomes.

MATERIALS AND METHODS

Materials: The dried rhizomes of ginger and turmeric were purchased from Spice Bazaar, Istanbul. The powdered samples (400 g each) were macerated 24 h with 1500 mL of 80% MeOH. Then, the macerates were extracted. Then, the macerates were continuously extracted with a rotatory evaporator at 45 °C using the same solvent for 4 h, then filtered and lyophilized.

In Vitro Digestion Method: The in vitro digestion method consisting of two successive steps was modified from previously published study [3].

Antioxidant Activity: The antioxidant activities were measured by various methods such as DPPH-radical and H2O2 -scavenging tests, total antioxidant capacity and cupric ion reducing capacity tests [2]

RESULTS AND DISCUSSION

Based on our method, the composition of extracts are affected by digestion process. Digested and undigested extracts have different antioxidant activity.

CONCLUSIONS

According to our study, ginger and turmeric have antioxidant activity and ginger has more potential than turmeric.

REFERENCES


P-292: ACETYLCOLINESTERASE AND BUTYRYLCOLINESTERASE INHIBITORY ACTIVITY OF WIEDEMANNIA MULTIFIDA L. BENTHAM

E. Sezen Karaoglan1, K.O. Yerdelen2, M. Şengül Koseoglu3, U. Özgen4

1 Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 2Department of Pharmaceutical Chemistry, 3Faculty of Science, Department of Biology, Erzurum, TURKEY, 4Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY

INTRODUCTION

Wiedemannia genus is used instead of ballibaba (Lamium sp.) in Anatolia [1]. Methanol extract of Wiedemannia multifida L. Bentham (Lamiaceae) has shown strong antioxidant and antibacterial activity [2]. Alzheimer’s disease is the frequent cause of dementia affecting people, and is associated with loss of cholinergic neurons in parts of the brain[3]. Therefore, the present study was planned to determine the in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of the aqueous, n-butanol, ethyl acetate, dichloromethane, hexane extracts of the aerial parts of W. multifida L.

MATERIALS AND METHODS

A general procedure for extractions: The air-dried aerial parts of W. multifida L. were extracted with methanol at 40 °C (3× 2 L). The methanol extract was concentrated in vacuo which was dissolved in water and consecutively partitioned by n-hexane, dichloromethane, ethyl acetate, and n-butanol.

Inhibition Studies on AChE and BuChE: AChE, BuChE, 5,5-dithiobis-(2-nitrobenzoic acid) DTNB, acetylthiocholine iodide (ATCI) and butyrylthiocholine iodide (BTCI) were purchased from Sigma Aldrich. Inhibitory activities of AChE and BuChE of the test compounds were evaluated by colorimetric Ellman’s method[4] with some modifications using commercially available neostigmine bromide[5] as the reference compound. The test compounds were dissolved in dimethylsulphoxide and then diluted in 50 mM Tris buffer (pH 8.0) to provide a final concentration range. In a 96-well plate, the assay medium in each well consisted of 50 μL of a Tris buffer, 125 μL of 3 mM DTNB, 25 μL of 0.2 U/mL enzyme (AChE or BuChE) and 15 mM substrate (ATCI or BTCI). The assay mixture containing enzyme, buffer, DTNB and 25 μL of inhibitor compound was preincubated for 15 min at 37° C, before the substrate was added to begin the reaction. Neostigmine bromide and all test compounds were prepared at four different concentrations such as 12.5, 25, 50 and 100 μg/mL. The absorbance of the
reaction mixture was then measured three times at 412 nm every 45 s using a microplate reader (Bio-Tek ELx800, USA).

RESULTS AND DISCUSSION
The extracts had low inhibition towards AChE and BuChE. The ethyl acetate extract (22% inhibition, 100 µg/mL) showed higher AChE inhibition activity and the hexan extract (37% inhibition, 100 µg/mL) showed higher BuChE inhibition activity than other extracts.

Fig. 1. A picture of Wiedemannia multifida L. Bentham

CONCLUSIONS
Isolation studies should be available about ethyl acetate and hexan extracts of W. multifida L. The pure compounds may be more effective on AChE and BuChE inhibitions.

REFERENCES

P-293: BIOLOGICAL ACTIVITIES AND CHEMICAL COMPOSITION OF FERULAGO IDAEA

E. Erçin Aslan1, D. Dincel1, B. Çakar Haberdar2, G. Topçu2

1Bezmialem Vakif University, Faculty of Pharmacy, Department of Pharmacognosy & Phytochemistry, Istanbul
2Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry, 34469, Maslak, Istanbul

The genus Ferulago W. Koch. (Apiaceae) is represented by 49 species in the world and 34 species in the flora of Turkey; 18 of these are endemic [1].

INTRODUCTION
Ferulago species belong to one of the aromatic flowering plants families, Apiaceae (=Umbelliferae). Since ancient times Ferulago species have been traditionally used as sedative, digestive, stimulative and aphrodisiac. Anatolia, which is one of the widely growing places of Ferulago species where Turkish local names for Ferulago are known as ‘Çaşır’ or ‘ Çağır’, and their roots are used as aphrodisiac. Ferulago idaea is endemic to Mount Ida and was first identified in 2000 [2].

MATERIALS AND METHODS
Preparation of plant extracts: Ferulago idea was collected from Mount Ida by Dr. Tuncay Dirmenci. Ferulago idea was dried at room temperature and ground to fine powder in a mechanic grinder. Then it was successively extracted with dichloromethane, and methanol. After filtration of each solvent, the organic phases were independently concentrated under vacuum by evaporating to dryness. The rest of the extract was fractionated on a silica gel column by using first petroleum ether (PE) and then dichloromethane (DCM) with increasing polarity to CH2Cl2 until 100% and finally methanol (MeOH) was added in the same way. The fractions were compared by TLC and similar fractions were combined. The combined fractions were separated by repeated preparative TLC procedures.

Activity Methods: Total phenolic contents of Ferulago idea extracts were determined with Folin-Ciocalteu’s reagent. Total flavonoid contents were determined with Aluminium Nitrate Method. In this study, antioxidant activities of dichloromethane and methanol extracts of Ferulago idaea were determined by two different methods. Antioxidant capacity were investigated by β-carotene-linoleic acid method and radical scavenging activity by DPPH test systems. In addition, total phenolics and total flavonoid contents of these extracts were determined as equivalent to pyrocatechol and quercetin, respectively. 

Anticholinesterase activity of the extracts of Ferulago idea were investigated against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes by modified Ellman method.

RESULTS AND DISCUSSION
Ferulago idaea which have not been studied yet chemically. In this study the aerial parts of Ferulago idaea were studied and two furocoumarins have been isolated. From these compounds isomeratorin, 3’-epidecursin are known compounds. Their structures were elucidated by 1D- and 2D-NMR and mass
spectroscopic techniques. Below the structure of isoimperatorin (Fig. 1) is given.

Based on the both antioxidant activity methods, the methanol extract of *Ferulagoidea* is found to be moderately active, but the dichloromethane extract is not active. As isolated pure compounds, none of them are found to be active by DPPH radical scavenging activity test, however isoimperatorin and 3'-epidecursin are active by β-carotene-linoleic acid method.

Anticholinesterase activities of dichloromethane and methanol extracts of *F.idea* and their pure compounds are moderately active compared to the standard of galanthamine. As pure compounds isoimperatorin and 3'-epidecursin have anticholinesterase activity particularly against AChE enzymes.

**CONCLUSIONS**

This is the first study that reports isolation of furocoumarins from *Ferulagoidea* extracts. In this study, we have investigated antioxidant and anticholinesterase activity of extracts and the isolated furocoumarins. Our studies are still going on the isolation of other furocoumarins from *Ferulagoidea*.

**REFERENCES**


**MATERIALS AND METHODS**

**Plant Materials:** The chamomile cultivars of Bona, Bodega and Zloyt Lan varieties and one chamomile population from Yalova having different sowing dates (early November, early December and late December) were obtained from Atatürk Central Horticultural Research Institute (Yalova, Turkey).

**HPTLC Method:** Separation was performed on the silica gel 60H254 HPTLC glass plates (20 x 10 cm) using the developing solvent system of ethyl acetate-formic acid-acetic acid-water (30:1.5:1.5:3, v/v/v/v). For the visual documentation, the plates were heated at 100 °C and dipped into NP/PEG 400 solutions, respectively. After derivatization, documentation of the plates was performed by the Camag TLC visualizer at 366 nm. All these instruments were operated by winCATS program (version 1.4.8, Camag) [2].

**HPLC Method:** HPLC analysis described in the ‘Matricaria flower’ monograph (Ph. Eur.) were carried out by Agilent Technologies 1100 series (Santa Clara, California, USA) coupled with a vacuum degasser, quaternary pump, auto-sampler, thermo-stated column compartment, and diode array detector which was operated by ChemStation 10.01 software. Separations were achieved on an Agilent Zorbax Eclipse Plus C18 ODS column (5 μm, 250 mm x 4.6 mm, i.d.). The mobile phases A and B used in this study were water-o-phosphoric acid (99.5:0.5, v/v) and acetonitrile-o-phosphoric acid (99.5:0.5, v/v), respectively. The following gradient pattern was used: 25% B (0-9 min), 25-75% B (9-19 min), 75% B (19-24 min). The flow rate was 1 mL/min, injection volume was 20 μL and the detection was monitored at 340 nm [1].

**RESULTS AND DISCUSSION**

**HPTLC analysis:** In hydroalcoholic chamomile extract other than apigenin 7-O-glucoside, mono- or diacetylated derivatives of apigenin 7-O-glucoside are also found [3]. HPTLC analysis showed that apigenin 7-O-glucoside was found in all chamomile cultivars.
Besides, addition of sodium hydroxide during sample preparation for HPLC analysis caused ester hydrolysis of acetylated derivatives of apigenin 7-O-glucoside by heat yielding totalapigenin 7-O-glucoside. These steps were followed by HPTLC.

**HPLC analysis:** The resolution ($R_s$) between the standards of apigenin 7-O-glucoside and 5,7-dihydroxy-4-methylcoumarin was found as $7.54 \pm 0.03$ ($n = 3$), indicated the suitability of the system according to the Ph. Eur. [1].

The identity of the apigenin 7-O-glucoside in the sample solutions was verified by comparing the retention time ($t_R$) with the reference apigenin 7-O-glucoside solution at 25 °C and found as $6.50 \pm 0.01$. In addition, the percentage content of totalapigenin 7-O-glucoside in chamomile cultivars was range from 0.35 to 0.82%.

**CONCLUSIONS**

In conclusion, totalapigenin 7-O-glucoside content in 12 different chamomile cultivars was higher than the proposed limit in the Ph. Eur., 0.25%. Besides, the highest totalapigenin 7-O-glucoside amount was found in Bodegold type chamomile cultivar sowing in early November.

**ACKNOWLEDGMENTS**

Etil Guzelmeric would like to state her deep gratitude to the Scientific and Technological Research Council of Turkey (TUBITAK) for the scholarship provided during the Ph.D. program.

**REFERENCES**


**P-295: EVALUATION OF RUSCOGENINS IN RUSCUS ACULEATUS L. FROM DIFFERENT LOCATIONS IN MARMARA REGION, TURKEY**

E. Guzelmeric$^1$, G. Ozer$^2$, E. Sezik$^3$, E. Yesilada$^3$

$^1$Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy and Phytotherapy, Istanbul, TURKEY
$^2$Marmara Forest Research Institute, Istanbul, TURKEY

**INTRODUCTION**

The underground parts (rhizomes) of *Ruscus aculeatus* L. (Butcher’s broom, Liliaceae) are mostly used to alleviate the symptoms of chronic venous insufficiency and hemorrhoids [1]. Rhizomes as an herbal drug should contain minimum 1% of total sapogenins expressed as ruscogenins (mixture of neoruscogenin and ruscogenin) according to the European Pharmacopoeia (Ph. Eur.) [2]. Butcher’s broom is one of the most commonly and excessively exported medicinal plants in Turkey for years. However, excessive nature picking of rhizomes may endanger the plant distribution. Therefore, studies on cultivation should put an emphasis both for the controlled production and yielding standard active ingredients of Butcher’s broom. In this study, ruscogenins content in Butcher’s broom rhizomes collected from different locations in Marmara region (Turkey) was comparatively determined in order to define optimum suitability and growth conditions for the plant cultivation.

**MATERIALS AND METHODS**

**Plant Materials**

Butcher’s broom rhizomes were obtained from 18 different locations in Marmara region. The codes and localities of materials: R1 (Sogutlu, Adapazari); R2 (Poyrazlar, Adapazari); R3 (Akyazi, Adapazari); R4 (Hendek, Adapazari); R5 (Bandırma, Balikesir); R6 (Gonen, Balikesir); R7 (Yenice, Canakkale); R8 (Biga, Canakkale); R9 (Kanlica, Istanbul); R10 (Sile, Istanbul); R11 (Bahcekoy, Istanbul); R12 (Tekirdag); R13 (Catalca, Istanbul); R14 (Vize, Tekirdag); R15 (Mustafakemalpasa, Bursa); R16 (Yalova); R17 (Inegol, Bursa) and R18 (Karacabey, Bursa).

**TLC Method:** TLC analyses were performed according to the ‘Butcher’s broom’ monograph in the Ph. Eur. [2]. Separation was performed on the silica gel 60 F$_{254}$ HPTLC glass plates using the developing solvent system of dichloromethane-methanol (93:7, v/v). For the visual documentation, the plates were sprayed with vanillin reagent and heated in an oven at 100 °C.

**HPLC Method:** HPLC analyses described in the ‘Butcher’s broom’ monograph (Ph. Eur.) were carried out by Agilent Technologies 1100 series (Santa Clara, California, USA) coupled with a vacuum degasser, quaternary pump, auto-sampler, thermo-stated column compartment, and diode array detector which was operated by ChemStation 10.01 software. Separations were performed on an Agilent Zorbax Eclipse Plus C$_{18}$ ODS column (5 μm, 250 mm x 4.6 mm, i.d.). The mobile phases A and B used in this study were water and acetonitrile, respectively. The following gradient pattern was used: 60% B (0-25 min), 60-100% B (25-27 min), 100% B (27-37 min). The flow rate was 1.2 mL/min, injection volume was 20 μL and the detection was monitored at 203 nm [2].
RESULTS AND DISCUSSION

TLC analysis: TLC analysis indicated that all Butcher’s broom rhizomes include both stigmasterol and rusco genins and this has also been stated in the criterion of the Ph. Eur. HPLC analysis: According to the Ph. Eur., the relative retention time (tR) of rusco genin to neoruscogenin should be approximately 1.2 [2]. In this study, it was found as 1.24 ± 0.01 (n=3), indicated the suitability of the system.

The identity of the neoruscogenin and rusco genin in test solutions were verified by comparing the tR with the reference solution at room temperature which was found as 15.5 ± 0.1 and 19.3 ± 0.1, respectively. Moreover, the total content of neoruscogenin and rusco genin expressed as rusco genins in R2, R6, R8, R9, R11, R17 and R18 was found not less than 1%.

CONCLUSIONS

Among the investigated 18 Butcher’s broom rhizome samples, those collected from Adapazari (R2), Balikesir (R6), Canakkale (R8), Istanbul (R9-11) and Bursa (R17-18) were found to be the most suitable samples for cultivation.

ACKNOWLEDGMENTS

Etil Guzelmeric would like to state her deep gratitude for the scholarship provided by the Scientific and Technological Research Council of Turkey (TUBITAK) for the Ph.D. program. This project is funded by Republic of Turkey Ministry of Forestry and Water Affairs (Project no: 10.7702).

REFERENCES


P-296: QUANTIFICATION OF MAJOR ANTIOXIDANTS DETERMINED BY HPTLC-DPPH® METHOD IN CYNARA SCOLYMUS L. AND ITS PRODUCTS

E. Guzelmeric1, I. Vovk2-3, E. Yesilada1

1Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy and Phytotherapy, Istanbul, TURKEY
2National Institute of Chemistry, Laboratory for Food Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia
3EN-FIST Centre of Excellence, Trg Osvobodilne fronte 13, SI-1000 Ljubljana, Slovenia

INTRODUCTION

The leaf of artichoke (Cynara scolymus L., Asteraceae) as an herbal drug has long been used in folk medicine mostly for its hepatoprotective and cholagogue activities. Besides, many studies have been conducted in recent years to establish its antihyperlipidemic, anticarcinogenic and antioxidant activities etc. [1]. It should contain at least 0.6% chlorogenic acid content as stated in the European Pharmacopoeia (Ph. Eur.) [2]. In the present study, we aimed to determine the major antioxidant constituents in artichoke leaf and quantify their content in artichoke leaf and its parts (bract, receptacle and stem) and also in marketed products.

MATERIALS AND METHODS

Plant Materials: Artichoke leaf and its parts (bract, receptacle and stem) belong to central and axillary heads were obtained from Canakkale province of Turkey in different stages (before maturity and marketing stage). Commercial artichoke products were purchased from pharmacies and spice shops, respectively.

HPTLC-DPPH® Method: TLC analysis was performed according to the ‘Artichoke leaf dry extract’ monograph in the Ph. Eur. [2]. Separation was performed on the silica gel 60 F 254 HPTLC glass plate using the developing solvent system of ethyl acetate-acetic acid-formic acid-water (100:11:11:26, v/v/v/v). The plate was immersed for 5s in freshly prepared 0.2% methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH®). After documentation, the images were evaluated by Camag VideoScan TLC Evaluation Softwer (version 1.02.00).

HPLC Method: Separation was performed on the silica gel 60 F 254 HPTLC glass plates (pre-developed with methanol-dichlorometane (1:1, v/v) containing 0.5% triethyamine) using the developing solvent system of ethyl acetate-acetic acid-formic acid-water (35:2:2:5, v/v/v/v). For the visual documentation, the plates were heated at 100 °C and dipped into NP/PEG 400 solutions, respectively. After derivatization, documentation of the plates was performed by the Camag TLC visualizer at 366 nm. All these instruments were operated by winCATS program (version 1.4.8, Camag).

RESULTS AND DISCUSSION

HPTLC-DPPH® analysis: The active antiradical constituents in artichoke leaf appeared on the densitogram as yellow zones on the purple background were rutin, chlorogenic acid, luteolin 7-O-glucoside and cynarin. However, two compounds showed the major antioxidant activity; chlorogenic acid and cynarin.

HPTLC analysis: The presence of the chlorogenic acid, caffieic acid, rutin, luteolin 7-O-glucoside and cynarin in all samples were comparatively evaluated. Eventually artichoke leaf had a unique chemical fingerprinting profile other than its bract, receptacle and stem. In addition, chemical fingerprinting of...
HPLC analysis: According to the HPTLC-DPPH results, chlorogenic acid and cynarin contents were quantified in all samples. As a result, the highest chlorogenic acid content was found in artichoke leaf as 6%. In addition, the highest cynarin content was detected in artichoke inner bract belong to central artichoke head in early stage of maturity as 12%. Besides, chlorogenic acid content in artichoke products purchased from pharmacies was found not less than 0.6%, as stated in the Ph. Eur. On the other hand, chlorogenic acid content in artichoke extract obtained from spice shop was lower than the proposed limit in the Ph. Eur.

CONCLUSIONS
As a result of this investigation, it is concluded that in order to reduce the oxidative stress level in human body, use of artichoke leaf as well as bract would be highly beneficial. Besides, artichoke products purchased from pharmacies possess higher efficiency comparing to those purchased from spice shops.

ACKNOWLEDGMENTS
Etil Guzelmeric would like to state her deep gratitude to the Scientific and Technological Research Council of Turkey (TUBITAK) for the scholarship provided during the Ph.D. program. She is also thankful to Jelena Trifković (Faculty of Chemistry, University of Belgrade, Belgrade, Serbia) for her great contributions during the study.

REFERENCES

P-297: CYTOTOXIC COUMARINS FROM THE ROOTS OF NEOCRYPTODISCUS PAPILLARIS

F. Tosun1, F. Mihoğlugil1, A. Duran2, M. Miski3

1Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY, 2Selçuk University, Faculty of Science, Department of Biology, Konya, TURKEY, 3Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, TURKEY

INTRODUCTION
The aim of present study is to investigate the cytotoxic activity of Neocryptodiscus papillaris (Boiss.) Herrnst.& Heyn (Apiaceae) [1,2]. Plants of the Apiaceae are rich sources of biologically active coumarins, terpenes and phenolic compounds with antibacterial, antiviral, anti-inflammatory and cytotoxic activities [3-4].
P-298: STUDY ON THE COMPOSITION OF THE ESSENTIAL OILS OF ASTER SUBULATUS MICHX. FROM TURKEY AND THEIR ANTIMICROBIAL ACTIVITY

F. Ayaz¹, N. Küçükboyaci¹, B. Demirci²

¹Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Ankara, ²Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, TURKEY

INTRODUCTION

The large genus Aster L. belongs to the family Asteraceae and consists of about two hundred fifty species native to North and South America, Europe, Africa and Asia [1,2]. Aster species have long been used for the treatment of colds, fever, tonsillitis, bronchitis, snake bites and bee stings in traditional Chinese medicine[3]. Several biological activities such as allelopathic, antifungal and anti-inflammatory have been reported on the essential oils from Aster species. Until now, only one study on A. subulatus is found, in which the chemical constituents of the essential oil were identified by gas chromatography [4].

MATERIALS AND METHODS

In our study, the essential oils from the aerial parts and the roots of A. subulatus collected from Selçuk, İzmir, Turkey at the flowering-fruit stage were obtained using a Clevenger type distillation apparatus. The chemical composition of the essential oils was analyzed by GC and GC-MS, simultaneously.

RESULTS AND DISCUSSION

Major components of the aerial parts oil were found to be elemol (21.5%), β-eudesmol (6.3%), caryophyllene oxide (5.2%), α-eudesmol (5.0%), α-muurolene (4.1%), humulene epoxide II (3.0%) and spathulenol (3.7%). The root oil contained hexadecanoic (33.0%), tetradecanoic (5.3%) and octadecanoic (4.6%) acids. In addition, antimicrobial activity of the essential oils was investigated by TLC-bioautography method on some bacterial and fungal strains. Both of the oils showed antimicrobial activity against the strains of Staphylococcus aureus ATCC 6558 and Candida albicans ATCC 90028, while there is no efficient compound in the oils against the strain of Escherichia coli NRRL B-3008.

CONCLUSION

To the best of our knowledge, this is the first report on the chemical composition of essential oils and preliminary antimicrobial activity of the essential oils of A. subulatus from Turkey.

REFERENCES


In our previous study, we reported that the n-hexane, chloroform, n-butanol and remaining water subextracts sequentially obtained from the methanol (80%) extracts of three Chrysophthalmum species growing in Turkey were tested against various human cancer cell lines such as MCF-7 and MDA-MB-231 breast cancer cell lines, LNCaP prostate cancer cell line, PC3 lung cancer cell line and HT-29 colon cancer cell line by Sulforhodamine B (SRB) assay and the % viability was found to change in a dose-dependent manner after 48 hours of the treatment. The most active subextracts of these species were found to be chloroform subextracts which have remarkable antigrowth effects at 20 μg/ml concentration with approximately 20% viability on all tested cell lines [3].

MATERIALS AND METHODS

In this study, we aimed to identify the compounds responsible for the anti-growth activity of the chloroform subextract from C. montanum through bioactivity-guided fractionation and isolation procedures. The chloroform subextract was subjected to column chromatographic separation on silica gel procedures. The chloroform subextract was subjected bioactivity-guided fractionation and isolation chloroform subextract from responsible for the anti-growth activity of the

RESULTS AND DISCUSSION

According to the results, Fr. A (Fr. 9) and Fr. B (Fr.13-14) eluted with CHCl₃:acetone (80:20) were found to be the highest antigrowth effects on HT-29 colon cancer cell line by SRB assay with viability 19.47% and 20.09%, respectively. The isolation and identification of the phytochemical constituents responsible for the anti-growth activity of C. montanum by bioactivity-guided isolation techniques are ongoing.

CONCLUSION

As a conclusion, we firstly defined the most active fractions from C. montanum against the human cancer cell lines. In this presentation, the preliminary results of anti-growth activity of C. montanum that has been found until now will be shown.

REFERENCES


P-300: SAPONINS FROM ASTRAGALUS ISAURICUS

D. Parlak¹, F. Nuray Yalçın¹, S. Piacente², H. Duman³, I. Çalış⁴

¹Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey
²Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, Via Ponte Don Melillo, I-84084 Fisciano, Italy
³Gazi University, Faculty of Arts and Sciences, Department of Biology, Ankara, Turkey
⁴Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia, N. Cyprus

INTRODUCTION

The genus Astragalus is represented by 445 species 224 of which are endemic in Turkish flora [1-2]. Astragalus species have been used to obtain “gum tragacanth” for many years. Also, many Astragalus species have been used for their hepatoprotective, diuretic, cardioprotective, anti-diabetic, antioxidant, anti-inflammatory, immunostimulant and antiviral properties [3]. Astragalus species contain triterpenoid saponins, polysaccharides, flavonoids, organic acids and alkaloids. Cycloartanes from Astragalus genus are found to possess cardiotonic, hypocholesteremic, anti-depressive and antiblastic actions as well as immunomodulatory activity. It has been aimed that isolation and structure elucidation of secondary metabolites especially cycloartane saponins from the roots of Astragalus sauricus which is endemic in Turkey. As a result of chromatographic studies on Astragalus sauricus root an amino acid [tryptophan] and three cycloartane triterpenoid [astragaloside I, astragaloside II and astragalosideIV] were isolated [4, 5].

MATERIALS AND METHODS

The MeOH extract from air dried and powdered roots of the plant was prepared by Dionex ASE 100 extractor. Dried extract was applied to several column chromatographic systems for isolation of cycloartane saponins. The structure elucidation was performed by spectroscopic methods.

RESULTS AND DISCUSSION

The compounds isolated from the MeOH extract were determined as an amino acid [tryptophan] and three cycloartane saponins [astragaloside I, astragaloside II and astragalosideIV] by spectroscopic methods.

CONCLUSION

It has been determined that, Cycloartanes from Astragalus genus are found to possess cardiotonic,
hypcholesteremic, anti-depressive and antiblastic actions as well as immunomodulatory activity. In this study tryptophan, astragaloside I, astragaloside II and astragaloside IV compounds were isolated from the roots of *Astragalus isauricus* which is endemic in Turkey.

**REFERENCES**


P-301: AN IN VITRO INVESTIGATION ON CHOLINESTERASE AND TYROSINASE INHIBITORY AND ANTIOXIDANT ACTIVITY OF FIVE *SCROPHULARIA L.* SPECIES

F. Ş. Şenol¹, G. Renda², B. Yaylı², S. Türküş³, E. Uzunhisarcıklı³, İ. Erdoğhan Orhan¹

¹Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY
²Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY
³Ordu University, Faculty of Art and Science, Department of Biology, Ordu, TURKEY
⁴Gazi University, Faculty of Science, Department of Biology, Ankara, TURKEY

**INTRODUCTION**

*Scrophularia* L. genus that belongs to Scrophulariaceae family has 60 species including 23 endemic species in Turkey [1]. Some *Scrophularia* species are used in folk medicine for the treatment of different skin inflammatory diseases, constipation, and neuritis [2, 3]. In the literature iridoid glycosides isolated from *Scrophularia buergeriana* have been reported to have neuroprotective activity [4]. In the present study, five *Scrophularia* L. species; *S. chrysantha* Jaub&Spach, *S. catarifolia* Boiss. &Heldr., *S. scopoli* (Hoppe ex) Pers.var. *adenocalyx* Somm. et Lev., *S. kotschyanana* Bentham, *S. cinarescens*Boiss. have been investigatedfor their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the sister enzymes linked to Alzheimer’s disease. Antioxidant potential of the methanol extracts of these species were determined using five types of in vitro assays.

**MATERIALS AND METHODS**

The *Scrophularia* species studied were collected throughout Turkey. The dried and powdered aerial parts of the plant materials were extracted with methanol. Cholinesteraseactivity of the extracts were measured using ELISA microtiter assay [5].

Since oxidative damage is associated with neurodegenerative diseases, the extracts were tested for their antioxidant effect by *in vitro* assays; 1,1-diphenyl-2-‘-picryl-hydrazyl (DPPH), and *N,N*-dimethyl-p-phenylenediamine (DMPD) radical scavenging, metal chelation, ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) assays [5]. Total phenol and flavonoid contents in the extracts were determined spectrophotometrically [5].

**RESULTS AND DISCUSSION**

Our results revealed that none of the tested methanol extracts showed cholinesterase inhibitory activity at 200 µg/ml. Among all samples screened, DPPH scavenging effect of the methanol extracts of *S. scopoli* var. *adenocalyx* and *S. cinarescens*has been found as 47.70±3.41% and 51.95±1.55%. In general, the extracts possessed weak to moderate antioxidant activity.

Total phenolics determined in the methanol extracts according to Folin-Ciocalteau method were 29.51 mg/g, 59.24 mg/g, 113.41 mg/g, 42.34 mg/g, 101.19 mg/g expressed as gallic acid equivalent respectively, while their flavonoid content was 13.98 mg/g, 27.13 mg/g, 59.24 mg/g, 113.41 mg/g, 42.34 mg/g and 101.19 mg/g expressed as quercetin equivalent, respectively.

**REFERENCES**

P-302: INHIBITION OF MICROBIAL ORAL PATHOGENS BY EUCALYPTUS SP. EXTRACTS FROM TURKEY

G. Göger*1,2, J. Bueno3, N. Karaca1,2, F. Göger2, F. Demirci2,4
1Graduate School of Health Sciences, Anadolu University, 26470-Eskişehir, TURKEY
2Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470-Eskişehir, TURKEY
3Bioprospecting Development and Consulting, Bogota, COLOMBIA
4Faculty of Health Sciences, Anadolu University, 26470-Eskişehir, TURKEY

INTRODUCTION
Plaque-related infectious diseases are relatively common, which can spread in the human body causing coronary thrombosis and stroke type complications. There is a rising trend in new treatment approaches for this condition using antimicrobial phytochemicals [1,2]. Thus, the aim of this research work was to evaluate the antimicrobial and antibiofilm activity [3] potential against Streptococcus mutans, Staphylococcus aureus, Candida albicans, Bacteroides fragilis and Streptomyces griseolus oral human pathogen selection using Eucalyptus extracts sourced from Turkey.

MATERIALS AND METHODS
Plant material: Six different sources were used as test samples: Eucalyptus grandis L., E. camaldulensis Dehnand E. globulus L. leaves were collected from Akdeniz and Ege Regions of Turkey and extracts were prepared using n-hexane (HEX), ethyl acetate (EtOAc) and methanol (ME) maceration at 25 °C for 24 h.

Phenolic compound determination: LC-MS/MS (Applied Biosciences) was used for the identification of antimicrobial active extract fractions.

Antimicrobial activity: Was determined using Clinical and Laboratory Standards Institute (CLSI) methods with adaptations (M07-A7 and M11). Additionally, broth microdilution method for fungi described by the European Committee of Antimicrobial Susceptibility Testing (EUCAST E.DEF 7.2) was employed. [MIC50]: Minimum inhibitory concentration that inhibit 50% of microbial growth. [MBIC50]: Minimal Biofilm Inhibitory Concentration that inhibit 50% of biofilm growth were determined spectrophotometrically in duplicate experiments).

Antibiofilm activity: Was determined using the CLSI method using a modified spectrofluorometric (Biotek, Synergy H1) static biofilm resazurin assay by duplicate experiments.

RESULTS AND DISCUSSION
Gallic acid, 3-p-coumaroylquinic acid, 4-pcoumaroylquinic acid and roseoside were determined in the ethyl acetate extracts of E. Grandis; while gallic acid, 3-p-coumaroylquinic acid, 5-caffeoylquinic acid, roseoside, quercetin-3-glucuronide, luteolin glucuronide and quercetin were determined as major compounds in E. camaldulensis methanol extract.

Eucalyptus extracts were found active against S. aureus and S. griseolus (MIC50 =31.25 μg/mL). Biofilm inhibition was observed against S. mutansS. aureus and B. fragilis strains. Whereas, S. griseolus biofilm formation was not inhibited as seen in the Results Table.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Eucalyptus sp.</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. grandis</td>
<td>E. camaldulensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>MIC 50</td>
<td>500</td>
<td></td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>MBIC 50</td>
<td>≤125</td>
<td>≤125</td>
<td>≤125</td>
<td>≤125</td>
</tr>
<tr>
<td>Streptomyces griseolus</td>
<td>MIC 50</td>
<td>≤31.25</td>
<td>≤31.25</td>
<td>≤31.25</td>
<td>≤31.25</td>
</tr>
<tr>
<td></td>
<td>MBIC 50</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>MIC 50</td>
<td>31.25</td>
<td>≤31.25</td>
<td>≤31.25</td>
<td>≤31.25</td>
</tr>
<tr>
<td></td>
<td>MBIC 50</td>
<td>125</td>
<td>125</td>
<td>NA</td>
<td>125</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>MIC 50</td>
<td>125</td>
<td>≤31.25</td>
<td>≤31.25</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>MBIC 50</td>
<td>≤125</td>
<td>≤125</td>
<td>≤125</td>
<td>≤125</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>MIC 50</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>MBIC 50</td>
<td>2000</td>
<td>1000</td>
<td>NA</td>
<td>1000</td>
</tr>
</tbody>
</table>

NA: Not active

CONCLUSIONS
It is suggested that Eucalyptus sp. extracts need higher concentrations to destroy C. albicans biofilm formation, due to complex matrix composition possessing glucose. However, Eucalyptus sp. extracts may be used as a promising alternative prevention agent against oral pathogens in planktonic as well as the biofilm state.

ACKNOWLEDGMENTS: Tübitak Bideb 2221 (sabbatical) for JB, Tübitak SBAG 114S740.

REFERENCES
P-303: ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF COREOPSIS TINCTORIA NUTT. ESSENTIAL OIL AGAINST UPPER RESPIRATORY HUMAN PATHOGENIC MICROORGANISMS

F. Demirci1,2, G. Sener1,2, J. Bueno3, B. Demirci2,4, K.H.C. Başer5,6

1 Faculty of Health Sciences, Anadolu University, Eskişehir, TURKEY
2 Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskişehir, TURKEY
3 Bioprospecting Development and Consulting, Bogota, COLOMBIA
4 Graduate School of Health Sciences, Anadolu University, Eskişehir, TURKEY
5 College of Science, Department of Botany and Microbiology, King Saud University, Riyadh, SAUDI ARABIA

INTRODUCTION

Coreopsis tinctoria Nutt. is a medicinal plant belonging to the family Asteraceae. This species is cultivated as an ornamental plant, especially in Xinjiang area, China. It is also used by the native Americans against diarrhoea and as an emetic. The pharmacological activities such as antidiabetic, hypolipidemic, antihypertensive, antioxidant, anticoagulating and antiinflammatory have been reported (1).

The aim of this research is to evaluate the antimicrobial and antibiofilm forming activity (2, 3) of C. tinctoria essential oil against the upper respiratory system human pathogens Streptococcus pyogenes, Moraxella catarrhalis and Candida albicans.

MATERIALS AND METHODS

Plant Material: C. tinctoria was purchased from a local market in China, in 2013. Essential oil was obtained by hydrodistillation using a Clevenger apparatus (yield 0.36 %). The resulting essential oil was analyzed by both GC-FID and GC-MS, simultaneously.

Antimicrobial activity: Minimum inhibitory concentration of the oil that produces 50% of inhibition (MIC50) was determined using Clinical and Laboratory Standards Institute (CLSI) methods with adaptations for aerobic microorganisms (M07-A7), also European Committee of Antimicrobial Susceptibility Testing (EUCAST E.DEF 7.2) was used.

Antibiofilm activity: Minimal Biofilm Inhibitory Concentration of the oil that produces 50% of inhibition (MBIC50) was determined using the CLSI method by a modified spectrofluorometric (Biotek, Synergy H1) static biofilm resazurin assay in duplicate experiments (2).

RESULTS AND DISCUSSION

The major compounds of C. tinctoria essential oil were identified as limonene (56.7 %), p-cymene (10.1 %), α-pinene (4.8 %), carveol (3.2 %) and trans-carveol (2.3 %). The antimicrobial activity (MIC50 and MBIC50) against S. pyogenes ATCC 19615 (0.125 and >2 mg/mL), M. catarrhalis ATCC 23245 (0.3125 and 2 mg/mL) and C. albicans ATCC 24453 (0.5 and >2 mg/mL) were observed, respectively.

CONCLUSIONS

C. tinctoria essential oil showed antimicrobial activity (0.125 - 2 mg/mL) against the selected upper respiratory system pathogens. The activity is relatively lower against the biofilm form of the same microorganisms.

It is important to highlight that the microorganisms in the respiratory system infections are in biofilm state. Further comparative antimicrobial evaluations using microbial cells both in planktonic and biofilm state are needed.

KEYWORDS

Coreopsis tinctoria Nutt., Essential Oil, Antimicrobial, Antibiofilm

ACKNOWLEDGMENTS

The authors would like to thank Tübitak BİDEB 2221 Sabbatical Programme for supporting JB.

REFERENCES


P-304: ANTIOXIDANT AND ANTIINFLAMMATORY POTENTIALS OF THE SECONDARY METABOLITES FROM SCUTELLARIA HASTIFOLIA

Hilal Bardakci1,6, Helen Skaltsa2, Tanja Milosevic-FACTIS 5, Diamanto Lazarid3, Dimitra Hadjipavlou-Litina4, Erdem Yeşilada4, Hasan Kirmizibekmez2
INTRODUCTION
As a continuation of our work on Scutellaria species (Lamiaceae), with respect to the results of in vitro tests mentioned in our previous studies, along with the minority of investigations conducted on S. hastifolia, this plant was chosen for further phytochemical and bioactivity (antioxidant and antiinflammatory) studies. In total, 15 secondary metabolites including a new one, S. hastifolia (hastifolioside) were isolated from the aerial parts of this plant. All secondary metabolites isolated from S. hastifolia were tested for their DPPH radical scavenging activities, inhibition of linoleic acid lipid peroxidation and soybean LOX.

MATERIALS AND METHODS
The compounds belonging to ethylcyclohexane derivatives (isorengyol, isorengyoside, cleroidin B, cleroidin F), phenylethanoid glycosides (cornoside, calceolaroside D, hastifolioside, neocalceolaroside D, calceolaroside B, verbascoside), flavonoids (apigenin 7-ȕ-D-glucopyranoside, scutellarein 7-ȕ-D-glucopyranoside, hispidulin 7-ȕ-D-glucopyranoside, hispidulin 7-ȕ-D-glucuronopyranoside), and sugar ester (6-O-cafeoylglucopyranose) were isolated from S. hastifolia. The DPPH reducing abilities and the inhibition of the AAPH induced lipid peroxidation of the secondary metabolites were evaluated by using the method described by Neochoritis et al. [1]. Moreover, LOX inhibition activity of the metabolites was determined by using the method described by Pontiki and Hadjipavlou [2].

RESULTS AND DISCUSSION
Among the ethylcyclohexane derivative compounds cleroidin F and isorengyol elicited the highest DPPH antioxidant activity, whereas the derivative of isorengyl, namely isorengyoside did not show any activity. With the exception of hastifolioside and neocalceolaroside D, all the phenylethanoid glycosides highly reacted with DPPH. Among the four isolated flavonoids, scutellarein 7-ȕ-D-glucopyranoside exhibited the highest DPPH antioxidant activity. On the other hand, hispidulin aglycone with different sugar moieties, elicited similar degrees of DPPH scavenging activity. The results from the inhibition of the AAPH induced linoleic acid lipid peroxidation showed that the ethylcyclohexane derivatives were weak inhibitors. Among the phenylethanoid glycosides, only calceolaroside D exerted moderate inhibition while hastifolioside did not show any activity. Among the isolated flavonoids, apigenin 7-ȕ-B-D-glucopyranoside displayed the highest inhibition on lipid peroxidation, whereas hispidulin 7-ȕ-B-D-glucuronopyranoside was not active under these experimental conditions. Among the ethylcyclohexane derivatives, none of the molecules showed any remarkable LOX inhibition in comparison to the reference substance NDGA. Only isorengyoside displayed a moderate LOX inhibition. Additionally, phenylethanoid glycosides showed very low inhibition. Among the isolated flavonoids, apigenin 7-ȕ-B-D-glucopyranoside elicited the highest LOX inhibition, whereas hispidulin 7-ȕ-B-D-glucuronopyranoside did not show any activity under the experimental conditions. In our previous study, the MeOH extract of S. hastifolia showed significant LOX inhibition, whereas the isolated compounds did not show any remarkable activity on LOX enzyme. This result can be explained by the synergistic activity of the compounds present in the MeOH extract of the title plant.

ACKNOWLEDGEMENTS
This study was funded by TUBITAK-SBAG (Project no: 109S480) and GSRT.

REFERENCES

P-305: ISOLATION AND IDENTIFICATION OF KAEMPFEROL-3-O-GLUCOSYL (1-2) RHAMNOSIDE FROM LATHYRUS ARNUMENS L.

H. Heydari1, G. Saltan2, O. Bahadir Acikara2, M. Tekin2

1 Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY
2 Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sivas, TURKEY

INTRODUCTION
Genus Lathyrus L., is a member of the Viciae tribe (family Leguminosae), consists of about 160 species and many of them are economically important and
used as forage, human food, ornamental plants, soil nitrifiers and model organisms for genetic and ecological research [1]. In Turkey, *Lathyrus* is represented by 65 genera and 75 taxa. *Lathyrus armenus* L. is endemic for Turkey [2].

Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development. Plant phenolics include simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans, and lignins. *Lathyrus* species contain various flavonoids, such as quercetin, kaempferol, luteolin, myricetin and their glycoside. They also contain fatty acids, such as linoleic and linolenic acid [3] and, as well as proanthocyanidins, cyanogenic glucosides [4], phytoecdisteroids, triterpene saponins [5].

**MATERIALS AND METHODS**

The whole plants of *L. armenus* were collected during flowering period from Sivas, Turkey (M. Tekin 1278). The 900 gr powdered dried plant was refluxed with MeOH for 24 h at 80 °C (Soxhlet). The MeOH extract was dried under reduced pressure and then partitioned consecutively between H2O and hexane, CHCl3, EtOAc. The EtOAc fraction (5,81 gr) was subjected to column chromatography over silica gel with EtOAc: MeOH: H2O. 45 fractions were obtained, 7. fraction (0,1339 mg) was re-chromatographed on reverse phase TLC plates to purify. The structure of the compound was identified by 1H, 13C-NMR and 2D-NMR analysis.

**RESULTS AND DISCUSSION**

Methanol extract of the whole plants of *L. armenus* revealed the isolation of a compound, by means of 1H, 13C-NMR as well as 2D-NMR (HSQC, HMBC, COSY; TOCSY) analysis.

**CONCLUSIONS**

The results of present study revealed the presence of kaempferol-3-O-glicosyl (1-2) rhamnoside in *L. armenus*. The partitioned sample can be further used for determination of various biological activity and nutritive values of *L. armenus*. Several studies suggest that the consumption of plant foods containing dietary phenolics may significantly contribute to human health.

**REFERENCES**


P-306: WOUND-HEALING ACTIVITY OF *COTINUS COGGYRIA* IN DIABETIC RATS

**H. Aksoy1*, A. Sen2, M. Sancar3, D. Akak4, L. Bitis5, F. Uras3, S. Kultur5, F.V. Izzettin3**

Marmara University, Faculty of Pharmacy, 1Department of Biochemistry, 2Department of Pharmacognosy, 3Clinical Pharmacy Department, 4Marmara University, Faculty of Medicine, Histology-Embryology Department, 5Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, TURKEY

**INTRODUCTION**

Wound-healing in diabetics is frequently a therapeutic challenge, lasting for weeks despite adequate and appropriate care [1]. Various herbal ointment used in the folk medicine have been reported to be effective in wound healing [2]. The aim of present study is to determine wound healing activity of the ethanol extract of *Cotinus coggyria* leaves used for wound treatment in traditional medicine in diabetic rats.

**MATERIALS AND METHODS**

*Cotinus coggyria* Scop. (Anacardiaceae) leaves were identified and collected from the Hamidiye village of Kirkclareli province of Turkey, in June 2002 by Sukran Kultur. Voucher specimens have been deposited in the herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 80926). The preparation of ethanol extract (CCE), its ointment and wound-healing activity assay were performed as described in our previous study [2].
RESULTS AND DISCUSSION

In this study, we investigated biochemical and histological effects of CCE on excision wound model in diabetic rats to provide scientific evidence for the traditional use. In the present study, the hydroxyproline content in granulation tissue of rats significantly increased in the treatment group when compared with the control group after the 3rd and 7th days (p<0.05). A statistically significant elevation in glutathione (GSH) at the end of 3rd, 7th, and 14th days and a statistically significant decrease in malondialdehyde (MDA) level at the end of 7th day were determined in the CCE treatment group when compared with the control group. These results were supported with histological analyzes (Figure 1).

CONCLUSIONS

These results indicate that CCE accelerated the cutaneous wound healing process in diabetic wounds and also confirm the traditional use of these plants.

Table 1. The amount of topical ointment application and groups in excision wound

<table>
<thead>
<tr>
<th>Three Day Experiment</th>
<th>Seven Day Experiment</th>
<th>Fourteen Day Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>Control (n=6)</td>
<td>Control (n=6)</td>
</tr>
<tr>
<td>Simple Ointment</td>
<td>Simple Ointment</td>
<td>Simple Ointment</td>
</tr>
<tr>
<td>0.5-1 g</td>
<td>0.5-1 g</td>
<td>0.5-1 g</td>
</tr>
<tr>
<td>Treatment (n=6)</td>
<td>Treatment (n=6)</td>
<td>Treatment (n=6)</td>
</tr>
<tr>
<td>5% (w/w)</td>
<td>5% (w/w)</td>
<td>5% (w/w)</td>
</tr>
<tr>
<td>CCE ointment</td>
<td>CCE ointment</td>
<td>CCE ointment</td>
</tr>
<tr>
<td>0.5-1 g</td>
<td>0.5-1 g</td>
<td>0.5-1 g</td>
</tr>
</tbody>
</table>

Table 2. Comparison of hydroxyproline contents between control and treatment groups at different days in wound tissue

<table>
<thead>
<tr>
<th>Day</th>
<th>Hydroxyproline content (mg/g tissue) Median/SD (25-75 percentile)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>15.33 ± 2.94 (11.00-19.00)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2nd day</td>
<td>11.83 ± 2.14 (10.00-15.00)</td>
<td></td>
</tr>
<tr>
<td>3rd day</td>
<td>19.67 ± 1.75 (17.00-21.00)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>7th day</td>
<td>15.67 ± 2.73 (14.00-21.00)</td>
<td></td>
</tr>
<tr>
<td>14th day</td>
<td>17.50 ± 3.27 (13.00-22.00)</td>
<td></td>
</tr>
<tr>
<td>14th day</td>
<td>14.67 ± 2.34 (12.00-18.00)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results were expressed as median ± SD (25-75 percentile).
* P<0.05 when compared with control group; NS: non-significant

Figure 1. Representative skin wound sections from both experimental groups on days 3 (A, B), 7 (C, D) and 14 (E, F). Large amount of inflammatory infiltrate consisting predominantly of neutrophils (arrows) in control (A) and treated (B) groups on day 3. Marked accumulation of exudate (*) and hemorrhage (**) can be observed. Diffuse granulation tissue (*) and completely disrupted areas in the dermis (**) in the controls (C) and dilated neovessels (arrow) in the treated group (D) are observed at day 7. Epithelial regeneration (arrowheads) is apparent. Almost complete epithelization of wound sites on day 14 are observed. Ongoing granulation tissue in the subepidermal region (*) is noted in the controls (E) compared to almost totally healed wound site in the treated rats (F). Hematoxylin and Eosin (H&E) stain, Bars: 20 μm (A, B); 100 μm (C, D); 50 μm (E, F).

Table 3. Comparison of other biochemical parameters between control and treatment groups at different days in wound tissue

<table>
<thead>
<tr>
<th>Day</th>
<th>GSH (μmol/g tissue)</th>
<th>MDA (μmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd day</td>
<td>5.13±2.13 **</td>
<td>2.77±1.25</td>
</tr>
<tr>
<td>3rd day</td>
<td>1.58±0.58</td>
<td>2.09±1.08</td>
</tr>
<tr>
<td>7th day</td>
<td>4.72±1.68 **</td>
<td>1.84±0.64 *</td>
</tr>
<tr>
<td>7th day</td>
<td>1.88±0.98</td>
<td>4.49±2.10</td>
</tr>
<tr>
<td>14th day</td>
<td>3.83±1.00 *</td>
<td>3.19±0.99</td>
</tr>
<tr>
<td>14th day</td>
<td>1.88±0.79</td>
<td>5.75±2.86</td>
</tr>
</tbody>
</table>

Results were expressed as median ± SD; * P<0.05 when compared with control group; ** P<0.01 when compared with control group

ACKNOWLEDGMENTS: This study was supported by Marmara University Scientific Research Projects Committee under Grant (file no. SAG-A-030114-0004)

REFERENCES

P-307: ANTIOXIDANT POTENTIALS OF FOUR SCUTELLARIA SPECIES FROM THE FLORA OF TURKEY

H. Bardakci & H. Kirmizibekmez

aYeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, TURKEY
bCurrent address: Kemerburgaz University, School of Pharmacy, Department of Pharmacognosy, Istanbul, TURKEY

INTRODUCTION

The term phenolics involve approximately 8000 naturally occurring compounds. Phenolic acids and flavonoids and tannins are the few examples of the naturally occurring plant phenolics. The best expressed property of phenolics is the ability to trap free radicals. They act both as hydrogen and electron donors and have the ability of chelating metal ions [1]. Referring the importance and uses of Scutellaria (Lamiaceae) species such as antitumor, antinflammatory, antiviral, anticonvulsant, hepatoprotective in different folk medicines [2], this study was designed to evaluate the total antioxidant capacity, total phenolic and flavonoid contents of acetone, MeOH and aqueous methanol [MeOH:H2O (5:1)] extracts prepared from the aerial parts of S. hastifolia L., S. velenovskiyi Rech., S. albida
L. ssp. albida and S. orientalis L. ssp. pinnatifida growing wild in Turkey.

MATERIALS AND METHODS

The collection sites of plant materials are as follows; S. hastifolia from Sakarya, S. velenovskyi from Karabük, S. albida ssp. albida from Istanbul and S. orientalis ssp. pinnatifida from Eskisehir. The air-dried and powdered plant materials were subjected to subsequent extraction with acetone, MeOH and MeOH:H₂O (5:1), respectively. Total antioxidant capacities, phenolic contents and flavonoid contents of the extracts were measured by using the previously published methods with slight modifications [3-5].

RESULTS

The results elicited that among the acetone and MeOH extracts, S. hastifolia (470.19±1.01 and 249.84±5.00 mg AAE/g extract, respectively) showed the highest ascorbic acid equivalent total antioxidant capacity. In terms of total phenolic contents, S. orientalis (158.20±1.93 mg GAE/g extract) displayed the highest gallic acid equivalent total phenolic content among the MeOH extracts. Among the MeOH:H₂O extracts, S. hastifolia (127.94±1.33 mg GAE/g extract) elicited the highest amount of total phenol content. Additionally, total flavonoid content test results exhibited that among the acetone extracts, S. velenovskyi (147.22±0.67 mg QE/g extract) displayed the highest quercetin equivalent total flavonoid content. Among the MeOH extracts, S. orientalis (47.83±0.50 mg QE/g extract) showed the highest total flavonoid content and S. hastifolia (36.95±1.15 mg QE/g extract) was following S. orientalis. Among the MeOH:H₂O extracts, S. hastifolia (53.33±1.53 mg QE/g extract) showed the highest total flavonoid content.

CONCLUSION

Scutellaria species have long been the subject of many phytochemical, analytical and biological studies. In this study, four Scutellaria taxa were screened for their antioxidant profiles by calculating their total antioxidant capacities as well as total phenol and total flavonoid contents. Taken together the results of all assays, S. hastifolia seems to be more promising species with its total phenolic, flavonoid content as well its and antioxidant activities.

REFERENCES


P-308: CHEMICAL COMPOSITION OF ONONIS NARIS SUBSP. ANGUSTISSIMA AND EUPHORBIA GUYONIANA ESSENTIAL OILS

H. Laouer1, T. Benmeddour2, S. Amira3, S. Akka4 and G. Flaminig5

1Universit of Setif 1; Laboratory of Valorization of Natural and Biological Resources, ALGERIA
2University of Biskra, Department of Nature and life sciences, ALGERIA
3University of Setif 1; Laboratory of phytotherapy applied to chronic diseases, ALGERIA
4University of Constantine, Laboratoire de Phytochimie et Analyses Physico-chimiques et Biologiques, ALGERIE
5Dipartimento di Farmacia, Via Bonanno 33, 56126 Pisa, ITALY

INTRODUCTION

Our study lies within the scope of the valorization of algerian medicinal and aromatic plants having the aim of discovering new bioactive natural products. In addition, A biological antioxidant has been defined as any substance that is present at low concentrations compared to an oxidizable substrate and significantly delays or prevents the oxidation of that substrate [1]. The screening of essential oils permits the discovery of new bioactive antibacterial and antioxidant. Our study contributes to the knowledge of chemical composition of essential oils of two plants from Algeria, namely Ononis natrix ssp angustissima and Euphorbia guyoniana.

MATERIALS AND METHODS

The oils were extracted by hydrodistillation and their chemical composition was analysed by Gas-Chromatography (GC/FID) and Gas-Chromatography coupled to Mass-spectrometry (CPG/SM). Identification of essential oil components cmponents of the oil were identified by comparison of their mass spectra with those of a computer library (Wiley 275). Further confirmation was done from retention index data generated from a series of alkanes retention indices (relative to C5-C28 on the DB-5 column. Additionally, the in vitro antimicrobial effects for the oils were evaluated by the diffusion method agar [2] against Staphylococcus aureus, Escherichia coli and Candida albicans.
RESULTS AND DISCUSSION
Thirty-four and nineteen compounds represented 91.6% and 92.3% respectively for O. natrix ssp angustissima and E. guyoniana essential oils. hexahydrofarnesylacetone (14.8%), α-cadinol (6.4%), β-eudesmol (6.6%), μ-cadinol and linalol (6.2%) were the major compounds of O. natrix ssp angustissima, but n-pentadecane (35.5%), and n-heptadecanee (11.3%) were the major compounds in E. O of E. guyoniana. The E. guyoniana oil presented the highest growth inhibition for all the microorganisms tested.

Fig. 1. Photos of Ononis natrix ssp angustissima and Euphorbia guyoniana

CONCLUSIONS
These results indicate that Ononis natrix ssp angustissima is a good source of hexahydrofarnesylacetone (14%, 8%), whereas E. guyoniana has two main constituents: n-pentadecane (35%, 5%), and n-heptadecane (11%, 3%). Ononis natrix ssp angustissima essential had the weakest antibacterial activity.

ACKNOWLEDGMENTS
The authors are grateful to the Ministry of Higher Education and Scientific Research for the financial support.

REFERENCES

P-309: NF-κB INHIBITORY FRACTIONS FROM SAMBUCUS NIGRA

Atay L.¹, İliter AZ.², Bağatur Y.², Telci D.², Kirmızıbekmez H.³, Yesilada E.³

¹İstanbul Medipol University, School of Pharmacy, Department of Pharmacognosy, 34810, Beykoz, İstanbul
²Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, 34755, Ataşehir, İstanbul
³Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy and Phytotherapy, 34755, Ataşehir, İstanbul

INTRODUCTION
Nuclear factor kappa B (NF-κB) is a transcription factor that regulates the expression of several molecules which have critical roles in inflammatory responses such as interleukines, TNFα, iNOS and COX-2 [1]. NF-κB pathway involves in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis and asthma as well as several cancers (2,3). For that reason NF-κB pathway have become an important target for drug discovery and development. Sambucus nigra (mürver) (Adoxaceae) is a perennial plant which is used traditionally against rheumatisms disorders in Anatolia [4]. The aim of this study is to isolate and identify the NF-κB inhibitory components of S. nigra by in vitro activity guided fractionation.

MATERIALS AND METHODS
Plant Material: The leaves of S. nigra were collected from Uludag-Bursa (Turkey) in June, 2009. The plants were identified by Prof. Dr. Erdem Yeşişala (Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey). A voucher specimen S. nigra (YEF 09021) was deposited at the Herbarium of Yeditepe University.

Extraction and solvent-solvent fractionation: The dried and powdered leaves of S. nigra (131 g) were extracted by maceration with methanol (MeOH; 0.9 L) for 24 hr’s. The MeOH extract was evaporated to dryness under reduced pressure to give the crude MeOH extract of S. nigra (SN-MeOH 37.31 g, yield: 12.8%). SN-MeOH was fractionated by successive solvent extractions with n-hexane (14x100 ml), CHCl₃ (3x100 ml), EtOAc (4x100 ml), and n-butanol saturated with H₂O (3x100 ml). Each of subextract after solvent extractions was evaporated to dryness under reduced pressure to give crude MeOH of S. nigra (SN-MeOH 37.31 g, yield: 12.8%). SN-MeOH was fractionated by successive solvent extractions with n-hexane (14x100 ml), CHCl₃ (3x100 ml), EtOAc (4x100 ml), and n-butanol saturated with H₂O (3x100 ml). Each of subextract after solvent extractions was evaporated to dryness under reduced pressure to yield “n-hexane subextract” (SN-Hex 9.5 g, yield: 42.2%) “CHCl₃ subextract” (SN-CHCl₃ 780 mg, yield: 3.5%), “EtOAc subextract” (SN-EtOAc 1.2 g, yield: 5.3%), “n-BuOH subextract.” (SN-n-BuOH 2.5 g yield: 1.1%), and “remaining aqueous subextract” (SN-R-H₂O 7.7 g, yield: 34.2%), respectively.

Cells and Cell Culture: The Raw 264.7 macrophages were obtained from American Type Culture Collection (ATCC TIB-71). The cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 4 mM L-glutamin, 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO2.

WST-1 Cell Viability Assay: The non-toxic concentrations of the samples were determined by WST-1 reagent (Roche Diagnostics; 05 015 944 001) according to the manufacturer’s instructions.
Electromobility Shift Assay: Activity guided fractionation of extracts were achieved by monitoring in vitro NF-κB inhibitory effects. Raw 264.7 macrophage cells were incubated with extracts or subextracts and then induced with LPS. Nuclear and cytosolic fractions of the cells were isolated. Nuclear fractions were submitted to EMSA as described by Telci et al [5].

Chromatographic Methods: Open Column Chromatography was used to fractionate active hexane subextract using silica gel and sephadex LH-20 as adsorbent.

RESULTS AND DISCUSSION
The crude MeOH extract of S. nigra and all subextracts were investigated for their inhibitory effects on NF-κB transcription factor on lipopolysaccharide (LPS) induced Raw 264.7 macrophages. S. nigra hexane subextract inhibited 30% of NF-κB activation. Therefore this subextract was further fractionated by in vitro activity guided fractionation.

Hexane subextract of S. nigra applied to Silica gel Column Chromatography (CC) to yield four fractions (Fr.1-6, Fr.7-10, Fr.11-16, Fr.17-25). Only Fr.11-16 and Fr.17-25, exerted 70% and 79% NF-κB inhibitions at 100 μg/ml concentrations. A fraction was obtained from Fr.11-16 and named as SNH-1 by Sephadex CC. Silica gel CC of Fr.17-25 yielded a fraction which was named as SNH-2. These fractions provided more than 70% inhibition at 50 μg/ml concentrations. SNH-1 applied to Raw 264.7 cells at 12, 25 and 50 μg/ml concentrations and provided 42,25%, 62,00% and 73,35% inhibitions. SNH-2 caused 66,00%, 77,69% and 78,38% inhibitions at the same concentrations. All tested concentrations were non-toxic to Raw 264.7 cells.

CONCLUSIONS
In this study two active fractions were obtained from S. nigra hexane subextract and their concentration dependent inhibitory activities on NF-κB were also investigated.

ACKNOWLEDGMENTS
This study is supported by TUBITAK (project no: 110S197) research grant.

REFERENCES
4. Telci, D.; Collighan, R. J.; Basaga, H.; Griffin, M. Increased TG2 Expression can result in induction of transforming growth factor β1, causing increased synthesis and deposition of matrix proteins, which can be regulated by nitric oxide. J Biol Chem, 2009, 284 (3), 29547–29558.

P-310: IN VITRO ANTI-INFLAMMATORY EFFECTS OF NF-κB INHIBITORY FRACTIONS OF SAMBUCUS NIGRA HEXANE SUBEXTRACT

Atay I., 1, lter AZ., 2, Bağatür Y., 2, Telci D., 2, Kirmızibekmez H. 3, Yesilada E. 3

1 İstanbul Medipol University, School of Pharmacy, Department of Pharmacognosy, 34810, Beykoz, İstanbul
2 Yeditepe University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, 34755, Ataşehir, İstanbul
3 Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy and Phytotherapy, 34755, Ataşehir, İstanbul

INTRODUCTION
Sambucus nigra (mürver) (Adoxaceae) is a perennial plant which is used traditionally against rheumatismal disorders in Anatolia [1]. The aim of this study is to investigate the in vitro anti-inflammatory effects of NF-κB inhibitory components of S. nigra and to elucidate the mechanism underlying the effects. The effects of the fractions on nitric oxide (NO), prostaglandine E2 (PGE2), tumor necrozis factor (TNFα) and several inflammatory interleukins (IL-1α, IL-1β), IL-2, IL-6) and the effects on iNOS, COX-2 protein levels as well as phosphorylation levels of mitogen activated protein kinases (MAPK)(ERK1/2, JNK, p38) and I kappa B alpha (IκBα) were investigated.

MATERIALS AND METHODS
Plant Material: The leaves of S. nigra were collected from Uludağ-Bursa (Turkey) in June, 2009. The plants were identified by Prof. Dr. Erdem Yesilada (Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey).

Extraction and solvent-solvent fractionation: The dried andpowdered leaves of S. nigra (131 g) were extracted with methanol (MeOH) and MeOH extract was fractionated by solvent-solvent extractions to obtain n-hexane, chloroform, ethyl acetate, n-butanol and remaining water subextracts.

Chromatographic Methods: Open Column Chromatography was used to fractionate active hexane subextract using silica gel and sephadex LH-20 as adsorbent.

Cells and Cell Culture: The Raw 264.7 macrophages were (ATCC TIB-71) grown in DMEM supplemented with 10% FBS, 4 mM L-glutamin, 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO2.
RESULTS AND DISCUSSION
Two active fractions (SNH-1 and SNH-2) were obtained from the hexane subextract of the methanol extract of *Sambucus nigra* L. leaves by *in vitro* activity-guided fractionation. These fractions inhibited the activation of NF-κB transcription factor by 78% at 50 μg/ml, it exerted no effects on other *in vitro* inflammatory parameters.

CONCLUSIONS
Consequently, it was revealed that the NF-κB inhibitory active fractions of *S. nigra* hexane subextract has *in vitro* anti-inflammatory effects on several inflammatory parameters.

ACKNOWLEDGMENTS
This study is supported by TUBITAK (project no: 110S197) research grant.

REFERENCES
2. Telci, D.; Collighan, R. J.; Basaga, H.; Griffin, M. Increased TG2 Expression can result in induction of transforming growth factor β1, causing increased synthesis and deposition of matrix proteins, which can be regulated by nitric oxide. *J Biol Chem*, 2009, 284 (43), 29547–29558.

P-311: PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF CAPSELLA BURSA-PASTORIS FROM ANATOLIA

M. Boğa,⁵ A. Ertaş,² M. Öztürk,³ M.A. Yılmaz,⁴ S. Demirci,⁵ H. Temel⁶

Dicle University, Faculty of Pharmacy
¹Department of Pharmaceutical Technology, ²Department of Pharmacognosy, ³Muğla Sıtkı Koçman University, Faculty of Sciences, Department of Chemistry, Muğla TURKEY, ⁴Dicle UniversityResearch and Application of Science and Technology Center (DUBTAM), Diyarbakır, TURKEY, ⁵Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Adana, TURKEY

Phytochemical analysis and biological activities of the extracts obtained from whole parts of *Capsella bursa-pastoris* were studied. Petroleum ether extract of *Capsella bursa-pastoris* showed good butyrylcholinesterase inhibitory activity. The main constituents of fatty acid and essential oil were identified as palmitic acid and Z-8-octadecen-1-ol acetate, respectively. Qualitative and quantitative determination of chemical composition of the methanol extract was determined by LC-MS/MS.

INTRODUCTION
*Capsella bursa-pastoris* has analgesic and anti-diarrheal properties. *C. bursa-pastoris* is used for the treatment of stomach cramps, both internal and external bleedings, wounds, burns, premenstrual syndrome, malignant ulcers, stomach and uterine cancers, tumors and fibromas [1].
MATERIALS AND METHODS

Plant materials: Capsella bursa-pastoris was collected from Mardin-Kızıltepe in 2012 and was identified by Dr. Serpil Demirci (ISTE 97147).

Methods: Phenolics, flavonoids, essential oils and fatty acids profiles of C. bursa-pastoris were determined by LC-MS/MS and GC-MS. Total phenolic and flavonoid contents were determined as pyrocatechol and quercetin equivalents. DPH free radical, ABTS cation radical scavenging and CUPRAC activity assays were studied to determine antioxidant activity. Ellman method was used for anticholinesterase activity [2, 3].

RESULTS AND DISCUSSION

Twenty-seven compounds including (L)-malic acid, quercetin, protocatechuc acid, chrysin, rutin, hesperetin, naringenin, rosmarinic acid, vanillin, p-coumaric acid, caffeic acid, chlorogenic acid, hyperoside, myricetin, coumarin, kaempferol, quinic acid, tr-aconitic acid, 4-hydroxybenzoic acid, fisetin, gallic acid, tannic acid, salicylic acid, hesperidin, luteolin, apigenin, rhamnetin have been investigated by LC-MS/MS for the first time in this study.

Z-8-octadecen-1-ol acetate and 1-hexacosanol were determined as major compounds for essential oil of C. bursa-pastoris. Palmitic and behenic acids were determined as major fatty acids of petroleum ether extract of C. bursa-pastoris. The phenolic contents of petroleum ether extract of C. bursa-pastoris were investigated by LC-MS/MS and GC-MS. Total phenolic and flavonoid contents, antioxidant and anticholinesterase activities of C. bursa-pastoris from Anatolia were studied for the first time in this study.

CONCLUSIONS

Phenolic and flavonoid profile of C. bursa-pastoris were investigated by LC-MS/MS for the first time. Essential oil and fatty acid contents were determined with GC-MS analysis. Total phenolic and flavonoid contents, antioxidant and anticholinesterase activities of C. bursa-pastoris from Anatolia were studied for the first time in this study. Further investigation should be done for isolation and identification of anti-butyrlycholinesterase active constituents of the petroleum ether extract.

ACKNOWLEDGMENTS

The authors are thankful to Dicle University Science and Technology Research and Application Center (DUBTAM) for its support.

REFERENCES


P-312: CHEMICAL AND BIOLOGICAL INVESTIGATION OF CALYSTEGIA SILVATICA

M. Boğa1, A. Ertas2, M.A. Yılmaz3, H.Temel3,4, Y. Yeşil5, U. Kolak6

Dicle University, Faculty of Pharmacy
1Department of Pharmaceutical Technology, 2Department of Pharmacognosy, 3Dicle University Research and Application of Science and Technology Center (DUBTAM), 4Department of Pharmaceutical Chemistry, Diyarbakır, TURKEY

Istanbul University, Faculty of Pharmacy,
5Department of Pharmaceutical Botany, Istanbul TURKEY,
6Department of Analytical Chemistry, Istanbul, TURKEY

Chemical composition and biological activities of various extracts obtained from whole parts of Calystegia silvatica were studied. Methanol and water extracts of Calystegia silvatica showed good ABTS cation radical scavenging activity assay. The main constituents of essential oil and fatty acid were identified as heptacosanol and palmitic acid, respectively. Qualitative and quantitative determination of chemical composition of the methanol extract was determined by LC-MS/MS.

INTRODUCTION

Calystegia silvatica is known as “boytan sarmaşık, bürük” in Turkish. Roots and branches with flowers of Calystegia species are used as cholagogue and purgative in traditional medicine in Turkey (Baytop, 1999). In this study, we aimed to determine chemical (phenolics, flavonoids and essential oils) profile and biological activities of C. silvatica.
MATERIALS AND METHODS

Plant materials: Calystegia silvatica was collected from Istanbul-Belgrad in 2012 and was identified by Dr. Yeter Yeşil (ISTE 98063).

Methods: Phenolics, flavonoids, essential oils and fatty acids profiles of *C. silvatica* were determined by LC-MS/MS and GC-MS. The amounts of phenolic and flavonoid contents in the crude extracts of *C. silvatica* were expressed as pyrocatechol and quercetin equivalents. DPPH free radical, ABTS cation radical scavenging and CUPRAC activity assays were studied to determine antioxidant activity. Ellman method was used for anticholinesterase activity [2, 3].

RESULTS AND DISCUSSION

Qualitative and quantitative determination of twenty-seven compounds including (L)-malic acid, quercetin, protocatechuic acid, chrys, rutin, hesperetin, naringenin, rosmarinic acid, vanillin, p-coumaric acid, caffeic acid, chlorogenic acid, hyperoside, myricetin, coumarin, kaempferol, quinic acid, tr-aconitic acid, 4-caffeic acid, chlorogenic acid, hyperoside, myricetin, naringenin, rosmarinic acid, vanillin, p-coumaric acid, protocatechuic acid, chrysin, rutin, hesperetin, seven compounds including (L)-malic acid, quercetin, and flavonoid contents in the crude extracts of *C. silvatica* were expressed as pyrocatechol and quercetin equivalents. DPPH free radical, ABTS cation radical scavenging and CUPRAC activity assays were studied to determine antioxidant activity. Ellman method was used for anticholinesterase activity [2, 3].

CONCLUSIONS

Phenolic and flavonoid profile of *C. silvatica* was investigated with LC-MS/MS for the first time. Essential oil and fatty acid contents were determined with GC-MS analysis. Total phenolic and flavonoid contents, antioxidant and anticholinesterase activity of *C. silvatica* studied for the first time in this study. Further investigation should be done for isolation and identification of antioxidant active constituents of the extracts, especially methanol and water extract.

ACKNOWLEDGMENTS

The authors are thankful to Dicle University Science and Technology Research and Application Center (DUBTAM) for its support.

REFERENCES


P-313: INVESTIGATION OF ENZYME ACTIVITY/INHIBITION OF PRUNUS SPINOSA L. EXTRACTS

M. Kurt, M. Ay

Çanakkale Onsekiz Mart University, Faculty of Sciences and Arts, Department of Chemistry, Natural Products and Drug Research Laboratory, Çanakkale, Turkey

INTRODUCTION

Nowadays, many medicinal raw materials are obtained from plants which have biological activities such as antimicrobial, antiviral, antioxidant, anti-tumor. Plant extracts are a useful resource for many important therapeutic drugs derived from natural products. *Prunus spinosa* L. comprises important components that are major in terms of human health and having anticancer, antioxidants, antimicrobial properties of phytochemicals such as anthocyanins, glycosides, flavonoids, coumarins and phenolic compounds [1,2].

MATERIALS AND METHODS

For this purpose, hexane, dichloromethane, ethyl acetate and methanolextracts of the fruit, leaves and flower parts of *Prunus spinosa* L. were checked for enzyme-activity/inhibition studies with proteases (*α*-Chymotrypsin, trypsin, papain and carboxypeptidase A), tyrosinase, *β*-lactamase enzymes out successfully. 10 mg/mL and 1 mg/mL solutions of fruit, leaf and flower extracts of plant of have been used for performing enzyme inhibition assays[3].

The inhibition studies of the extracts have been carried out at 25 °C with tyrosinase and *β*-lactamase, at 37 °C with *α*-chymotrypsin, trypsin and papain enzymes for 10 minutes, respectively. The absorbances were measured at 410 nm for each one and at 254 nm for carboxypeptidase A.

366
The absorbances of tyrosinase and penicillinase studies were measured at 475 and 495 nm with a microplate reader, respectively. The graphs have been obtained against the PMSF (Phenyl methane sulphonyl fluor) inhibitor.

RESULTS AND DISCUSSION

When examined the result of inhibition of tyrosinase enzyme and proteases enzyme (including papain, α-chymotrypsin and trypsin); while the concentration of extracts were increasing, inhibition values in general seemed to be increasing in extracts of fruit, leaf and flower. Although, while the concentration of extracts were increasing for penicillinase enzyme; the inhibition values in general seemed to be increasing in extracts of fruit, leaf and flower.

EtOAc fruit (22.24 %), DCM leaf (23.93%) and MeOH flower (22.67%) of extracts have the high values of % inhibition than the other extracts for α-Chymotrypsin enzyme, fruit (64.19%), leaf (92.10%), flower (95.21%) of EtOAc extracts have the high values of % inhibition than the other enzyme extracts; the inhibition values in general seemed to be increasing for concentration of 10 μL/10 mg/mL but inhibition values in general seemed to be decreasing for concentration of 50 μL/10 mg/mL in extracts of fruit, leaf and flower.

CONCLUSIONS

The ethyl acetate extracts may be used potential papain enzyme inhibitors.

ACKNOWLEDGMENTS

We thank to ÇOMÜ BAP (FYL 2014/212) for financial support and to GTE (Ayhan Çelik) for contributions during enzyme studies.

REFERENCES


P-314: ALPHA GLUCOSIDASE INHIBITION AND ANTIOXIDANT POTENTIAL OF SOME RUMEX ACETOSELLA L. EXTRACTS

N. Ozenver¹, L.O. Demirezer¹

Hacettepe University, Faculty of Pharmacy, ²Department of Pharmacognosy, 06100, Ankara, TURKEY

INTRODUCTION

According to World Health Organization (WHO), the global prevalence of diabetes was estimated to be 9% among adults aged 18+ years in 2014 and type 2 diabetes comprises 90% of people with diabetes around the world.WHO also projects that diabetes will be the 7th leading cause of death in 2030.

Oxidative stress has pivotal role in the development of complications of diabetes [1]. Alpha glucosidase inhibitors delaying breakdown of complex carbohydrates in small intestine and slowing glucose absorption are also significant to prevent development of hyperglycemia [2].

Rumex acetosella L. (Polygonaceae) has been commonly used for diabetes, stomach and heart diseases in traditional medicine [3]. In this study some root and herb extracts of R. acetosella (HUEF: 13005) were searched for their α glucosidase enzyme inhibition and antioxidant profile and thereby their antidiabetic potential.

MATERIALS AND METHODS

Preparation of methanol and 70% methanol extracts of herbs and roots of R. acetosella: 5 g of dried powdered herbs and roots were extracted two times with 50 ml methanol for methanol extracts and 70% methanol for 70% methanol extracts for six hours respectively. The extracts were filtered, evaporated to dryness in vacuo.

Herb extracts were separated from their lipids and pigments with petroleum ether extraction. Then the extracts were dissolved in buffer solution to prepare different concentrations.

Preparation of chloroform extracts of herbs of R. acetosella:
5 g of dried powdered herbs were extracted two times with 50 ml chloroform for six hours stirring in a water bath at 40 °C, respectively The extracts were filtered, evaporated to dryness in vacuo and then dissolved in DMSO: buffer mixture to prepare different concentrations.

Preparation of chloroform extracts of roots of R. acetosella:
5 g of dried powdered roots were extracted two times with 50 ml chloroform for six hours stirring in a water bath at 40 °C. The extract was filtered, evaporated to dryness in vacuo and then dissolved in DMSO: buffer mixture to prepare different concentrations.
Alpha glucosidase enzyme inhibition assay: A glucosidase enzyme inhibition assay was performed according to the method previously described by Bachhawat et al. [4] with slight modifications.

Antioxidant assays: DPPH, ABTS, NO scavenging and phosphomolybdate antioxidant assays were carried out according to the procedures reported before with slight modifications.

RESULTS AND DISCUSSION
α glucosidase inhibition potential of chloroform extract of R. acetosella roots is lower than its methanol and 70% methanol extracts. IC50 values of methanol and 70% methanol extracts of the roots were lower than standard Acarbose. Additionally, 70% methanol extracts of R. acetosella herbs also had similar results with 70% methanol extracts of the roots. Methanol and 70% methanol extracts of the herbs had also better inhibition than standard Acarbose.

Antioxidant capacity of methanol and 70% methanol extracts were significant and good in contrast to chloroform extracts of herbs and roots of R. acetosella having from moderate to low when compared to standard antioxidants in all studied methods.

CONCLUSIONS
Methanol and 70% methanol extracts of both roots and herbs of R. acetosella may be significant sources to be inhibitors to prevent or treat type 2 DM.

ACKNOWLEDGMENTS
This study was supported by grants from Hacettepe University Scientific Research Projects Coordination Unit (Project No: 1216).

REFERENCES

P-315: BIOASSAY GUIDED ISOLATION OF WOUND HEALING ACTIVE CONSTITUENTS FROM ECHIUM SPECIES FROM TURKEY
N. Eruygur1, O. Üstün2, G. Yilmaz3

1Cumhuriyet University, Faculty of Pharmacy, Department of Pharmacognosy, Sivas, 2Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, 3Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

In this study, four Echium species namely: E. italicum L., E. vulgare L., E. angustifolium Miller, E. parviflorum Moench, growing in Turkey, has been investigated by biological activity and chemical approaches. Biological activity guided fractionation are conducted on total extracts that were prepared from plant material with Ethanol. Wound healing effect of crude extracts were assessed in Linear incision wound model test in mice.

INTRODUCTION
The roots and root barks of Echium sp. have been used to treat ulcer, burn and wounds in traditional Turkish medicine [1-2]. In order to evaluate this traditional use, four Echium species which is used as wound healing in Turkish folk medicine have been investigated.

MATERIALS AND METHODS
Plant materials: The aerial parts and root of the four Echium species were collected in June 2012 from Bolu and Antalya, Turkey
Extraction: Extraction proceed on air dried aerial parts and roots of plants separately with ethanol.
Animal: Healthy, male Swiss albino mice weighing 20-25g were used for Linear incision wound healing method. Madecassol® was used as reference.

RESULTS AND DISCUSSION
The ethanol extracts of roots and herbs of four Echium species were investigated for their in vivo wound healing potential.

The tensile strength of the progression healing of wounds on incision wound model for vehicle, negative control, alcohol extract and reference drug treated groups were shown in Table 1.

In previous studies reported that Echium species, as the members of Boraginaceae family, have shikonin derivatives. There have been a lot of reports about multiple pharmacological activites of shikonin derivatives.
Table 1. Linear incision wound healing test with vehicle, negative control, extract and reference drug

<table>
<thead>
<tr>
<th>Material</th>
<th>Kullanma n kısım</th>
<th>Dosag e (mg/g)</th>
<th>Tensile strength h ± SD (% Wound intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>5.27 ± 0.35</td>
<td>8.44</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>4.86 ± 0.71</td>
<td>7.97</td>
</tr>
<tr>
<td>E. italicum L. root</td>
<td>10</td>
<td>7.24 ± 1.74</td>
<td>37.38**</td>
</tr>
<tr>
<td>E. v novel L. root</td>
<td>10</td>
<td>7.43 ± 0.28</td>
<td>40.97**</td>
</tr>
<tr>
<td>E. angustifolium Miller root</td>
<td>10</td>
<td>7.13 ± 0.67</td>
<td>35.29**</td>
</tr>
<tr>
<td>E. parviflorum Moench root</td>
<td>10</td>
<td>5.59 ± 0.96</td>
<td>6.07</td>
</tr>
<tr>
<td>Madecassol®</td>
<td></td>
<td>7.68 ± 0.57</td>
<td>45.73**</td>
</tr>
</tbody>
</table>

CONCLUSIONS
In conclusion, our results verified the beneficial ethnopharmacological effects of topically administered *Echium sp.* extracts on linear incision wound model. Moreover, the phytochemical investigation of *Echium* species in Turkey was achieved for the first time. Further research is designed to isolate active pure compounds from extracts as well as from active fractions by bioassay guided isolation method.

ACKNOWLEDGMENTS
This study was supported by Gazi University Research Foundation (BAP 02-2010/2011).

REFERENCES

P-316: SPME/GC-MS ANALYSIS OF THREE STACHYS SPECIES FROM TURKEY

G. Renda¹, N. Yanci², G. Tosun², B. Yaylı³, S. Sevgi³, N. Yaylı³

¹Karadeniz Technical University, Faculty of Pharmacy Department of Pharmacognosy, Trabzon, TURKEY
²Karadeniz Technical University, Faculty of Science, Department of Chemistry, Trabzon, TURKEY

INTRODUCTION
The genus *Stachys* (Lamiaceae) is represented by 90 species and 115 taxa in Turkey; 54 of the 115 taxa are endemic [1]. *Stachys* species were used as folk medicine in Turkey for the treatment of cold, cough, stomachache and as antipyretic [2]. In this study; a solid phase micro extraction (SPME) method with gas chromatography-mass spectrometry (GC-MS) was used for the analysis of volatile compounds of three *Stachys* species; *Stachys macrantha* (C. Koch) Stearn, *Stachys sylvatica* L., *Stachys annua* ssp. *annua* var. *annua* L.

MATERIALS AND METHODS
Flowered plants were collected from Black Sea Region of Turkey [1]. Fresh plant materials (1 g, each) were grounded and placed in a 10 mL vial sealed with a silicone-rubber septum cap. A polydimethylsiloxane/divinylbenzene fiber was used for the extraction of the volatile components. The fiber coating was placed to the head space for temperature and times (incubation and extraction times) values set according to the experiment. The fiber containing the extracted aroma compounds were then injected into the GC-MS injector (split mode). The components of the essential oils were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature [3].

RESULTS AND DISCUSSION
A comparison of volatile compounds was made among three *Stachys* species. A total of 76 compounds were identified and different volatile compounds were determined within the species. The major volatile constituents of the investigated essential oils of *Stachys* species were; α-Pinene (11.2%), p-cymene (18.2%), carvacrol (28.8%) in *S. macrantha*, γ-murolene (10.2%) α-cedrene (11.2%), limonene (37%) in *S. sylvatica*, ando-pinene (11.4%), β-pinene (23.1%), (E)-β-ocimene (24.8%) in *S. annua* ssp. *annua* var. *annua*. Analyses revealed that SPME/GC-MS method is appropriate for the analysis of volatile compounds of *Stachys* species.

CONCLUSIONS
Comparison studies showed that sesquiterpenes; α-cedrene (11.2%) and γ-murolene (10.2%) were found to be two of the main components of *S. sylvatica*; it is found that monoterpenes were present in highest percentage in all *Stachys* species studied; *S. annua* ssp. *annua* var. *annua* and *S. sylvatica*, *S. macrantha*(36.2%, 49.9%, 51.6% respectively).

REFERENCES
P-317: IN VITRO ANTIBACTERIAL EFFECTS OF SYNERGISTIC TERPENE COMBINATIONS WITH ANTIBIOTICS AGAINST RHINOSINUSITIS PATHOGENS

N. Karaca¹, B. Demirci¹-², and F. Demirci²-³

¹Graduate School of Health Sciences, Department of Pharmacognosy, ²Faculty of Pharmacy, Department of Pharmacognosy, ³Faculty of Health Sciences, Anadolu University-Eskişehir, TURKEY

INTRODUCTION

Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa are the most encountered bacterial pathogens in rhinosinusitis. It is well known that antibiotic-resistant infections caused by unconscious and excessive use of antibiotics, high treatment costs and side effects of synthetic drugs complicate the treatment of rhinosinusitis. This requires the search for new sources to control the disease [1]. Natural antimicrobial substances have withdrawn attention, where essential oils and their main components have shown a potential for a long time [2].

In this present study we aimed to reduce the amount of antibiotics used in the treatment of rhinosinusitis and resistance factors. For this purpose, antimicrobial synergistic effect of terpenes and amoxicillin/clavulanate combinations were investigated against human pathogenic standard strains.

MATERIALS AND METHODS

Synergistic antibacterial effect of pure 1,8-cineole, α-pinene, L-carvone and p-cymene combined with amoxicillin/clavulanate (5:1) at 20:90, 30:80, 40:60, 50:50, 60:40, 70:30 and 80:20 (V:V) ratios were studied using the microdilution method [3, 4] against S. pneumoniae ATCC® 10015, H. influenzae ATCC® 49247 and M. catarrhalis ATCC® 23245 standard strains. Resulting antibacterial effects were evaluated for their fractional inhibitory concentrations (FICs).

RESULTS AND DISCUSSION

Several combinations of L-carvone and 1,8-cineole with amoxicillin/clavulanate were determined as synergistic against H. influenzae. Additive and ineffective combinations were determined in the MIC range of 0.04-20 mg/mL against all tested pathogenic microorganisms.

CONCLUSIONS

The results may suggest that the rational combination of selected terpenes and antibiotics are a good way for the reduction of antibiotic concentrations as well as resistant infection risks in the case of rhinosinusitis [5].

KEYWORDS

Rhinosinusitis, microdilution method, 1,8-cineole, α-pinene, L-carvone and p-cymene.

ACKNOWLEDGMENTS

This work was financially supported by the Tubitak 117S740 project.

REFERENCES

hypertension, cirrhosis, throat diseases, leukaemia and uterine cancer. [2-4].

This presentation is a part of our ongoing research project on A. sibthorpi anus.

MATERIALS AND METHODS
A. sibthorpi anus (Endemic) was collected from ~2100 m, Kirkpınarlar locality, Uludağ Mountain National Park, Bursa, Turkey in June 2012 identified by Prof. Dr. Gülcan Güleryüz (Fig 1.).

Fig 1. A. sibthorpi anus Boiss. and its location

The air-dried and powdered whole plant materials were exhaustively macerated with MeOH. The concentrated MeOH layer was extracted with hexane to remove oils and fats. After fractionation by BuOH:H2O, the saponin rich BuOH extract was obtained.

The extract was fractioned by vacuum liquid chromatography using RP-18 SiGel and stepwise MeOH–H2O gradient (20–100% MeOH). Fractions from VLC were subjected to open column chromatography using SiGel as stationary phase. Elution with CHCl3:MeOH:H2O/ 61:32:7 and 85:15:1,5 respectively gave Compound 1 and 2.

RESULTS AND DISCUSSION
The structures of 1 (Fig 2) and 2 (Fig 3) were determined by detailed NMR studies. The aglycone and sugar moieties was identified as olean with two sugar units for 1 and β-sitosterol with one sugar unit for 2 from 1H, 13C, DEPT and HSQC analysis. All connectivity within 1 and 2 were also confirmed by HMBC spectrum.

CONCLUSIONS
In the present work, respectively, a triterpene and steroid glycoside namely 3-O-[β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl]-3β,22β,24-tri hydroxyolean-12-ene and 3-O-β-D-glucopyranosyl-β-sitosterol (Daucosterol) were purified by various chromatographic techniques and their structure were investigated by spectroscopic techniques.

To the best of our knowledge, our study is the first phytocemical study on A. sibthorpi anus Boiss.

ACKNOWLEDGMENTS
This study was supported by Uludağ U., Scientific Research Projects Div. (KUAP(F)-2013-85)

REFERENCES

P-319: CHARACTERIZATION OF COMPOUNDS ISOLATED FROM ZOSIMA ABSINTHIIFOLIA AS POTENTIAL INHIBITORS OF ACETYLCHOLINESTERASE

Ö. Bahadir Acıkarın, S. Dall’Acqua, K. Smejkal, G. Saltan

1Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey 2Department of Università degli Studi di Padova, Dipartimento di Scienze Farmaceutiche, Padova, ITALY 3University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Pharmacy, Department of Natural Drugs, Brno, CZECH REPUBLIC

INTRODUCTION
Coumarines are naturally occurring compounds have important biological activities including anti-inflammatory, antifungal, antiviral, anticoagulant, anti-tumor [1]. Many plants belonging to Umbelliferae family are found to be rich in coumarine derivatives [2]. Zosima absinthifolia L. from Umbelliferae family, grows in central and southwestern areas of Asia widely [3]. Coumarines such as (–)-deltoin, zosimin, bergapten, isobergapten, pimpinellin, isopimpinellin, imperatorin, sphondin and umbelliferone as well as flavonoids and alkaloids have been isolated from Z. absinthifolia. Antimycobacterial, cytotoxic, antioxidant, antibacterial, hepatoprotective and anti-inflammatory activities of Z. absinthifolia have been reported previously [4]. Current study is aimed to evaluate acetylcholinesterase inhibitory activities of Z.
absinthifolia roots and aerial parts as well the coumarines; deltoin, columbianadin and methoxsalen isolated from the aerial parts.

MATERIALS AND METHODS
Z. absinthifolia was collected from Bolu, Turkey. Identification of the plant sample was confirmed by Prof. Hayri Duman, a plant taxonomist from Gazi University. Voucher specimen was kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No: 23847). Aerial parts of the plant were dried and extracted with n-hexane under reflux for 5 h. The extract was evaporated to dryness and subjected to column chromatography to obtain deltoin, columbianadin and methoxsalen by elution n-hexane:ethylacetate mixture. The characterization of the isolated compounds was performed by NMR spectrometric techniques (1D and 2D) and measurements of mass spectrometry. The anti-acetylcholinesterase activities of extracts and compounds were evaluated by the Ellman test, assay by thin layer chromatography and spectrophotometric assay.

RESULTS AND DISCUSSION
A significant fact is that all isolated compounds, albeit at high doses compared to galantamine, are able to inhibit the enzyme. All compounds exhibit IC$_{50}$ values in the order of 0.082 to 0.228 mM and then are modest inhibitors of the enzyme. From the IC$_{50}$ values obtained, the most active compound is deltoin. The coumarin derivatives that have a furan cycle and a substituent hydroxy-isopropyl and the esterification with the acid tiglic (deltoin and columbianadin) appear to be more active than the compound without this group.

CONCLUSIONS
Identification of coumarin derivatives as moderate anti-AChE is in agreement with some recent works that demonstrate for some furan or pyran coumarins moderate activity with IC$_{50}$ values between 28 and 600 mM [5].

ACKNOWLEDGMENTS
We are grateful to Prof. Hayri Duman for plant identification.

REFERENCES

P-320: POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF GLOBULARIA AYLPUM ACETONE EXTRACT

R.Belhattab, A. Abirez and K.Djemmal
Department of Biochemistry, Faculty of Nature and Life Sciences, University F.A. Setif 1, 19000, Setif, Algeria

INTRODUCTION
Globularia alypum (Globulariaceae) is a medicinal plant largely used in Algerian folk medicine to cure several pains related to diabetes, cardio vascular deases and digestive disorders.

MATERIALS AND METHODS
1- Plant material : The aerial parts of G. alypum, were collected during April 2012 in Batna, Algeria.
2- Preparation of extracts :Acetone extract of the was obtained with Soxhlet apparatus (1).
3- Phytochemical screening and thin layer chromatography: Aliquots from acetone extract were subjected to different chemical revelators. For the separation of the constituents, two different elution systems were used:toluene/ethyle acetate/ formic acid (5/4/1) and chloroform/acetic acid/ formic acid (5/4/1). The revelation was conducted with ethanol solutions of FeCl$_3$ 2% and AlCl$_3$ 1% and visualization under UV light (366nm) before and after heating at 105°C. The Rf values were then determined and compared to control.
4- Total polyphenol content: The determination of polypphenol content was achieved according to Folin- Ciocalteu method (2).
5- Total flavonoid content : This determination was carried using AlCl$_3$ method (3).
6- Antioxidant activity : Linoleic acid/β-carotene bleaching test, the scavenging effect (DPPH test) and iron chelating capacity (4) were performed.

RESULTS AND DISCUSSION
The extraction yield was 14,66 % (w/w), the chemical screening and TLC led to the identification of a few secondary metabolites (fig.1).
Fig.1. Chemical screening of *G. alypum* acetone extract

1-terpenoids, 2- quinone ; 3- anthraquinone, 4- flavonoids , 5- polyphenols , 6- control

Total polyphenol content

The equation of the curve is \( y = 0.007 x - 0.074; \ R^2 = 0.998 \).

*G. alypum* extract contains 150.14mg GAE/g DW which corresponds to 2.2 g GAE/100g fresh material (Fig.2).

Flavonoid content

The equation of the curve is: \( y= 0.033 x^5 \); \( R^2 = 0.999 \).

*G.alypum* flavonoid content was 7.84mg QE/g DW which corresponds to 115mg QE/100g fresh material.

Antioxidant activities

a-β-carotene/linoleic acid bleaching test: the effect was dose dependent

b-Scavenging effect using DPPH: The scavenging effect of acetone extract obtained from *G. alypum* and BHA (positive control) were estimated to have an IC50 of 137.7 μg/ml and 15μg/ml respectively.

c-Iron chelating capacity: The ferrous chelating test using acetone extract obtained from *G. alypum* (10μg/ml) showed a high effect (6.28 μg EDTAE/ ml).

CONCLUSIONS

These findings support the traditional uses of this plant However; more investigations are needed with other extraction solvents and antioxidant tests.

ACKNOWLEDGMENTS

We would like to thank the Laboratory of Applied Microbiology (LMA) at Setif-1 University; Setif and the Ministry of High Education and Scientific Research (MESRS) of Algeria for financial support (CNEPRU Project N°F01220110079).

REFERENCES

3-Bahorun T., Gressier B., Trotin F., Brunet C., Dine T., Luyckx M., Vasseur J., Cazin M., Cazin J.C., Pinkas M..Arzneimittle Forsching 1996, 46 (11), 1086-1089

P-321: THE PHENOLIC PROFILES AND ANTIOXIDANT ACTIVITY POTENTIALS OF BOTH GREEN COFFEE AND TURKISH COFFEE AFTER A TWO-STEP IN VITRO DIGESTION

S. Akyüz1, E. Celep2

Yeditepe University, Faculty of Pharmacy, 1Department of Pharmacognosy, Istanbul, TURKEY

INTRODUCTION

In cell, oxidative stress occurs when antioxidant potential is not enough to scavenge reactive oxygen species and it leads to many diseases like cancer, cardiovascular diseases, neurological disorders, among others. The antioxidant potentials of beverages and their content have been gaining so much interest in the scientific community, recently. Coffee is nearly the most widely consumed beverages in the world and it has many active substances that have antioxidant activity. In coffee, antioxidant activity is generally associated with the levels of indigenous phenolic compounds contained therein, as well as Maillard reaction products, the latter being generated during roasting [1]. In this study, we investigated antioxidant capacity of Turkish coffee and compared to green coffee.
MATERIALS AND METHODS

Materials: Both green coffee seeds and roasted Turkish coffee samples were obtained from a local market in Istanbul, Turkey.

In Vitro Digestion: In vitro digestion simulation method was performed according to the methodology, previously described by McDougal with some slight modifications [2]. In vitro digestion method has two steps including both gastric and intestinal phases.

Phenolic Profile: The total phenolic, flavonoid and proanthocyanin contents of both samples were measured using previously published methods [3].

Antioxidant Activity: The antioxidant capacities were evaluated by using the following methods: DPPH radical scavenging activity, CUPRAC (metal reducing activity) and total antioxidant capacity [3].

RESULTS AND DISCUSSION

Changes in the phenolic composition of coffee were observed following the in vitro digestion procedure on both green coffee and Turkish coffee extracts. In addition, the results of the antioxidant activity tests of Turkish and green coffee were different as well.

CONCLUSIONS

According to our study, antioxidant activity of digested Turkish and green coffee are close to each other. When we compared to undigested extraction, some differences were observed.

REFERENCES


P-322: QUANTITATIVE ANALYSIS OF THYMOQUINONE IN NIGELLA SATIVA L. (BLACK CUMIN) SEEDS AND SEED OILS FROM LOCAL MARKETS OF TURKEY

S. Isik1, S. Aslan-Erdem2, M. Kartal2

Ankara University, 1Biotechnology Institue, 2 Faculty of Pharmacy, Department of Pharmacognosy; 06100 Tandoğan, Ankara, TURKEY

INTRODUCTION

Medicinally and economically important plant Nigella sativa L., popularly known as çörek otu in Turkey is grown in many parts of Turkey. The most important component of its essential oil is thymoquinone. Thymoquinone is used in alternative medicine to treat cancer, rheumatism, headaches, and many diseases for thousands of years. In this study, 10 different seeds and seed oil samples were supplied from local markets in Ankara and their thymoquinon contents were investigated by using HPLC (High Performance Liquid Chromatography).

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human diseases. N. sativa L., a spicy plant, is cultivated in various parts of the world. The seeds, also known as “black cumin” or “black caraway”, as well as “çörek otu” in Turkey, have been used in many Middle Eastern, and Far Eastern Countries as a natural remedy for over 2000 years. In Turkey it is cultivated in Afyon, Burdur and Isparta provinces [1]. N. sativa is an annual plant. It grows up to 15–30 cm, is branched, more or less furry with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually coloured white, with five petals. The seeds are three-sided and black color [1], [2]. The seeds and/or its oil is believed to be carminative, diuretic, lactagogue and vermifuge [3]. Pharmacologically, active constituents of seed oils are known as; Thymoquinone (TQ), Dithymoquinone (DTQ), Thymohydroquinone (THQ) and Thymol (THY) [4].

Black cumin seeds are used in food industry as preservative and spice.
**HPLC Conditions:** Samples were analyzed with Agilent Technologies 1200 series high pressure liquid chromatography (HPLC), including a binary pump, vacuum degasser, auto-sampler, diode array detector. Column: Eclipse XDB-C18 column (150 mm x 4.6 mm, 5μm). Analysis conditions was as follows: Water:Methanol:2-propanol (50:45:5) solution was used for separation at a flow rate of 0.9 mL min⁻¹. Analysis time was 28 min. and detection wavelength was set at 254 nm for thymoquinone. Injection volume was 10 μL for each sample and standard solutions. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas.

**RESULTS AND DISCUSSION**

Thymoquinone was observed in all samples. The amount of the thymoquinone found in the seeds and seed oils are given in Table 1 and Table 2 with both concentration and percentages, respectively. According to the tables given below, sample NS1 has the highest and sample NS4 has the lowest percentage of thymoquinon. For oil samples; sample NO9 has the highest and sample NO4 has the lowest percentage of thymoquinone.

**CONCLUSIONS**

The percentages of thymoquinone are varies for each sample. When compared, the amount of the almost equal in both seed and seed oil samples.

**REFERENCES**


### Table 1. Concentrations and percentages of thymoquinone in seeds

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Concentration (ppm)</th>
<th>Percentage of Thymoquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS1</td>
<td>376.192</td>
<td>0.376</td>
</tr>
<tr>
<td>NS2</td>
<td>100.579</td>
<td>0.101</td>
</tr>
<tr>
<td>NS3</td>
<td>15.190</td>
<td>0.152</td>
</tr>
<tr>
<td>NS4</td>
<td>9.806</td>
<td>0.099</td>
</tr>
<tr>
<td>NS5</td>
<td>20.959</td>
<td>0.021</td>
</tr>
<tr>
<td>NS6</td>
<td>107.062</td>
<td>0.107</td>
</tr>
<tr>
<td>NS7</td>
<td>84.782</td>
<td>0.085</td>
</tr>
<tr>
<td>NS8</td>
<td>14.278</td>
<td>0.014</td>
</tr>
<tr>
<td>NS9</td>
<td>106.614</td>
<td>0.106</td>
</tr>
<tr>
<td>NS10</td>
<td>167.061</td>
<td>0.167</td>
</tr>
</tbody>
</table>

### Table 2. Concentrations and percentages of thymoquinone in seed oils

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Concentration (ppm)</th>
<th>Percentage of Thymoquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO1</td>
<td>84.217</td>
<td>0.079</td>
</tr>
<tr>
<td>NO2</td>
<td>126.521</td>
<td>0.138</td>
</tr>
<tr>
<td>NO3</td>
<td>96.467</td>
<td>0.099</td>
</tr>
<tr>
<td>NO4</td>
<td>18.121</td>
<td>0.017</td>
</tr>
<tr>
<td>NO5</td>
<td>226.276</td>
<td>0.213</td>
</tr>
<tr>
<td>NO6</td>
<td>177.964</td>
<td>0.215</td>
</tr>
<tr>
<td>NO7</td>
<td>69.931</td>
<td>0.082</td>
</tr>
<tr>
<td>NO8</td>
<td>55.848</td>
<td>0.053</td>
</tr>
<tr>
<td>NO9</td>
<td>175.454</td>
<td>0.216</td>
</tr>
<tr>
<td>NO10</td>
<td>51.837</td>
<td>0.063</td>
</tr>
</tbody>
</table>
P-323: TOTAL PHENOLIC CONTENTS, ANTIOXIDANT POTENTIAL AND PHENOLIC COMPOUND PROFILES OF THREE ACHILLEA SPECIES

S. Sabanoğlu, A. Gökbület, M.L. Altun
Ankara University, Faculty of Pharmacy Department of Pharmacognosy, 06100, Ankara, TURKEY

INTRODUCTION
The genus Achillea (Asteraceae) comprises more than 100 species widespread in Northern hemisphere. Achillea species have been characterized by a high content and diversity of terpenes, flavonoids, coumarins, phenolic acids, lignans and essential oil some of which are responsible for the antioxidant, estrogenic, antispermatogenic, antiulcerogenic, antimicrobial, antiviral, antispasmodic, immunosupresif, antitumor and antidiabetic activities [1,2].

In this study, methanolic extracts of A. biebersteinii Afan., A. setacea Waldst. Et Kit. and A. wilhelmsii C. Koch were investigated for their total phenolic contents and antioxidant activities with the main focus on phenolics.

MATERIALS AND METHODS
Achillea species were collected near Yahyali-Kayseri in their flowering stage (2014). All the analytical standards and solvents were purchased from either Sigma or Merck.

The air-dried and milled flowers, leaves and roots of plants were extracted separately with methanol for 6 hours by magnetic stirrer at room temperature. Total phenolic content of methanolic extracts was estimated by the Folin-Ciocalteu method using gallic acid as standard. The antioxidant activity of the extracts was evaluated using DPPH and ABTS assays using caffeic acid and trolox as standarts. RP-HPLC method with UV detection was used to analyse the phenolics.

RESULTS AND DISCUSSION
The total phenolic content varied widely in different parts of the three tested Achillea species, ranging from 113.2 ± 1.9 to 178.4 ± 9.5 mg GAE/g extract. Both the DPPH and ABTS assay results revealed that the radical scavenging activity of A. biebersteinii leaf extract is remarkably high with IC50 values of 0.377 ± 0.011 and 0.016 ± 0.002 mg/ml, respectively. Among all the investigated extracts, flower extract of A. wilhelmsii has the least antioxidant potential together with A. setacea flower extract. Phenolic components, such as gallic acid, chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid, rutin, quercetin, luteolin, apigenin and kaempferol were analyzed by HPLC-DAD in the methanol extracts. It is clear that the significant antioxidant properties of the extracts are due to the phenolics.

CONCLUSIONS
Total phenolic contents, antioxidant potential and phenolic compound profiles of three Achillea species picked up from Inner Anotolia were studied. In conclusion, all the extracts, especially the leaf extracts, contain high amount of total phenolics which support our antioxidant activity test results. The methanolic extracts are also rich in phenolic acids and flavonoids, thus Achillea species could be evaluated as serious antioxidant sources.

REFERENCES

P-324: PRE-MONOGRAPH ANALYSIS OF NIGELLA SATIVA

O. Bagci1, C. Aka1, U. Koca Caliskan2
Gazi University, Faculty of Pharmacy, 1Department of Phytopharmacy, 2Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION
Nigella sativa is an anual herbaceous plant, which belongs to Ranunculaceae family. It is native to South West Asia and cultivated in countries of Middle Eastern and Mediterrenean regions, like Turkey, Syria, Saudi Arabia, India. For thousands of years, many cultures have traditionally used N. sativa as a spice, food additive, preservative, as well as herbal remedy for various diseases and conditions such as asthma, diarrhea, headache, toothache, nasal congestion, and several types of cancer [1]. Researchs have showed that seeds of N. sativa had anti-inflammatory, anti-tumour , anti diabetic , anti ulcerogenic, antioxidant, antitumor , analgesic, hepatoprotective, immunomodulator, antimicrobial and anthelmintic activities [2]. N. sativa is one of the important plants with its seeds, and seed oil which are used for both healing and nutritional purposes.

Although there are a lot of increasing studies currently, there is no pharmacopeia for N. sativa. So, the aim of the study to analyze the seed samples collected from five different regions of Turkey in order to prepare monograph of N. sativa which is compatible to European Pharmacopoeia.
MATERIALS AND METHODS

*N. sativa* seed samples were collected from different regions of Turkey like Isparta, Afyon, Denizli and purchased from Hindistan. They were analyzed according to European Pharmacopoeia (7th edition, 2011). They were analyzed macroscopic and microscopic analysis, foreign materials, water and ash rates, loss on drying, essential oils and fixed oil assay, acidity index, saponification index, peroxide index and TLC. Even though the phytochemical structure of the seed hasn’t explained yet, studies about it is still ongoing.

RESULTS AND DISCUSSION

When the macroscopic properties were investigated. There is no differences between seeds. In microscope, endosperm, testa epidermis, fat, abundant starch and aleurone were determined. Loss on drying was found between 6.18% and 5.74%. Foreign materials was found between 0.21% and 0.28%. Water rates was found between 3.99% and 5.96%. Total ash rates was found between 3.97% and 4.64%. Acidity index was found between 5.90% and 8.39%. Essential oils was found 0.97% and 1.82%. And also *N. sativa* fixed oil content greater than 30%.

Fig. 1. A picture of Nigella sativa

CONCLUSIONS

In conclusion, microscopic and chemical analysis have showed that samples have common characteristics. But this study is not enough to form a monograph. So, seeds of Nigella sativa, which were proven effectiveness in terms of therapeutic, are must be examined in detail. The scope of study should be expanded by increasing the number of samples. And also results will be possible to obtain more reliable and accurate.

REFERENCES


P-325: ISOLATION AND QUANTIFICATION OF QUERCETIN GLYCOSIDES IN EUPHORBIA CHARACIAS L.

S. Ozbilgin1, G. Saltan1, O. Bahadır Akıkara1, İ. Suntar2, E. Kupeli Akkol2

1Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY
2Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION

Euphorbia is the largest genus in Euphorbiaceae family, comprising more than 2000 species [1] that have various biological activities including cytotoxic, antitumor, antibacterial, anti-inflammatory and anti-HIV [2]. Some of the species are used in folk medicines to cure skin diseases and as wound healer. Euphorbia species contain diterpenoids, flavonoids, volatile compounds and tannins [3]. The first aim of this study is to isolate the active compounds from active fraction of Euphorbia characias L., determined by anti-inflammatory and wound healing activity tests. The second aim of the study is to elucidate the chemical structures of the compounds that isolated from active fractions and quantification of these isolated compounds by using HPLC.

MATERIALS AND METHODS

In this study, n-hexane, ethylacetate and methanol extracts which were prepared successively from aerial parts of Euphorbia characias were evaluated for wound healing [4] and anti-inflammatory activities [5] to isolate the active compounds. Methanolic extract of *E. characias* herba, that the most potent extract, was fractionated by bioassay-guided fractionation technique. Column chromatography was used to separation procedure. Fractions that show similar chromatographic profile were mixed and tested for their activities. The most active fractions were applied to further chromatographic techniques to obtain active compounds. The chemical structures of the compounds were determined by using spectroscopic techniques (1H, 13C and 2D-NMR, MS).

HPLC analyses were carried out using Agilent LC 1200 model chromatograph (Agilent Technologies, California, USA). The diode array detector (DAD) was set at wavelength 254 nm and peak areas were integrated automatically by computer using Agilent Software. Separation was carried out using a ACE 5 C18 (250 mm×4.6 mm; 5 μm) column. The mobile phase was made up of 0.2% phosphoric acid in water (A), acetonitrile (B) and in gradient elution: initial 0 min, A–B (90:10, v/v); then 0–20 min, linear change
from A–B (90:10, v/v) to A–B (40:60); 20-25 min the linear isocratic elution is from A–B (0:100). The flow rate was 1 ml/min and column temperature was maintained at 40°C. The sample injection volume was 10 μl.

Standard stock solutions were prepared as 0.1 mg/ml for quercetin-3-O-rhamnoside, quercetin-3-O-galactoside and quercetin-3-O-arabinoside. All compounds were weighed in 10 ml volumetric flask, dissolved in methanol:water (80:20) mixture and adjusted to the final volume separately. Different concentration levels were prepared by diluting the stock solution. Triplicate 10 μl injections were performed for each standard solution. Peak area of each solution was plotted against the concentration to obtain the calibration curves.

Methanol:water (80:20) mixture was used for the extraction of plant sample for HPLC analyses and the sample was filtered through 0.45 μm membrane filter. Triplicate 10 μl injections were performed for plant sample.

RESULTS AND DISCUSSION

According to bioassay-guided fractionation technique, three flavonoids were isolated from active fraction as active compounds. The chemical structures of the compounds were determined as quercetin-3-O-rhamnoside, quercetin-3-O-galactoside and quercetin-3-O-arabinoside. The quantitative analyses of isolated compounds were performed by using HPLC. The results revealed that E. characias contain 1.2238% quercetin-3-O-rhamnoside, 0.3525% quercetin-3-O-galactoside and 0.1194% quercetin-3-O-arabinoside.

REFERENCES


P-326: DETERMINATION OF PHENOLIC COMPOUNDS IN FIVE SCROPHULARIA L. SPECIES BY HPLC-DAD AND THEIR ANTIMICROBIAL ACTIVITY

G. Renda1, S. Sevgi1, B. Yayı̇1, N. Yazıcı2, S. Yıldırım2, A. Reis3, S. Türki̇ş4, E. Uzunhisarcıklı̇5, A. Yaşar2, İ. Tosun3

Karadeniz Technical University, Faculty of Pharmacy
1Department of Pharmacognosy, 2Department of Analytical Chemistry, Trabzon, TURKEY
3Karadeniz Technical University, Faculty of Medicine, Department of Medical Microbiology, Trabzon, TURKEY
4Ordu University, Faculty of Art and Science, Department of Biology, Ordu, TURKEY
5Gazi University, Faculty of Science, Department of Biology, Ankara, TURKEY

INTRODUCTION

Scrophularia L. genus that belongs to Scrophulariaceae family has 60 species including 23 endemic species in Turkey [1]. Some Scrophularia species are used in folk medicine for the treatment of different skin inflammatory diseases, constipation and neuritis [2,3].

In the present study some phenolic components were analyzed by reverse phase high performance liquid chromatography (RP-HPLC) to determine the phenolic profiles of five Scrophularia L. species; S. kotschyanana Bentham, S. cinarescens Boiss., S. catarifolia Boiss. & Heldr., S. chrysantha Jaub&Spach, S. scopolori var. scopolori (Hoppe ex) Pers. Also antimicrobial activities of the methanolic extracts of the species were determined.

MATERIALS AND METHODS

Scrophularia species were collected from different parts of Turkey. Dried and powdered aerial parts of plant materials were extracted with methanol. An HPLC-DAD method was validated for the qualitative and quantitative determination of the phenolic compounds (gallic acid, proto-catechuc acid, proto-catechuic aldehyde, p-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, vanillin, syringic aldehyde, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid) [4].

Microtiter-plate assay was used to study the antibacterial activity of Scrophularia extracts against common pathogen bacteria Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 35218, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Candida albicans ATCC 60193 [5].

RESULTS AND DISCUSSION

Our results revealed that main secondary metabolites in the thirteen tested phenolic standarts were syringic aldehyde, sinapic acid and benzoic acid for S. chrysantha; ferulic acid, sinapic acid and benzoic acid for...
P-327: ANTIFEEDANT ACTIVITY OF ROOT EXTRACTS OF SOME Vincetoxicum Taxa AGAINST DESTRUCTIVE PESTS PODOPTERA LITTORALIS AND LEPTINOTARSA DECEMLINEATA

S. Guzel1, R. Pavela2, A. Ilcim3, G. Kokdil1

1 Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenisehir Campus, 33169 Mersin, TURKEY
2 Crop Res Inst, Prague 16106 6, Ruzyné, Czech Republic
3 Mustafa Kemal University, Faculty of Arts and Science, Department of Biology, 31000 Antakya-Hatay, TURKEY

INTRODUCTION
The genus Vincetoxicum N.M. Wolf (Apocynaceae; subfamily Asclepiadoideae) is represented approximately 100 species distributed throughout Asia and Europe [1,2]. The Vincetoxicum genus comprises about 10 taxa that three of them are endemic to Turkey [3]. Some species of the genus have been used as expectorant, diuretic, emetic and laxative in folk medicine [4,5]. Bioactivity studies indicated that some species of the genus have antifeedant activity and growth inhibition effects against some pests [5]. Vincetoxicum species contain steroids, triterpenoids, alkanols, glycosides, alkaloids, flavonoids, saponins and phenolic compounds [4,5]. In the present study root extracts of five Vincetoxicum taxa (V. canescens (Wild.) Decne. subsp. canescens, V. canescens subsp. pedunculata Browicz, V. fuscatum subsp. fuscatum (Hornem) Reichb., V. fuscatum subsp. boissieri (Kusn) Browiczand V. parviflorum Decne.) were investigated for their insect antifeedant activities. The insect antifeedant activities were tested against polyphagous pest Spodoptera littoralis Bois. (Boisdual) (Lepidoptera:Noctuidae) and oligophagous pest Leptinotarsa decemlineata Say. (Coleoptera: Chrysomelidae) which caused destructive damage to many economically important crops.

RESULTS AND DISCUSSION
The root extracts displayed significant differences of the all tested plants were in the range of 100-92.7% against S. littoralis larvae. Additionally, extracts caused growth inhibition and larval mortality against S. littoralis larvae.

CONCLUSIONS
All tested extracts had different level of antifeedant activity against larvae of S. littoralis and L. decemlineata: The test larvae were obtained from a colony renewed annually with collecting wild adults from potato fields, fed on potato Solanum tuberosum. Second instar larvae of S. littoralis and L. decemlineata were used for the experiments. The antifeedant activity of four different polarity extracts of five Vincetoxicum taxa were tested by using the leaf disc no-choice method against two destructive pests [5].
decemlineata. Based on the present findings further investigation is needed to find constituents which responsible from these activities and elucidate the antifeedant action of these plants for using as commercial pesticide.

REFERENCES
1. Heywood, VH.; Brummitt, RK.; Culham, A.; Seberg, O., Flowering plant families of the world, Firefly Books, 2007, 38-40

P-328: TWO NEW STEROID GLYCOSIDES FROM DIGITALIS CARIENSIS

S. Avunduk1, Ö. VarolP, A.C. Mitaine-Offer3, T. Miyamoto4, C. Tanaka4, and M.A. Lacaille-Dubois3

1Vocational School of Health Care, Mugla University, Marmaris, Mugla, 48187 Turkey, 2Department of Biology, Science Faculty, Aksaray University, 3Laboratoire de Pharmacognosie, EA 4267 FDE/UFC, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne D’Arc, BP 87900, 21079 Dijon Cedex, France, 4Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

INTRODUCTION
The genus Digitalis was traditionally placed in the family Scrophulariaceae, but recent reviews of phylogenetic research have placed it in the much enlarged family Plantaginaceae [1]. The genus Digitalis(Plantaginaceae) is represented by 9 species in the flora of Turkey, of which D. cariensis Boiss. ex Jaub. & Spach is endemic for Turkey and East Mediterranean element [2]. The plant is known as “Mugla foxglove” and distributed in southern Anatolia [3]. The leaves of Digitalis species contains digitalic glycosides and used as cardiotonic. [3] Previous phytochemical studies on D. cariensis resulted in the isolation of pregnane and furostanol glycosides along with phenyl ethanoid glycosides [4]. This presentation described the isolation and structure elucidation of additional steroid glycosides from the aerial parts of this plant.

MATERIALS AND METHODS
A methanolic extract of the aerial parts was submitted to successive solid/liquid preparative chromatographic methods, i.e. vacuum liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC) over silica gel and RP 18 yielding four compounds (1-4). Their structures were elucidated by using by 1D and 2D NMR spectroscopic techniques (1H-1H COSY, NOESY, HSQC, HMBC), and FABMS.

RESULTS AND DISCUSSION
The phytochemical study on D. cariensis resulted in the isolation and characterisation of one new spirostan-type glycoside, (25R)-5a,2a,3β,23β-trihydroxy-spirostan-3-yl-3-O-β-D-xylpyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, one new cardenolide glycoside, 3β,14β,16β-trihydroxy-card 20(22)-enolide 3-O-β-D-glucopyranosyl-(1→4)-β-D-diglotxyopyranoside together with two known steroid saponins described for the first time in D. cariensis, (25R)-5a,2a,3β,14β,16β-trihydroxy-card 20(22)-enolide 3-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and (25R)-2a,3β,5a,22β furostane-2,3,22,26 tetról 3-O-β-D-xylpyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl 26-O-β-D-glucopyranoside [5].

REFERENCES
5. Matsuo Y., Akagi N., Hashimoto C., Tashikawa F., Momaki Y. Steroid glycosides from the bulbs of Besseria elegans Phytochemistry 2013, 96, 244-256.

P-329: ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC AND FLAVONOID CONTENT OF THREE ENDEMIC TRIGONELLA L. SPECIES

S. S. Uras Gungor1, A. Ilcim2, G. Kodikil1

1Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Mersin, TURKEY
INTRODUCTION
The genus *Trigonella* L. (Fabaceae) includes about 135 species distributed from the Mediterranean regions, Southeastern Europe, Western Asia, North and South Africa [1,2]. *Trigonella foenum-graecum* L., commonly called fenugreek, is the most widely used species in *Trigonella* genus for culinary and medicinal purposes. Its seeds have been used as a carminative, tonic, aphrodisiac in Ayurvedic, Chinese and Unani systems of medicine [1-3]. It was known that the plant contains flavonoids, alkaloids, saponins, fixed oil, polysaccharides, minerals and proteins [3]. *T. foenum-graecum* has been extensively studied but there is little information about other species of the genus in the literature. In Turkey, the genus *Trigonella* represented by 54 taxa.T. kotschyi Fenzl, T. ciliicica Hub.-Mor. and T. synnnea Boiss. are three endemic species that have not been studied phytochemically and pharmacologically. The aim of the present study was to determine antioxidant potential, and total phenolic and flavonoid contents of these three endemic *Trigonella* species.

MATERIALS AND METHODS
Aerial parts and seeds of three endemic *Trigonella* species were obtained from different regions of Turkey during the seedling period in 2011 and 2012. The voucher specimens were deposited in the Herbarium of the Faculty of Sciences and Arts, Mustafa Kemal University (MKUH). The total phenolic and flavonoid contents of the aqueous methanolic extracts of plant materials were determined by Folin Ciocalteu method and AlCl₃ assay, respectively [4]. Radical scavenging activity of the plant materials were investigated by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging method.

RESULTS AND DISCUSSION
All seeds showed higher radical scavenging activity than the aerial parts. *T. synnnea* seed exhibited the highest antioxidant activity (76.18±0.03 % inhibition). The seeds had total phenolic content ranging from 145.33±0.83 to 166.06±1.45 mg/g GAE (gallic acid equivalents). *T. ciliicica* seed showed the highest flavonoid content of 118.89±0.03 mg/g RE (rutin equivalents).

CONCLUSIONS
We report total phenolic and flavonoid content, and antioxidant activity of *T. kotschyi*, *T. ciliicica* and *T. synnnea* for the first time. All seeds have high content of total phenolics and flavonoids. Among them, *T. synnnea* have strong free radical scavenging activity. Further studies are needed to evaluation of this plant in health and food industry.

REFERENCES

P-330: CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF RHODODENDRON CAUCASICUM

S. Fandakli¹, A.Yaşar², A. Bozdeveci ³, N. Yaşlı²

¹Department of Chemistry, Faculty of Sciences, Karadeniz Technical University,Trabzon, Turkey
²Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Turkey
³Department of Biology, Faculty of Arts and Sciences, Rize University, Recep Tayyip Erdoğan, Turkey

INTRODUCTION
There are more than 600 Rhododendron species were known in worldwide, but only five rhododendron species growing in Turkey, especially in the East Blacksea Region of Turkey, which were namely *Rhododendron luteum* Sweet, *R. ponticum* L., *R. smirnovii* Trautv., *R. caucasicum* Pall. and *R. ungerii* Trautv.¹ Rhododendron species were used in Turkish folk medicine. Previous studies on the *Rhododendrum* species have shown various natural compounds, like phenolic compounds, iridoid monoterpenes, flavonodis, saponins, ursolic acid, and triterpenes.¹ *R. caucasicum* was used as tea for weight loss, wellness and longevity and it was used in Europe and Asia for the treatment of heart disease, arthritis, gout, neuroses and other conditions.²

MATERIAL AND METHODS
Plant Material: *Rhododendrum caucasicum* were collected in September 2014 from Uzungöl, Trabzon-Turkey. The plant was authenticated by Prof. S. Terzioglu³. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 12171), Karadeniz Technical University, Turkey.
Hydro distillation Apparatus and Procedure
The fresh plant material (100 g) were grounded into small pieces and submitted to hydrodistillation (HD) using a Clevenger-type apparatus with cooling bath (-15 °C) system (4h) (yield (v/w): 0.035%). The obtained oil was extracted with HPLC grade n-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 °C in a sealed brown vial.

**Analysis of Essential Oil Composition by Hydro distillation GC/MS:**
GC-MS analysis was performed using a Shimadzu QP20104 Ultra and an AOC-5000 plus Auto Samper.

**RESULT of DISCUSSION**
In this study; sesquiterpene hydrocarbon and diterpene components were found as the major group of compounds in *R. caucasia*, constituted 46.13, 25.93 % in of the essential oil, respectively. Calarene 46.13% and sandaracopimaradiene 25.93 % were identified as the main components of the essential oil of *R. caucasia*.
The antimicrobial activity of the isolated essential oil of *R. caucasia* was also investigated, and it showed moderate antimicrobial activities against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus* and *Mycobacterium smegmatis*.

**ACKNOWLEDGMENTS**
We thank Prof. Dr. Salih Terzioglu for the identification of plant material.

**REFERENCES**

**P-331: MYRISTICIN-RICH ESSENTIAL OIL FROM DAUCUS SAHARIENSIS GROWING IN ALGERIA**
T. Smaili1, A. Zellagui2, K. Rebbas1, A. Belkassam3, G. Flamenti3, M. Öztürk4, M. Emin Duru4

1Department of Life Science and Nature, Faculty of Science, University of M'sila, Algeria.
2Faculty of Exact Science, University of Larbi Ben M'hidi, Oum Elbooughi, Algeria.
3Dipartimento di Farmacia, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy.
4Department of Chemistry, Faculty of science, Mugla Sitki Kocman University, 48121 Mugla, Turkey

The essential oils obtained by hydrodistillation from leaves and fruits of *Daucus sahariensis* Murb. were analyzed by GC/MS. The main constituents of the essential oil from the leaves were myristicin (34.3%), α-pinene (5.4%), *cis*-chrysanthenyl acetate (5.3%) and *epi*-α-bisabolol (4.8%), and those from the fruits myristicin (43.9%), α-pinene (13.1%), limonene (9.4%), and *cis*-chrysanthenyl acetate (7.4%). Myristicin, the main constituent of both essential oils, is generally absent in the oils from other *Daucus* species, permitting the hypothesis that this compound is a chemical marker of this Saharan species.

**Keywords:** *Daucus sahariensis*, Apiaceae, essential oil, leaves, fruits, myristicin.

**INTRODUCTION**
*Daucus* is a genus of the family Apiaceae (Umbelliferae), which includes about 60 species, most of which grow in Europe, Africa, West Asia and North America. In Algeria eleven species of *Daucus* can be found. Two of them, *D. sahariensis* Murb. and *D. biseriatus* Murb, grow in the Saharan territory [1]. *Daucus* species have been intensively studied mainly for their flavonoid and essential oil contents. It was observed to be the richest genus of the Apiaceae in essential oil. The composition of the essential oils was found very useful for the chemotaxonomic separation and characterization of the genus within the family [2].

**MATERIALS AND METHODS**
The leaves of *Daucus sahariensis* were collected during February 2009 near Bousaada M'sila (pre-Saharan area) on a sandy soil, approximately 250 km south-east of Algiers, Algeria. The fruits were collected during the ripening stage at the end of April 2009 from the same place. Fresh leaves and fruits were dried to constant weight at room temperature. The dried plant material (150 g for leaves and fruits) were hydrodistilled in a Clevenger-type apparatus for 2 h.

GC-MS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30m × 0.25 mm, coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection 0.2 μL (10% n-hexane solution); split ratio 1:30.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up.
from pure substances and components of known oils and MS literature data.

RESULTS AND DISCUSSION
Altogether, 97 compounds were identified in the two samples. In the leaf oil, 80 compounds were detected, representing 91.3% of the whole oil. The essential oil (yield 0.02%, w/w) was mainly composed of phenylpropanoids (37.1%), with myristicin (34.3%) as the major constituent. Other important classes of chemicals were monoterpene hydrocarbons (18.7%), oxygenated monoterpenes (16.0%), oxygenated sesquiterpenes (11.5%), and sesquiterpene hydrocarbons (5.7%). The main constituent of the essential oil from the leaves was myristicin (34.3%), l-\(\alpha\)-pinene (5.4%), \(\alpha\)-chrysanthenyl acetate (5.3%) and epi-\(\alpha\)-bisabolol (4.8%). The fruits yielded 0.54% of essential oil, in which 77 compounds were identified, accounting for 98.9% of the whole oil. The essential oil of the fruits was mostly constituted of phenylpropanoids (44.9%), mainly because of the high percentage of myristicin (43.9%).

CONCLUSIONS
The composition of the essential oil of the leaves and fruits of \textit{D. sahariensis} was very different from that of all the \textit{Daucus} species studied so far [3]. The compound that mostly characterized this species was myristicin.

REFERENCES
3. Ahmed, AA; Bishr, MM; El-Shanawany, MA; Attia, EZ; Ross, SA; Pare, PW. Rare trisubstituted sesquiterpene daucanes from the wild \textit{Daucus carota}. \textit{Phytochemistry}, 2005, 66, 1680-1684.

P-332: CHEMISTRY, ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITY OF THE ESSENTIAL OIL OF \textit{HIPPMARATHRUM LIBANOTIS KOCH}.

T. Smalli\textsuperscript{1}, A. Zellagui\textsuperscript{2}, K. Rebbas\textsuperscript{1}, A. Belkassam\textsuperscript{1}, GhadBane M\textsuperscript{1}, G. Flaminì\textsuperscript{1}, M. Öztürk\textsuperscript{2}, M. Emin Duru\textsuperscript{4}.

\textsuperscript{1}Department of Life Science and Nature, Faculty of Science, University of M’sila, Algeria.
\textsuperscript{2}Faculty of Exact Science and Life Science and Nature, University of Oum Elbouaghi, Algeria.

The essential oil obtained by hydrodistillation of the leaves of \textit{Hippomarathrum libanotis} Koch (Apiaceae) collected from Algeria were analyzed by GC/MS. 26 compounds were identified accounting for 94.8% of the total oil, the main constituents of the essential oil were \(\beta\)-pinene (17.9%) Sabine (17.8%), myrcene (12%) and \(\alpha\)-pinene (11.4%). In vitro antioxidant activity of the Essential oil were assayed using DPPH (1,1-diphenyl-2 picrylhydrazyl) radical scavenging assay. The anticholinesterase activity of the essential oil of the plant was investigated against acetylcholinesterase and butyrylcholinesterase enzymes by the Ellman method in vitro.

INTRODUCTION
The Apiaceae is a large family, with 455 genera and 3750 species, which are distributed throughout the world [1]. Many of the members of this family are aromatic and economically important species, used as foods, spices, condiments, and ornamentals [2]. In contribution to the phytochemical studies of Algerian apiaceous plants, we report here a phytochemical work on perennial plant \textit{Hippomarathrum libanotis} Koch.

MATERIALS AND METHODS
The leaves of \textit{Hippomarathrum libanotis} Koch. were harvested during May 2012 in M’sila, Algeria. The fresh leaves (700 g) were dried at room temperature. The dried material (150g) was hydrodistilled in a Clevenger’s apparatus for 2 h. The GC/MS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a Varian Saturn 2000 ion-trap mass detector and a DB-5 cap. column (30 m_0.25 mm i.d., film thickness 0.25 mm). The oven temp. was programmed rising from 60 to 2408 at 38/min; injector temp., 2208; transfer-line temp., 2408; carrier gas, He (1 ml/min); split ratio, 1 :30; injection volume, 0.2 ml (10% hexane soln.). The identification of the constituents was based on the comparison of the retention times (IR) with those of authentic samples and the computer matching of linear retention indices (LRI, determined rel.)
P-333: ANTIOXIDANT, CYTOTOXIC AND
ANTIMICROBIAL ACTIVITY OF THREE
LICHENS (RAMALINA FASTIGIATA,
XANTHORIA PARIETINA, ASPICILIA
INTERMUTANS)

T. Erkan¹, T. Taşkınlı, O. Bingol Ozakçınar², S. Birteksoz Tan³,
A. Şenkardeşler², F. Uras², L. Bitiş¹

Marmara University, Faculty of Pharmacy, ¹Department of
Pharmacognosy, ¹Department of Biochemistry; Istanbul
University, Faculty of Pharmacy, ²Department of
Pharmaceutical Microbiology, Istanbul; Ege University,
Faculty of Science ³Department of Biology, Izmir; TURKEY.

INTRODUCTION

Lichens are symbiotic organisms of fungi and algae or
cyanobacteria [1]. They produce characteristic
secondary metabolites, lichen substances, which
seldom occur in other organisms. Lichen and their
metabolites have various biological activities such as
antioxidant, antimicrobial, antiproliferative,
allelochemical, antiviral and antinsectivore [1,2].
Ramalina fastigiata (Pers.) Ach., (Ramalinaceae) was
most commonly used for medicinal, perfumery, and
cosmetics [3]. Xanthoria parietina (L.) Th. Fr. (Teloschistaceae)
have a broad ecological
amplitude and worldwide distribution and is,
therefore, suitable as a model pollution-resistant
species. Aspicilia intermutans is a rare crustose lichen
that it has never been investigated through
pharmacognosy. The aim of this study is to reveal
antioxidant, antimicrobial and cytotoxic activity of
acetone extractsof Ramalina fastigiata, Xanthoria
parietina and Aspicilia intermutans.

MATERIALS AND METHODS

The extracts were obtained by maceration method. The
antioxidant capacity of acetone extracts were assayed
with various methods, DPPH free radical scavenging
and ferric reducing antioxidant power (FRAP)
activity, including total phenolic compound contents
by Folin – Ciocalteu reagent (FCR). The obtained
results were compared with standard antioxidants such as
Ascorbic acid and BHT. Also, cytotoxicity of
acetone extracts was evaluated and screened against
human MCF-7 (breast), HeLa (cervical), A549 (lung)
and HT-29 (colon) cancer cell lines. In addition,
antimicrobial activity of acetone extracts from
Ramalina fastigiata, Xanthoria parietina and Aspicilia
intermutans were investigated.

RESULTS AND DISCUSSION

The A. intermutans extractshowed higher amount of
total phenolic compounds and DPPH free radical
scavenging activity than X. parietina and R.
fastigiataextracts.

R. fastigiataextract had stronger ferric reducing
antioxidant power activity than A. intermutans and X.
parietina extracts.

Also, extract of R. fastigiata at the concentration of
100 μg/mL showed mild cytotoxic activity against
only HeLa (growth inhibition of 61%). In addition,
extract of Ramalina fastigiata presented moderate
activity against Staphylococcus aureus (MIC: 312
µg/ml) and Staphylococcus epidermidis (MIC: 156
µg/ml).

CONCLUSIONS

The results show that Ramalina fastigiata are good
candidates for further activity-guided fractionation in
the search for new active antimicrobial and antitumor
compounds.

REFERENCES

1. Katalin, M.; Edit F., Current Results on Biological
Activities of Lichen Secondary Metabolites: a Review;
2. Soon-Os, O.; Hae-Sook, J.; Kwang-Mi, L.; Young-Jin,
K.; Jae-Seoun, H., Antifungal Activity of Lichen-Forming
Fungi Isolated from Korean and Chinese Lichen Species
381-385.
Analysis of Usnic Acid in Some Ramalina Species from
Anatolia and Investigation of their Antimicrobial Activities;;
P-334: FATTY ACID CONTENT OF RESEDA LUTEA VAR. LUTEA WITH ANTI-OXIDANT POTENTIAL

A. Ertaş1, T. Kuşman2, D. Dinçel2, B. Çulhaoglu2, Y. Yeşil3, H. Fidan Soruhan2, M. Boğa3, G. Topçu2

1 Dicle University, Faculty of Pharmacy, Department of Pharmacognosy, Diyarbakır, TURKEY
2 Bezmialem Vakıf University, Faculty of Pharmacy, Department of Pharmacognosy & Phytochemistry, Istanbul, TURKEY
3 İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, İstanbul, TURKEY

The aerial parts of the Reseda lutea var. lutea were separately extracted with petroleum ether to afford 13 fatty acids which composed mainly of oleic, palmitic, linoleic and linolenic acids.

INTRODUCTION
About 60 species of Reseda genus are known to be distributed in the world, but in Turkey there are only 15 of them [1]. In our country, Reseda lutea var. lutea known as ‘‘sari muhabbet çiçeği’’ which belongs to the family Resedaceae (Kuzuotugiller) and is one of the most important medicinal plants. Its decoction is used to treat stomach diseases [2].

MATERIALS AND METHODS
Preparation of plant extracts: Whole plants of Reseda lutea var. lutea (100 g) were dried, powdered, and then sequentially macerated with petroleum ether, acetone, methanol, and water for 24 h at 25°C. After filtration, the solvents were evaporated to obtain crude extracts.

Esterification of total fatty acids with GC/MS conditions: A hundred milligram of the petroleum ether extract was refluxed in 0.1 M NaOH solution in 2 mL of methanol during 1 h, the solution was cooled and 5 mL of water was added. The aqueous mixture was neutralized with 0.5 mL of HCl solution, it was extracted with diethyl ether: hexane (3.5: 1: 1 mL). The separating organic phase was washed with 10 mL of 0.1 M NaOH solution, it was neutralized with 0.5 mL of HCl solution, and 5 mL of water was added. The aqueous mixture was extracted with diethyl ether to afford 13 fatty acids, separated and identified by GC/MS analysis.

RESULTS AND DISCUSSION
In this study Reseda lutea var. lutea investigated for its fatty acid content. The petroleum ether extract afforded fatty acids, composed mainly of linolenic acid (47.5%), oleic acid (14.6%), palmitic acid (11.6%), and linoleic acid (15.7%), while stearic acid (4.4%), arachidic acid (0.7%) and others were present in lesser amounts. Below the structure of main component (Fig. 1) of these fatty acids is given.

CONCLUSIONS
Determination of total phenolic and flavonoid contents and antioxidant activities (ABTS**, CUPRAC,DPPH) of the petroleum ether, acetone, methanol, and water extracts will be carried out following our studies.

REFERENCES

P-335: CYTOTOXICITY, ANTI-OXIDANT AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF SATUREJA CILICICA P.H. DAVIS FROM TURKEY

T. Arabaci1, G. Uzay1, U. Kelestemur2, MG. Karaaslan2, S. Balcıoğlu2, B. Ates2

1 İonné University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 44280, Malatya, TURKEY
2 İonné University, Faculty of Science and Arts, Department of Chemistry, 44280, Malatya, TURKEY

INTRODUCTION
Satureja L. is an aromatic and medicinal genus of Lamiaceae, including about 38 species in the Mediterranean region, North Africa, Morocco and Libya. The genus is represented in Turkey by 40 species (42 taxa), of which 18 are endemic in Turkey [1]. The genus Satureja is being used worldwide as herbal beverages, spices, food additives and flavoring, perfume and cosmetic industries. Traditionally, in folk medicine to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases [2]. Satureja species are usually subject of antibacterial, antifungal, antioxidant, cytotoxicity, anti-diabetes, anti-hyperlipidemic, reproduction stimulatory, expectorant and vasodilatory activity studies [2-5]. In this study, it was aimed to determine the essential oil composition of Satureja cilicica P.H. Davis and antioxidant properties and cytotoxic activity of this essential oil.
MATERIALS AND METHODS

The aerial parts of *S. ciliicica* were collected at flowering stage from Osmaniye province, in Turkey (Collector number: Arabaci 2947). The specimens were dried by air dried method. The essential oil was obtained from the aerial parts by hydrodistillation for 3 h with Clevenger-type apparatus. GC/MS analyses were performed for the identifying the essential oil composition. The identified constituents of the essential oils and their relative percentages are determined. Antioxidant properties of essential oil obtained from *S. ciliicica* were determined by using DPPH and ABTS scavenging assay and reducing power methods. Cytotoxicity of essential oil on MCF-7 (breast cancer cells) was measured of MTT assay.

RESULTS AND DISCUSSION

The yield of essential oil of *S. ciliicica* was calculated as 0.69% (w/w) on per weight of the dried plant material. The numbers of the identified compounds are 29 and representing 91.80% of the oil. Durenol (20.31%), p-cymene (14.16%), thymol (13.24%) and γ-terpinene (10.79%) are identified as the major components of the essential oil in this study. Antioxidant activities of the essential oil was found 328.28±2.41 mg trolox equivalent per hundred milliliter of essential oil in term of DPPH radical scavenging. ABTS radical scavenging and reducing power of essential oil were measured as 238.15±3.59 and 39.76±3.66 mg trolox equivalent per milliliter of essential oil, respectively. ABTS radical scavenging power of essential oil was higher almost 100 times than DPPH radical scavenging activity and was the parallel with reducing power. Cytotoxic activity of essential oil of *S. ciliicica* was determined by MTT assay after 24 h treatment of MCF-7. Results of MTT assay are shown in figure as % inhibition. In addition, IC50 value of essential oil was determined. Essential oil of *S. ciliicica* exhibit low cytotoxic activity, with IC50 values, 268 μg/mL.

CONCLUSIONS

In another study made on *S. ciliicica*, carvacrol (37.58%), p-cymene (14.38%), γ-terpinene (13.38%) and thymol (7.41%) reported as the main compounds [5]. In our study, Durenol (20.31%) is most abundance while carvacrol is represented with 0.36%. Antioxidant activities of essential oils were the parallel previous study of *S. ciliicica*. The essential oil of *S. intermedia* showed great potent for antimicrobial and cytotoxic activities. Thymol (34.5%), γ-terpinene (18.2%) and p-cymene (10.5%) were the main components of the essential oil [4]. The essential oil of *S. montana* ssp. *pisidica* demonstrated significantly better results against HeLa and MDA-MB-453 cell lines. The major compounds of this oil were carvacrol (37.6%), thymol (24.5%), carvacrol methyl ether (11.8%) and β-linalool (15.2%) [3]. As far as our knowledge, this is the first cytotoxic activity studies on the essential oil of *S. ciliicica*.

ACKNOWLEDGMENTS

The authors want to thank Dr. Tuncay Dirmenci for helps during the field studies.

REFERENCES


P-336: GERMACRANE AND BORNEOL ESTERS FROM *FERULA RIGIDULA* DC.

T. Idug1, M. Miski2

1Istanbul Medipol University, School of Pharmacy, Department of Pharmacognosy, 2Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, TURKEY

INTRODUCTION

Old world genus *Ferula* is the third largest genus of Apiaceae family. Many *Ferula* species are used to treat various diseases including asthma, gastrointestinal disorders, intestinal parasites etc. in traditional medicine. In addition, various biological activities of sesquiterpenoids isolated from *Ferula* species such as antispasmodic, hypotensive, anticonvulsant, antibacterial, anticarcinogenic, cancer chemopreventive, anticoagulant activities and reproductive toxicity were reported in the literature [1-2].

MATERIALS AND METHODS

Dried roots of *Ferula rigidula* DC. were extracted with dichloromethane. Two germacrane esters, tovarol vanillate and tovarol *p*-hydroxybenzoate, and two monoterpene esters, tschimganin (bornyl vanillate) and tschimgin (bornyl *p*-hydroxybenzoate), were isolated from the dichloromethane extract by chromatographic techniques [2]. Structure of these...
esters were identified by spectroscopic techniques and direct comparison with the reference compounds where available.

RESULTS AND DISCUSSION
Previously, we have investigated another population of *F. rigidula* collected from the Eastern Taurus Mountains near Çiftehan-Niğde in Turkey, this population exclusively yielded daucane type sesquiterpene esters [2]. In contrast, we now report isolation and structure elucidation of two germacrane and two borneol esters from another population of *F. rigidula* collected from Hasan Dağı ca. 80 km NE of the previous collection site near Aksaray. Monoterpene esters, tshimgin and tshimginian, exhibit various biological activities, such as antibacterial activity and cytotoxic activity against

CONCLUSIONS
Isolation of completely different types of terpenoid esters from the Aksaray population of *F. rigidula* DC. vs. the Eastern Taurus Mountain population in Turkey as well as completely different sesquiterpene ester profile of *F. rigidula* DC. collected from the type specimen location in Adzberaijani [5] suggest the presence of taxonomical issues within this species and warrants further taxonomical and chemical examination of the *F. rigidula* DC. populations through out its distribution areas in the Central and Eastern Anatolia to clarify intra- and interspecies level taxonomic status of this species.

ACKNOWLEDGMENTS
This work was supported by Istanbul University Scientific Research Projects (Project No. 40361).

REFERENCES

P-337: DETERMINATION OF ASCORBIC ACID AND CATECHIN IN THE ROOTS AND FRUITS OF *ROSA PIMPINELLIFOLIA* BY HPLC

S.Ö. Şener¹, M. Badem¹, L. Güven², U. Özgen¹

¹Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY, ²Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, TURKEY

INTRODUCTION
*Rosa* (Rosaceae) genus has 24 species and is known as “Gül, Kuşçurumu” in Turkey [1,2]. *R. pimpinellifolia* is known as “kuşçurumu, koyungözü” and is used for for hemorrhoid and internal infections [3]. In this study, the catechin and ascorbic acid contents of the fruits and roots of *R. pimpinellifolia*were quantitatively determined using by HPLC.

MATERIALS AND METHODS
Plant Materials: *R. pimpinellifolia* were collected from Erzurum Province (Küşk Village, October 2012, 1900 m) in Turkey and identified by Dr. Ufuk ÖZGEN.

Preparation Standard Solutions: In this study, catechin and ascorbic acid were used as standards. Previously, a stock solution of each standard (100 mg/L) was prepared and filtered through 0.45 μm membrane filters. To make calibration curve and to determine quantitatively for catechin and ascorbic acid, the stock solutions were diluted in the concentrations range of 5-100 mg/L.

Preparing Sample Solutions: The roots and fruits of *R. pimpinellifolia* were extracted in methanol for 12 h at room temperature and solvent was removed under vacuum. The extracts were redissolved in HPLC grade methanol (10 mg/mL) and filtered through 0.45 μm membrane filter.

HPLC Conditions
Ascorbic Acid Determination: The new method running only 7 minutes was development for ascorbic acid determination. A C18 column was used with a gradient elution of 0.5% (v/v) acetic acid in HPLC-grade water/acetonitril (50:50) and 2% (v/v) acetic acid in HPLC-grade water as mobile phase at a flow rate of 1.2 mL/min, injection volume 20 μL for the method. UV detection was performed at 270 nm. Ascorbic acid solutions were run at this method. Methanolic extract of the plant runs three times at the same method.

Catechin Determination: The modified method were run 18 minutes to determine catechin. A C18 column was used with a gradient elution of acetonitril and 0.1% (w/v) o-phosphoric acid in HPLC-grade water as mobile phase at a flow rate of 1.0 mL/min, injection volume of 20 μL, column compartment temperature of 35 °C for the method. UV detection was performed at 280 nm. Catechin solutions, and methanolic extract of
the plant were run at this method. The isolated and identified catechin from the roots of *R. pimpinellifolia* was run with the same method.

**RESULTS AND DISCUSSION**

As shown figures, *R. pimpinellifolia* roots contains significantly catechin and ascorbic acid. Methanolic extract of the plant runs three times.

\[
\begin{align*}
\text{Conc. [10}^\text{3} \text{]} & = 7.59605e-005 * x + 4.03319 \\
R_1 & = 0.9998739 \quad R_2 = 0.9997476 \\
\text{Fig. 1A: The Calibration Curve of Ascorbic acid} \\
\end{align*}
\]

\[
\begin{align*}
\text{Conc. [10}^\text{3} \text{]} & = 0.000108888 * x + 0.90201/3 \\
R_1 & = 0.9999218 \quad R_2 = 0.9998437 \\
\text{Fig. 1B: The Calibration Curve of Catechin} \\
\end{align*}
\]

According to our study, besides the concentration of ascorbic acid was 1710.19 mg/L (85.509*x20**) for root; 100.93 mg/L (50.3963*x20**), the concentration of catechin was 478.48 mg/L (47.848*x10**) for root; 71.87 mg/L (7.187*x10**) for the fruits of *R. pimpinellifolia*.

*The average concentration from three replicate analyses of the same sample

**Dilution factor**

**CONCLUSIONS**

In this study, the amounts of ascorbic acid and catechin were determined and developed a new HPLC method for ascorbic acid determination, modified a method for catechin determination.

**REFERENCES**

P-338: HPLC – FINGERPRINT ANALYSIS AND QUANTIFICATION OF PHENOLIC COMPOUNDS IN SOME ENDEMIC CIRSIUM SPECIES

U. Özgen1, R. Aliyazıcıoğlu2, S. Ö. Şener3, M. Badem1, S. Yıldırım3, N. Ulaş3, T. Dirmenci4, A. Yaşar4

1Karadeniz Technical University, Faculty of Pharmacy, 2Department of Pharmacognosy, 3Department of Biochemistry, 4Department of Analytical Chemistry, 61080, Trabzon, TURKEY, 4Balıkesir University, Faculty of Science, Department of Biology, Balıkesir, TURKEY

INTRODUCTION
Cirsium (Asteraceae) genus is known as “Köy göğüren” and used for treatment of hemorrhoid, peptic ulcer, bronchitis traditionally in Turkey [1]. It has 66 species (78 taxon) in Turkey [2]. In this study, it has been aimed to analysed phenolic fingerprints of 4 endemic Cirsium species; C. trachylepis, C. dirmilense, C. sipyleum, C. aytatchii. A simple method was modified using RP-HPLC for fingerprint and quantitative determination of phenolic compounds in methanolic extract of aerial part of these species. 13 phenolic compounds, namely gallic acid, protocatechuic acid, protocatechaldehyde, p-hydroxy benzoic acid, chlorogenic acid, caffeic acid, vanillin, syringic aldehyde, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid were quantitatively determined. Calibration plots were linear over the concentration ranges 5 - 50 μg/mL for mixture of phenolic standards.

MATERIALS AND METHODS
Plant Materials: Cirsium species were collected from different region in Turkey and identified by Dr. Tuncay Dirmenci: C. dirmilense: Karaman, C. sipyleum: Denizli, C. aytatchii: Adana, C. trachylepis: Trabzon.

Preparing of the Standard Solutions: In this study, 13 phenolic compounds, gallic acid, protocatechuic acid, protocatechaldehyde, p-hydroxy benzoic acid, chlorogenic acid, caffeic acid, vanillin, syringic aldehyde, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid were used as standards. Previously, a stock solution of each standard (100 mg/L) was prepared and filtered through 0.45 μm membranes. A stock solution was prepared by mixing all standard solutions (100 mg/L). To make calibration curve, the stock solutions of mixed standards were diluted in the concentrations range of 5-50 mg/L.

Preparing Sample Solutions: The aerials part of Cirsium species were extracted in methanol for 12 h at room temperature and the solvent was removed under vacuum. The extracts were redissolved in HPLC grade methanol (10 mg/mL) and filtered through 0.45 μm membranes.

HPLC Conditions: The modified method was run 41 minutes to determine 13 phenolic compounds and prepare fingerprints of these species. A C18 column was used with a gradient elution of 0.5% (v/v) acetic acid in HPLC-grade water/acetonitril (50:50) and 2% (v/v) acetic acid in HPLC-grade water as mobile phase at a flow rate of 1.2 mL/min, injection volume 20 μL for the method. UV detection was performed at 270 nm.

RESULTS AND DISCUSSION
As shown figures, all species contain signficantly phenolic compounds and the most amount of phenolic compounds was determined on C. trachylepis.

![Fig. 1: The HPLC Chromatogram of the Mixture of the 13 Phenolic Standards](attachment:image)

According to our study, C. aytachii and C. sipyleum include all phenolic compounds, while C. trachylepis includes all phenolic compounds except for gallic acid and C. dirmilense includes all phenolic compounds except for p-coumaric acid. Benzoic acid is the major phenolic compound all studied Cirsium species. The concentration of benzoic acid is 2.860, 6.406, 69.074, 23.673 mg/L for C. aytachii, C. dirmilense, C. trachylepis, C. sipyleum, respectively.

![Fig. 2: The Calibration Curve of Benzoic Acid Standart](attachment:image)
**Fig.3**: The HPLC Phenolic Fingerprint Chromatogram of *C. trachylepis*

**REFERENCES**


**P-339: ACETYL- AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY STUDIES ON SOME ENDEMIC CIRSIUM SPECIES**

U. Ozgen1, K.Ö. Yerdelen2, Ş. Emiroğlu1, G. Sanaloğlu1, M.E. Abanoz1, G. Seçkin1, T. Dirmenci3

1Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY, 2Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 3Balıkesir University, Faculty of Science, Department of Biology, Balıkesir, TURKEY

**INTRODUCTION**

*Cirsium* (Asteraceae) genus is represented by 66 species (78 taxa) and thirty of these species are endemic for Turkey [1]. Some *Cirsium* species have been used in traditional medicine in Turkey [2]. Alzheimer’s disease is the frequent cause of dementia affecting people, and is associated with loss of cholinergic neurons in parts of the brain[3]. Therefore, the present study was planned to determine the *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the metanolic extracts of the aerial parts *C. dirmilense*, *C. aytachii*, *C. sipyleum*, and *C. trachylepis*.

**MATERIALS AND METHODS**

*Plant material*: The aerial parts of the plants were collected from different locations: *C. dirmilense*: Karaman, *C. sipyleum*: Denizli, *C. aytachii*: Adana, *C. trachylepis*: Trabzon. They were identified by Dr. Tuncay Dirmenci.

*A general procedure for extractions*: The air-dried aerial parts of the plants were extracted with methanol at 40 °C (3× 2 L). The methanol extract was concentrated *in vacuo*.

**Inhibition Studies on AChE and BChE**: AChE, BuChE, 5,5-dithiobis-(2-nitrobenzoic acid) DTNB, acetylthiocholine iodide (ATCI) and butyrylthiocholine iodide (BTDI) were purchased from Sigma Aldrich. Inhibitory activities of AChE and BuChE of the methanolic extracts were evaluated by colorimetric Ellman’s method[4] with some modifications using commercially available neostigmine bromide[5] as the reference compound. These extracts were dissolved in dimethylsulphoxide and then diluted in 50 mM Tris buffer (pH 8.0) to provide a final concentration range. In a 96-well plate, the assay medium in each well consisted of 50 μL of a Tris buffer, 125 μL of 3 mM DTNB (Ellman’s reagent), 25 μL of 0.2 U/mL enzyme (AChE or BuChE) and 15 mM substrate (ATCI or BTDI). The assay mixture containing enzyme, buffer, DTNB and 25 μL of inhibitor compound was preincubated for 15 min at 37°C, before the substrate was added to begin the reaction.

Neostigmine bromide and all samples were prepared at four different concentrations such as 12.5, 25, 50 and 100 μg/mL. The absorbance of the reaction mixture was then measured three times at 412 nm every 45 s using a microplate reader (Bio-Tek ELx800, USA).

**RESULTS AND DISCUSSION**

The extracts did not show any AChE inhibition activity but showed BChE inhibition activity (15-29% inhibition) at 100 μg/mL concentration.

**CONCLUSIONS**

Isolation studies on the plants are still in progress. The pure compounds may be more effective on AChE and BChE inhibitions.

**REFERENCES**

P-340: PHYTOCHEMICAL STUDIES ON THE ROOTS OF ROSA PIMPINELLIFOLIA

L. Güven1, S.Ö. Şener2, M. Badem2, U. Özgen2, H. Seçen3

1 Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, TURKEY, 2 Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY, 3 Atatürk University, Faculty of Science, Department of Chemistry, Erzurum, TURKEY

INTRODUCTION

Rosa (Rosaceae) genus has 24 species and is known as “Gül, Kuşburnu” in Turkey [1,2]. R. pimpinellifolia is known as “kuşburnu, koyungözü” and is used for for hemorrhoid and internal infections [3]. In this study, phytochemical studies were carried out on the methanolic extract of the roots of R. pimpinellifolia.

MATERIALS AND METHODS

Plant Materials: The roots of R. pimpinellifolia were collected from Erzurum Province (Koşk Village, October 2012, 1900 m) in Turkey and was identified by Dr. Ufuk ÖZGEN.

Equipments and chemicals: 1H-NMR and 13C-NMR spectra were recorded with a Varian Mercury spectrometer at 400 MHz and 100 MHz. Sephadex LH-20 (Sigma-Aldrich), Silica gel (Kiesel gel 60, 0.063-0.2 mm and 0.040-0.063 mm Merck and LiChroprep RP-18, 25-40 µm, Merck 9303) for column chromatography, and silica gel 60 F254 (Merck, 05554) for TLC were used. TLC spots were detected with a UV lamp and 1% Vanilin/H2SO4 for 2 min. Solvents used in isolation and solvent system are methanol, chloroform, ethyl acetate, formic acid, toluene, n-butanol

Extraction and isolation: The roots of R. pimpinellifolia were dried and powdered (350 g) and were extracted with methanol (3 × 2 L). Methanolic extract was concentrated and dried under reduced pressure to give a residue. Methanolic extract was dissolved in H2O:MeOH (9:1) and partitioned with chloroform and then ethyl acetate. (+)-catechin and kajiichigoside F1 were isolated from the remaining aqueous phase using by several chromatographic techniques such as silica gel column chromatography, vacuum liquid chromatography, jel permeation chromatography, and thin layer chromatography.

RESULTS AND DISCUSSION

Fig. 1. Compounds isolated from the roots of R. pimpinellifolia

CONCLUSIONS

Phytochemical studies are still in progress on the methanolic extract of the roots of R. pimpinellifolia.

REFERENCES


P-341: SELECTIVE CYTOTOXIC ACTIVITIES OF SOME DIGITALIS SPECIES ON HELA CELL LINE

V.M. Kutluay, I. Saracoglu

Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION

In the Flora of Turkey, the genus Digitalis (Plantaginaceae) is represented by nine species [1]. Digitalis species contain biologically active compounds such as cardenolides, phenylethanoid glycosides, flavonoids, and anthraquinones [2]. They are most known among plants which contain cardioactive glycosides. Especially, D.lanata and D.purpurea are very important for their cardioactive glycosides. Our previous research, on the aerial parts and roots of three different Digitalis species;
**INTRODUCTION**

Geranium species have been employed in folk medicine as tonic, antidiabetic, antidiarrheal, antihemorrhoidal, diuretic and for the treatment of cough, fever, tonsillitis, urticaria, kidney pain and gastrointestinal ailments [1,2]. The leaves of some Geranium species are consumed in traditional Turkish cousin as salad or added to pastry [1,3].

In the present study, different extracts of Geranium stepporum and G. psilostemon were investigated for their radical scavenging and cytotoxic activities in addition to determination of their phytochemical content. Major polyphenolic compounds of extracts were determined by HPLC.

**MATERIALS AND METHODS**

The extracts from the aerial parts of the plants were prepared with water, n-BuOH and EtOAc. SO, NO and ABTS radical scavenging activities of extracts tested. Cytotoxic activities of extracts against KB human epidermoid carcinoma cell line tested by MTT method. Total phenolic, flavonoid and flavonol contents were estimated and major polyphenolic compounds of extracts were determined by HPLC.

**RESULTS AND DISCUSSION**

Phytochemical contents and bioactivities of G. psilostemon and G. stepporum were found to be very similar to each other. Total phenolic contents were found to be the highest for the EtOAc extracts, while total flavonoid and flavonol contents were found to be the highest for the n-BuOH extracts. While EtOAc extracts showed strong radical scavenging activity against SO, NO and ABTS radicals (IC50 of EtOAc...
extracts of *G. psilostemon* 29.4, 98.4, µg/ml for SO and NO respectively and TEAC value 0.371 ± 0.29 µM TE) cytotoxic activity against KB cell line was observed for the water extracts of tested species. Major compounds of the extracts were determined as gallic acid, methyl gallate and pusilagin.

CONCLUSIONS
The results showed that gallic acid derivatives are important for the bioactivities of *Geranium* species. According to our results, since *G. psilostemon* and *G. stepporum* are rich sources of phenolic constituents, these species may be considered a good source of natural antioxidants for application in food industry. Additional investigations are in progress for future development of antioxidants from *Geranium* species by different mechanisms.

ACKNOWLEDGMENTS
US Harput has been supported by TUBA-GEBIP (USH/2013) Award program.

REFERENCES

P-343: BIOACTIVE COMPOUNDS FROM *SCUTELLARIA SALVIIFOLIA* BENTH.

Z. Dogan, I. Saracoglu

Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, TURKEY

INTRODUCTION
*Scutellaria* L. is a geographically widespread and diverse genus of the Lamiaceae family of herbaceous plants commonly known as skullcaps. It has been estimated that there are over 300 species distributed throughout the world [1]. *Scutellaria* species and their active principles possess antioxidant, antitumor, anti-angiogenesis, hepatoprotective, anticonvulsant, antibacterial and antiviral activities. Investigations on *Scutellaria* genus were resulted isolation of flavonoids, iridoid glucosides, phenylethanoid glycosides, diterpenes, triterpenoids, alkaloids and essential oils [2]. There are 25 taxa of *Scutellaria* species in Turkish flora and 14 taxa of which are endemic [3]. In this study, one of the endemic species, *S. salviifolia* Benth., was selected for bioactivity guided phytochemical studies.

MATERIALS AND METHODS
The aerial parts of the plant were collected from Mamak, Ankara in June, 2012 in stony slopes. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey [HUEF 12003]. Air-dried aerial parts of *S. salviifolia* were extracted with MeOH several times at 40°C. The combined extracts were evaporated under vacuum to give crude MeOH extract. Crude extract was dissolved in H2O and partitioned with petroleum ether to remove lipophylic compounds. Aqueous fraction of MeOH extract was applied to polyamide column chromatography to get five sub-fractions, Frs. A-E, using increasing concentrations of methanol (0-100%). Eluted fractions of polyamide column and the aqueous extract were tested for their radical scavenging activities against DPPH, SO and NO radicals spectrscopically. Cytotoxic activity experiments were also conducted on the extract and the fractions against HEp-2 (human larynx epidermoid carcinoma) cell line based on MTT assay. Since the flavonoid-rich fractions (Frs. D-E) showed significant antioxidant and cytotoxic activities, isolation studies were focused on these fractions. Fr. D, eluted 75% methanol through polyamide column, was applied to silica gel column chromatography using different concentrations of CH2Cl2:CH3OH as a mobile phase. Fraction of 5 % methanol (Fr. D3) was applied to Sephadex LH-20 column using CH3OH as a mobile phase to give compound 1 in pure form. Fr. D2, eluted 9 % methanol of silica gel column was applied to Sephadex LH-20 column to give compound 2 in pure form. Fr. E, eluted 100 % methanol of polyamide column was applied to MPLC using 0–100% methanol as a solvent system to give compound 3 in pure form.

RESULTS AND DISCUSSION
DPPH, NO and SO radical scavenging activities of the fractions were tested in the concentrations of 50 and 100 µg/mL. Fr. D scavenged 85 % of DPPH, 29 % of NO and 62 % of SO radicals at 100 µg/mL concentration. Fr. E scavenged 88 % of DPPH, 30 % of NO and 65 % of SO radicals at 100 µg/mL concentration. In cytotoxicity assay, IC50 values of Fr. D and Fr. E were found to be 116, 4 µg/mL and 33,1 µg/mL, respectively.

Three pure compounds were isolated from active fractions. Structure of the isolated compound 1 was identified as apigenin and compound 3 as luteolin-7-O-β-glucopyranoside on the basis of 1D and 2D NMR spectral data. Structure elucidation studies of compound 2have still been continuing.

CONCLUSIONS
A bioactivity guided isolation study, based on cytotoxic and antioxidant activities, was performed on the endemic *S. salviifolia* Benth.
Extract and the flavonoid-rich fractions showed concentration dependent significant antioxidant and cytotoxic activities. Three pure compounds were isolated from the active fractions. Structures of two compounds were identified as apigenin and luteolin-7-O-β-glucopyranoside on the basis of extensive NMR analysis.

ACKNOWLEDGMENTS
This study was supported by The Scientific and Technological Research Council of Turkey (Program No: 2211-C) and Hacettepe University Scientific Research Projects Coordination Unit (Project No: 014-D08 301 001).

The authors are thankful to Prof. Toshiaki Makino for recording of NMR spectra.

REFERENCES

P-344: INVESTIGATION OF BIOLOGICAL ACTIVITIES AND CHEMICAL ANALYSIS OF HELIANTHEMUM CANUM L. BAUMG (CISTACEAE)

A. Baldemir1, N. Göksen2, N. Ildız3, G. Şeker Karatoprak2, M. Koşar2

Erciyes University, Faculty of Pharmacy 1Department of Pharmaceutical Botany, 2Department of Pharmacognosy, 3Department of Pharmaceutical Microbiology, Kayseri, TURKEY

INTRODUCTION
In Turkey, Helianthemum Miller is represented by nineteen taxa of which include sixteen species and three subspecies [1]. Most of them are traditionally used as both in our country (constipation, styptic) [2] and several countries (gastrointestinal problems, antiinflammatory, antiluercerogenic, wound healing, antiparasitary, antimicrobial, analgesic, cytotoxic and vasodilator remedies) [3]. The aims of the study are to evaluate antibacterial and antioxidant activities and carry out chemical analysis of Helianthemum canum L. Baumg 70% methanolic (HCMOH) and water extracts (HCW).

MATERIALS AND METHODS
Plant materials were collected from province Kayseri (Pınarbaşı) in August-September, 2013.

Preparation of Extracts: Water and 70 MeOH extracts were prepared from aerial parts of H. canum.

Spectrophotometric analysis: Total phenols, flavonoids and flavonols of extracts were analyzed.

Antioxidant activity: The used methods to evaluate antioxidant activities of HCMOH and HCW extracts are as follows: 1,1-diphenyl-2-picrylhydrazyl (DPPH*), Trolox equivalent antioxidant capacity (TEAC), β-carotene and lipid peroxidation.

Antibacterial activity: Gram negative (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhi NCTC 8394, Haemophilus influenza ATCC 4447) and Gram positive (Staphylococcus aureus ATCC 25923, Enterococcus faecium NJ-1, Streptococcus pyogenes ATCC, Bacillus subtilis ATCC 11778, Bacillus subtilis ATCC 6633, Listeria monocytogenes ATCC 7644 19615) microorganisms were used for disc diffusion and microdilution methods. Gallic acid, catechin and quercetin were used as positive controls in all activity studies.

High Pressure Liquid Chromatography (HPLC): Qualitative and quantitative compositional analyses of HCMOH and HCW extracts of H. canum were performed with reversed-phase HPLC.

RESULTS AND DISCUSSION
Results of Spectrophotometric Analysis:

Table. Yields and phenolics of H. canum extracts.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Yield [%]</th>
<th>Total Phenol [mgGAE/g extract]</th>
<th>Total Flavonoid [mgRE/g extract]</th>
<th>Total Flavonols [mgRE/g extract]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCMOH</td>
<td>17.74</td>
<td>284.13±0.30</td>
<td>13.13±0.10</td>
<td>40.88±0.60</td>
</tr>
<tr>
<td>HCW</td>
<td>4.83</td>
<td>244.55±0.35</td>
<td>14.01±0.06</td>
<td>41.54±0.11</td>
</tr>
</tbody>
</table>

Results of Antioxidant activity: HCMOH and HCW extracts showed scavenging activity on DPPH*. IC50 values were calculated as 0.19±0.013 and 0.33±0.026 mg/ml. TEAC values for HCMOH extract were 0.99±0.065 (0.25mg/mL) and 1.45±0.110 (0.5 mg/ml); TEAC values for HCW extract were 0.59±0.037 (0.25mg/mL) and 1.030±0.070 (0.5 mg/ml). IC50 values for lipid peroxidation experiments are as follows: 0.72±0.002 mg/mL for HCMOH; 0.49±0.009 mg/mL for HCW. In β-carotene/linoleic acid co-oxidation assay, the inhibition percentages of both extracts are nearly the same and BHA is the most active positive control.

Results of Antibacterial activity: Bacillus cereus ATCC 11778 and Bacillus subtilis ATCC 6633 have broad-spectrum antibacterial activity to HCMOH and HCW plant extracts and standards.

Results of HPLC: The quantities in the HCMOH and HCW extracts of gallic acid, 3,5-di-OH-benzoic acid, 3,4-di-OH benzoic acid, methyl gallate, 4-OH-benzoic acid, apigenin-7-O-glucoside, luteolin-7-O-glucoside, luteolin and isorhamnetin were calculated.
CONCLUSIONS
To the best of our knowledge there are no studies that demonstrate the effectiveness of *H. canum* extracts in vitro antibacterial and antioxidant activities. In addition, this is the first study demonstrating the plant’s chemical contents. However, further studies are required to confirm if these in vitro activities can be supported in vivo. Natural products are often a source for bioactive compounds which have great potential for developing novel therapeutic agents. *H. canum* can be introduced as a new plant source for antibacterial and antioxidant agents.

ACKNOWLEDGEMENTS
This work was supported by Scientific Research Projects Coordination Unit of Erciyes University [Project number: TYL-2015-5642]. We would like to thank Erciyes University Scientific Research Projects Coordination Unit and Pharmacy Faculty.

REFERENCES

P-345: BOTANICAL PESTICIDES THAT USED ASALTERNATIVE TO CHEMICAL METHODS FIGHTING BACK AGAINST HERBARIUM PESTS
B. Büyükkılıç, O. Alınbaşak, E. Akalin
Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, TURKEY

INTRODUCTION
Protecting the herbarium specimens from herbarium pests requires constant attention. Especially in the tropics or subtropics regions, the most dangerous threat for the herbarium specimens is the herbarium pests. In the herbaria, there are two methods (chemical and physical) to prevent infestation of pests. Poisonous compounds and chemical insecticides are the materials used as chemical method to prevent infestation of pests in the herbarium. Even though chemical methods are the important to fight against infestation, they have disadvantages. The most important disadvantage is the harmful effects for the public health. Also chemical methods can be expensive and the pests can evolve tolerance to chemical insecticides [1].

Literature survey revealed that several pesticide plants have been reported. Many essential oils and their components have insecticidal or repellent activity. Extracts and oils from garlic have already been marketed as pest control products [2]. Plant and extracts are good resources in the development of pest control agents. The essential oils can be easily obtained by steam distillation. Moreover, because of the high vaporization of plant essential oils, there is little concern about their residue when applied in the field [3].

MATERIALS AND METHODS
This study is a literature screening of botanical pesticides against herbarium pests.

RESULTS AND DISCUSSION
A total of 89 plant species belonging to 24 families were screened against 8 herbarium pests’ species. The kind of plant family is the most density Lamiales, Myrtaceae, Apiaceae. Also the most encountered pest species in literature are *Lasioderma serricorn*, *Blattella germanica*, *Liposcelis bostrychophil*, *Periplaneta americana*.

CONCLUSIONS
The methods were used in the studies for pesticide activities are contact toxicity, fumigant toxicity and repellency. In this study, essential oils are the most commonly used extract to fight back against herbarium pests.

We hope that botanical pesticides mentioned in this review can be alternatives to the chemical methods at the herbaria in our country and abroad.

REFERENCES

P-346: QUANTITATIVE DETERMINATION OF ICARIIN, EPIMEDIN A, B AND C IN *EPIMEDIUM PUBIGERUM* (DC.) MOREN & DECAISNE (BERBERIDACEAE) GROWING IN TURKEY*
D. Cicek Polat¹, M. Coskun¹
¹Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, TURKEY
INTRODUCTION

*Epimedium* L. (Berberidaceae) is a genus of approximately 52 taxa. A large part of this genus is endemic and found in the southwestern part of China. More than 15 species in this genus have a long history of use in traditional Chinese medicine (TCM) (Shahnawaz et al., 2013). *Epimedium* spp. have been used to treat sexual dysfunction, pro spermia, hyper diuresis, osteoporosis, menopause syndrome, rheumatic arthritis, hypertension, and cardiovascular disease (Huiping et al., 2011). *Epimedium* species are known as “Keşiklülahi” ve “Tekeru” in Turkey (Güner, 2012). The genus *Epimedium* is rich in flavonoids. Especially, icariin, epimedin A, epimedin B and epimedin C are known to be biologically active flavonoid glycosides from this genus. Quantitative determination of these compounds in *E. pubigerum* (DC.) Moren & Decaisne [EP] growing in Turkey has not been studied. In this study, determination of icariin, epimedin A, epimedin B and epimedin C in *E. pubigerum* growing naturally in Turkey, collected from two different locations, were performed.

MATERIALS AND METHODS

*E. pubigerum* was collected from Uzungol and Macka, Trabzon. Voucher samples of *E. pubigerum* are deposited in the Herbarium of Faculty of Pharmacy, Ankara University (AEF).

### Voucher Samples

<table>
<thead>
<tr>
<th>Voucher Samples No</th>
<th>Date</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEF 26332!</td>
<td>28.04.2013</td>
<td>Uzungol</td>
</tr>
<tr>
<td>AEF 26335!</td>
<td>03.10.2013</td>
<td>Uzungol</td>
</tr>
<tr>
<td>AEF 26336!</td>
<td>29.04.2013</td>
<td>Macka</td>
</tr>
<tr>
<td>AEF 26337!</td>
<td>03.10.2013</td>
<td>Macka</td>
</tr>
</tbody>
</table>

Aerial parts and underground parts of *E. pubigerum* were air-dried. Dried aerial parts and underground parts were powdered and sample was extracted with 70% ethanol by soxhelet for 3 hr, then concentrated and lyophilized.

Standard compounds of icariin, epimedin A, epimedin B and epimedin C were provided from Chromadex. Chromatographic separation of compounds was achieved using a Discovery HS C18 (15cmx 4.6mm, 5μm) with column temperature 40°C. Elution was performed at a flow rate of 1ml/min in a gradient mode. Mobile phase consisted of 0.1% formic acid (A) and acetonitrile (B). Chromatograms were acquired at 270 nm with UV detector. Method validation was performed.

RESULTS AND DISCUSSION

Highest values; Icariin 0.651%, Epimedin A 0.128%, Epimedin B 0.111%, Epimedin C 0.056%.

Maximum amount of icariin and epimedin A were found in aerial parts of *E. pubigerum* during flowering period. But amount of epimedin C was found to be maximum in underground parts of *E. pubigerum* during non-flowering period. Highest values were obtained from materials collected from Uzungol.

<table>
<thead>
<tr>
<th></th>
<th>EP MACKA (fw*)</th>
<th>EP MACKA (dhw**)</th>
<th>EP UZUNGOL (fw*)</th>
<th>EP UZUNGOL (dhw**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial Parts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icariin</td>
<td>0.486%</td>
<td>0.119%</td>
<td>0.651%</td>
<td>0.120%</td>
</tr>
<tr>
<td>Epimedin A</td>
<td>0.128%</td>
<td>0.070%</td>
<td>0.127%</td>
<td>0.085%</td>
</tr>
<tr>
<td>Epimedin B</td>
<td>0.090%</td>
<td>0.047%</td>
<td>0.082%</td>
<td>0.059%</td>
</tr>
<tr>
<td>Epimedin C</td>
<td>0.049%</td>
<td>0.040%</td>
<td>0.044%</td>
<td>0.056%</td>
</tr>
<tr>
<td>Underground Parts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Icariin</td>
<td>0.102%</td>
<td>0.011%</td>
<td>0.194%</td>
<td>0.222%</td>
</tr>
<tr>
<td>Epimedin A</td>
<td>0.036%</td>
<td>0.010%</td>
<td>0.044%</td>
<td>0.059%</td>
</tr>
<tr>
<td>Epimedin B</td>
<td>0.052%</td>
<td>0.056%</td>
<td>0.057%</td>
<td>0.111%</td>
</tr>
<tr>
<td>Epimedin C</td>
<td>0.034%</td>
<td>0.029%</td>
<td>0.045%</td>
<td>0.041%</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Quantitative determination of icariin, epimedin A, epimedin B and epimedin C in *E. pubigerum* was achieved in the present study. Method was also validated with respect to linearity, intra-day and inter-day precision, recovery, and limit of detection and quantification. The contents of these four flavonoids in several *Epimedium* species have been reported (Huiping et al., 2011). This is the first report on the quantification of these flavonoids in *E. pubigerum* which is growing naturally in Turkey.

ACKNOWLEDGMENTS

This study is financially supported by Ankara University Research Fund (13L3336005).

REFERENCES


* This research is a part of PhD thesis.
P-347: COMPARATIVE STUDY OF IN VITRO CYTOTOXIC AND ANTI-CYTOTOXIC ACTIVITIES OF EXTRACTS OF CALAMINTHANEPETA(L.) SAVI SUBSP. GLANDULOSA (REQ.) P.W. BALL (LAMIACEAE)

F. Zilifdar1, E. Foto1, E. B. Yeşilyurt2, B. Büyük3, N. Diril1

1Hacettepe University, Faculty of Science, Molecular Biology Department, Beytepe, Ankara, Turkey
2Hacettepe University, Faculty of Science, Botany Department, Beytepe, Ankara, Turkey
3Ankara University, Faculty of Pharmacy, Pharmaceutical Botany Department, Tandogan, Ankara, Turkey

INTRODUCTION

Calaminthanepeta subsp. glandulosabelong to the genus Calamintha represented with 12 taxa. It is traditionally used for its diaphoretic, expectorant, febrifuge and stomachic properties, treatment of respiratory and gastroenteric diseases and used for the disinfection and cicatrization of wounds and this herb used as a mint like spice in recipes as food flavouring[1]. Leaves, flowers, herba and roots of plants are used traditionally for pharmacological benefits. Therefore they are the source of lots of studies about biological and pharmacological activity. Researchers try to determine their composition and effective molecules of them. Some plants are advised to patients for including some effective molecules. However, they may contain some undesirable molecules and cause side effects. For the reason of that their various biological activities should be evaluated before using. The present study aims to evaluated both the cytotoxicity and anti-cytotoxicity of C. nepetasubsp. glandulosa extracts on a non-cancerous cell line (L929).

MATERIALS AND METHODS

The plant materials were collected from Ankara-Güdülin Turkey. After drying them at room temperature, plant extracts (Water; Methanol (MeOH); n-Butanol (BuOH); Ethyl acetate (EtOAc); Dichloromethane (DCM)) were prepared from herba. Water extract was prepared by decoction and methanol extract by maceration method as described [2]. n-BuOH, EtOH and DCM extracts were fractionated from MeOH extract. Water extract were dissolved in water and other extracts were dissolved in DMSO at 100mg/ml and then were used required concentration for experiments.

We performed the sulforhodamine B (SRB) assay for evaluating cytotoxic and anti-cytotoxic effects of extracts on L929 cell line. Cells were seeded on 96 well-plate at 1x10⁴ cell per well. Following 24h incubation period, various concentrations of extracts were added to wells for cytotoxic activity assay while 4-Nitroquinoline 1-oxide (4NQO) were added to wells with non-cytotoxic concentrations of extracts for anti-cytotoxicity test. Then cells were incubated further 48 hours. Cells were fixed on plate by 10% TCA and then incubated with SRB dye. After removing excessive dye, protein bound-dye was solubilized in analkali buffer and OD values were measured at 510nm. IC₅₀ values of compounds were calculated from % growth by S-probit analysis. Data in this study are presented as mean values obtained from two independent experiments.

RESULTS AND DISCUSSION

According to IC₅₀ values of tested extracts, it was shown that water, n-BuOH and MeOH extracts have weak cytotoxic effects (~500-1000μg/ml) against L929 cells. However EtOAc and DCM fractions have significant cytotoxic effects with the IC₅₀ values of 58.9 and 77.1 μg/ml, respectively. On the other hand, none of tested extracts recovered cytotoxic effect of 4NQO at any concentration.

CONCLUSION

In conclusion, we determined that Calaminthanepeta subsp. glandulosodon’t have significant cell damage protective compounds while it included some cytotoxic compounds. So we advise to be careful during their pharmacological usage.

REFERENCES


P-348: THE IN VITRO ANTIBACTERIAL ACTIVITY OF SOME EXTRACTS AND FRACTIONS OF CALAMINTHA NEPETA (L.) SAVI SUBSP. GLANDULOSA (REQ.) P.W. BALL (LAMIACEAE)

E. Foto1, F. Zilifdar1, E. B. Yeşilyurt2, B. Büyük3, N. Diril1

1Hacettepe University, Faculty of Science, Molecular Biology Department, Beytepe, Ankara, Turkey
2Hacettepe University, Faculty of Science, Botany Department, Beytepe, Ankara, Turkey
3Ankara University, Faculty of Pharmacy, Pharmaceutical Botany Department, Tandogan, Ankara, Turkey
INTRODUCTION

Calamintha species are perennial aromatic plants which belong to the family Lamiaceae. Some of them have been used in folk medicine, as antiseptics, spasmsolytics, stimulants, diuretics, carminatives and tonics, and some are also known as spices. *C. nepeta* (L.) Savi is mainly distributed in Anatolia and is used in folk medicine as a stimulant, stomachic and antiseptic [1]. Beyond limited literature, there is little known about its biological activity. This study aims to evaluate antibacterial activity of this plant extracts and some fractions from herba against some Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

The plant materials were collected from Ankara-Güdüll in Turkey. After drying them at room temperature, plant extracts (Water; Methanol (MeOH); n-Butanol (BuOH); Ethyl acetate (EtOAc); Dichloromethane (DCM)) were prepared from herba. Water extract was prepared by decoction and methanol extract by maceration method as described [2]. n-BuOH, EtOH and DCM extracts were fractionated from MeOH extract. Antibacterial activity of the them were performed against Gram positive bacteria *Bacillus subtilis* and Gram negative bacteria *Escherichia coli* and *Salmonella typhimurium* by using 2-fold dilution method in 96 well plates. Extracts dissolved in DMSO were dispensed in different wells with various concentrations (0.006-6 mg/ml) and incubated at 37 °C for 24 hours. The control wells were loaded with medium consisting 1% DMSO (negative control) or Tetracycline was considered as positive control. Minimum inhibition concentrations (MICs) were then recorded in mg/ml. 50% inhibition concentrations (IC50) were additionally calculated by S-probit analysis. All experiments were made in replicate and data were evaluated by Student’s t-test.

RESULTS AND DISCUSSION

The results indicated that the plant extracts showed antibacterial activities at variable degrees with MIC and IC50 values varying from 0.3 to >0.6 mg/ml and from 0.42 to 16.9 mg/ml, respectively. These results are in accordance with many researchers. Results showed that most of extracts and fractions exhibited the best antibacterial activity against Gram positive bacteria, *B. Subtilis*. While only water extract was much more effective than MeOH extract, fractions showed greater bactericidal effect than tested extracts. DCM fraction was the most potent through the other fractions against Gram negative bacteria, E. coli and S. typhimurium, with the IC50 values of 1.03 and 0.42 mg/ml, respectively. On the other hand, like water extract (IC50:0.56 mg/ml), only BuOH fraction was effective against Gram positive bacteria, *B. Subtilis* (IC50:0.58 mg/ml). However, methanolic extract and EtOAc fraction failed to inhibit all tested bacteria at dose as high as 6 mg/ml. On comparing with standard antibiotic, their activities against all bacteria was much less than Tetracycline (IC50:<0.003 mg/ml).

CONCLUSION

The present study provides some of the first data in showing bactericidal potentials of some extracts and fractions of *C. nepeta* (L.) Savi. Even though the results suggest that they have a broad spectrum of antibacterial activity, they are not as efficient as tested reference. On the contrary, pharmacological tests are necessary to isolate and characterize their active compounds. Moreover, they should be investigated in vivo to better understand their safety, efficacy and properties.

REFERENCES


P-349: INHIBITORY EFFECTS OF ENDEMIC SPECIES OF GENUS MICHUXIA L’HERIT., NATIVE TO TURKEY, ON EUCARYOTIC DNA TOPOISOMERASE I

E. Fata1, F. Zilifdar2, M.M. Hürkul2, A. Köroğlu2, N. Diril1

1Hacettepe University, Faculty of Science, Molecular Biology Department, Beytepe, Ankara, Turkey
2Ankara University, Faculty of Pharmacy, Pharmaceutical Botany Department, Tandogan, Ankara, Turkey

INTRODUCTION

Five species of the genus *Michuxia* locally known as “keçi biçiği”, a member of Campanulaceae family, are indigenous to Turkey [1]. *M. tchihatchewii* and *M. thyroidea* are endemic to Turkey and the endemic ratio is 40%. It is known that root and herba of *M. campanuloides* and *M. tchihatchewii* have been used as vegetables and in traditional medicine [2]. Due to such kinds of uses, they require some scientific investigations.

DNA topoisomerases are ubiquitous molecules that manage the topological state of DNA in the cell during the process such as replication, transcription and recombination and so provide genomic stability. Due to its high expression level in cancer cells, enzyme inhibition affect these important processes and cell can be directed to apoptosis. Therefore DNA topoisomerase inhibitors represent an important group of anticancer agents. In this study, we aimed to evaluate inhibitory effects of water extracts obtained
from herba and root and metanol extracts obtained from herba of these endemic species.

MATERIALS AND METHODS
Plants were collected from Karaman and identified. Water extract was prepared by decoction and metanol extract by maceration method as described [3]. Inhibitory effects of DNA topoisomerase I were tested with relaxation assay. pBR322 plasmid DNA as substrat and recombinant human topoisomerase I enzyme was incubated in the appropriate buffer system. After electrophoresis, DNA band intensity was measured and estimated % inhibition values. Experiments were duplicated and data were evaluated by Student’s t-test.

RESULTS AND DISCUSSION
Using relaxation assay, it was found that water extract obtained from root and metanol extract obtained from herba of M. thyrsoides inhibited eukaryotic DNA topoisomerase I with the IC50 values of 3.8 and 10 mg/ml, respectively. However, any extracts of M. tchihatchewii had topoisomerase I inhibitory activity.

CONCLUSION
Our previous studies related the other three species of the genus Michuxia grown in Turkey (M. campanuloides, M. laevigata, M. nuda) had showed strong inhibitory activity on topoisomerase I. Our present in vitro study demonstrate that endemic species of this genus have no significant inhibitory effect on topoisomerase I. With following studies, we have planned to test whether extracts have topoisomerase II inhibitory activity and to detect active metabolite of the extracts inhibited topoisomerase I.

REFERENCES

P-350: ENDEMIC PLANTS AND THEIR THERAPEUTIC USES IN TURKEY

A. Ç. Ülgen1, E. Sönmez2, Y. B. Köse2

1Ülgen Eczanesi, Isparta, TURKEY
2Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Eskişehir, TURKEY

Turkey has rich plant diversity. Turkish flora consists of about 10,000 vascular plant taxa, and one third of this flora is endemic [1]. The aim of this study is to determine Turkish endemic plants and their uses in the folk medicine. For this purpose, a list has been done from Turkish Plants Data Service (TÜBİVES). For determining endemic plants’ uses in the folk medicine, data has been obtained from book of Baytop “Treatment with Plants in Turkey”, and other ethnobotanical studies from literature.

126 endemic plant remedies determined from 30 plant families are reported with used parts, methods of preparing remedies and therapeutic uses. The results show that these endemic plants are used for respiratory system disorders (39), gastrointestinal disorders (47), urinary disorders (27), inflammatory disorders (7), gynecological problems (5), dermatological problems (30), infections (12), cardiovascular (12), rheumatism (8), diabetes (9), lung and liver diseases (7), nerve relaxing (11), malaria (4), tumors (2), and for cancers (4) without specifying the type.

INTRODUCTION
Turkey has a rich biodiversity with different climatic and biogeographical regions. Turkey has three different climates; Continental, Mediterranean and Black Sea climates, and three different geographical regions; at north Euro-Siberian, in Central Anatolia and Eastern Anatolia Iran-Turan, and in Southern Mediterranean geographical regions [2]. These properties are the result of diversity of plant species in Turkey. Turkey is represented by more than 10,000 taxa belonging to 173 families and 1,225 species [3, 4, 5]. 15 genera are endemic, and these genera have more than 2,650 endemic species. Endemism rate of Turkey is 30%. The plant families which have the most endemic species are Scrophulariaceae (52%), Campanulaceae (49%), Lamiaceae (Labiatea) (44%), and Asteraceae (Compositae) (38%). Addition to these plant families, also there are some genera which have the greatest number of endemic species are Ebenus (100%), Verbascum (80%), and Sideritis (78%) [6]. This study has been made for determining endemic plants in Turkey and their uses in the folk medicine.

MATERIALS AND METHODS
A list of endemic plants in Turkey has been established from Turkish Plants Data Service (TÜBİVES). For determining endemic plants’ uses, book of Baytop ”Treatment with Plants in Turkey”, and other ethnobotanical studies from literature also several books have been obtained.

Research results are presented on the table in the results section.
RESULTS AND DISCUSSION

126 endemic plant remedies have been determined from 30 plant families are reported with used parts, methods of preparing remedies and therapeutic uses. These endemic plants are used for respiratory system disorders (39); cough, shortness of breath, common cold, asthma, bronchitis, gastrointestinal disorders (47); gastric symptoms, stomach ache, ulcers, indigestion, hemorrhoids, constipation, urinary disorders (27); diuretic, kidney stone/or sand, urinary sand, urinary inflammations, inflammatory disorders (7); allergy, pain, gynecological problems (5); menstrual pain, uterine inflammations, dermatological problems (30); wound healing, abscess maturation, hairloss, infections (12); tuberculosis, anthelmintic, agricultural parasites, oral infections, cardiovascular (12); hypertension, blood purifying, rheumatism (8), diabetes (9), lung and liver diseases (7); jaundice, nerve relaxing (11), malaria (4), tumors (2), and cancers (4) without specifying the type. Results are shown on the table.

CONCLUSIONS

Endemic plants are the richness of a country. These are not only important for flora, but also for folk medicine. Folk medicine is a tradition for Turkish people and this knowledge should be transferred to the young generations. In addition to this, protecting biodiversity and endemic plants should not be forgotten.

REFERENCES (A part of it)


P-351: VOLATILE COMPOSITION OF THREE LIMONIUM SPECIES GROWING IN TURKEY

F.P. Turkmenoglu¹, G. Akaydin², B. Demirci³

Hacettepe University, ¹Faculty of Pharmacy, Department of Pharmaceutical Botany, ² Faculty of Education, Division of Biology, Ankara, ³Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, TURKEY

INTRODUCTION

The genus Limonium Mill., (Plumbaginaceae), is represented by 22 taxa in Turkey [1]. Various species of the genus have been used in traditional medicine in different countries and investigated for their antitumor, immunomodulator, hepatoprotective, cardioprotective, antioxidant, antibacterial and antiviral potential. However, data on essential oil composition of the genus is limited. In this study, we have investigated volatile compounds of three Limonium species growing in Turkey, L. lilacinum (Boiss. et Bal.) Wagenitz var. lilacinum, L. iconicum (Boiss. et Heldr.) Kuntze and L. pycnanthum (C. Koch) Kuntze

MATERIALS AND METHODS

Plant material, collection sites, dates and number:

L. lilacinum (Boiss. et Bal.) Wagenitz var. lilacinum: B4 Ankara, Şereflikoçhisar, Tuz gölü çevresi, tuzlu bataklık, 940 m. 29.06.2013. Akaydin 14960. L. iconicum (Boiss. et Heldr.) Kuntze, B4 Ankara, Şereflikoçhisar, Tuz gölü çevresi, tuzlu bataklık, 940 m. 29.06.2013. Akaydin 14962

L. pycnanthum (C. Koch) Kuntze, B4 Aksaray, Aksaray Univ. Çevresi, tuzlu step, 950 m. 29.06.2013. Akaydin 14966

Isolation of essential oil: The air-dried aerial parts of the plant were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in n-hexane. The obtained volatile samples were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analysed and tested.

GC-MS analysis: The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

GC analysis: The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 °C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) [2,3] and in-house “Baser Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data [4,5], was used for the identification.
RESULTS AND DISCUSSION

Three *Limonium* essential oils were obtained by hydrodistillation from air dried aerial parts and subsequently analyzed by GC and GC/MS systems. 50 compounds were identified from *Limonium* oils which constituted 95.5% to 98.5% of the total oil.

In the oil of the *L. lilacinum* var. *lilacinum* 38 components were characterized representing 95.5% of the total oil. Heptacosane (28.7%), tricosane (17.8%), pentacosane (14.9%), nanocosane (14.4%) and hexadecanoic acid (6.4%) were found as main constituents.

A total of 34 compounds were characterized in *L. iconicum* essential oil, representing 95.5% of the total oil with nanocosane (39.5%), heptacosane (39.4%), pentacosane (5.1) and tricosane (3.3%).

The main components of *L. pycnatum* oil were determined as heptacosane (33.3%), nanocosane (33.3%), pentacosane (20.5%), pentacosane (12.8%), and tricosane (11.3%). 31 components were identified representing 96.8% of the total *L. pycnatum* essential oil.

CONCLUSIONS

We would like to highlight the high content of long chain hydrocarbons, such as heptacosane, nanocosane, pentacosane and tricosane were found to be the most abundant compounds in essential oil of three *Limonium* species. Our results are in accordance with the previous data indicating hydrocarbon rich essential oil composition of *Limonium* species.

REFERENCES


P-352: EVALUATION OF HERBAL USAGE AMONG TURKISH ELDERS AND POSSIBLE HERB-DRUG INTERACTIONS

F. P. Turkmenoglu1, Yesim Gökce-Kutsal2, A. Dolgun3, N.Y. Diker1, T. Baydar4

Hacettepe University
1Faculty of Pharmacy, Department of Pharmaceutical Botany, 2Faculty of Medicine, Department of Physical Medicine, 3Faculty of Medicine, Department of Biostatistics, 4Faculty of Pharmacy, Department of Toxicology, Ankara, TURKEY

INTRODUCTION

The use of herbal products among older adults is an important concern for healthcare professionals. Because of the high consumption of prescribed medications, the risk of potential herb-drug interactions is higher in geriatrics who have a higher incidence of illness and chronic conditions. Moreover, older adults are an especially vulnerable population since many of the human body’s physiological activities, such as renal and hepatic detoxification and clearance usually decrease with age. Therefore, it is important essential to know the prevalence of herbal use among older adults [1,2]. However, no data exists regarding herbal use among Turkish elderly population.

The present study aimed to determine prevalence of use and documentation of herbal remedies by individuals aged 65 years and over in Turkey and also to evaluate possible adverse reactions and herb-drug interactions.

MATERIALS AND METHODS

A total of 1418 elderly people from 12 provinces in different geographical regions of Turkey were included in this study. A face-to-face interview was applied by the physicians to the elderly who admitted to the Physical Medicine and Rehabilitation polyclinics of the medical schools or hospitals in each province. During patient registration and data recording, a questionnaire was constructed. The questions in the questionnaire were oriented mainly in three parts: (i) individual demographic characteristics of the elderly, (ii) current diagnosis of elderly, (iii) herbal product use and (iv) conventional prescribed drug use. Demographical parameters, the first part of the study, were province, gender, age, marital status, level of education, status of retirement, presence of social insurance, presence of income, place of living, presence of ongoing diagnosed disease and presence of continuously used herbas. The IBM SPSS Version 20 was used for all calculations and statistical significance was set at p<0.05.

RESULTS AND DISCUSSION

1418 participants (aged between 65 and 95 years) were recruited to complete the questionnaire. The prevalence of herbal use among older adults was 30% (n=426). 64.3% of these participants (n=274) reported using more than one prescribed medications. Prevalence of polyherbacy was 48.4% (n=202). Herbals which are known to interfere with some conventional drugs related with geriatric diseases such as cardiac glycosides, diuretics, anticoagulants, antidiabetics etc. were used by elderly. However, majority did not discuss herbal use with their health care providers.
Our results indicated that most of the participants follow recommendations by neighbors, friends, relatives, herbal drug shops etc. Only 4.7% sought information from pharmacists. More than half of the elderly who use herbals stated that they could neither assess whether concomitant use of herbs and drugs can cause serious interactions, nor assess what side effects might arise because of herbal use, and hence, they do not discuss herbal use with their healthcare providers. Only 12.3% were aware of possibly harmful drug interactions or side effects.

In many of those cases physicians were also insufficiently informed about the proper use of herbals. However, the risk of potential herb-prescription drug interactions is even higher in older adults (65 years old and over) because of the high consumption of prescription medications within this age category, as compared with adults less than 65 years of age [2,3]. Therefore, physician’s awareness of polypharmacy in the elderly and concomitant use of herbs and prescription drugs is necessary in order to prevent or minimize consequences of possible herb-drug interactions.

CONCLUSIONS
Increased patient awareness about safe herbal product use is important considering that most Turkish older adults are being informed by neighbors, relatives and friends. In the interest of good patient care, it is also important for health care professionals to be aware of the possibilities of health complications due to the concomitant use of herbs and diverse medications. Pharmacists, who are often the first point-of-contact for patients with health inquiries, should play an active role in creating awareness and advising the patients as well as physicians about the rational, safe and proper use of herbals in order to better serve elderly patients.

REFERENCES

P-353: MUTAGENIC AND ANTIMUTAGENIC EFFECTS OF SOME EXTRACTS OF THE ENDEMIC PLANT, MICHAUXIA THYRSOIDEA BOISS. & HELDR., INDIGENOUS TO TURKEY

F. Zilifdar¹, E. Foto², N. Diril³, M.M. Hürkul², A. Koroglu²

INTRODUCTION
It is known that fresh stem and roots of some species of genus Michauxia L.’Hérit., a member of the family Campanulaceae represented by five species locally known as “Keçi bıçığı” in Turkey, have been used as a vegetable [1]. Detection of the biological activities of them is important to clarify their beneficial or harmful effects on metabolism. M. tchihatchewii and M. thyrsoida are endemic to Turkey and their endemism ratio is %40. For this purpose mutagenic and antimutagenic activities of different extracts of varying polarities, extracted from the stem and roots parts of the species M. thyrsoida, were investigated with this study.

MATERIALS AND METHODS
The plant materials were collected from Karaman and descriptions of specimens were performed by Hürkul. For preparation of water extracts, decoction method was used and maceration method was used for methanol extracts. Ethyl acetate, n-Butanol and dichloromethane extracts were additionally fractionated from the methanol extracts.

In the experiments were used to standard plaque incorporation test described by Dr. Bruce Ames [2]. Firstly, genetic markers of strains were verified. The test concentrations of the samples were determined with cytotoxicity assay by use S.typhimurium strain TA100.

1, 2, 0.5 mg/plate doses of the extracts were used in experiments using Salmonella typhimurium strains, TA98 and TA100. In mutagenicity test, into histidine supplemented soft agar was added 0.1 ml bacteria culture and maximum 0.1 ml sample with non-cytotoxic doses and for anti-mutagenicity test was added positive control mutagens (daunomycine for TA98 and sodium azide for TA100) to system. This mixture was spread on minimal agar plate. After incubation of 48h histidine revertant colonies were counted. All determinations were made in triplicate plates.

Experimental data were evaluated with statistical analysis method, Student’s t-test at a 95-99% confidence interval.

RESULTS AND DISCUSSION
It was observed that only ethyl acetate root extract led to a weak mutagenic activity at 2 mg/plate for TA98. In evaluating antimutagenic effects, extracts were added in addition with the compounds used as positive controls for the test system. As a result of antimutagenicity test, dichloromethane extracts of both stem and root showed antimutagenic activity and statistically significant differences at 2,1 and 0.5mg/plate concentrations for TA98, while n-butanol
and dichloromethane stem extracts showed the same activities at the 2 and 1mg/plate concentration for TA100 (p<0.05).

CONCLUSION
It is well known that extracts of natural source, especially herbal source have activities like antimutagenic, antitoxic and antioxidative. So traditional plants, which have such as these features are used frequently in alternative medicine. In this study, we were determined n-butanol and dichloromethane extracts of M. thyrsoidea have antimutagenic potentials and they can be subjected further studies about determination active compounds of their contents.

REFERENCES

P-354: ISOLATION OF ACTIVE COMPONENTS FROM THE ESSENTIAL OIL OF SATUREJA BOISSIERI AND DETERMINATION OF THEIR ANTIMICROBIAL AND ANTICANCER PROPERTIES

Fevza Oke-Altuntas1, Ibrahim Demirtas2, Ali Riza Tufekci2, Serkan Koldas2, Fatih Gül3, Lutfi Behcet2, Halil İbrahim Gecibesler4

1 Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey
2 Department of Chemistry, Faculty of Science, Cankiri Karatekin University, 18100 Cankiri, Turkey
3 Department of Biology, Faculty of Science and Art, Bingöl University, 12000, Bingöl, Turkey
4 Department of Chemistry, Faculty of Science and Art, Bingöl University, 12000 Bingöl, Turkey

INTRODUCTION
Researchers have introduced many drug active compounds from natural sources into public use. These originated from either plants, e.g. paclitaxel, etoposide and vinblastine, or microorganisms, e.g. dactinomycin and bleomycin (Cragg and Newman, 1999).

*Satureja boissieri* Hausskn. ex Boiss.(Lamiaceae) is known as Catli/Kekik and used as a condiment and herbal tea in the kitchen of local people in Turkey (Satil et al., 2008). The aim of this study was to isolate the active components from the essential oil of *Satureja boissieri* (EO) and to investigate the antimicrobial and anticancer effects of these components.

MATERIALS AND METHODS
Essential oil of *S. boissieri* was obtained by hydrodistillation and analyzed by gas chromatography/mass spectrometry (GC/MS). The components were separated by column chromatography. The anticancer activity of the components was determined using the xCELLigence system (Real Time Cell Analyzer). Antimicrobial activities of the compounds were determined by agar disc and agar well diffusion methods.

RESULTS AND DISCUSSION
The major components of EO were found to be *p*-cymene (23.15%), *γ*-terpinene (22.84%), thymol (18.96%), and carvacrol (21.25%). *p*-Cymene, thymol, and spathulenol were isolated as pure terpenoids. Among the components, thymol:carvacrol (3:97%) exhibited the highest inhibitory effect against HeLa cell line. Isolated pure oxygenated sesquiterpene;spathulenol (L) exhibited low antiproliferative effect in a dose depended manner. The mixture of *p*-cymen-8-ol:*α*-terpineol:spathulenol:isospathulenol (40:20:20:20%) exhibited significant antiproliferative activity at the high concentrations. Also, thymol, thymol:carvacrol (40:60%), and terpinene-4-ol:carvacrol (25:75%) exhibited significant antimicrobial activity against food borne pathogen *S. aureus*. Among all the components, thymol was the most active against *S. aureus* ATCC 25923 (25.41 ± 0.12 mm) and *S. aureus* ATCC 29213 (27.08 ± 0.22 mm).

![Fig. 1. Antiproliferative effect of thmol:carvacrol (3:97)](image1)

![Fig. 2. Antiproliferative effect of β-mycrene5%: p-cymene 80%:γ-terpinene15%](image2)
CONCLUSIONS
In this study, the most of the isolated components exhibited significant antiproliferative and antimicrobial activities. The synergistic and antagonistic effects were found in combinations of the components. According to the results of this study, *S. boissieri* may be suggested as a new potential source of anticancer and antimicrobial agent for pharmacy industry.

REFERENCES

P-355: BIOACTIVITY EVALUATION OF THREE COMPOUNDS ISOLATED FROM *MILICIA EXCELSA* WELW. C. C. BERG (MORACEAE)

F. Oke-Altuntas¹, W. F. G. D. Kapche², J. L. Nantchouang Ouete³, I. Demirtaş⁴, M.B. Koc⁴, B. T. Ngadjui⁴

¹Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey
²Department of Chemistry, Higher Teacher Training College, University of Yaoundé I, P.O. Box 47, Yaoundé, Cameroon
³Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon
⁴Department of Chemistry, Faculty of Science, Çankırı Karatekin University, Çankırı, Turkey

INTRODUCTION
*Milicia excelsa* Welw. C. C. Berg (Moraceae) is an important commercially grown timber species of Western, Central, and Eastern Africa where it is sold under the trade name Iroko [1]. Studies on new chemical structures from herbal extract for new anticancer drugs have attracted great interest. About 25% of drugs in the modern pharmcopoeia are derived from plants, including several anticancer drugs currently in clinical use such as vinblastine, vincristine, paclitaxel, podophyllotoxin, camptothecin and combretastatin [2]. This study was aimed to investigate the anticancer, antioxidant, and antimicrobial activities of three compounds isolated from the methanolic extract of *Milicia excelsa* (Moraceae) namely cudraxanthone I (1), neocyclomorusin (2) and (9βH)-3β-acetoxylanosta-7,24-diene (3).

MATERIALS AND METHODS
The structures of the pure compounds were established on the basis of their spectral data and by comparison with those reported in the literature. The anticancer activity of the compounds was determined using the xCELLigence system (Real Time Cell Analyzer). The antioxidant capacities of the compounds were evaluated by free radical scavenging and metal chelating activity assays. Antimicrobial activities of the compounds were determined by disc diffusion method.

RESULTS AND DISCUSSION
Among the compounds, cudraxanthone and neocyclomorusin exhibited significant anticancer effect against HeLa cell lines. Neocyclomorusin (5) showed the highest radical scavenging (IC₅₀ = 0.73 ± 0.01 mg/mL) and metal chelating activity (83.5 ± 0.6 % at 1 mg/mL). Cudraxanthone I exhibited moderate antimicrobial activity against *S. aureus* ATCC 25923.

Fig. 1. Anticancer effect of neocyclomorusin at the tested concentrations.

CONCLUSIONS
This study supports the documented medicinal effects of the compounds especially neocyclomorusin and open up the possibilities of pharmaceutical applications.

ACKNOWLEDGMENTS
GDKWF acknowledge The Scientific and Technological Research Council of Turkey (TÜBİTAK), through the program 2221, for a 3-months maintenance grant to the Çankırı Karatekin University, Turkey; The Faculty of Science of the Çankırı Karatekin University is also acknowledged for providing research facilities.

REFERENCES
P-356: FATTY ACID COMPOSITION, PHENOLIC PROFILE AND ANTIOXIDANT CAPACITY OF CENTAUREA DERDERIFOLIA WAGENITZ

F. Bulüt¹, F. Oke-Altuntas², I. Demirtas¹

¹ Department of Chemistry, Faculty of Science, Cankiri Karatekin University, 18100 Cankiri, Turkey
² Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey

INTRODUCTION

Centaurea is widely spread in Turkey with 180 species, 109 of which are endemic [1,2]. Various species of Centaurea are used as herbal remedies for their digestive, tonic, expectorant, antipyretic and antidiarrheal effects in traditional medicine [3]. Centaurea derderiifolia WAGENITZ is an endemic species and frequent locally in the Elazığ, Tunceli, Kahramanmaraş, and Sivas provinces. To the best of our knowledge, in the literature there is no any information about antioxidant activity of C. derderiifolia. This study was undertaken in order to examine the fatty acid composition, phenolic profiles and antioxidant activities of seed and leaf extracts from C. derderiifolia (CD).

MATERIALS AND METHODS

Methanol (M), dichloromethane (D), chloroform (C), hexane (H) and methanol-chloroform (MC) extracts were prepared from the seed (S) and leaf (L) parts of C. derderiifolia.

Phenolic contents of the extracts were determined qualitatively and quantitatively by high performance liquid chromatography/time of flight-mass spectrometry (HPLC/TOF-MS).

Fatty acid composition of the hexane extract was performed by GC-MS.

The antioxidant capacities of the extracts were evaluated by various methods including the total phenolic content, free radical scavenging and metal chelating activity assays.

RESULTS AND DISCUSSION

According to GC-MS analysis results; the main fatty acid component of the C. derderiifolia leaf hexane extract was found to be lupeol (36.91%) and the main component of the seed hexane extract was found to be linoleic acid (51.41%). Twenty-six phenolic compounds of the extracts were identified by HPLC/TOF-MS.

DPPH scavenging activity of the samples decreased in the order: BHA > BHT > CD/SM > CD/SMC > CD/SD > CD/LMC > CD/LM > CD/SC > CD/LC > CD/LD > CD/SH > CD/LH. The highest DPPH radical scavenging effect was detected in C. derderiifolia seed methanol extract (IC₀₅₀ = 0.127 ± 0.001 mg/mL). The water and methanol extracts of the species showed significant metal chelating capacity.

Fig.1. Antioxidant activities of the extracts from C. derderiifolia

CONCLUSIONS

It could be suggested that C. derderiifolia may be used a potential source of natural antioxidant in food and pharmacy industries.

REFERENCES


P-357: PHYTOCHEMICAL SCREENING, ANTI PROLIFERATIVE AND ANTIOXIDANT POTENTIALS OF ORIGANUM ACUTIDENS

M.A. Demirci¹, F. Oke-Altuntas², A. Sahin Yaglioglu¹, I. Demirtas¹

¹ Department of Chemistry, Faculty of Science, Cankiri Karatekin University, 18100 Cankiri, Turkey
² Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey

INTRODUCTION

All over the world, people depended on herbs for the treatment of various ailments before the advent of modern medicine. Medicinal plants constitute an arsenal of chemicals that could be exploited by human to prevent or cure various infections. They have been a major source for drug development [1]. Origanum spp. have been used for thousands of years as spice and in ethnomedicine [2]. Origanum acutidens is an endemic species growing in northeastern Turkey.

The aim of this study was to investigate the detailed phytochemical analysis, antioxidant, and antiproliferative effects of Origanum acutidens (Hand.-Mazz.) Ietswaart.
MATERIALS AND METHODS
In this study, extracts of *Origanum acutidens* in different solvents were prepared. The ethylacetate extract was fractionated by flash chromatography. Phenolic contents of the extracts and the sub-fractions from ethyl acetate extract were determined qualitatively and quantitatively by HPLC/TOF-MS.

The ethylacetate extract (OA/E) and its sub-fractions were investigated for their antiproliferative activities against human cervix tumor (HeLa) cell line by using real time cell analyzer and BrdU ELISA assay. The antioxidant activities of the OA/E and the subfractions were determined using 2,2-diphenylpicrylhydrazyl and metal chelating assays. Total phenolic contents of all the fractions were also determined. Moreover, essential oil of *O. acutidens* was obtained by hydrodistillation and analyzed by gas chromatography/mass spectrometry (GC/MS).

RESULTS AND DISCUSSION
OA/E exhibited the highest antiproliferative activity against HeLa cell line. OA/E and the some sub-fractions displayed higher radical scavenging activity compared to synthetic antioxidant BHT. A significant relationship between antioxidant activity and total phenolic content of the fractions was observed. The main component of the essential oil was carvacrol (65.51%). Rosmaniric acid and 4-hydrobenzoic acid were found in OA/E and the all of the subfractions.

CONCLUSIONS
The ethyl acetate extract of *Origanum ocutidens* may be considered a potential source of a natural antioxidant or anticancer agent in pharmacy industry.

REFERENCES

P-358: MORPHO-ANATOMICAL INVESTIGATIONS ON *MOMORDICA CHARANTIA* L. (CUCURBITACEAE)

I. Eroz Poyraz1, N. Saltan1, G. Derdovski1

Anadolu University, Faculty of Pharmacy1Department of Pharmaceutical BotanyEskişehir, TURKEY

It was reported that *Momordica charantia* L. (Cucurbitaceae) used for some medicinal purposes like antidiabetic, anticancer, antiviral and treat to gastritis. In this study morphological and anatomical properties of *Momordica charantia* L. collected from Kosovo/Prizren was investigated. Morphological studies was supported by morphometric measurements and drawings of male and female flowers, fruit and seeds of the species. In anatomical studies cross sections of stem and leaf, upper and lower surface sections of leaves were evaluated. It is detected that the stem has a typical anatomical properties of a climbing dicotyledon plant. The leaves are amphistomatic and have plenty of cyctoliths on lower surface. The stomata are anomocytic and they situated at the lower surface much more then upper.

INTRODUCTION
*M. charantia* L. (Cucurbitaceae) was collected from Kosovo/Prizren in September 2012 (Fig. 1).

Morphological and anatomical properties of stem and leaf of *Momordica charantia* was determined for support investigations of the species.

MATERIALS AND METHODS
Plant material was collected from Kosovo/Prizren/Nashec village. Voucher specimens were deposited at the Herbarium of Anadolu University, Faculty of Pharmacy Eskişehir (ESSE 14681). Macromorphological observations were carried out under a binocular stereomicroscope, Olympus SZX12 with drawing tube. Live material was stored in 70% ethanol for anatomical studies. Cross sections of the plant stem and cross and surface sections of the leaves were made by hand and stained with Sartor solution. Olympus BX51 binocular light microscope and camera were used for photographs.

RESULTS AND DISCUSSION
Morphology: Annuals, 3.42–1.15 m, climbing herbs, stem with scarcely covering hairs. Leaves alternate, simple, extipulate, petiol 93–45 mm; reniform, 113-73 x 120-76 mm, cordate, palmatilobate, with 5-7...
lobes, lobes sinuate, glabrous at upper surface, scarcely with covering hairs on the veins at the lower surface. Flowers born at leaf axils usually solitary, unisexual and with 5-merous. Female flowers petiol 33-26 mm, calyx green, tubulate, sepals 6-4 x 2-1 mm; petals 14-8 x 10-7 mm, brilliant yellow, stigma with 3 lobes; male flowers petiol 66-55 mm, calyx green, sepals 8-6 x 2-1 mm, petals 18-12 x 12-9 mm, free, obovate, yellow-orange, stamens 3 and with staminal colon. Fruits 128-70 x 62-37 mm, ovoid-ellipsoid, yellow-orange, a fleshy capsula, large tuberculate, stalk 37-28 mm. Seeds oblong, number in the fruit 24-41, 14-11 x 8-6 x 3-2 mm, white and with a sculptured testa.

Fig. 1. *Momordica charantia* L. (Kosova/Prizren/Nashec village, September, 2012).

**Anatomy:** Stem was with 5 corners. Anatomical tissues was cuticle, epidermis, collenchyma at the corners. 10 bicollateral vascular bundles was situated in the stem. Bundles were separated by parenchymatic rays which had druses and prismatic crystals. Pith was parenchymatic. Upper and lower leaf of epidermis was in a line with rectangle, ovoid cells. It was situated some collenchymatic cells under the xylem. Dorsiventral leaves with amphistomatic stomata. Lower epidermis cells walls were crinkly and had a lot of double, triple and scarcely sested cystoliths.

**CONCLUSIONS**

It was aimed by this study to improve the morpho-anatomical knowledge about *M. charantia*.

The detailed morphological properties of the species was given by present study. It was reported that *M. charantia* is a cultivar in Izmir (Ozbakis Dengiz and Gursan 2005) and Bursa (Yesilada et al. 1999), Turkey. It was very limited data about the species enrolled as “balsam elması” in Flora of Turkey (Davis, 1972).

Cross sections of stem and leaf and upper and lower surface sections of the leaves. Stems observed in five angles. Yasuda (1903) reported that the old stems of the species have sharply ridged angles collected from Japan. Our plant stems did not have any covering or secretory trichomes on stem while conic covering trichomes with blunt at apex plants collected from Japan. Collenchyma developed only at the rigdes. Secondary fibro-vascular bundles were situated under the collenchyma. It was also observed selerenchyma, phloem and xylem. Ten bicollateral vascular bundles seen in the stem anatomy which were separated by parenchymatic rays bearing druses and prismatic crystals. Pith was parenchymatic. Our outputs are corresponded to results of Yasuda (1903).

It was observed by us that the leaves were dorsiventral and amphistomatic with anomocytic stomata. Gill and Karatela (1982) reported that *M. charantia* from Nigeria has dorsiventral leaves with anomocytic stomata and bearing cystoliths on the leaf epidermis as also observed by us. The plant was mentioned with covering simple secretory hairs, two or four head cells, long or short stalks which could not observed by us.

**REFERENCES**


**P-359: ANTI-ACETYLCHOLINESTERASE ACTIVITY OF STERNBERGIA CANDIDA**

M. Z. Haznedaroglu1, G. Gokce2

Ege University, Faculty of Pharmacy
1Department of Pharmaceutical Botany, 2Department of Pharmacology Ankara, TURKEY

Acetylcholinesterase inhibitory (AChEI) activity of *Sternbergia candida* was evaluated in both bulbs and leaves in comparison with lycorine. Methanol and chloroform extracts of the plant show AChEI activity.

**INTRODUCTION**

Studies on Alzheimer’s disease have been highlighted due to increasing prevalence of this disease. Oldest hypothesis about the pathophysiology strengthens the research of cholinesterase inhibitors for treatment. Amaryllidaceae plants are well known for alkaloids showing cholinesterase inhibiting activity. Among them, *Sternbergia* species gained attention as a source...
of metabolites of these alkaloid contents. Studies have focused mainly on the bulbs of these plants. In this study the potential acetylcholinesterase (AChE) inhibitory activity of endemic *Sternbergia candida* Mathew & T. Baytop (*Sc*) species was evaluated in both bulbs and leaves in comparison with lycorine.

**MATERIALS AND METHODS**

*Plant Material*: *Sc* was collected from Mugla, Turkey; was identified at the Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, Izmir. Voucher specimens are kept at IZEF Herbarium (IZEF5798).

*Preparation of Extract*: Extraction was performed by sonication with chloroform (CHCl₃), ethyl acetate (EtOAc) and methanol (MeOH). All extracts were examined by TLC on silica plates by use of adequate mobile phases. Detection was performed with Dragendorff reagent. Alkaloids were not found in EtOAc extracts. Therefore, alkaloid enrichment extraction was carried out with CHCl₃ and MeOH extracts.

*TLC bioautography and Microplate assay*: TLC bioautography-screening assay was performed for AChEI activity based on Ellman’s method [1]. Lycorine (Lyc) was used as a positive control. The AChEI activities of the samples were measured by a quantitative colorimetric assay based on Ellman’s method [1] in a 96-well plate.

**RESULTS AND DISCUSSION**

We report for the first time that MeOH and CHCl₃ leaf extracts of the plant show AChEI activity. MeOH extract was much more potent than CHCl₃ extract by means of AChE inhibition. Although IC₅₀ values for MeOH extract was found to be lower than reference drug lycorine; this value of inhibition did not reach to a statistically significant level. Future studies aiming at investigation of the AChEI activity could be considered using leaves of the plant.

**CONCLUSIONS**

Methanolic bulb extract of *Sc* (SCBM) showed the most potent AChEI activity (IC₅₀: 103.6 μg/mL); AChEI activity of MeOH leaf extract (SCLM) was 74% higher than the reference drug lycorine.

**ACKNOWLEDGEMENTS**

Further detailed data is presented in the manuscript “Acta Biologica Hungarica 65(4), pp. 396–404 (2014)”. The authors are thankful to Prof. Dr. Ulvi Zeybek, Prof. Dr. Levent Ustunes, Ernst Mach Grants and OEAD for supports.

**REFERENCES**


**P-360: THE SCREENING PHYTOCHEMICAL COMPONENTS OF MUSCARI AND BELLEVALLA SPECIES GROWING IN KAHRAMANMARAS AND REVIEW OF PHYTOCHEMICAL STUDIES**

S. Demirci1, Esra Ergolu Ozkkan2, Neriman Ozhatay3

1 Çukurova University, Faculty of PharmacyDepartment of Pharmaceutical Botany, 01330, Adana, TURKEY
2Istanbul University, Faculty of Pharmacy 3Department of Pharmacognosy, 3Department of Pharmaceutical Botany 34116, Istanbul, TURKEY

**INTRODUCTION**

Geophytes are specialized underground organs including rhizomes, corms and bulbs. About 1042 geophytes taxa of known naturally grow in Turkey. Almost all of them are economically and medically valuable plants. Taxonomically *Bellevallia* is closely related to *Muscari* which are both bulbous genera belonging to petaloid monocots family (Hyacinthaceae). In Turkey, *Bellevallia* is represented with 24 taxa, 13 of which are endemic, on the other hand *Muscari* is represented by 32 taxa, 20 of which are endemic. The species of *Muscari* and *Bellevallia* have long been used in traditional medicine for a wide range of medicinal applications for the treatment of rheumatism, cardiac diseases, urinary infections, dermatological problems, stomach diseases, hemorrhoid and prostate [1, 2].

In this study, phytochemical components from herba and bulbs of *Muscari* and *Bellevallia* species collected from Kahramanmaraş were studied and compared with the phytochemical studies in the literature [3]. Studied species are as follows: *Bellevallia gracilis, B. macrobotrys, B. tauri, Muscari anaotilicum, M. armeniacum, M. aucheri, M. azureum, M. babachii,*
M. comosum, M. neglectum, M. parviflorum, M. tenuiflorum.

MATERIALS AND METHODS
This study is a part of a PhD thesis entitled “Pharmaceutical Botanical Studies on the Hyacinthaceae Family in Kahramanmaraş”. Dried and powdered herba and bulbs of Muscari and Bellevalia species were separately infused with hot water for 30 minutes. Anthracene glycosides, flavone glycosides, saponins, alkaloids, cardiotonic glycosides and mucilage derivates of the extract were determined according to the color reactions.

RESULTS AND DISCUSSION
Phytochemical components of 12 taxa were studied and phytochemical studies of 5 taxa and 14 different references of these were scanned. Flavones and cardiotonic glycosides from herba and tannins and cardiotonic glycosides from bulbs of Bellevalia species were observed. Flavones, cardiotonic glycosides, tannin, mucilage, and saponins from herba and bulbs of Muscari species were determined. The difference between this study and other studies is that alkaloids and homoisoflavanones were found in the other studies.

ACKNOWLEDGMENTS
This work was supported by the Scientific Project Research Unit of Istanbul University (BAP) (Project No: 26188).

REFERENCES

P-361: COMPOSITION OF THE ESSENTIAL OILS OF JUNIPERUS OXYCEDRUS L. SUBSP. OXYCEDRUS GROWING IN EŞİŞEHİR

S. Alan¹, M Kürşüoğlu², G. Şener¹

¹Anadolu University, Faculty of Pharmacy, Pharmaceutical Botany, 26470-Eskişehir, Turkey
²Anadolu University, Department of Pharmacognosy, Faculty of Pharmacy, 26470-Eskişehir, Turkey

INTRODUCTION
The genus Juniperus L. belongs to the Cupressaceae family, representing about 70 species all over the world, and widely distributed throughout the forests of the temperate and cold regions of the northern Hemisphere, from the far north to the Mediterranean. In the present work, leaves, berries and twig essential oils of Juniperus oxycedrus L. subsp. oxycedrus were investigated to chemical compositions of plants collected with samples collected from different locations. In the study the oils were obtained from the leaves, berries and twigs of the plant by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) techniques. Several studies have reported the chemical composition of leaves, berries and twigs the essential oils of J. oxycedrus subsp. oxycedrus [1, 2, 3, 4, 5].

MATERIALS AND METHODS
Plant Material: J. oxycedrus subsp. oxycedrus was collected in March and April from Eskişehir Hekimdağ and location. Isolation of essential oils: The essential oil of dried leaves, berries and twigs of J. oxycedrus were isolated by hydrodistillation for 3h using Clevenger apparatus. Essential oil analysis: The oils were analyzed by capillary GC and GC/MS using a Agilent GC-MSD system. The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done by using the same column and appropriate operational conditions. FID temperature was 300°C. Identification of Compounds: The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

RESULTS AND DISCUSSION
Leaves, berries and twigs of the plant were water distilled for 3 h using a Clevenger-type apparatus to yield oil in the following percentages: 0.02 %, 2.12 % and 0.01 %, resp. Manoyl oxide (32.8 %), caryophyllen oxide (11.9 %) and Germacrene D (5.7 %) are the major components of the leaves; Myrcene (44.6 %), 3-pinene (15.5 %) and Germacrene D (15.5 %) are the major components of the berries; Manoyl oxide (35.4 %), caryophyllen oxide (16.8 %) and Caryophyllenol II (4.5 %).

CONCLUSIONS
In the study the oils were obtained from the leaves, berries and twigs of the plant by hydrodistillation and analyzed by gas chromatography (GC) and gas
P-362: ANATOMICAL STUDIES ON THREE ENDEMIC VERBASCUM L. SPECIES FROM TURKEY: VERBASCUM PESTALOZZAE BOISS., VERBASCUM PYNOSTACHYUM BOISS. & HELDR AND VERBASCUM BELLUM HUB. & MOR.

S. Alan1, R. S. Göktürk2, M.B. Gökçe3

1Anadolu University, Faculty of Pharmacy, Pharmaceutical Botany, 26470-Eskişehir, Turkey
2Akdeniz University, Faculty of Science and Arts, Department of Biology, 07058, Antalya, Turkey

INTRODUCTION

The genus Verbascum L. (Scrophulariaceae) known as “Şiğir Kuyruğu” in Anatolia is represented by 245 species, 194 of them endemic. These species are distributed in Anatolia and Mediterranean phytogeographical area [1-3]. These species are widely used in folkloric medicine due to their antimicrobial, expectorant, sedative and treatment of dysmenorrhea and rheumatalgia, and anticarcinogenic properties. It was also notified the usage for healing wounds in animal care [4]. The roots, flowers and leaves of Verbascum are also used as anodyne, antiseptic, astringent, analgesic and antihistaminic [5]. In addition, anatomical structures of root, stem and leaves of three Verbascumspecies (Verbascum pestalozzaeBoiss., Verbascum pynostachyum Boiss. & Heldr and Verbascum bellum HUB. & Mor.) are given in this study for the first time.

MATERIALS AND METHODS

Plants Material: The flowering aerial parts of Verbascum pestalozzae; C3 Antalya: Konyaalti, Doyran, between Sakkhlent and Karçukuru (36° 49’ 01” N, 30° 21’ 54” E), on limestone rocks, 2100 m, 17.07.2008, Göktürk 7338; Verbascum pynostachyum, C3 Antalya, between Korkuteli and Fethiye (37° 02’ 53” N, 30° 06’26” E), steppe, 1370 m above the sea level, at the end of July 2007, Göktürk 6093; Verbascum bellum; C2 Antalya: Kaş, Gömbe, between Uçarsu and İkizgöl (36° 34’ 20” N, 29° 36’ 34” E), rocky area, 1967 m, 14.06.2008, Göktürk 7208 were collected in Turkey. Voucher specimens of V. pestalozzae; V. pynostachyum and V. bellum are deposited in the Herbarium of the Biology Department, Akdeniz University in Antalya, Turkey and Herbarium of the Faculty of Pharmacy, Anadolu University in Eskişehir, Turkey.

Anatomical: For anatomical studies, samples have been collected from their natural habitats and kept in 70% alcohol. In the research, root, stem and leaves of mature and flowered plants have been used. Investigations were performed on the cross-sections of the root, the flowering stem and leaf.

RESULTS AND DISCUSSION

Root: Root in all the species is formed by periderm on the outside and felloderm where 4-5 radial row are broken down and felloderm with 2-3 row tissues. Outer felloderm cells are broken up or tissue residues belonging to primer cortex is time to time crushed are found. Secondary floem formed of elliptical-shapeless, round shaped, irregular-arranged and 4-6 row cells under the periderm is taken part. Cambium is uncertain. Seconder xylem part cover a large area and consist of tracheal elements with big and small sizes in a sclerenchymatic tissue. Pith branches are 2-3 row cells.

Stem: When the cross sections are taken on the stems of three Verbascum species, secondary development have been observed. Epidermis is formed by singlerow, thick membrane elliptic or round cells. Covering hair and glandular trichomes are observed. Parenchymatic cortex in 8 or 10 rows is found in all species under the epidermis. Collenchyma cells under the epidermis in primer cortex are seen, on the other hand parenchyma cells including oval-shaped chloroplast inside the epidermis are found. Endodermis consisting of flattened cells can hardly be distinguished from the cortex parenchyma. It has been seen that there are sclerenchymatic bunches such as big, small and discontinuous, Cambium is uncertain. Brunches in all types of seconder xylem are especially getting narrow towards primer xylem. Sclerenchymatic cells in this part formed from trache
and tracheids in all types have been created regular rows in radial direction.

Leaf: In cross-section of main and intervascular tissues outer and inner layers of V. pestalozzae; V. pynostachyum and V. bellum are clearly protrusive. Epidermis includes single flattered row, rectangular, round or oval shaped cells. Covering hairs and glandular trichomes are the same as the stem and are seen in both epidermis. Stoma (amphistomatic) are found in both surfaces of the leaf are much denser on the lower surface. They are on much upper limit of epidermis cells in crosssection (hygromorphic stoma). Mesophyll in all types has been arranged as a two or three-row under the outer epiderm. It has been formed with plenty of chloroplasted palisade parenchyma and three or five rows sponge parenchyma which is under it (bicellular leaf). Tracheal elements in xylem are arranged like radial are found parenchymatic cells with thin walls. Phloem is placed under the xylem.

CONCLUSIONS
The sections taken from root and stem leaves of V. pestalozzae; V. pynostachyum and V. bellum are examined and the anatomic features belonging to plants are determined, compared.

REFERENCES

P-363: THE EFFECTS OF LYOPHILIZED EXTRACT OF ROOTS OF FERULAGO TRACHYCARPA (FENZL) BOISS. (APIACEAE) ON CANCER CELL PROLIFERATION

S. Karakaya1, F. Bakár2, C.S. Kheıı1

1 Ankara University Faculty of Pharmacy, Department of Pharmaceutical Botany, Tandoğan, Ankara, TURKEY
2 Ankara University Faculty of Pharmacy, Department of Biochemistry, Tandoğan, Ankara, TURKEY

INTRODUCTION
Ferulago W. Koch. (Apiaceae) genus is represented by approximately 50 taxa throughout the world. Ferulago species are known as “Çaşır” or “Çaşır” in Turkey and mostly known for their aphrodisiac effects. However recent reports emphasize the activity of various Ferulago species against cancer, as well. The aim of this study was to investigate the effect of lyophilized extract of roots of F. trachycarpa (Fenzl) Boiss. (Apiaceae), a species endemic for Turkey, on cancer cell proliferation.

MATERIALS AND METHODS
For the extraction procedure, roots were grounded and macerated with distilled water each for 4 h at temperatures between 30-35°C. The effects of lyophilized extract human MCF-7 breast carcinoma cells were performed via MTT test. Plant extracts were applied in a concentration range between 1000 µg/ml to 0.01 µg/ml.

RESULTS AND DISCUSSION
Lyophilized extract obtained from the roots exhibited potent inhibitor effects on cell proliferation. The cell viability was 35.13±4.72 % for 1000 µM extract treated group, potent effect compared to the roots. When we review the literature on the cytotoxic and anticancer effects of various Ferulago species, we can see that different extracts prepared from different parts of Ferulago species (flower, leaves and roots) possess promising biological activities (1,2,3,4,5).

CONCLUSIONS
In conclusion, this species has promising effects against the proliferation of cancer cells, and may represent a herbal alternative to synthetic drugs. However, further studies are necessary to elucidate the mechanisms underlying these effects and also to determine the responsible constituent(s).

REFERENCES
5. Karimian, H., Moghadamtousi, M. S., Golbabapour, S., Razavi, M., Hajrezaie, M., Arya, A., Abdulla, M.A., Mohan,

P-364: ESSENTIAL OIL COMPOSITION OF ACHILLEA CUCULLATA BORN. M.

T. Arabacı, S. Köstekci

Inönü University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Malatya, TURKEY

INTRODUCTION

The genus Achillea L. is a member of the Asteraceae and comprises of 110-140 species, which are centred in SW Asia and SE Europe, with extensions through Eurasia to North America [1]. The genus is represented in Turkey by 48 species, 24 of which are endemic to Anatolia [2]. Achillea cucullata Born. is endemic species of the genus. The aerial parts of Achillea species are widely used in folk medicine such as anti-inflammatory, antioxidant, diuretic, emmenagogue, wound healing, stomachache, diarrhea and antispasmodic [3-5].

MATERIALS AND METHODS

Aerial parts of Achillea cucullata were collected from Malatya province, in Turkey (Collector number: Arabacı 2784). The specimens were dried by air dried method. The aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus. The oils were analysed by GC/MS. Library search was carried out using Wiley7N, Nist02, Nist05 and Adams. Relative percentages were calculated. The identified constituents of the essential oils and their relative percentages are listed in Table 1.

RESULTS AND DISCUSSION

The amount of oil calculated per weight of the dried plant material (w/w) was 0.22%. The numbers of the identified compounds are 26 and representing 89.71% of the oil. According to our results, 1,8-cineole (5.07%) are the major components of the oil. According to our results, 1,8-cineole is major component in both studies. Camphor, nerolidol, β-pinene, myrtenal, isoborneol are represented with different ratio as major components in the oil of A. cucullata that grown from different localities.

ACKNOWLEDGMENTS

The authors want to thank İnönü University-IBTAM for GC-MS analyses and Onur Özgül for assistance during GC-MS analyses.

Table 1. Chemical composition, Retention indices (RI), percentage (%) composition of the essential oil of Achillea cucullata.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>Composition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>1023</td>
<td>1.51</td>
</tr>
<tr>
<td>Camphene</td>
<td>1063</td>
<td>0.35</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>1115</td>
<td>7.62</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1253</td>
<td>25.57</td>
</tr>
<tr>
<td>P-Cymene</td>
<td>1314</td>
<td>3.10</td>
</tr>
<tr>
<td>Cyclohexanol</td>
<td>1494</td>
<td>0.38</td>
</tr>
<tr>
<td>Campholenic aldehyde</td>
<td>1519</td>
<td>0.35</td>
</tr>
<tr>
<td>Camphor</td>
<td>1541</td>
<td>2.72</td>
</tr>
<tr>
<td>Linalool</td>
<td>1555</td>
<td>2.39</td>
</tr>
<tr>
<td>Pinen-3-one</td>
<td>1580</td>
<td>2.15</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1602</td>
<td>1.28</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>1625</td>
<td>5.07</td>
</tr>
<tr>
<td>Isoborneol</td>
<td>1674</td>
<td>2.48</td>
</tr>
<tr>
<td>Myrtenol</td>
<td>1757</td>
<td>3.25</td>
</tr>
<tr>
<td>2-Cyclohexen-1-ol</td>
<td>1804</td>
<td>0.24</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>1818</td>
<td>0.49</td>
</tr>
<tr>
<td>cis-Lasmone</td>
<td>1978</td>
<td>0.64</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>2083</td>
<td>2.86</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>2210</td>
<td>13.35</td>
</tr>
<tr>
<td>γ-Cadinene</td>
<td>2238</td>
<td>0.07</td>
</tr>
<tr>
<td>4-Isopropylbenzyl alcohol</td>
<td>2275</td>
<td>1.00</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>2329</td>
<td>2.70</td>
</tr>
<tr>
<td>Eugenol</td>
<td>2361</td>
<td>0.82</td>
</tr>
<tr>
<td>Bicyclosesquiphellandrene</td>
<td>2388</td>
<td>3.61</td>
</tr>
<tr>
<td>β-Eudesmol</td>
<td>2447</td>
<td>1.90</td>
</tr>
<tr>
<td>8-Hexadecyn-1-ol, 14-methyl-</td>
<td>2602</td>
<td>3.81</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>89.71</td>
</tr>
</tbody>
</table>

REFERENCES

P-365: COMPOSITION OF THE ESSENTIAL OIL OF SALVIA CAESPITOSA MONTBRET & AUCHER EX BENTH.

T. Arabacı, H. Ügülü

İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Malatya, TURKEY

INTRODUCTION

Salvia L. (tribe Mentheae, Lamiaceae) is displayed a remarkable range of variation and represented with an enormous and cosmopolitan assemblage of nearly 1000 species [1]. The genus is represented in Turkey by 99 species [2]. Salvia caespitosa Montbret & Aucher ex Benth. is endemic species of the genus. Salvia spp. are used as herbal tea, for food flavouring, in cosmetics, perfumery and the pharmaceutical industry [3-4].

MATERIALS AND METHODS

Plant material of this study was collected from Malatya province, in Turkey (Collector number: Arabacı 2791). The aerial parts of Salvia caespitosa were dried by air dried method and hydrodistilled for 3 h using a Clevenger-type apparatus. The oils were analysed by GC/MS. Library search was carried out using Wiley7N, Nist02, Nist05 and Adams. Relative percentages were calculated. The chemican composition of the essential oils and their relative percentages are listed in Table 1.

RESULTS AND DISCUSSION

In this study, 35 components were identified in the oil constituting 93.00% of the total components detected. The amount of oil calculated per weight of the dried plant material (w/w) was 0.18%. β-pinene (32.84%), α-pinene (17.51%), 1,8-cineole (10.81%) and myrtenal (6.02%) are found the major components of the essential oil of Salvia caespitosa (Table 1). The results are compared with the other chemotypes given in previous studies [4-5].

CONCLUSIONS

The essential oil composition of Salvia caespitosa was identified in previous studies. α + β-thujones (24.0%), 1,8-cineole (14.8%) and α-pinene (7.3%) were found to be the major components by Tanker et al. (1993) whereas, β-pinene (22.7%), 1,8-cineole (10.3%) and α-pinene (6.8%) were characterized as main components by Demirci et al. (2003) [4-5]. When we compare these chemotypes we can see that, the main components in our study were the same with incompatible ratios with Demirci et al. (2003), but α + β-thujones was come out as major component in the study given by Tanker et al. (1993) [4-5].

ACKNOWLEDGMENTS

The authors want to thank İnönü University-IBTAM for GC-MS analyses and Onur Özgül for assistance during GC-MS analyses.

Table 1. Chemical composition, Retention indices (RI), percentage (% ) composition of the essential oil of Salvia caespitosa.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>Composition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santene</td>
<td>1001</td>
<td>0.10</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>1026</td>
<td>17.51</td>
</tr>
<tr>
<td>Camphene</td>
<td>1065</td>
<td>1.28</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>1127</td>
<td>32.84</td>
</tr>
<tr>
<td>Myrcene</td>
<td>1188</td>
<td>1.05</td>
</tr>
<tr>
<td>α-Terpine</td>
<td>1210</td>
<td>0.23</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1245</td>
<td>10.81</td>
</tr>
<tr>
<td>Cis ocimene</td>
<td>1275</td>
<td>0.24</td>
</tr>
<tr>
<td>γ-Terpine</td>
<td>1289</td>
<td>0.92</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1312</td>
<td>1.30</td>
</tr>
<tr>
<td>Terpinolene</td>
<td>1330</td>
<td>0.26</td>
</tr>
<tr>
<td>3-Octanol</td>
<td>1427</td>
<td>0.23</td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>1473</td>
<td>0.76</td>
</tr>
<tr>
<td>α-Campholenic aldehyde</td>
<td>1518</td>
<td>0.35</td>
</tr>
<tr>
<td>d-Camphor</td>
<td>1540</td>
<td>1.14</td>
</tr>
<tr>
<td>Pinocarvone</td>
<td>1579</td>
<td>3.18</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1602</td>
<td>0.57</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>1624</td>
<td>6.02</td>
</tr>
<tr>
<td>Isoborneol</td>
<td>1673</td>
<td>2.38</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1715</td>
<td>2.38</td>
</tr>
<tr>
<td>Germacrene B</td>
<td>1738</td>
<td>0.22</td>
</tr>
<tr>
<td>Myrtenol</td>
<td>1756</td>
<td>1.74</td>
</tr>
<tr>
<td>cis-carveol</td>
<td>1804</td>
<td>0.35</td>
</tr>
<tr>
<td>p-cymen-8-ol</td>
<td>1818</td>
<td>0.15</td>
</tr>
<tr>
<td>Geranylactone</td>
<td>1846</td>
<td>0.28</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>2079</td>
<td>1.91</td>
</tr>
<tr>
<td>Humulene epoxide II</td>
<td>2199</td>
<td>0.60</td>
</tr>
<tr>
<td>Teresantalol</td>
<td>2260</td>
<td>0.08</td>
</tr>
<tr>
<td>Cuminic alcohol</td>
<td>2275</td>
<td>0.18</td>
</tr>
<tr>
<td>Spathulenol</td>
<td>2326</td>
<td>0.45</td>
</tr>
<tr>
<td>Eugenol</td>
<td>2362</td>
<td>0.33</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>2413</td>
<td>0.08</td>
</tr>
<tr>
<td>β-Eudesmol</td>
<td>2442</td>
<td>0.56</td>
</tr>
<tr>
<td>Isopimariadiene</td>
<td>2544</td>
<td>1.94</td>
</tr>
<tr>
<td>1,3-Propanediol</td>
<td>2768</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>93.00</td>
</tr>
</tbody>
</table>

REFERENCES

P-366: QUANTITATIVE DETERMINATION OF PARTHENOLIDÊ IN TANACETUM TAXA GROWING IN ANTALYA BY USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Z.C. Arıtuğ1, A. Doğan2, N. Ezer1, A.M. Gençler-Özkan3

Hacettepe University, Faculty of Pharmacy, 1Department of Pharmaceutical Botany, 2Department of Analytical Chemistry
Ankara University, Faculty of Pharmacy, 3Department of Pharmaceutical Botany, Ankara, TURKEY

INTRODUCTION

Parthenolide, a germacranolide-type sesquiterpene lactone, is the major constituent of Tanacetum parthenium (L.) Schultz Bip. and several other members of the Asteraceae family [1]. It’s also an important compound because of its biological activities including antiinflammatory, antimicrobial, antiulcer and cytotoxic [2]. In the present study, we analyzed 8 Tanacetum taxa growing in Antalya for their parthenolide contents by using High Performance Liquid Chromatography (HPLC) method described in the European Pharmacopoeia [3]. Table 1 shows the names of the plants investigated, their locality and the voucher numbers. Voucher specimens were deposited in the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Turkey (HUEF).

Table 1. Tanacetum taxa analyzed for parthenolide contents.

<table>
<thead>
<tr>
<th>Plant names</th>
<th>Localities</th>
<th>HUEF no</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. argenteum (Lam.) Willd. subsp. canum (C.Koch) Grierson var. pumilum Grierson</td>
<td>Alanya</td>
<td>13026</td>
</tr>
<tr>
<td>T. argenteum (Lam.) Willd. ssp. flabellifolium (Boiss.&amp; Heldr.) Grierson</td>
<td>Gazipaşa</td>
<td>13024</td>
</tr>
<tr>
<td>T. armenum (DC.) Schultz Bip.</td>
<td>Gündoğmuş</td>
<td>13016</td>
</tr>
<tr>
<td>T. cadmeum (Boiss.) Heywood subsp. cadmeum</td>
<td>Gazipaşa</td>
<td>13023</td>
</tr>
<tr>
<td>T. ciliicum (Boiss.) Grierson</td>
<td>Manavgat</td>
<td>13019</td>
</tr>
<tr>
<td>T. parthenium (L.) Schultz Bip.</td>
<td>Kaş</td>
<td>14062</td>
</tr>
<tr>
<td>T. praeteritum (Horwood) Heywood subsp. massicyticum Heywood</td>
<td>Kaş</td>
<td>13021</td>
</tr>
<tr>
<td>T. praeteritum (Horwood) Heywood subsp. praeteritum</td>
<td>Kaş</td>
<td>13022</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The results of our analysis showed that only three of total eight taxa collected from Antalya have parthenolide contents. The amounts of parthenolide (%) of the flowers, leaves and stems of each taxon characterized by parthenolide contents of their aerial parts by preliminary analysis were given in the Table 2.

Table 2: Parthenolide amounts (%) of plant parts

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Parthenolit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAF-flower</td>
<td>0,28</td>
</tr>
<tr>
<td>TAF-leaf</td>
<td>0,29</td>
</tr>
<tr>
<td>TAF-stem</td>
<td>0,03</td>
</tr>
<tr>
<td>TACP-flower</td>
<td>1,04</td>
</tr>
<tr>
<td>TACP-leaf</td>
<td>0,74</td>
</tr>
<tr>
<td>TACP-stem</td>
<td>0,25</td>
</tr>
<tr>
<td>TPA-flower</td>
<td>1,02</td>
</tr>
<tr>
<td>TPA-leaf</td>
<td>0,16</td>
</tr>
<tr>
<td>TPA-stem</td>
<td>0,01</td>
</tr>
</tbody>
</table>

CONCLUSIONS

According to the “Tanaceti parthenii herba” monograph of European Pharmacopoeia 7.0 parthenolide content must be higher than 0.2%. In our study, the parthenolide contents of the aerial parts of three of eight taxa collected from Antalya were found higher than this pharmacopoeial limit. Additionally,
the amount of parthenolide in *T. argenteum* subsp. *canum* var. *pumilum* was found rather higher than the amount found in *T. parthenium*, the officinal taxon. The separate analysis of the different parts (flower, leaf and stem) of these three parthenolide containing *Tanacetum* species showed that; the parthenolide contents of all parts of *T. argenteum* subsp. *canum* var. *pumilum* and flowers and leaves of the other two species (*T. parthenium*, *T. argenteum* ssp. *flabellifolium*) were above 0.2%. The results showed that *Tanacetum* species growing naturally in Antalya Turkey presage further comprehensive investigations.

ACKNOWLEDGEMENTS

This study was supported by AUBAB (Project no: 13L3336007).

REFERENCES


P-367: EFFECT OF THE ETHANOLIC EXTRACT OF A MIXTURE OF PLANTS ON THE ALLERGIC REACTION

Bendjeddou D1, ; Zuerguine K1, ; Lalaoui K3, ; Bensakhri 21, ; Amouzou TY1, ; Maiga IH1.

1 Laboratory of "Biology, Water and Environment" Faculty of Natural and Life Sciences, Earth Sciences and Universe. University 8 Mai 1945 –Guelma 24000- Algeria
2 Laboratory of "Molecular cellulaire biology" Faculty of Natural and Life Sciences. University Mentouri Constantine - Algeria

ABSTRACT

Allergic or inflammatory diseases due to allergens are in constant growth. However, the effectiveness of conventional treatment is not complete. That’s why, new therapeutic tools are considered, for example herbal medicine.

MATERIALS AND METHODS

Thus, this study was carried out to demonstrate the effect of a mixture of the plants on allergic inflammatory reaction using the mice as a model. using determination of number of lymphocytes and neutrophils.

RESULTS AND DISCUSSION

This study revealed a significant increase in the rate of lymphocytes and a significant recruitment of neutrophils reflecting an inflammatory reaction which was confirmed by histological analysis of lung. This reaction was deleterious in the mice that received only the allergen while it was protective in mice treated with both the allergen and the ethanol plants extract. So the mixture of plants under study, promoted an adaptive response and inflammatory reaction in order to eliminate the allergen and prevent the adverse effects of inflammation on the lungs. This observation justifies the empirical success of this extract against inflammatory disorders or other affections. So the above extract which named “Swedish elixir” could be used to enhance the efficiency of conventional therapeutic methods.

REFERENCES


P-368: LC-MS/MS DETERMINATION OF PHENOLIC COMPOUNDS OF MATURE GALLS FROM SALVIA TRILOBA L.

F Göger1, K.H.C.Baser2, N.Kirmer1

1 Anadolu University, Faculty of Pharmacy Department of Pharmacognosy Eskişehir, TURKEY
2 King Saud University, College of Science, Department of Botany and Microbiology, Riyadh, Saudi Arabia

INTRODUCTION

The genus *Salvia*, known as sage, represented in the world with more than 900 species while there are 99 species in the. *Flora of Turkey* 2, *S. fruticosa* Miller which is the synonym of *S. triloba* L is consumed as herbal tea. Moreover, dried leaves of this species are an important export commodity. A pathological gall which occurs on the leaves and branches of the native
Salvia fruticosa, is called apple locally are eaten in some regions while fresh.

MATERIAL AND METHODS
Plant material: Salvia triloba galls were collected from Marmara Island on August 2013. Powdered dried galls were macerated with methanol % 70 at 25 °C for 24h. After evaporation of the methanolic part, the aqueous parts freeze-dried and the dry extract was used for LC-MS/MS analysis.

Phenolic compound determination: Experiments were performed with a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC-MS/MS instrument equipped with an ESI ion source was used in the negative ionization mode. Separations were performed on a ODS 150 x 4.6 mm, i.d., 3 μm particle size, octadecyl silica gel analytical column operating at 40°C at a flow rate of 0.3 mL/min.

RESULTS AND DISCUSSION
According to LC-MS/MS results methyl gallate, luteolin, luteoline glucuronide, rosmarinic acid, rosmanol, galactronic and shikimic acid were determined.

CONCLUSIONS
Phenolic compounds of the herbal parts of S. fruticosa has been well studied but to the best of our knowledge this is the first report on the galls of S. fruticosa from Turkey. It was not possible to compare immature galls to mature ones. But the phenolic profile of leaves (not presented here) showed similarity to those of galls.

REFERENCES

P-369: ANTIMICROBIAL ACTIVITY OF SOME ALIMENTARY AND MEDICINAL PLANTS
S. Akroumi1, L Korrichi2
PhD. Departement of Molecular and cellular biology Jijel university Algeria
Prof. Laboratory of Molecular and Cellular biology Constantine 1 University Algeria

INTRODUCTION
Several plants were used for many generations for their therapeutic virtues, and this was before knowing the exact origin of their benefits. In fact, they were used for treating infections1, malaria2, burns, edema, allergies and prevent several diseases. Vicia faba L, Vaccinium macrocarpon, Punica granatum, Lavandula officinalis, Artemisia absinthium, Linum capitatum and Camellia sinensis were frequently used in our alimentation. In this study, we have tested the antimicrobial activity of their ethanolic and methanolic extracts on some pathogen bacteria, then their ability to in vivo inhibit the growth of Streptococcus pneumoniae.

METHOD
The plants were picked from different localities of Algeria. The tested bacteria came from the Constantine UHC (University Hospital Center, Constantine, Algeria). The ethanolic extracts were obtained by maceration of the plant material during 24 h in aqueous ethanol (80%). And the methanolic extracts were prepared using the Soxhlet apparatus with the pure methanol as solvent.

Patches of 6 mm in diameter were cut up on the Watman No. 1 paper and sterilized. The bacteria suspensions were normalized at 10^6 UFC/ml. Petri dishes containing the Muller-Hinton medium were tested extract were put in their surface.

RESULTS AND DISCUSSION
The phytochemical screening has given the composition of the most active extracts. According to the obtained results, the ethanolic extract of Lavendula officinalis and A absinthium has shown an inhibition of all the tested strains of bacteria. The ethanolic extract of L. officinalis has given the highest activity against S. pneumoniae, followed by the methanolic extract of C. sinensis and P. granatum. the phytochemical screening showed that the most active extracts contained mainly naturels compounds.
REFERENCES

P-370: THE LIGNAN, (-)-SESAMIN REVEALS CYTOTOXICITY TOWARD CANCER CELLS: PHARMACOGENOMIC DETERMINATION OF GENES ASSOCIATED WITH SENSITIVITY OR RESISTANCE

M. Saeed1, H. Khalid2, Y. Sugimoto3, T. Efferth1*
1 Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany
2 Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research, Khartoum, Sudan
3 Division of Chemotherapy, Faculty of Pharmacy, Keio University, Tokyo, Japan

INTRODUCTION
(-)-Sesamin is a lignan present in sesam oil and a number of medicinal plants. It exerts various pharmacological effects, such as prevention of hyperlipidemia, hypertension, and carcinogenesis. Moreover, (-)-sesamin has chemopreventive and anticancer activity in vitro and in vivo. Multidrug resistance (MDR) of tumors leads to fatal treatment outcome in many patients and novel drugs able to kill multidrug-resistant cells are urgently needed. P-glycoprotein (MDR1/ABCB1) is the best known ATP-binding cassette (ABC) drug transporter mediating MDR. ABCB5 is a close relative to ABCB1, which also mediates MDR (1).

MATERIALS AND METHODS
Resazurin cell growth inhibition assay. The resazurin reduction assay was used to assess the cytotoxicity of (-)-Sesamin towards multidrug resistant tumor cells. Statistical analysis (WinSTAT). COMPARE analyses were performed to produce rank-ordered lists of genes expressed in the NCI cell lines. hierarchical cluster analysis (WARD method) was done to obtain a dendrogram which it merge each individual cluster with another depending on closeness of their gene expression features.

RESULTS AND DISCUSSION
We found that the mRNA expressions of ABCB1 and ABCB5 were not related to the 50% inhibition concentrations (IC50) for (-)-sesamin in a panel of 55 cell lines of the National Cancer Institute, USA. Furthermore, (-)-sesamin inhibited ABCB1- or ABCB5-overexpressing cells with similar efficacy than their drug-sensitive parental counterparts. In addition to ABC transporter-mediated MDR, we attempted to identify other molecular determinants of (-)-sesamin resistance. For this reason, we performed COMPARE and hierarchical cluster analyses of the transcriptome-wide microarray-based mRNA expression of the NCI cell panel. Twenty-three genes were identified, whose mRNA expression correlated with the IC50 values for (-)-sesamin. These genes code for proteins of different biological functions, i.e. ribosomalproteins, components of the mitochondrial respiratory chain, proteins involved in RNA metabolism, protein biosynthesis, or glucose and fatty acid metabolism. Subjecting this set of genes to cluster analysis showed that the cell lines were assembled in the resulting dendrogram according to their responsiveness to (-)-sesamin. In conclusion, (-)-sesamin is not involved in MDR mediated by ABCB1 or ABCB5 and maybe valuable to bypass chemoresistance of refractory tumors. The microarray expression profile, which predicted sensitivity or resistance of tumor cells to (-)-sesamin consisted of genes, which do not belong to the classical resistance mechanisms to established anticancer drugs.

Figure. Chemical structure of (-)-sesamin and mean log10IC50 values for (-)-sesamin of the NCI cell lines. Mean values and S.E.M. of log10IC50 were grouped according to the tumor type of the cell lines.
CONCLUSIONS
The concept of personalized medicine in clinical oncology is to determine responsiveness of tumors before chemotherapy to optimize therapy protocols with the most active drugs for each individual patient. The definition of an expression profile which correlated to cellular response towards (-)-sesamin indicates that the concept of prediction of chemosensitivity may also be applied to cytotoxic natural compounds.

REFERENCE

P-371: CYTOTOXICITY OF THE LIGNAN HONOKIOL AGAINST MULTIPLE DRUG-RESISTANT TUMOR CELLS
M. Saeed¹, V. Kuete²,3, O. Kadioglu¹, J. Börtzler¹, H. Khalid¹, H. Johannes Greten⁴,5, T. Efferth¹
1 Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany
2 Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon
3 Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research, Khartoum, Sudan
4 Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal
5 Heidelberg School of Chinese Medicine, Heidelberg, Germany

INTRODUCTION
A main problem in oncology is the development of drug-resistance. Some plant-derived lignans are established in cancer therapy, e.g. the semisynthetic epipodophyllotoxins, etoposide and teniposide. Honokiol is biphenolic lignin derived from Magnolia officinalis and other species of this genus and is traditionally used in Chinese and Japanese medicine. Recent studies in vitro and in vivo reported various pharmacological activities of honokiol, e.g. anti-arrhythmic, anti-inflammatory, anti-thrombotic, anxiolytic, antimicrobial and other activities (1). Interestingly, honokiol inhibited growth of xenograft transplants derived from various tumor types in nude mice (2, 3, 4).

MATERIALS AND METHODS
Resazurin cell growth inhibition assay. The resazurin reduction assay was used to assess the cytotoxicity of honokiol towards multidrug resistant tumor cells. Statistical analysis (WinSTAT), COMPARE analyses were performed to produce rank-ordered lists of genes expressed in the NCI cell lines. hierarchical cluster analysis (WARD method) was done to obtain a dendrogram which it merge each individual cluster with another depending on closeness of their gene expression features.

RESULTS AND DISCUSSION
We investigated the bisphenolic honokiol derived from Magnolia officinalis. P-glycoprotein-overexpressing CEM/ADR5000 cells were not cross-resistant to honokiol, but MDA-MB-231BRCP cells transfected with another ABC-transporter, BCRP, revealed 3-fold resistance. Further drug resistance mechanisms analyzed study was the tumor suppressor TP53 and the epidermal growth factor receptor (EGFR). HCT116 p53−/− did not reveal resistance to honokiol, and EGFR-transfected U87.MEGFR cells were collateral sensitive compared to wild-type cells (degree of resistance: 0.34). To gain insight into possible modes of collateral sensitivity, we performed in silico molecular docking studies of honokiol to EGFR and EGFR-related downstream signal proteins. Honokiol bound with comparable binding energies to EGFR (−7.30 ± 0.01 kcal/mol) as the control drugs erlotinib (−7.50 ± 0.30 kcal/mol) and gefitinib (−8.30 ± 0.10 kcal/mol). Similar binding affinities of AKT, MEK1, MEK2, STAT3 and mTOR were calculated (kcal/mol) compared to corresponding control inhibitor compounds for these signal transducers. This indicates that collateral sensitivity of EGFR-transfectant cells towards honokiol may be due to binding to EGFR and downstream signal transducers. COMPARE and hierarchical cluster analyses of microarray-based transcriptomic mRNA expression data of 59 tumor cell lines revealed a specific gene expression profile predicting sensitivity or resistance towards honokiol.

CONCLUSIONS
We concluded that, if honokiol would find its way into the clinics, it can be expected that this compound will be rather used as part of a combination therapy than as monotherapy. Silencing EGFR and its downstream signaling routes by honokiol may, therefore, be a valuable strategy to re-sensitize drug-resistant tumors.

REFERENCES
P-372: ANTI-OXIDANT ACTIVITY OF PHENOLIC COMPOUNDS FROM LIMONIUM BONDELLI

O. Benaissa1, A. Amrani1, S. Bicha2, D. Zamaa2, A. Bentamen1, F. Benayache1 and S. Benayache1

Constantine University, Faculty of Exact Sciences.
1Unité de recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques (VARENBIOMOL), Département de Chimie, Faculté des Sciences Exactes, Université Constantine 1, 25000 Constantine, Algeria

INTRODUCTION

Limonium bonduelli is a half-hardy annual plant which is endemic to the septentrional Sahara. It’s considered as a subspecies of the Mediterranean species Limonium sinuatum [1]. Aerial parts of this species were collected on April 2011 at Mogheul near Bechar in the South West of Algeria. We report our results on Limonium bonduelli, which has not been previously investigated.

MATERIAL AND METHODS

Extraction and isolation: After extraction of aerial parts of this plant with a [MeOH–H2O: 70/30] solution and separation of the ethyl acetate extract using different chromatographic methods, we isolated three flavonoids (Eriodictyol, Apigenin, Luteolin) and acid phenolic (4-hydroxy-3-methoxy-benzoic). All these results were in good agreement with the literature data [2-3].

DPPH radical-scavenging activity assay: The capacity of ethyl acetate extract and the phenolic compounds from L. Bonduelli to reduce the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was assessed following the method described by Braca et al. (2001) [4]. 3 ml of methanol DPPH solution (0.004%) was added to various concentrations (1, 2.5, 5, 10, 15, 20, 30, 50, 75 μg/ml) of the test samples in methanol. After 30 min incubation at room temperature absorbance was measured at 517 nm. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

\[ I\% = \left( \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \right) \times 100. \]

RESULTS AND DISCUSSION

DPPH radical scavenging activity: Ethyl acetate extract of Limonium bonduelli and isolated flavonoids have DPPH radical scavenging activities at various degrees. It is shown that chemical structure has an important impact on radical scavenging activity. The higher antioxidant activity depends on their molecular structure, and the number and position of the hydroxyl groups. The scavenging effect of ethyl acetate extract of Limonium bonduelli, pure flavonoids and Vitamin C on the DPPH radical decreased in the order of Vitamin C > Eriodictyol > Ethyl acetate extract > Luteolin > Apigenin at the concentration of 20 μg/ml. Inhibition of lipid peroxidation: The percentage inhibition of lipid peroxidation by 100 μg/ml ethyl acetate extract of Limonium bonduelli and eriodictyol were found to be 58% and 69.25% respectively. The ratio at this concentration for vitamin C was found to be 86.95% inhibition of lipid peroxidation. The IC50 of ethyl acetate extract of Limonium bonduelli, eriodictyol and vitamin C (standard) were 20.88, 22 and 20 μg/ml respectively.

CONCLUSION

The results clearly indicated high antioxidant potential of ethyl acetate extract of Limonium bonduelli and isolated flavonoids. Eriodictyol exhibited the highest level of radical scavenging properties at all concentrations among tested samples followed by ethyl acetate extract of Limonium bonduelli, luteolin and apigenin. This study demonstrated that DPPH radical scavenging activity and inhibition of lipid peroxidation depend on the number and order of OH groups in the flavonoids structure. All this compounds were isolated for the first time in this plant.

ACKNOWLEDGMENTS

we are grateful to MESRS (Ministry of Scientific Research, Algeria) for financial support.

REFERENCES

P-373: EFFECT OF VERBASCUM INULIFOLIUM EXTRACT OF METHANOLE ON WOUND HEALING OF IMPAIRED DIABETIC AND INJURED RATS


1Department of Medical Biology, Faculty of Medicine, Adiyaman University, Adiyaman, Turkey
2 Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey
3Adiyaman University Faculty of Medicine, Department of Pathology, Adiyaman, Turkey
4Department of Biology, Faculty of Arts and Sciences, Dumlupinar University, Kütahya, Turkey
5Yenimahalle AOÇ Community Health Center, Ankara, Turkey
6Gediz Voc. School of Health Services, Dumlupinar University, Kütahya, Turkey
7Adiyaman University Training and Research Hospital, Department of Pathology, Adiyaman, Turkey

In this study, we aim to observe the effect of Verbascum inulifolium herb extract of methanol on healing of the excision and incision wounds of impaired diabetic and injured rats.

INTRODUCTION

Wound healing initiates with trauma which triggers the process of systematic, cellular and biochemical procedures that ends up with new tissue formation. Wound healing mechanism presents in four principles. These are; inflammation, contraction of the wound, epithelization and formation of granulation tissue. Diabetes decreases the hydroxyprolin levels and wound tension. For this reason, wound healing is difficult in diabetic people. [1-2].

Verbascum genus (Scrophulariaceae) comprises approximately 360 species distributed throughout the northern hemisphere. The genus is represented by 233 species in Turkey and 185 of them are endemic. One of the endemic species is V. inulifolium. Verbascum species known as Mullein have been used in folk medicine as antiseptic, astrigent, demulcent, emollient, expectorant, sedative and diuretic. Many internal and external uses of the leaves and flowers of several Verbascum species have been documented in many societies in Europe, Asia, Africa and Northern America. In this study wound healing activity of V. inulifolium on diabetic rats were investigated. [3-5].

MATERIALS AND METHODS

In this study, 56 Wistar albino rats which weighed 200-250gr were used. Streptozotocin (STZ) was given to rats and blood glucose levels were measured after 48 hours. If the blood glucose level was equal or higher than 200mg/dl, the rat was recruited in the study. Excision and incision wounds were formed at the dorsalis of the rats. The experiment was divided into four groups as seven and fourteen days. Groups were as follows; Group 1 was diabetic controls (n=14), Group 2 was placebo (n=14), Group 3 was rats which were given %0.5 V.inulifolium (n=14) lotion, Group 4 was rats which were given %1 V.inulifolium (n=14) lotion.

At the end of seven and fourteen days, macroscopic, hystopathologic, mechanic and biochemical evaluations were done in the tissue samples. SPSS version 16.0 was used for statistical analysis. p<0.05 accepted as statistically significant.

RESULTS AND DISCUSSION

In macroscopic evaluation, we observed statistically significant difference in the treatment groups compared to controls in terms of excision and incision wounds. In biomechanical evaluation incision wounds were monitored and no statistically significant change was found in wound resistance (p>0.05). In biochemical assessment, hydroxyproline levels were statistically higher in excision wounds in both dosage and for incision wounds hydroxyproline levels were statistically increased on the 7th day compared to controls (p<0.05). For pathologic investigation, hematoxylin eosin, van gieson and toluidine blue were used.

CONCLUSIONS

V.inulifolium herb extract of methanol has a positive effect on excision and incision diabetic wound healing compared to controls. Rehabilitation depends on the dosage and time course of the appliance.

ACKNOWLEDGMENTS

This study was done with the approval of Dumlupinar University Animal Ethics Committe Opinion 2012/8.1 and the funding was supported from Ahi Evran University Scientific Research Center BAP 2011-12.

REFERENCES

5. Klimek, B.; Stepień, H., P17 Effect of some constituents of Mullein (Verbascum sp.) on

**P-374: THE INVESTIGATION OF WOUND HEALING EFFECT OF THERANEKRON® IN RATS**

I. YILMAZ, A. CETİN, S. BINDAK, M. BADEM

1Inonu University, Faculty of Pharmacy, Department of Pharmacology, Malatya, TURKEY.
2Inonu University, Faculty of Medicine, Department of Histology-Embryology, Malatya, TURKEY.
3Pharmacist, State Hospital, Cizre/Şırnak, TURKEY.
4Karadeniz Technical University, Faculty of Pharmacy, Department of Pharmacognosy, Trabzon, TURKEY.

**INTRODUCTION**

Theranekron® (Richter Pharma AG, Austria) is an alcoholic extract of *Tarantula cubensis* which has been used in veterinary practice for 30 years. Antiphlogistic, demarcative, necrotizing actions were described for Theranekron (1, 2). In this context, it can be successfully used in cows for demarcation of necrotic tissue, enhanced granulation of infection, necrotic wounds and especially in the retention of placenta and pododermatitis circumspecta (1-3). And also (*Cuban tarantula*) have been used to treat abscesses gangrene, support of demarcation in necrotic processes, septicemia, toxemia, and stop growth of canine mammary tumors (4, 5), the treatment of bovine cutaneous papillomatosis, in prevention of retentio secundinarium and the treatment of chronic endometritis in dairy cows and in the treatment of foot and mouth disease lesions and bluetongue disease (2, 3).

The aim of present study was to investigate wound healing effect of Theranekron (Theranekron®, Richter Pharma, Austria) in rats.

**MATERIALS AND METHODS**

In this study, 28 male Sprague Dawley rats were used (n=7/groups). Group 1; (control), for the wound to heal spontaneously and there were not applied any drug or chemical agent. Group 2; (Thr. s.c.) every three days injection of 0.8 ml/rat dose Theranekron injectable solution were administered subcutaneously. Group 3; (Thr. drp.) 0.8 ml/rat dose of Theranekron injectable solution were topically applied every days and group 4; (Madec.) every days topically Madecassol pomade were applied. The present study were carried out 10 days.

**RESULTS AND DISCUSSION**

When we compared all the groups, we observed some histological alterations. These histological alterations are mononuclear cell infiltration, hemorrhage, vascular congestion, fibrinoid necrosis areas, granulation, multifocal areas of ulceration (inflammatory infiltrate). The epidermis and dermis displays images of exuberrant fibrosis. Thr-Sc group showed improved histological appaearance compared with others. In Madec and Thr-drp groups, we observed these alterations. In control group, wound and normal areas are prominent.

**CONCLUSIONS**

In conclusion, most of the observed changes in serum biochemical parameters were not statistically and clinically significant. Thus it seems administration of Theranekron® has not any adverse reaction in experimental rats. We conclude that further different studies (as wound healing effects of Theranekron® in diabetic or immflamed wounds of rats) may be useful effects of Theranekron®.

**ACKNOWLEDGMENTS**

This study is supported as a student research project by TUBITAK (No: 2013/2209). We particularly thank for their support of this project to TUBITAK, to the Richter Pharma AG Austria's company representative in Turkey, and for statistical contribute to Dr. Harika BAG.

**REFERENCES**


**P-375: CHRYSN PREVENTS BRAIN DAMAGE CAUSED BY GLOBAL CEREBRAL ISCHEMIA/REPERFUSION IN A C57BL/J6 MOUSE**

M.A. DURAK, O. Ciftci, M.N. ÖZTANIR, A. ÇETİN, N. BASAK, A. ONDER

1Inonu University, Faculty of Medicine, Department of Neurosurgery, Malatya, TURKEY.
2Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, TURKEY.
levels in the SH group. At result, our study demonstrates that chrysin treatment effectively prevents oxidative and histological damage in the brain caused by global I/R.

CONCLUSIONS
In conclusion, it is suggested that treatment with chrysin can positively affect the neural system in mice, and it can be used for the treatment of global cerebral ischemia/reperfusion.

ACKNOWLEDGMENTS
We acknowledge the support of IUBAP (Scientific Research Fund of Inonu University) under Grant 2013/204.

REFERENCES

P-376: EVALUATION OF ANTIOXIDANT EFFECTS IN RAT LIVER AND ESSENTIAL ELEMENT CONTENTS OF CHERRY LAUREL (LAUROCERASUS OFFICINALIS ROEM.)

A. Eken1, A. Baldemir2, B. Ünlü-Endirlik3, E. Özger4, S. İlgün2

1Erciyes University, Faculty of Pharmacy
2Department of Pharmaceutical Toxicology, 3Department of Pharmaceutical Botany, Ankara, TURKEY
INTRODUCTION
Cherry laurel (Laurocerasus officinalis Roem., syn: Prunus laurocerasus L.) is grown as a native fruit crop locally called “Taflan” or “Karayemiş” in the coasts of the Black Sea region of Turkey [1]. The fruits and seeds of the cherry laurel are widely utilized as herbal medicine in Turkey for the treatment of stomach ulcers, digestive system problems, bronchitis, eczemas, hemorrhoids, anti-diabetic, analgesic on local pain, and as a diuretic [2,3]. Cherry laurel fruits rich in phenolics and has a good source of natural antioxidant protecting humans from several diseases caused by oxidative stress [4].

The present study was designed to investigate the beneficial effects of cherry laurel fruit on altered oxidative stress parameters evaluating the total oxidant and antioxidant status of rat liver. Furthermore, we investigated the content of trace elements and toxic metals in its fruit and seed collected from Akçaabat in Trabzon province of Turkey.

MATERIALS AND METHODS
Sixty male Wistar albino rats were obtained from the Experimental and Clinical Research Centre of Erciyes University, Kayseri, Turkey. The protocol for the use of experimental animals was approved by the Institutional Review Board of the National Institute of Health and Local Committee on Animal Research. The animals were divided randomly into six groups consisting of ten rats each and were treated by oral gavage for 60 days as follows: Group I served as control was given saline (0.9 % NaCl) daily, Group II was administered with dimethoate to induce hepatotoxicity, Group III was received fruit extract (70% methanol), Group IV was co-treated with fruit extract and dimethoate, Group V was co-treated with 100 mg/kg/day Vitamin C and dimethoate, Group VI was firstly treated with dimethoate and 30 minutes later fruit extract. Dimethoate and cherry laurel fruit extract were applied at 20 mg/kg/day and 4 mg/kg/day doses, respectively. All animals were sacrificed 24 h after the last treatment under ketamine and xylazine anesthesia. The liver homogenate was prepared in ice-cold Tris-HCl buffer with KCl (pH 7.6) and the total oxidant status (TOS) and total antioxidant status (TAS) were measured in supernatants of liver homogenate by using commercially available kits. The assay is calibrated with H$_2$O$_2$ and the results of TOS are expressed in terms of μmol H$_2$O$_2$ equivalent per liter. The reaction rate is calibrated with Trolox standard (an analog of vitamin E) and the results of TAS are expressed as mmol Trolox equivalent per liter. For metal analysis, the dried fruit and seed materials were digested in microwave digestion unit. After then, the content of toxic metals and essential elements in samples was evaluated by using inductively coupled plasma mass spectrometry (ICP-MS).

RESULTS AND DISCUSSION
Cherry laurel fruit showed protective/therapeutic effects on biochemical parameters in the liver of all experimental groups. The hepatic TAC level decreased (p<0.05) in the dimethoate administered group. On the contrary, TOS level was increased by the effect of dimethoate and significantly decreased in cherry laurel fruit extract treated groups (p<0.05). The control hepatocytes maintained optimal value of the antioxidant status. The average values of major essential elements in the fruits were K (67431±2685 μg/g), Mg (10454±217 μg/g), Ca (210±33 μg/g), Na (24±1.35 μg/g). The concentrations of essential trace elements were Fe (7.94±0.37 μg/g), Cu (11.99±0.54 μg/g), Zn (3.75±0.19 μg/g), Ni (2.46±0.41 μg/g), Mo (0.35±0.0 μg/g), Co (0.06±0.01 μg/g), Cr (0.11±0.01 μg/g). The contents of major essential elements in the seeds were K (54553±1472 μg/g), Mg (21471±465 μg/g), Ca (442±69.61 μg/g), Na (2.49±0.58 μg/g). The average values of essential trace elements were Fe (29.24±0.64 μg/g), Cu (13.76±1.49 μg/g), Zn (22.81±2.24 μg/g), Ni (2.49±0.54 μg/g), Mo (0.24±0.06 μg/g), Co (0.1±0.03 μg/g), Cr (1.25±0.06 μg/g). Furthermore, toxic metals such as Hg, Cd, As, Pb, Ag, and Al were not detected in the fruit and seeds of cherry laurel.

CONCLUSIONS
The present findings showed that treatment with cherry laurel fruit extract repaired the liver damage induced by dimethoate. Therefore, it may be useful for the prevention of oxidative stress-induced hepatotoxicity. Required amounts of trace elements must be in human diet to pursue good healthy life. Our results showed that the fruit and seeds of cherry laurel contented plentiful essential elements, especially Na, K, Mg, Ca, Fe and Cu, which are necessary for human health. Therefore, cherry laurel might be used as a source of natural mineral supplementation.

ACKNOWLEDGMENTS
This research was financially supported by Research Fund of the Erciyes University Scientific Research Project Unit (Project number: TCD-2013-4127).

REFERENCE
P-377: EFFECTS OF AKR1B1 SILENCING IN HCT-116 COLON CANCER CELLS

B. Tağkoperan1, M.Ş. Ceyhan1, S. Tunçer1, M. Stefek2, S. Banerjee1

1METU Department of Biological Sciences, Ankara, TURKEY 2Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia

AKR1B1, aldo-keto reductase family 1, member B1, is a NADPH-dependent oxidoreductase which is implicated in many types of cancer. It can mediate generation of lipid peroxidation metabolic products, leading to induction of inflammatory responses. Since colorectal cancer is highly associated with chronic inflammation, we hypothesized that AKR1B1 might have a role in colon cancer progression. Herein we show that silencing of AKR1B1 gene through shRNA technology in HCT-116 colorectal cell line leads to a decrease in cell proliferation and delay in cell cycle progression.

INTRODUCTION

In this study, we aimed to obtain AKR1B1 knockdown HCT-116 cells to analyze the effects of AKR1B1 loss in colon cancer cells in terms of mitogenic marker levels, proliferation and cell cycle progression. In order to observe the effects of AKR1B1, we have used an artificial quercetin derivative, CHNQ, (2-chloro-3-hydroxy-[1,4]naphthoquinone), which was designed as a potent AKR inhibitor with potential antioxidant activities [1].

MATERIALS AND METHODS

Cell transfection, culture conditions and treatments
SureSilencing shRNA Plasmids Kit (Qiagen) was used for the generation of HCT-116 cells with stably silenced expression of AKR1B1. Cells transfected with the scrambled shRNA served as the control. The stably silenced HCT-116 cells were grown in RPMI-1640 supplemented with 10% FBS, Pen/Strep and L-glutamine, in the presence of G418, the selective antibiotic. CHNQ treatments were done under serum free conditions. The cells were grown with humidified atmosphere of 5% CO2 at 37 ºC.

Proliferation Assays: IC50 (half maximal inhibitory concentration) values of CHNQ and relative proliferation activities of the cells were determined by Cell Proliferation ELISA, BrdU Kit (Roche).

Protein isolation and Western Blotting: The cells were treated with the different concentrations of CHNQ for 24h and the proteins were collected for western blot analysis.

Synchronization and Cell Cycle Analysis: Overnight serum starvation was done to for cell cycle synchronization. After replacement of the starvation medium with serum containing medium, cells were stained with Propidium Iodide for the indicated time points. The results were analyzed with BD Accuri C6 Flow Cytometer (Becton, Dickinson).

RESULTS AND DISCUSSION

The treatment of the cells with CHNQ led to cell cycle arrest at G2/M phase which was accompanied by the increased activation of MAPK (Mitogen-activated protein kinases) pathway, especially ERK1/2. On the other hand, AKR1B1 silencing itself inhibited this pathway. Moreover, we observed a delay in cell cycle progression in the AKR1B1 silenced cells compared with the scrambled shRNA cells.

CONCLUSION

We show that the novel quercetin derivative compound, CHNQ, causes G2/M arrest in HCT-116 colon cancer cells, and this effect is not dependent on AKR1B1 activity. However, AKR1B1 silencing itself decreased cell proliferation, probably because of the time delay in cell cycle progression which may be related with the inhibition of MAPK pathway, at least partly. Taken together, these results suggest a tumorigenic role for AKR1B1 in colon cancer. Further studies are aimed for the better understanding of the molecular mechanisms underlying the anti-proliferative effects CHNQ and tumor-related functions of AKR1B1 in colon cancer.

ACKNOWLEDGMENTS

This project is supported by TUBITAK-SAS Collaboration Program (113S006).

REFERENCES


P-378: EFFECT OF MELATONIN AND RESVERATROL ON CARBONTETRACHLORIDE- INDUCED ACUTE PANCREAS INJURY IN RATS

B. Yiğitcan1, M. Güll, S. Güll, C.C. Güll2, N. Bayat2

Inonu University Medical Faculty Department of Histology and Embryology, MALATYA

INTRODUCTION

Carbontetrachloride (CCl4) has been commonly used in animal models to study chemical toxin-induced tissue injury. Pineal-gland-derived melatonin (N-Acetyl 5-Methoxytryptamine), which is a known antioxidant...
hormone, is known to prevents oxidative damage at the level of cells, tissues, organs and organisms(1). Resveratrol (trans-3,4',5-trihydroxystilbene, C14H12O3), a natural polyphenol found in various plants and Chinese herbs, has been proposed as an ideal chemopreventive agent because of its relatively low toxicity and its capacity to target multiple signaling molecules that collectively promote cancer cell survival and tumor growth (2). We aimed to study the protective effect of resveratrol and melatonin on carbontetrachloride induced acute pancreas injury.

MATERIALS AND METHODS

Wistar rats weighing 150-180 g were divided into 5 groups each group consisted of 8 rats.

1. grup: saline, 0.5 ml
2. grup: oliveoil, 0.5 ml
3. grup: oliveoil-CCl4 , 1 ml/kg/day (1/1),
4. grup: oliveoil- CCl4 , 1 ml/kg+Resveratrol 10 mg/kg
5. grup: oliveoil- CCl4, 1 ml/kg+Melatonin 20 mg/kg

In this investigation, applications were injected with intraperitoneal for 4 days and on fourth day pancreas tissue samples were collected for histological analysis. Tissue samples of the pancreas were fixed in 10% formaldehyde and embedded with paraffin. Four micrometer thick sections were stained with hematoxylin-eosin and tricrom. Then examined under a lyc a DFC280 Light microscope by Leica Q Win and Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

RESULTS

Control and oliveoil groups presented no histological alterations. In grup 3, some histopathological changes such as acinar cell degeneration, hydropic degeneration in endocrine pancreas cells, vascular congestion showed. Melatonin and resveratrol injection reduced this histological changes compared with carbontetrachloride group.

CONCLUSIONS

The results of the present study suggest that melatonin and resveratrol can be used an agent the protect the pancreatic tissue from CCl4 induced toxicity.

REFERENCES


P-379: INVESTIGATION OF THE TiO2 SOLID NANOPARTICLES SAFETY FOR HUMAN HEALTH BY USING IN VITRO TESTS

C.O. YALCIN, A. USTUNDAG, O. ULKER CEMILOGLU, Y. DUYDU, A. KARAKAYA

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology

INTRODUCTION

Nanoparticles (NP) are particles between 1 and 100 nanometers in size. Metal oxide nanoparticles especially titanium dioxide being used increasingly for various cosmetics (especially sunscreens), food and drug products. Because of widespread environmental exposure to metal oxide nanoparticles, it is urgent to elucidate their effects on human health. Recently, various toxic effects of NPs have been widely investigated in cell lines using different endpoints. On the other hand the results of these studies are not compatible with each other. We aimed to investigate the toxic effects of three different nanosize (20 nm, <100 nm and <150 nm) of TiO2 NPs using in cosmetics by in vitro toxicological studies. The cytotoxic and genotoxic effects of TiO2 NPs was investigated on human keratinocyte cell line HaCaT (Human adult low Calcium high Temperature).

MATERIALS AND METHODS

The NPs characterized for their size by using transmission electron microscopy (TEM) and zeta potential by using Zeta Sizer. Penetration of TiO2 NPs to the human keratinocyte cells investigated by using confocal microscopy prior to the genotoxicity by using comet assay and cytotoxicity test by using Neutral Red Uptake (NRU) assay.

RESULTS AND DISCUSSION

NRU assay results:
The tested concentrations of TiO2 NPs sizes of 20 nm, <100 nm and <150 nm by using NRU assay has no cytotoxic effect. However at <100 nm and <20 nm size of TiO2 NP’s IC20 concentration.

Comet assay results:
Comet scores obtained from TiO2 NPs exposed cells showed that 20 nm TiO2 NPs induced DNA damage in all tested concentrations of 5, 10, 50, 100 μM. Size of <100 nm TiO2 NPs induced DNA damage at 5 and 50 μM concentration. And size of <150 nm TiO2 NPs induced DNA damage at 50 μM concentration.

CONCLUSIONS

Our results demonstrate that especially in high concentrations and small particle size of TiO2 NP’s show genotoxic effect in HaCaT.
ACKNOWLEDGMENTS
This project was supported by TUBITAK (Project no: 113S229).

REFERENCES

P-380: CYTOTOXIC EFFECTS OF SODIUM ARSENITE AND CADMIUM CHLORIDE IN VERO CELLS: A PRELIMINARY STUDY
D. Pasl, A. Gürbay
Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, TURKEY

INTRODUCTION
Sodium arsenite (NaAsO₂) and cadmium chloride (CdCl₂) are two environmental and industrial pollutants with well-known carcinogenic and mutagenic effects. Although kidney is a target organ for arsenic and cadmium, underlying mechanisms of their nephrotoxic effects are poorly understood. In the present study, possible cytotoxic effects of NaAsO₂ and CdCl₂ were determined in African green monkey kidney epithelial (Vero) cells.

MATERIALS AND METHODS
For this purpose, Vero cells were exposed to various concentrations of NaAsO₂ (0.05-120 μM) or CdCl₂ (0.05-300 μM) for 24, 48 and 72 h and cytotoxicity was determined by MTT assay. Protective effects of N-acetylcysteine (NAC), glutathione (GSH) and catalase against cytotoxic effects of selected concentrations of two substances were also examined following 48 h.

RESULTS AND DISCUSSION
Our results showed that when the cells were exposed to ten different concentrations of NaAsO₂, dose- and time-dependent decreases in cell survival were noted. For all incubation periods, survival percent of cells were found ≤ 43% at 20-40 μM concentrations of NaAsO₂. A dose- and time-dependent decreases were also found in Vero cells exposed to eleven different concentrations of CdCl₂. Decreases in cell survival percent were found 40% following 24 and 72 hours, and 50% following 48 h incubation periods. Pretreatment of cells with NAC (1 mM), GSH (0.6 g/l) or catalase (50 and 100 U/ml) for 4 h provided different degrees of protection against NaAsO₂- or CdCl₂-induced cytotoxicity: NAC, GSH and 50 U/ml catalase were found protective against (40 μM, 48 h) NaAsO₂-induced cytotoxicity. On the other hand, pretreatment of cells with GSH caused marked protection against CdCl₂-induced cytotoxicity following 48 h. However, NAC or 50 U/ml catalase pretreatment provided slight protection. These preliminary results, in accordance with the literature [1, 2], suggest that oxidative stress might be involved in the cytotoxicity mechanism of NaAsO₂ and CdCl₂ in kidney cells.

CONCLUSION
The results obtained in this study need further investigations in order to understand exact mechanism of cytotoxicity of NaAsO₂ and CdCl₂ in Vero cells.

ACKNOWLEDGMENTS
This study was performed in Hacettepe University, Faculty of Pharmacy, cell culture laboratory of Department of Toxicology which was established by a project supported by H. U. Research Foundation (04A301003).

REFERENCE

P-381: MUTAGENICITY OF 4-ETHYL-6-SUBSTITUTED-2H-3,4-DIHYDRO-1,4-BENZOXAZIN-3-ONE DERIVATIVES AND THEIR METABOLITES THAT INHIBITED HUMAN TOPOISOMERASE I
E. Foto, F. Zilifdar, N. Diril, S. Yilmaz, E. Aki-Yalcin
1Molecular Biology Department, Faculty of Science, Hacettepe University, Ankara, Turkey
2Pharmaceutical Chemistry Dept Faculty of Pharmacy, Ankara University, Ankara, Turkey

INTRODUCTION
1,4-benzoxazin-3-one derivatives have become an important class of compounds due to their promising biological properties, such as anti-inflammatory, antimicrobial and anticancer activities. In addition to these, some of them showed inhibitory activity against DNA topoisomerases required essentially for many biological processes. DNA topoisomerase I targeted cytotoxic drugs show great promise as potentially important agents for cancer chemotherapy. Due to their mechanism of action, they may also be potential mutagens. However, their mutagenicities still remain to be elucidated. In this study, we investigated some ethyl-substituted-1,4-benzoxazine-3-one derivatives and their metabolites which we previously reported...
that some of these compounds were human topoisomerase I (hTopo1) inhibitor and poison [1, 2].

MATERIALS AND METHODS

The plate incorporation assay was carried out with Salmonella typhimurium tester strains TA98 and TA100. These strains are commonly used for screening on mutagenicity because of indicating both frameshift (TA98) and basepair (TA100) mutations. Both cultures were purified from spontaneous His+ revertants before use.

For all of the compounds three doses (50, 100 and 200 µg/per plate) were tested in both the presence and absence of metabolic activation system (S9) obtained from Sprague Dawley rat liver. Each dose was plated in triplicate. Following incubation, revertant colonies were counted. Results were evaluated with Student’s-T test.

RESULTS AND DISCUSSION

The results of these independent mutagenicity assays showed that no increases in His+ revertants frequencies per plate induced by BS14, hTopo1 poison, were observed with TA98 and TA100 in both presence and absence of metabolic activation. However, BS15, hTopo1 catalytic inhibitor, induced small increase in the frequencies of His+ revertants on TA98 strain in the S9 mix absence. Moreover, other hTopo1 inhibitors, BS16 and BS17, showed very strong mutagenic effects on both strains in both presence and absence of the metabolic activation system. When we compared increases in revertants frequencies induced by BS16 and BS17 with the positive controls (sodium azide for TA100 and daunomycin for TA98), it was noticed that BS16 and BS17 showed similar mutagenicity with the positive controls in low concentrations.

CONCLUSIONS

BS15, BS16 and BS17, previously found as hTopo1 catalytic inhibitor, interacted with DNA and form mutagenic alterations. Furthermore, while BS15 induced only base-pair substitution; BS16 and BS17 induced both base-pair substitution and frameshift mutations. Additionally, these mutagens can be used used as positive controls for the test system. However, further studies are needed for clarifying the mechanism of mutagenesis of these compounds. Structure-Activity Relationships of tested compounds indicated that mutagenicity was enhanced by the introduction of −NO2 and Cl group to 4-ethyl-6-substituted-2H-3,4-dihydro-1,4-benzoxazin-3-one ring.

ACKNOWLEDGMENTS

We thank the Research Fund of Hacettepe University (Grant No:012D06601007 and Grant No:014D03 601001) for the financial supports of this research.

REFERENCES


P-382: EVALUATION OF IN VITRO GENOTOXIC POTENTIALS OF 4-METHYL-6-SUBSTITUTED-2H-3,4-DIHYDRO-1,4-BENZOAZIN-3-ONE DERIVATIVES ON HeLa CELLS

E. Photo1, F. Zilifdar1, S. Yilmaz2, N. Diril2, I. Yalcin2, I. Yildiz2, E. Aki-Yalcin2

1Molecular Biology Department, Faculty of Science, Hacettepe University, Ankara, Turkey
2 Pharmaceutical Chemistry Dept Faculty of Pharmacy, Ankara University, Ankara, Turkey

INTRODUCTION

Substituted benzoxazine derivatives have become of great importance due to their wide range of biological activity including antitumor, analgesic, anti-inflammatory, anti-fertility and anti-bacterial. Besides, we previously reported that some of 1,4 benzoxazines were human topoisomerase I (hTopo1) inhibitor and poison [1,2]. The versatility of the benzoxazine skeleton, in addition to its relative chemical simplicity and accessibility, makes these chemicals amongst the most promising sources of bioactive compounds. As a part of our interest on the synthesis of biologically active benzoxazines, the present investigation aims to evaluate in vitro genotoxicity of 4-methyl-6-substituted-2H-3,4-dihydro-1,4-benzoxazin-3-one derivatives.

MATERIALS AND METHODS

We previously evaluated cytotoxicity of these compounds (BS8-BS13) with using sulforhodamine B (SRB) assay and determined IC50 values by S-probit analyis [3]. We next evaluated their potentials of generating DNA strand breaks on a cancer cell line (HeLa) with alkaline comet assay in this present study. Cells seeded in 24-well plate and then incubated 48 hours in the presence of various non-cytotoxic concentrations of the test compounds. Following unwinding, electrophoresis and staining procedures, slides were scored by comet assay IV analysis program and calculated % tail intensity, tail moment, tail length and tail migration. The results were statistically
RESULTS AND DISCUSSION

Our results indicated that all of the tested compounds which inhibited hTopoI caused increase of comet tail at different levels and triggered DNA strand breaks compared with control. According to the comet assay parameters, BS10 and BS11, hTopoI poisons, was determined as the most genotoxic compounds against Hela cells (p<0.05).

CONCLUSIONS

From the combined results of both cytotoxicity and genotoxicity of the tested compounds against human epithelial carcinoma cells, ethyl-6-chloro-4-methyl-3-oxo-3,4-dihydro-2H-1,4-benzoxazine-2-acetate (BS10) was identified as the most promising compounds. It can act as an important drug candidate for researchers to develop newer benzoxazine derivatives that may prove to be better agents in terms of cancer treatment.

These results also indicated that the compound-stimulated topoisomerase I DNA cleavage was strongly associated with the cyto/genotoxic potency of topoisomerase I poisons. Further studies are ongoing to investigate their molecular mechanism.

ACKNOWLEDGMENTS

We thank the Research Fund of Hacettepe University (Grant No: 0801601009 and Grant No: 013 D10 601 006) for the financial supports of this research.

REFERENCES

3. Foto, E., Zilifdar F., Diril, N., Yildiz, I., Yalcin, I., Aki-Yalcin E., In-Vitro Antiproliferative Activity of 4-Methyl-6-Substituted-2H-3,4-Dihydro-1,4-Benzoxazine-3-One Derivatives That Inhibited Human Topoisomerase I, 3rd Anticancer agent development congress, 2015, Dokuz Eylül University, Faculty of Medicine, İzmir, Turkey, In press.
CONCLUSIONS
All of the tested compounds showed statistically significant antioxidant activity when they are challenged with H$_2$O$_2$. None of the compounds were found to have cytotoxic effect and they also exhibited increasing activity on cell proliferation. Antioxidant efficiency of two newly synthesized groups were approximately similar.

ACKNOWLEDGMENTS
This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) Grant 112S599. Cell culture facility was established with the TÜBİTAK Grant 108S202.

REFERENCES

P-384: PROTECTIVE EFFECTS OF DEXPANTHENOL ON THIACETAMIDE-INDUCED CARDIAC INJURY IN RATS

E. Taslidere$^1$, E. Ozerol$^2$, A Yildiz$^1$, N. Vardi$^1$

$^1$Inonu University Medical Faculty Department of Histology and Embriyology, MALATYA
$^2$Inonu University Medical Faculty Department of Biochemistry, MALATYA

INTRODUCTION
Thioacetamide (TAA), an organo sulfur compound is formally used in leather processing, laboratories, textile and paper industries. It is reported that TAA can hurt different organs, including the liver, lungs, intestine, kidneys, spleen, thymus, pancreas and heart (1). Dexpanthenol (DXP), an alcoholic analogue of pantothenic acid (PA), is oxidized to PA within tissues (2). The present study aimed to examine the possible ameliorative effects of dexpanthenol against cardiac toxicity induced by TAA in rats.

MATERIALS AND METHODS
The study was carried out on Wistar rats divided into four groups: (1) Control group, (2) DXP group, (3) TAA group, and (4) DXP + TAA group. At the end of the study, for histological evaluation, heart samples were fixed in 10% formalin. Sections of heart tissue were cut at 5 µm, mounted on slides, stained with hematoxylin-eosin (H-E). An overall score of cardiac damage severity was semiquantitatively assessed as follows: Cell with eosinophilic cytoplasm and
pyknotic nuclei, cytoplasmic vacuolization, and congestion. The microscopic score of each tissue was calculated as the sum of the scores given to each criteria. Scores were given as 0, none; 1, mild; 2, moderate and 3, severe for each criteria. Tissues were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

RESULTS
The control and DXP groups showed no detectable histological abnormalities. The nucleus and cytoplasm of cardiac muscle cells exhibited normal histological structure in both of groups. There was no statistically significant difference between these groups in the term of histopathological damage score. However, the hearts of in the TAA treated rats showed morphological damage such as cells with eosinophilic cytoplasm and darkening nuclei and congestion. Moreover, some of the cardiomyocytes showed intracytoplasmic vacuoles. On the other hand, although cardiac damage was recognized as alleviated in DXP+TAA group, the lesions did not completely ameliorate. Milder degenerative alterations such as congestion still was present in some area in this group. Mean histopathological score was 0.14±0.14 in control group, 0.28±0.18 in DXP group, 1.85±0.14 in TAA group, 0.85±0.14 in DXP+TAA group. Significant differences were found between control and TAA group (p<0.05), TAA and DXP+TAA group (p<0.05).

CONCLUSIONS
This study shows that DXP can be regarded as a protective agent action against TAA induced cardiac damage.

REFERENCES

P-385: METHOTREXATE-INDUCED CARDIAC TOXICITY: ROLE OF DEXPANTHENOL IN TREATMENT
E. Taslidere1, E. Ozerol2, A Yildiz1, N. Vardi1
1Inonu University Medical Faculty Department of Histology and Embriyology, MALATYA
2Inonu University Medical Faculty Department of Biochemistry, MALATYA

INTRODUCTION
Methotrexate (MTX), a folic acid antagonist, is widely used as a cytotoxic chemotherapeutic agent for several neoplastic diseases such as osteosarcoma, solid cancers and non-neoplastic disease such as psoriasis rheumatoid arthritis. However, the efficacy of these agents is often limited by severe toxic side effects. Also, one of the serious side effects of the MTX is its cardiotoxic effect (1). Dexpanthenol (DXP), an alcoholic analogue of pantothenic acid (PA), is oxidized to PA within tissues. It is well established that PA play a major role in cellular defence and in the repair systems against oxidative stress (2). This experimental study was designed to investigate the protective effect of DXP on cardiac damage induced by MTX in rats.

MATERIALS AND METHODS
The study was carried out on Wistar rats divided into four groups: (1) Control group, (2) DXP group, (3) MTX group, and (4) DXP + MTX group. At the end of the study, for histological evaluation, heart samples were fixed in 10% formalin. Sections of heart tissue were cut at 5 μm, mounted on slides, stained with hematoxylin-eosin (H-E). An overall score of cardiac damage severity was semiquantitively assessed as follows: Cell with eosinophilic cytoplasm and pyknotic nuclei, cytoplasmic vacuolization and congestion. The microscopic score of each tissue was calculated as the sum of the scores given to each criteria. Statically significant differences were found between control and MTX group (p<0.05), TAA and DXP+TAA group (p<0.05).

RESULTS
The hearts of rats in the control group showed normal heart structure and there were no lesions. DXP alone treated group was similar to that of the control group. However in the MTX group, the hearts showed mild morphological damage. Cells with eosinophilic cytoplasm and pyknotic nuclei, cytoplasmic vacuolization and congestion were recognized in MTX group. Moreover, congestion were marked in some area. Mean histopathological score was 0.14±0.14 in control group, 0.28±0.18 in DXP group, 1.71±0.18 in MTX group, 1.00±0.21 in DXP+MTX group. Statically significant differences were found between control and MTX group (p<0.05), MTA and DXP+MTX group (p<0.05).

CONCLUSIONS
The results of the present study suggest that DXP may be used as an agent the protect the cardiac tissue from MTX induced toxicity.

REFERENCES
1. Antunes NL, Souweidane MM, Lis E, Rosenblum MK, Steinherz PG. Methotrexate leukoencephalopathy

P-386: BIOLOGICAL ACTIVITY OF A SERIES OF 2-(4-SUBSTITUTED-BENZYL)-5-AMINO-BENZOXAZOLE DERIVATIVES

F. Zilifdar¹, E. Foto², N. Dirili², T. Bolelli², I. Yalcin²
¹Hacettepe University, Faculty of Science, Molecular Biology Department, Beytepe, Ankara, Turkey
²Ankara University, Faculty of Pharmacy, Pharmaceutical Chemistry Department, Tandogan, Ankara, Turkey

INTRODUCTION
Benzoxazoles are heterocyclic compounds containing a benzene-fused oxazole ring. They can easily interact with organic molecules in the organism due to sharing structural similarities with DNA nucleotides. Therefore, these compounds are important subject for drug design studies. In this study, we aimed to evaluate biological activity of some newly synthesized 2-substituted-benzoxazole derivatives [1]. We investigated cytotoxic and genotoxic activities of these compounds on human cervix adenocarcinoma cell line (HeLa) and in vitro topoisomerase I inhibitory activity.

MATERIALS AND METHODS
The sulforhodamine B (SRB) assay was employed for evaluating cytotoxic activity of the compounds on HeLa cell line. Cells were treated with the test compounds at 37 °C for 48 h. Absorbance of wells was measured at 570 nm following cell fixation and SRB staining. IC₅₀ values of compounds were determined by S-probit analysis.

The alkaline Comet assay was used to investigate genotoxicity of tested compounds. Cells were treated with the IC₅₀ concentration of test compounds at 37 °C for 48 h. After lysis and electrophoresis, slides were stained and % tail intensity, tail moment, tail length and tail migration were measured with comet IV analysis system. Kruskal-Wallis and Mann–Whitney U-tests were used to compare treatment groups with control. To determine whether test compounds resulted in inhibiting human topoisomerase I enzyme activity relaxation assay was implemented. Following electrophoresis, distribution and intensity of bands were analyzed. Superciled DNA band intensity for each compound was compared with control and IC₅₀ values were estimated by S-probit analysis.

Data in this study were presented as mean values obtained from three independent experiments.

RESULTS AND DISCUSSION
According to the SRB assay results, IC₅₀ values of tested compounds ranged from 78 μM to 28 mM. 2-(4-chloro-benzyl)-5-amino-benzoxazole derivative was determined as the most cytotoxic compound against HeLa cells while 2-(4-flouro-benzyl)-5-amino-benzoxazole derivative showed much lower cytotoxic effect.

According to the comet assay data, all of the tested compounds caused increase of comet tail at different levels and triggered DNA strand breaks. By comet assay parameters, 2-(4′-methyl-benzyl)-5-amino-benzoxazole was determined most genotoxic compound by statistical analysis (p<0.05).

Relaxation assay results revealed that only one compound, 2-benzyl-5-amino-benzoxazole, inhibited human DNA topoisomerase I.

CONCLUSIONS
In conclusion, while 2-benzyl-5-amino-benzoxazole derivative was determined as a potent topoisomerase catalytic inhibitor, their inhibitor activity is not higher than positive control. Moreover it has genotoxic activity at low level. So, effective compounds of this study should be subjected to further biochemical and structure activity relationship studies.

ACKNOWLEDGEMENTS:
We thank the Research Fund of Hacettepe University (Grant No: 0801601009 and Grant No: 013 D10 601 006) for the financial supports of this research.

REFERENCES

P-387: TWO NEW 4-SUBSTITUTED-N-(2´-HYDROXY-4´-NITROPHENYL)-BENZENE ACETAMIDE DERIVATIVES AS HUMAN TOPOISOMERASE I CATALYTIC INHIBITORS

F. Zilifdar¹, E. Foto², N. Dirili², T. Bolelli², E. Aki-Yalcin²
¹Hacettepe University, Faculty of Science, Molecular Biology Department, Beytepe, Ankara, Turkey
²Ankara University, Faculty of Pharmacy, Pharmaceutical Chemistry Department, Tandogan, Ankara, Turkey

INTRODUCTION
Anticancer agents are the most interesting subject of drug design studies due to cancer of which is important deathly disease affecting so many people every year in the world. Topoisomerase inhibitors are one of the main classes of anticancer agents. Some new benzene acetamide derivatives were previously synthesized as
the candidate of topoisomerase I inhibitors [1]. Benzamides and benzene acetamide derivatives are known as the potential major metabolite of benzoxazole heterocyclic structures. Benzoxazoles can interact easily with organic molecules in organism due to the structural similarity with organic bases. So they and their metabolites are subjected to anticancer agent designing studies. In this study, we aimed to evaluate potentials of human topoisomerase I inhibition by a series of 4-substituted-N-(2-hydroxy-4'-nitrofenil)-benzene acetamide derivatives.

MATERIALS AND METHODS
We used relaxation assay to evaluate topoisomerase enzyme inhibition by the tested compounds. pBR322 plasmid DNA as substrate, human recombinant topoisomerase I enzyme and different concentration of tested compounds were incubated in appropriate buffer system at 37°C 1 hour. Following gel electrophoresis and EtBr staining, the rate of formation of the newly formed bands was used as a measure of enzyme activity. Moreover, topoisomerase I enzyme inhibitory effect was measured as supercoiled DNA band intensity for control and test compound wells. Intensities of each compound were compared with control band intensity and estimated 50% inhibitory concentration (IC50) by S-probit analyze. CPT was used as the reference. If the inhibition was not obtained at any concentration of a tested compound, it was assumed to have no inhibitory activity on eukaryotic DNA topoisomerase I.

RESULTS AND DISCUSSION
According to the relaxation assay results, only two derivatives of tested five compounds inhibited topoisomerase I enzyme at tested concentrations. According to the IC50 values, 4-chloro-N-(2′-hydroxy-4′-nitrophenyl)-benzene acetamide and 4-fluoro-N-(2′-hydroxy-4′-nitrophenyl)-benzene acetamide were found as topoisomerase I catalytic inhibitors. When chemical structures of compounds were investigated, it was found that both effective compounds have NO2 group at the benzene ring at 4th position.

CONCLUSION
Topoisomerases are ubiquitous enzymes for DNA metabolism such as replication, recombination, transcription and repair. By the inhibition of these enzymes in cancer cells, apoptosis can be triggered due to the interrupted essential biological functions of cells [2]. Compounds determined as topoisomerase catalytic inhibitor in this study should be investigated by future structure-activity studies.

REFERENCES

P-388: PREVENTIVE EFFECTS OF RESVERATROL AGAINST AZOXYMETHANE INDUCED TESTIS INJURY IN RATS

M. Kurus1, A. Bay Karabulut2, E. Taslidere1, O. Otlu2

1Department of Histological and Embryology, 2Department of Biochemistry
Malatya, TURKEY

INTRODUCTION
Azoxymethane (AOM) is a carcinogenic and neurotoxic chemical compound used in biological research (1). Resveratrol is a potent antioxidant and anti-inflammatory properties, which can play a crucial role against carcinogenesis (2). In the present study, we aimed to investigate the effects of resveratrol on testicular injury induced by azoxymethane.

MATERIALS AND METHODS
Six months old female rats were used in the study. Rats were allocated randomly into 4 groups, each consisting of seven (n:7) animals groups. Group I served as control, Group II (RES) was administered resveratrol (Twice a week for 7 weeks. 20 mg/kg of resveratrol). Group III was administered with AOM (Twice a week for 7 weeks, 5 mg/kg subcutaneous azoxymethan). Group IV (ARES) was administered AOM and resveratrol (Twice a week for 7 weeks, 5 mg/kg subcutaneous azoxymethan, 20 mg/kg of resveratrol). At the end of the study, the testis samples were fixed in 10% formalin and were embedded in paraffin. Tissue sections were cut at 5μm, mounted on slides, stained with hematoxylin-eosin (H-E) for general testicular structure. In each specimen 100 tubules were classified as intact, atrophic or degenerated. Sections were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). The testis specimens were taken for biochemical analysis of Total oxidative stress (TOS) and Total antioxidant status (TAS).

RESULTS
Germinal cells were organized in concentric layers and seminiferous tubules containing all stages of spermatogenesis were observed in the control and RES groups. However, degenerative changes in seminiferous tubules were observed in the experimental groups. Some of the tubules were atrophic. There was a significant loss in the number
germ cell in this seminiferous tubule. Arrested spermatogenic cells at various stages of division were observed in other some of the seminiferous tubules. In the AOM group, 70.71±6.07% of tubules were intact, 16.42±4.75% of tubules were atrophic, and 12.85±4.87% of tubules were degenerative. In the ARES group, 80.35±7.80% of tubules were intact, 10.71±4.49% of tubules were atrophic, and 7.14±2.67% of tubules were degenerative. In AOM group, number of affected seminiferous tubules were found to be significantly increased when compared with control group (P<0.05). On the other hand, the number of affected seminiferous tubules in ARES group was statistically significantly lower than in the AOM group (P<0.05). The TOS levels in AOM group were significantly higher than in the control group (p < 0.05) but no statistically differences were found between AOM and ARES groups. The TAS levels in ARES group were significantly higher than in the AOM group (p < 0.05).

CONCLUSIONS
We suggest that antioxidants like resveratrol may be useful for decreasing the harmful effects of AOM.

REFERENCES

P-389: THE COMPARISON OF THE EFFECTS OF ESTROGEN AND MELATONIN AGAINST CORNEAL DISORDERS IN OVARIECTOMIZED AND PINEALECTOMIZED RATS
E. Taslidere1, S Tasdemir2, M.Sagir3, A. Yildiz1, N. Vardi1

1- Inonu University, Faculty of Medicine, Department of Histology and Embryology, 2- Inonu University, Faculty of Medicine, Department of Pharmacology, Malatya, 3- Gaziosmanpasa University, Faculty of Medicine, Department of Pharmacology, Tokat, Turkey

INTRODUCTION
Women experience instabilities in sex steroids hormonal changes throughout their life span in association with puberty, ovarian cycles, pregnancy, and aged menopause. Corneal thickness and curvature variations are indicated to be influenced by steroid hormonal levels during ovarian cycles (1). We investigated the impact of estrogen (E) and melatonin (M) against corneal disorders in ovariecctomized (Ovx) and pinealectomized (Px) rats.

MATERIALS AND METHODS
Fifty-six rats were divided into seven groups 1) Sham 2) Px 3) Bilateral Ovx 4) Ovx+Px 5) Ovx+Px+M (5 mg/kg melatonin (i.p.) 6) Ovx+Px+E (250 µg/kg βestradiol 17-cypionate s.c) 7) Ovx+Px+EM. At the end of the study eyes tissues were fixed in 10% formalin and was embedded in paraffin. Sections of tissue were cut at 5 µm, mounted on slides, stained with hematoxylin-eosin (H-E) general structure of the eyes. For semiquantitative evaluation, the mean thicknesses of the total cornea, corneal epithelium, stroma and descement membrane from three different areas of each cornea were measured using a Leica Q Win Plus Image Analysis System (Leica Micors Imaging Solutions Ltd, Cambridge, United Kingdom) at 40X.

RESULTS AND DISCUSSION
The stratified squamous epithelium, stroma and descement membrane were normal histologic appearance in the sham group. The mean thicknesses for the sham group were as follows: epithelium, 87.85 ± 16.9 µm; stroma, 280.21 ± 68.4 µm; descement membrane, 21, 53 ± 4.67 µm and total cornea, 397.93 ± 79.3 µm. Pinealectomy and ovariectomy caused an increase in the thickness of the epithelium, stroma, descement membrane and total cornea. The corneal thicknesses was higher in Ovx group than Px group. The mean thicknesses for the Px and Ovx groups were as follows: epithelium, 99.58 ± 29.7 and 144.55 ± 17.2 µm; stroma, 319.69 ± 64.7 and 396.69 ± 83.9 µm; descement membrane 24.05 ± 7.26 and 24.89 ± 2.62 µm; total cornea, 439.16 ± 101.6 and 562.79 ± 97.6 µm (respectively). Making of ovariectomy and pinealectomy together didn’t more affect the corneal thicknesses according to the individual. The mean thicknesses for the Ovx+Px group are as follows: epithelium, 100.12 ± 15.5 µm; stroma, 393.61 ± 40.2 µm; descement membrane 26.97 ± 4.33 µm, total cornea µm, 521.91 ± 45.2 µm. In Px, Ovx and CPx+Ovx groups, the stroma was highly edematous. Wide detachment in the stroma were observed. In addition, the lining of endotelium cells and shapes were destroyed in this groups. Also, vacualisation was seen in the cytoplasm of endothelium cells. The border of descement membrane distruption was detected especially in Ovx and Ovx+Px groups. The most obvious improvement among the treatment groups occurred in the Ovx+Px+E group (epithelium, 84.48 ± 19.7 µm; stroma, 343.74 ± 62.4 µm; descement membrane 19.01 ± 2.48 µm, total cornea µm, 455.62 ± 56.1 µm) followed by the Ovx+Px+EM (epithelium, 87.89 ± 20.6 µm; stroma, 385.96 ± 46.0 µm; descement membrane 21.88 ± 2.1µm, total cornea µm, 513.68 ± 54.9 µm) and Ovx+Px+M (epithelium, 109.77 ± 12.4 µm; stroma, 468.23 ± 35.0 µm; descement membrane 24.13 ± 4.80 µm, total cornea µm, 598.94 ± 42.9 µm) groups. Stroma detachment was not extensive in the estrogen-administered groups. Moreover, the layer of corneal
endothelium was almost intact and more arranged except slight vacuolisation in the Ovx+Px+E and Ovx+Px+EM groups. On the other hand, degenerative changes were still present in endothelium such as rupture between cells and desquamation in the Ovx+Px+M group.

CONCLUSIONS
Ostrogen administration was more effective than melatonin in respect to preservation of corneal structures.

REFERENCES

P-390: COMPARING THE REGENERATIVE EFFECTS OF SILYMARIN (SILYBUM MARIANUM) AND APRICOT (PRUNUS ARMENIACA L.) ON LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN RATS

INTRODUCTION
The liver is the largest internal organ of the human body. It has important roles in regulating the metabolic functions and in the immune system. Partial hepatectomy (PH) is performed for the treatment of mass lesions in the liver. Control of the hepatic regeneration is an important step in modern surgery (1). The aim of this study is to evaluate the regenerative effects of silymarin and apricot on hepatic regeneration in rats.

MATERIALS AND METHODS
Forty-six Sprague Dawley rats were divided into five groups: 1. Sham group, (n=6) were fed with standart rat chow and water were given ad libitum for seven days before laparotomy and ten days after laparotomy. 2nd group (n=8) PH (partial hepatectomy), were fed with standard rat chow and water were given ad libitum for seven days before and ten days after PH. 3rd group (n=10) PH+SLM (Silymarin), were fed standart rat chow and water were given ad libitum, and additionally they were given daily 100 mg/kg dose of SLM by intragastric gavage for seven days before and ten days after PH. 4th PH+ (SLM+ SDOA) group (n=8), were fed with 10% supplemented SDOA (Sun Dried Organic Apricot) to chow and water were given ad libitum and additionally they were given 100 mg/kg dose SLM by intragastric gavage for seven days before and ten day after PH. 5. PH+ SDOA group (n=8), were fed with 10% supplemented SDOA to chow and water were given ad libitum for seven days before and ten day after PH. At the end of the study (18th day), liver tissues were excised and weighed, and used for histological, immunohistological (proliferating cell nuclear antigen (PCNA) and tissue biochemical examinations (malondialdehyde (MDA), glutathione (GSH) superoxide dismutase (SOD) and catalase (CAT). Approximately 10 ml blood sample were used for the measurements of serum AST, ALT and ALP. Except control and SLM groups, two rats within other three groups (totally six) were died after two days of PH.

RESULTS
The liver weights: On the seventh day of study (PH operation day), the average resected liver weights of rats were determined as 5.48-6.26g. On the 10th day of PH, average regenerated liver weights which remnant liver of rats were determined as 7.64-8.77g. Histological evaluation: The sham group showed a normal appearance of the liver cells. However in the PH group, histological alterations were observed such as inflammatur cell infiltration around portal triad and central vein. In addition, congestion was determined in sinusoidal area. The histopathological score of PH group was found to be significantly increased when compared to the sham group (p= 0.02). In PH+SDOA, PH+SLM and PH+(SLM+ SDOA) groups although the liver tissue preserved its normal histological appearance, mild inflammatur cell infiltration still were marked in some areas. The histopathological score of PH+SLM and PH+(SLM+S-DOA) groups were significantly lower than that of PH+SDOA group (p=0.02). The number of PCNA (+) of silymarin treatment groups displayed a statistically significant difference according to other groups (p<0.05). Biochemical evaluation: Mean ALP levels were significantly increased in PH group when compared with the control group while mean AST and ALP levels of all the groups were similar. Significant increases in tissue levels of reduced glutathione (GSH) and decreased malondialdehyde (MDA) levels were observed in the PH group receiving SLM compared with the PH group. Mean tissue SOD and CAT activities of all the groups were similar, so no significant difference was detected.

CONCLUSIONS
We have suggested that hepatic regeneration was particularly higher in silymarin groups compared to SDOA group.
REFERENCES

P-391: IN VITRO AND IN VIVO TOXICITY ANALYSES OF BARE AND CHITOSAN COATED MNPS

G. Unsoy¹, S. Yalcin², R. Khodadust³, P. Mutlu³, T. Celik⁴, E. Macit⁵, K.G. Ulusoy⁶, O. Onguru⁵, U. Gunduz¹

¹ Middle East Technical University, Department of Biotechnology, ² Ahi Evran University, Department of Food Engineering, ³ Middle East Technical University, Central Laboratory, Molecular Biology and Biotechnology R&D Center, ⁴ GATA, Pharmacology, ⁵ GATA, Pathology, Ankara, Turkey

INTRODUCTION
Nanotechnology has become a part of our daily life, from novel materials to electronics, cosmetics, pharmaceutics¹, and medicine including targeted drug delivery², biomedical imaging³ and biosensing⁴. However, effect of these nanomaterials on humans and environment is not known in detail, yet. Chitosan (Cs) and superparamagnetic iron oxide nanoparticles (MNPs) are so popular in the biomedical and pharmaceutical applications. Therefore, in vitro and in vivo toxicity of bare MNPs and chitosan coated MNPs (CsMNPs) were evaluated.

MATERIALS AND METHODS
Bare MNPs and CsMNPs were synthesized through the method of Unsoy et al. (2012)⁵. The in vitro cytotoxicity of nanoparticles was investigated with XTT Cell Proliferation Assay. Also, their toxicity was evaluated in vivo, on rats by microscopic imaging of Haematoxylin/Eosin stained paraffin embedded rat liver tissues.

RESULTS AND DISCUSSION
It was found by in vitro cytotoxicity analysis that, bare chitosan coated magnetic nanoparticles (CsMNPs) were not cytotoxic up to 500 mg/ml concentrations. According to the pathology results, when we compare bare MNPs (2.5 mg/ml) and CsMNPs (2.5 mg/ml), a significant toxic effect was not observed on the liver tissues of the rats in vivo (Figure 1 and 2). However, when we double the application dose of CsMNPs, an inflammation at a minimal level, was observed on the liver tissues of rats (Figure 3). Additionally, there is no inflammation was observed, in the liver tissues of bare MNP given rats, even at the highest doses (5 mg/ml).

CONCLUSIONS
Consequently, synthesized bare MNPs and CsMNPs were not found toxic up to 2.5 mg/ml concentrations, both on in vitro and in vivo analyses.

REFERENCES

P-392: BIODISTRIBUTION OF MAGNETITE NANOPARTICLES CHEMICALLY MODIFIED WITH CHITOSAN IN MICE LIVER AND BRAIN TISSUE

G. Unsoy¹, S. Yalcin², P. Mutlu³, R. Khodadust³, U. Gunduz², B. Soykut⁴, O. Erdem⁵, C. Akay⁵, K.G. Ulusoy⁶, E. Macit⁵, T. Çelik⁶

¹ Middle East Technical University, Department of Biotechnology, ² Ahi Evran University, Department of Food
INTRODUCTION
The synthesis and characterization of magnetic nanoparticles have been the focus of an intensive research for more than 10 years. Biochemically functionalized magnetic nanoparticles can be used in many different biomedical applications, such as targeted drug delivery, hyperthermia, and MR imaging (Allen and Cullis 2004; Prabaharan and Mano 2005). These magnetic nanoparticles must be biocompatible, nontoxic and non-immunogenic for biomedical applications. In this study, chitosan coated magnetic nanoparticles (CS-MNPs) were synthesized for biomedical applications and characterized by FTIR, XRD, TGA, VSM, SEM and TEM methods of analysis (Unsoy et al., 2012). The biodistribution and elimination of bare magnetic nanoparticles on mice was studied in this work.

MATERIALS AND METHODS
Chitosan coated magnetic iron oxide (Fe\(_3\)O\(_4\)) nanoparticles were \textit{in situ} synthesized by the coprecipitation of Fe (II) and Fe (III) salts in the presence of chitosan molecules. We explored the magnetic nanoparticle biodistribution pattern in liver and brain of mice after tail vein administration. Iron levels were analyzed by atomic absorption spectrometry (AAS) analysis. All tissue samples were kept at \(-20\) \(^\circ\)C in a refrigerator until the analysis. Each sample was dried at \(75\) \(^\circ\)C for 24 h and weighed. Then, samples were dissolved in 10 ml of nitric acid and digested at 800 W, 220 \(^\circ\)C for 20 min in a microwave oven.

RESULTS
Accumulation of bare and chitosan coated magnetic iron oxide (Fe\(_3\)O\(_4\)) nanoparticles (at three different doses) in liver and brain tissues were determined, and the highest iron levels were observed on the liver of bare iron oxide applied mice group. Therefore, chitosan coating would decrease the accumulation of magnetic iron oxide nanoparticles in liver (Figure 1).

CONCLUSIONS
According to the liver and brain tissue accumulation analysis of bare and chitosan coated magnetic iron oxide (Fe\(_3\)O\(_4\)) nanoparticles; chitosan coated nanoparticle levels were lower than bare iron oxide nanoparticles.

REFERENCES

P-393: CHANGES IN SEMEN PARAMETERS OVER A 16 YEARS PERIOD IN 24693 TURKISH MEN

INTRODUCTION
There are growing evidence that genetic and epigenetic factors, life style factors (obesity, smoking) and environmental factors (endocrine disrupter chemicals) have critical roles for male reproductive health. Decrease in sperm quality is one of the most significant current threats to men’ reproductive health[1] and effected by one or more of these factors. The aims of this study, which is based on information on hospital admissions, are determined as follows: (i) to determine the current average sperm count in men in Turkey, (ii) to prepare a basis for further investigations into the reasons behind the differences between the detected values and values from previous studies by making comparisons (iii) to contribute to the evaluation of infertility cases in
Turkey (iii) to provide information on possible changes in sperm concentrations and quality in the future. Considering the high number of cases included and the duration studied, the present study is a first report about Turkish men semen quality.

MATERIALS AND METHODS
The study was based on the retrospective review of a total 24693 subjects. The medical records of men admitted to the Düzce Laboratories Group, Ankara Centre for infertility evaluation between January 1995 and June 2011 were reviewed. semen quality was evaluated by individual and age dependent (age groups: 18-25(n= 2085), 26-30(n=5577), 31-35(n= 7324), 36-40(n= 5624), 41-50(n= 4083) statistical analysis of semen volume, sperm concentration, total sperm count and mobility. Subjects with a total sperm count lower than 20 x10^6 /mL or higher than 250 x10^6 /mL and aged 50 years or above were excluded.

RESULTS AND DISCUSSION
Considering all age groups, mean sperm concentration was found to be 73.2x10^6/mL. Based on the yearly results, it was found that sperm concentration and total sperm count decreased by 0.99 % and 1.02 % respectively. The mean value (SD) for semen volume was 3.93 (1.93) mL and there was not found any significantly change over the 16 years (p>0.05). Statistically significant differences were not noted in the changes seen in A (+4), B(+3)and D (+1) mobility (p>0.05) values within years.C(+2) mobility parameter showed significant decrease in years, in all age groups as well as in the whole study (p<0.05).

In this study we demonstrated that sperm concentration decreased by 0.99 % yearly between1995–2001. The decrease in sperm quality and count over the years may be attributed to two factors: effects of environmental factors, including endocrine disruptors and lifestyle changes such as increase in body mass index (BMI), stress, nutrition or infections.

CONCLUSIONS
Studies reporting the sperm count as well as the sperm morphology and quality of Turkish men are limited with case reports and they do not build up a general consensus on this matter. Considering the high number of cases included and the duration studied, the present study is a first in that respect.

REFERENCES

P-394: URINARY COTININE LEVELS OF ELECTRONIC CIGARETTE (E-CIGARETTE) USERS
G. Göney,1 İ. Çok,1 U. Tamer,2 S. Burgaz,1 T. Şengezer3, A. A. Ali1
Ankara University, Faculty of Pharmacy
Gazi University, Faculty of Pharmacy, 1Department of Toxicology, and 2 Department of Analytical Chemistry, 06330, Ankara, 3Minister of Health, Sihhiye District Polyclinic, Sihhiye, Ankara, Turkey

INTRODUCTION
In recent years, as the number of smokers worldwide is reaching record highs and anti-smoking policies are proliferating, several new products are being launched by the industry of alternative smoking products with hopes for increasing market shares and revenues. One of the most popular products in the market is the electronic cigarette (e-cigarette). There is growing interest and concern about e-cigarettes in many countries because e-cigarettes have been widely advertised in many countries in the past few years, mostly through the Internet. Distributors of e-cigarettes promote the product as completely free of harmful substances. Nonetheless, some distributors present their products as an alternative to tobacco smoking, suggesting that e-cigarettes can be used to aid smoking cessation.

In this study we aimed to determination and comparison of the amount of nicotine exposure of e-cigarette users, cigarette smokers and passive smokers.

MATERIALS AND METHODS
Subjects: In order to determine cotinine (main metabolite of nicotine), spot urine samples were taken from three groups were exposed to tobacco smoke and tobacco products intentionally or unintentionally. These groups are: (1) E-cigarette users: The subjects selected for this study were 11 men and 2 women with cigarette smokers: 30 urine samples were collected from both male (n:21), female (n:9) (mean age 39.7±11.8) cigarette smokers who smoked at least ½ pack of cigarette per day. (3) Passive smokers: 13 subjects (7 male, 6 female) were chosen who exposed to smoke in different environment (mean age 22.8.±3.1). Cigarette smokers and e-cigarette users were not cigarette/ cigar/pipe smokers.

Measurement of cotinine levels in urine: Cotinine in urine samples was extracted using the method of Man et al,2006 [1]. Diphenylamine was used as an internal standard and cotinine levels determined by the Gas chromatography-mass spectrometry (GC-MS). GC–MS analyses were performed on Agilent 7820A GC coupled with Agilent 5977E mass spectrometer detector.
RESULTS AND DISCUSSION
The mean urinary cotinine concentration of e-cigarette users was 1953 ng/g creatinine and that of cigarette smokers was 1682 ng/g creatinine. No significant difference was found between e-cigarette users and cigarette smokers (p>0.05). The mean cotinine level of SDVVLYH VPRNHUV ZDV " QJJ FUHDWLQLQH

Significant differences were found between e-cigarette users and passive smokers (p<0.05). When cotinine values of subjects who smoked cigarette was compared to that of passive smokers, a statistical significant difference was obtained as well (p<0.05). These results prove that e-cigarette users are exposed to nicotine as much as cigarette smokers.

ACKNOWLEDGEMENTS
(This study is funded by TUBİTAK. Project No:113S837)

REFERENCES

P-395: ANTIOXIDANT CAPACITIES OF CURCUMIN, RESVERATROL AND ROSMARINIC ACID
M. Bacanlı1, H.G. Göktaş1,2, Z. Sangöl1, S. Aydın1, A.A. Başaran3, N. Başaran1
1 Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey
2 Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Çukurova University, 01330, Adana, Turkey
3 Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Ankara, Turkey

INTRODUCTION
Oxidative stress which is involved in the initiation and/or progression of several diseases such as inflammatory injury, ageing processes, cancer, neurodegenerative and cardiovascular diseases, is the state of imbalance between the level of antioxidant defence system and production of reactive oxygen species (ROS). Plant polyphenols may act as antioxidants by different mechanisms such as free radical scavenging, metal chelation and protein binding. Nevertheless, there are still many phenolic compounds with unclear or unidentified prooxidant and antioxidant properties. Curcumin, resveratrol and rosmarinic acid are the phenolic compounds which are known as antioxidants and commonly used for the prevention and treatment of oxidative stress related diseases.

MATERIALS AND METHODS
In this study, we determined the antioxidant capacities of these phenolic compounds by the trolox equivalent antioxidant capacity (TEAC) assay. TEAC assay, the most popular antioxidant activity screening method, described by Miller et al. (1993), is based on scavenging of long-lived, stable blue/green radical (ABTS•+) and converting it into a colourless product [1].

RESULTS AND DISCUSSION
Figure 1. Comparision of the antioxidant capacities of curcumin, resveratrol and rosmarinic acid to trolox.

The antioxidant capacities of curcumin, resveratrol and rosmarinic acid were shown in Figure 1. In our study, we found that resveratrol and rosmarinic acid have more antioxidant capacity than trolox whereas curcumin has less antioxidant capacity than trolox. Several studies have showed that fruits and vegetables rich diets can be associated with a markedly decreased risk of chronic diseases. This has been attributed to high levels of antioxidant compounds in these foods. Although curcumin has been found to have more antioxidant capacity in some studies, in this study, it showed less antioxidant activity at all tested concentration (except 10 µM) than well known antioxidant trolox[2]. Recently, Gülçin (2010) has clarified antioxidant and radical scavenging activities of resveratrol by different in vitro assays [3]. Similar to that, in our study, the antioxidant capacities of resveratrol at even a wide concentration range (2-200 µg/mL) were found to be significantly more than trolox. The antioxidant activity of rosmarinic acid was indicated several in vitro and in vivo studies[4]. Also we found that rosmarinic acid had significantly more antioxidant capacity when compared to trolox.

CONCLUSION
We found differences in the antioxidant capacity between these phenolic compounds being resveratrol and rosmarinic acid more active than curcumin.

REFERENCES
1. Miller NJ.; Rice-Evans C.; Davies MJ.; Gopinathan V.; Milner A, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant


P-396: CORRELATION BETWEEN SOME CYTOKINE GENE POLYMORPHISMS AND SUSCEPTIBILITY TO TYPE 2 DIABETES

**I. Ates**¹, D. Altuner², H. S. Süzen¹, A. Karakaya¹

¹ Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, TURKEY
² Erzincan University, Faculty of Medicine, Department of Medicinal Pharmacology, Erzincan, TURKEY

**INTRODUCTION**

Type 2 diabetes is a chronic and complex disease that is characterized by impaired pancreatic beta cell function and insulin resistance. Genetic and environmental factors are playing important role in the development of this disease. Cytokines and chemokines are the keystones in regulation of the homeostatic mechanisms such as inflammation and tissue repair. Thus, variations in their levels and structures are the reasons for the occurrence of various diseases. The single nucleotide polymorphisms (SNP) forming on the cytokine genes increase the risk of disease development. Recent studies showed the relationship between some cytokine and chemokine gene polymorphisms and the development of type 2 diabetes [1-4]. Based on this, our aim was searching and the evaluating the possible relations between the TNF-α, IL-1β, IL-6 and MCP-1 gene polymorphisms on the disease occurrence.

**RESULTS AND DISCUSSION**

In regards to our genotyping results, TNF-α, IL-1β, and IL-18 gene polymorphisms are significantly related with the development of Type 2 diabetes 3.11 fold, 2.01 fold and 1.75 fold respectively. On the other hand, there is a little but not significant effect of IL-6 and MCP-1 gene polymorphisms on the disease occurrence.

**CONCLUSIONS**

Considering closely to our results, it can be seen clearly that TNF-α, IL-1β, and IL-18 were the cytokines affecting the occurrence and development of Type 2 diabetes. Further extensive studies are needed to suggest our proposals about these cytokine gene polymorphisms.

**ACKNOWLEDGMENTS**

This study was supported by Research Fund of Ankara University with a project number of 12B3336005.

**REFERENCES**


P-397: THE ASSOCIATION OF SEROTONINE 2A RECEPTOR 102 T/C POLYMORPHISM AND NAUSEA IN CITALOPRAM TREATED PATIENTS

**M. Demirbügen**², B. Baskak², T. Kızılozlü², H. Devrimci Öğüven², H.S. Süzen¹

Ankara University
¹Faculty of Pharmacy, Department of Pharmaceutical Toxicology
² Faculty of Medicine, Department of Psychiatry
Ankara, TURKEY

**INTRODUCTION**

It is well documented that interindividual differences in drug response and adverse drug reactions are still important problem in pharmacotherapy. It is a trouble spot during the medication and it is important to eliminate these reactions according to genetic differences. Selective serotonin re-uptake inhibitors (SSRIs) are a class of antidepressants which are used for the treatment of depression. Citalopram is a widely prescribed agent of SSRIs. Despite, SSRIs are associated with less adverse drug reactions, nausea and
vomiting is observed 20% of the depression patients[1]. There are different mechanisms and factors for the occurrence of nausea and vomiting. Serotonin receptor polymorphisms might be one of these factors which contribute nausea and vomiting. Our aim was to evaluate potential associations between the serotonin 2A receptor (HTR2A) 102 T/C single nucleotide polymorphism and nausea in citalopram treated patients.

MATERIALS AND METHODS
Genomic DNA was isolated from 48 major depression disorder patients who were treated with daily citalopram. The HTR2A 102 T/C polymorphism analysed by using polymerase chain reaction coupled with restriction fragment length polymorphism techniques.

RESULTS AND DISCUSSION
In the full sample, HTR2A 102 T/C allele frequencies were C=0.52 and T=0.48. Genotype frequencies did not differ significantly from Hardy-Weinberg equilibrium (p=0.99).
For the full samples the genotype frequencies were as follows: wild type 13 (27.08%), heterozygous 24 (50.00%) and mutant 11 (22.92%) in our study group. According to our results the genotype frequencies in the patients who experienced nausea and vomiting were 3 (21.43%), 8 (57.14%) and 3 (21.43%) wild type, heterozygous and mutant, respectively. For the patients who were not experienced nausea and vomiting as a major side effect genotype frequencies were 10 (29.41%), 16 (47.06%) and 8 (23.53%) wild type, heterozygous and mutant respectively. We did not find statistically significant difference between patients with and without nausea in genotypic distribution associated with the 102 T/C polymorphism of the HTR2A gene (p= 0.797).

CONCLUSIONS
Our results demonstrate that serotonin 2A receptor 102T/C polymorphism may not be associated with nausea and vomiting in depressed patients treated with citalopram.
oxygen species (ROS) that induce lipid peroxidation and quench free radicals. Under this experimental setting, sub-acute melatonin treatment leads to significant changes in the biochemical parameters of rat livers.

CONCLUSIONS
Further investigation and detailed studies are necessary to examine the full impact of melatonin supplementation with regards to dose and duration.

ACKNOWLEDGMENTS
This study was partially supported by The Scientific Research unit of Hacettepe University (Project No. 953).

REFERENCES

P-399: COMPARISON OF BRAIN CYP2E1 IN MPTP-INDUCED SWISS ALBINO AND C57BL/6 MODELS OF PARKINSON’S DISEASE
R. Başaran, E.D. Özdamar, B. Can Eke

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology
Ankara, TURKEY

INTRODUCTION
Brain CYPs may also play a role in modulating brain activity, regulation and susceptibility to neurodegenerative diseases [1]. CYP2E1 is a substantial enzyme involved in the metabolism of xenobiotics in the brain and liver. It’s also known to generate reactive oxygen species and promote oxidative stress causing many diseases such as Parkinson’s disease [2]. A chemical neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) can lead to Parkinson’s disease in Swiss albino and C57Bl/6 mice. Previous findings indicate that CYP2E1 may induced by nicotine and MPTP-mediated neurotoxicity via CYP2E1 enzyme and a correlation between CYP2E1 activity and neurodegeneration in the brain [3]. The aim of our study was to compare CYP2E1 activities and expressions in the brain and liver between Swiss albino and C57Bl/6 mice. In addition, the mice were injected with nicotine and we investigated its effects on CYP2E1 activities and expressions in the brain and liver.

MATERIALS AND METHODS
Treatment of Animals: Swiss albino and C57Bl/6 male mice (12 weeks) were used throughout the experiments. Animals were randomly divided into four subgroups, were further treated with and without MPTP.HCI (30 mg/kg, i.p.) once in a day for 4 days and 21 days along with nicotine (1 mg/kg, i.p.). The animals were sacrificed via cervical dislocation after injections. Brain and liver tissues are taken immediately stored at -80°C.

Enzyme Assay: Microsomal brain and liver proteins were determined by the method of Lowry et al., (1951) [4] using bovine serum albumin as a standard. Microsomal CYP2E1 activity was assayed as describes by Reinke and Moyer (1985) [5].

Western Blotting: Microsomal proteins were separated by SDS-PAGE (4% stacking gel and 10% separating gel), transferred 2 hours onto nitrocellulose membrane which was incubated overnight at 4°C with the primary rabbit polyclonal antibody to CYP2E1 diluted 1:5000. The membrane was washed with TBS-T and then incubated the secondary antibody, horseradish peroxidase conjugate. Membrane protein bands were visualized the binding of the substrate ECL.

RESULTS AND DISCUSSION
The highest brain CYP2E1 activity was observed in nicotine+MPTP-induced group of C57Bl/6 mice. No significant alteration was observed in the liver CYP2E1 activity in both mouse species. Compared to the Swiss albino mice, brain CYP2E1 activity in C57Bl/6 mice was 1.8-, 1.3-1.3-, and 1.7-fold higher in the control group, MPTP group, nicotine group, and nicotine+MPTP group, respectively (p<0.05).

Brain CYP2E1 expressions in the control and treatment groups of the Swiss albino and C57Bl/6 species, a 24% statistically significant decrease was observed in the brain CYP2E1 band intensity of the Swiss albino mouse species’ nicotine and nicotine+MPTP groups in comparison to the control group. In C57Bl/6 mice, however, brain CYP2E1 expressions in the nicotine and nicotine+MPTP groups increased 40% and 65%, respectively (compared to the control group). We did not observe a significant difference between the mouse species considering both livers CYP2E1 expressions.
CONCLUSIONS

Brain CYP2E1 enzyme activities are induced by nicotine and MPTP; however, for different mouse species significant differences in the expressions of brain CYP2E1 enzyme were noted. The present results may help to understanding of the functional roles of brain and liver CYP2E1 in prevention and treatment of neurodegenerative diseases.

REFERENCES


MATERIALS AND METHODS

PHB-MNPs were synthesized through the method of Yalcin et al. (2014)[2]. The in vitro cytotoxicity of nanoparticles was investigated with XTT Cell Proliferation Assay. Also, their toxicity was evaluated in vivo, on mice by microscopic imaging of Haematoxylin/Eosin stained paraffin embedded mouse liver tissues.

RESULTS AND DISCUSSION

It was found by in vitro cytotoxicity analysis that, PHB-MNPs were not cytotoxic up to 1000 mg/ml concentrations (Figure 1).
According to the pathology results, when we compare bare MNPs (2.5 mg/ml) and PHB-MNPs (2.5mg/ml), a significant toxic effect was not observed on the liver tissues of mice (Figure 2).

INTRODUCTION

The nanoparticles are one of the most efficient drug delivery systems for cancer therapy. Nanoparticles, which commonly consist of various elements such as iron, nickel, and cobalt, titanium dioxide, alumina, zinc oxide, carbon are widely studied. However, nanoparticles can lead to production of reactive oxygen species (ROS), including free radicals. ROS and free radical production is an important mechanisms of nanoparticle toxicity and it may result in oxidative stress, inflammation, damage to proteins and lipids, membranes and DNA [1]. Therefore, in this study, in vitro and in vivo toxicity of Polyhydroxybutyrate coated magnetic nanoparticles (PHB-MNPs) were investigated in MDA-MB-231 cell line and mice, respectively.

CONCLUSIONS

In this study, synthesized PHB-MNPs were not found toxic up to 2.5 mg/ml concentrations in in vivo analyses. Moreover, in MDA-MB-231 cell line showed no toxicity up to 1000mg/ml. Consequently, PHB-MNPs can be used to target specific cancer cells in the presence of magnetic field.

REFERENCES

DOXORUBICIN LOADED DEXTRAN COATED MAGNETIC NANOPARTICLES ENHANCE CYTOTOXICITY IN DOXORUBICIN

S. Yalcin1*, G. Unsoy2, R. Khodadust2, U. Gunduz2,3

1 Ahi Evran University, Department of Food Engineering, Kırşehir, 2 Middle East Technical University, Department of Biotechnology, 3 Middle East Technical University, Department of Biology, Ankara, Turkey

INTRODUCTION
Doxorubicin is an anthracycline antibiotic. It is a commonly used anticancer agent in many cancer types, and its most serious side effect is damaging the heart cells. Therefore, targeting of this chemotherapeutic agent with a suitable nanocarrier-mediated drug delivery system is important. In this study, dextran coated magnetic nanoparticles (Dex-MNPs) were characterized by FTIR, XRD, TGA, VSM, SEM and TEM analyses. Doxorubicin was loaded onto the Dex-MNPs. In vitro cytotoxicity analysis of Doxorubicin loaded Dex-MNPs was performed on Doxorubicin-resistant MCF-7 cell line.

MATERIALS AND METHODS
Dextran coated magnetic iron oxide nanoparticles were in situ synthesized by the coprecipitation of Fe (II) and Fe (III) salts in the presence of dextran molecules. The in vitro cytotoxicity of nanoparticles was investigated with XTT Cell Proliferation Assay. According to the instructions of manufacturer, XTT reagent was added after the cells were exposed to Doxorubicin and Dox-Dex-MNPs, for 72 h. The cell viability of control groups was considered 100%. Amount of soluble product formazan was measured at 496 nm by microplate reader (Multiscan GO, Thermo Scientific).

RESULTS
The cytotoxic effect of Doxorubicin loaded Dex-MNPs on resistant MCF-7 cells was investigated by XTT cell proliferation assay, and IC50 values were calculated. In this study, empty Dex-MNPs were found not significantly cytotoxic up to 500 mg/mL. The IC50 value of free Doxorubicin and Doxorubicin loaded Dex-MNPs (300 μg/ml) were found as 183 and 25 μM on Doxorubicin-resistant MCF-7 cells, respectively.

CONCLUSIONS
According to these results, the synthesized dextran coated magnetic nanoparticles can be targeted to tumor cells under magnetic field and seems to be an efficient system to eliminate Doxorubicin resistance in MCF-7/Dox cells.

TP53 (ARG72PRO) POLYMORPHISM AND CLINICAL OUTCOME IN LUNG CANCER

S. Bilgen1, A. O. Ada1, C. S. Kunak2, M. Gulhan3, M. Iscan1

1 Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, TURKEY, 2 Department of Pharmacology, Faculty of Medicine, University of Ordu, TURKEY, 3 Atatürk Pulmonary Diseases and Thoracic Surgery Hospital, Sanatoryum, Ankara, TURKEY

INTRODUCTION
Gene polymorphisms of TP53 have been studied for the possible association with response to chemotherapy and survival rates of patients with non-small cell lung cancer (NSCLC). However, rather limited number of molecular epidemiological studies, some of which have controversial results, to date have considered determining the role of TP53 (Arg72Pro)
polymorphisms in this regard. Moreover, their combined impact on clinical outcome in NSCLC is lacking. In this study, \( TP53 \) (Arg72Pro) polymorphism and response to platinum based chemotherapy and survival in 137 (125 men and 12 women) advanced stage NSCLC patients have been investigated.

**MATERIALS AND METHODS**

The 137 patients with the mean age of 56 ± 9 (mean ± SD; range: 34-75) who had a histological diagnosis of primary NSCLC with stages III or IV, and who were treated with platinum based chemotherapy were enrolled in this study. The patients were treated only with a variety of standard platinum-based chemotherapy regimens as first-line treatments. The effect for chemotherapy was evaluated by the World Health Organization criteria. The \( TP53 \) (Arg72Pro) (rs1042522) gene polymorphism was determined by Real-Time PCR method modified from Talseth et al., (2006). Chi-square analysis and Fisher exact tests were used to compare the distribution of genotypes between subgroups and response to chemotherapy. Hazard ratios (HRs) were estimated from a multivariate Cox proportional hazards model with adjustment for age, gender, smoking status, chemotherapy regimen, tumor stage and tumor histology.

**RESULTS AND DISCUSSION**

No significant associations were noted between the gene polymorphisms and response to chemotherapy. However, we observed that the patients who had homozygous mutant genotypes of \( TP53 \) (Pro72Pro) were made likely to be resistant to chemotherapy than those with (Arg72Arg) variants (100% vs 66%) with marginal significance (P=0.066). Multivariate analysis revealed no significant altered adjusted hazard ratio of death associated with the genotypes of \( TP53 \) (Arg72Pro), (HR, 1.27; 95% CI, 0.68-2.36, P=0.456), (Pro72Pro), (HR, 0.22; 95% CI 0.02-2.57, P=0.226), (Arg72Arg+Pro72Pro), (HR, 1.14; 95% CI, 0.62-2.11, P=0.667).

**CONCLUSIONS**

These results show that the \( TP53 \) gene polymorphism is not likely to be associated with response to chemotherapy and survival in the patients with advanced NSCLC.

**ACKNOWLEDGMENTS**

Supported by Research Fund of Ankara University grant no: 2008-08-03-006HPD.

**REFERENCES**

1. Talseth, B.A. et al., *MDM2 SNP309 T>G alone or in combination with the TP53 R72P polymorphism does not appear to influence disease expression and age of diagnosis of colorectal cancer in HNPCC patients* Int J Cancer 2006, 118(10), 2479-84

**P-403: THE EVALUATION OF BIOMARKERS OF OXIDATIVE STRESS IN HASHIMOTO'S THYROIDITIS**

U. Yaman1,2, P. Erkekoglu1, Derya Bulus3, Nesibe Andiran3, B. Kocer-Gumusel1,*

1 Hacettepe University Faculty of Pharmacy Department of Pharmaceutical Toxicology Ankara, Turkey 2 Atatürk University Faculty of Pharmacy Department of Pharmaceutical Toxicology Erzurum, Turkey 3 Keçiören Training and Research Hospital, Pediatrics Clinic, Pediatric Endocrinology Unit, Ankara, Turkey;

**INTRODUCTION**

Hashimoto's thyroiditis (HT) is an autoimmune disease of thyroid gland. In the course of the disease, thyroid gland is attacked by a variety of cell- and antibody-mediated immune processes (1). High iodine intake, selenium deficiency, smoking, infectious diseases and some drugs may contribute the development of autoimmune thyroiditis. It is considered that oxidative events may increase in immune diseases like HT, and thyroid gland could not give an adequate response against oxidative stress depending on alterations in the antioxidant defense system (2). The aim of this study is to evaluate the biomarkers of oxidative stress in the children newly diagnosed by HT.

**MATERIALS AND METHODS**

**Study Groups**

1. HT group consists of newly diagnosed HT patients admitted to Keçiören Training and Research Hospital Pediatric Endocrinology Department. The children had no chronic, endocrine or genetic disease, were not taking any regular medication or supplementation, and not treated for HT (n=30, age=8-16 years).
2. Control group consists of the healthy children who were matched for age and gender with HT group. The children had no chronic, endocrine or genetic disease and were not taking any regular medication or supplementation, (n=30, age=8-16).

Blood samples from both the HT and control groups were collected in heparinized tubes and were transferred to Hacettepe University Faculty of Pharmacy Pharmaceutical Toxicology Laboratory in dry ice. Plasma samples were separated and erythrocyte packages were obtained after centrifugations.
As an indicator of lipid peroxidation, plasma malondialdehyde (MDA) levels were measured by using HPLC according to the method of Richard et al. (3).

Erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities were measured by using commercial kits (Cayman Chemicals, Ann Harbor, MI).

RESULTS AND DISCUSSION

Table. Erythrocyte SOD and CAT Activities and MDA Levels in Control and HT Groups.

<table>
<thead>
<tr>
<th></th>
<th>SOD (U/mg Hb)</th>
<th>CAT (nmol/min/mg Hb)</th>
<th>MDA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>4.13±2.23</td>
<td>332.08±104.88</td>
<td>1.37±0.39</td>
</tr>
<tr>
<td>HT</td>
<td>3.14±1.20*</td>
<td>327.04±143.08</td>
<td>1.29±0.33</td>
</tr>
</tbody>
</table>

*significantly different than the control

CONCLUSIONS

We did not observe any significant difference in MDA levels and CAT activities between HT and control groups (p>0.05). However, SOD activity was significantly lower in HT group compared to control (24%, p=0.02). Our results indicate that oxidative profile is altered in HT patients compared to control. Other oxidative stress parameters (glutathione, glutathione peroxidase, protein oxidation) will be evaluated in the future experiments in order to determine the complete oxidant/antioxidant status of HT patients. Future studies are needed to determine whether or not oxidative stress has a prognostic value in this pathology of HT.

REFERENCES


INTRODUCTION

Aldo-keto reductases (AKR) are NAD(P)H dependent oxidoreductases that have been best characterized as glucose reducing agents, and have been implicated in diabetic pathophysiology. The first enzyme of the polyol pathway, aldose reductase (AKR1B1) is involved in the molecular mechanisms of glucose toxicity leading to diabetic complications, including microvascular, neurological, and macrovascular disorders, which are responsible for significant increase in morbidity and mortality of diabetic patients.

These enzymes are also known to mediate an inflammatory response to lipid peroxidation metabolic products. Since chronic inflammation is associated with a number of pathological states, AKR1B1 has been implicated in various inflammatory diseases such as atherosclerosis, asthma, uveitis, sepsis, arthritis, periodontitis and several types of cancer related to chronic inflammation.

Recently novel aldose reductase inhibitors based on carboxymethylated mercapto-triazino-indole scaffold have been designed [1]. Among the novel compounds, cemtirestat (Fig. 1) was the most promising inhibitor, with an IC₅₀ in submicromolar range and high selectivity. Physicochemical parameters matching „the rule of five“, along with good water solubility, point to an excellent „drug-likeness“ of cemtirestat.

MATERIALS AND METHODS

Male Wistar rats (8 - 9 weeks, 230 - 250 g) were used. Aldose reductase was isolated from rat eye lenses and its activity was assayed spectrophotometrically by determining NADPH consumption. Experimental diabetes was induced by triple i.p. doses of streptozotocin (30 mg/kg) on three consecutive days. DPPH radical reaction was recorded by spectrophotometry. Hemolysis was evaluated spectrophotometrically on the basis of hemoglobin release. HCT-116 colon cancer cell proliferation was determined by BrdU incorporation assay.

RESULTS AND DISCUSSION

In rat aldose reductase inhibition, cemtirestat only mildly differentiated between glyceraldehyde and the inflammation mediator GS-4-hydroxynonenal used as substrates. In diabetic rats, cemtirestat (50 mg/kg/day, administered i.e. for 5 consecutive days) significantly inhibited accumulation of sorbitol in sciatic nerves.
In the cellular model of isolated erythrocytes, cemtirestat was readily taken up by the cells. Ability of cemtirestat to scavenge free radical species was proved in a DPPH test. The compound efficiently protected isolated erythrocytes against hemolysis induced by t-BuOOH, a model of pathology initiated by reactive oxygen species generated intracellularly. Osmotic fragility of the erythrocytes was not affected by cemtirestat.

In HCT-116 colon cancer cell lines cultivated in the presence of high glucose, antiproliferative effect of cemtirestat was recorded.

CONCLUSIONS
By affecting both aldose reductase and oxidative stress, the compound represents an example of a promising agent for multi-target pharmacology of pathologies related to glucose toxicity in diabetes, and inflammatory disorders related to lipid peroxidation.

ACKNOWLEDGMENTS
VEGA 2/0067/11, and TÜBİTAK 113S006

REFERENCES

P-405: A COMPERATIVE STUDY ON ANTIOXIDANT POTENTIALS OF THE VARIOUS SOLVENT EXTRACTS FROM DIFFERENT PARTS OF MOMORDICA CHARANTIA

S. Yılmaz¹, G. Çokşan², Ö. Bahadır Acıkara³, N. Artık², G. Saltan³, T. Çoban¹

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology¹
Faculty of Engineering, Department of Food Engineering², Faculty of Pharmacy, Department of Pharmacognosy³, Ankara, TURKEY

INTRODUCTION
Oxidative stress is an impaired balance between antioxidant defense system and free radical production resulting in several serious diseases such as asthma, cancer, diabetes and also other cardiovascular, neurological, retinal damages. Antioxidant-rich diet is a way to reduce oxidative stress and boost the immune system to fight free radicals. Momordica charantia is a plant generally found in tropical and subtropical regions of the world. It has several benefits for health and medicinal qualities such as antioxidant, anti-inflammatory, antiseptic, anti-rheumatic, anti-diabetic and anti-carcinogenic. These beneficial effects are probably due to the rich contents of phenolic compounds, triterpenes, proteins, steroids, alkaloids, inorganic compounds and lipids[1].

MATERIALS AND METHODS
This study was conducted to determine the antioxidant activities of the seed and fruit of different fractions of Momordica charantia by the DPPH scavenging and ABTS radical cation decolorization assays. Antioxidant activities of the lyophilized powder and various solvent extracts as methanol, ethyl acetate and hexane of the seed and fruit were analyzed. Each sample was dissolved in both DMSO, methanol and water (distilled/hot) and the results were compared. The radical scavenging activity was measured as a decrease in the absorbance of DPPH, and ABTS* radicals [2-3].

RESULTS AND DISCUSSION
Current study revealed that Momordica charantia have radical scavenger activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals Regarding the scavenging capacity of the ABTS radical, the strongest activity belongs to the methanolic aqueous extracts of fruit (IC₅₀=15 μg/ml) followed by seed extracts (IC₅₀=39 μg/ml) among the tested extracts. IC₅₀ values were calculated for all the extracts as well as trolox which is the reference standard for ABTS (IC₅₀=1.5 μg/ml). In comparison with trolox, the methanolic extract activity of fruit is 10% and seed is almost 25% lower. However, lyophilized aqueous seed extracts are (IC₅₀=117 μg/ml) more active than fruit extracts (IC₅₀=213 μg/ml). According to DPPH scavenging assay, the strongest antioxidant activity of fruit was found by methanolic aqueous extracts (IC₅₀=260 μg/ml) followed by lyophilized aqueous extracts (IC₅₀=416 μg/ml). The highest antioxidant activity of seed was established by lyophilized aqueous extracts (IC₅₀=342 μg/ml) followed by methanolic aqueous extracts (IC₅₀=465 μg/ml) among the tested extracts. IC₅₀ values were calculated for all the extracts as well as BHT which is the reference standard for DPPH (IC₅₀=11 μg/ml). It was generally observed that hexane extracts of seed showed minimum DPPH scavenging and ABTS radical cation decolorization capacity followed by the same extracts of fruit.
CONCLUSIONS
According to the results of our study, it was concluded that the methanolic extracts of the seeds and fruits are more effective than the other forms in the antioxidant activity generally. The noted differences among extracts, as regards the scavenging capacity of ABTS radical compared with DPPH, show the importance of the extraction solvents for the composition of the extracts. The solvents used determine a different report among substances in the extracts having an antioxidant effect, which is manifested through a different scavenging behavior of free radicals[4]. DPPH radical scavenging and ABTS radical cation decolorization activity of the extracts could be due to gallic acid, protocatechuic acid, gentisic acid, catechin, chlorogenic acid and epicatechin compounds in the extracts [5]. However, further studies are needed to investigate which components are exactly responsible for these effects of Momordica charantia.

REFERENCES
5. Horax R, Hettiarachchy N, Chen P. Extraction, quantification, and antioxidant activity of phenolics from pericarp and seeds of bitter melons (Momordica charantia) harvested at three maturity stages (immature, mature, and ripe). J Agric Food Chem. 2010, 14;58(7):4428-33

P-406: SAFE HANDLING OF ANTINEOPLASTIC DRUGS: NURSES’ COMPLIANCE AND AWARENESS

V. Karacaoğlan1, N. Taşdemir2, S. Çelik2, M. Çöplü3, M. Zor5, S. Akgül4

INTRODUCTION
The toxicities of antineoplastic drugs is well known. Occupational exposure to these drugs is highest among pharmacists and nurses that administer these agents. Antineoplastic drugs used in the treatment of cancer, making occupational exposure risk for nurses. Occupational exposure to these drugs which may result in adverse health outcomes. It is recommended that all healthcare workers who involve in manufacture, transport, distribution, receipt, storage, preparation, administration, and even waste management must follow national/international guidelines.

MATERIALS AND METHODS
This descriptive type study aimed to evaluate nurses’ compliance and awareness about standard safety guidelines during the preparation and administrations of antineoplastic medications. The study conducted in a university hospital in West Black Sea region in Turkey. The sample of the study was 52 nurses. Data collected by aself-reported questionnaire consisted 25 questions; socio-demographic characteristic of nurses (10 questions), compliance with national/international safety guidelines (6 questions), compliance with personal safety precautions (5 questions) and negative health outcomes of antineoplastic drugs (3 questions). Data were evaluated by descriptive statistics.

RESULTS AND DISCUSSION
Total 52 nurses enrolled the study. Of the nurses 84.6% were female with a mean age 29.7(±4.1) years (min:21, max:38). The administration rate of antineoplastic drugs was 3.36 (min:1; max: 15) per week. Only 32.7% of the nurses have training about the administration of antineoplastic drug. Ninety-two percent of nurses is not aware of national/international guidelines, 44.2% of them have information about institutional practice guidelines. Nearly half of the nurses (46.2%) indicated that they do not know about having a reporting system in case of any accidental chemotherapy problem.

Personal protective equipments usage evaluated in this study. Double gloving practice rate was 42.3%, protective gown usage rate was 3.8%, mask or face protection usage rate was 3.8% and eye protection equipment/ hair cover usage rate was 1.9%. Acute symptoms of antineoplastic drug exposure are headache, skin irritation, , hair loss, allergic reaction, nausea, and vomiting. In this study nurses
asked about this symptoms; nurses mainly reported that; 63.5%, hair loss (50.0%) skin irritation (44.2%).

CONCLUSIONS
This study aimed to evaluate nurses’ compliance and awareness about standard safety guidelines during the preparation and administrations of antineoplastic medications. Compliance with standard safety guidelines and wearing personnel protective equipments were low and negative health outcomes reported by nurses.

REFERENCES

P-407: CYP2C19*17 POLYMORPHISM AND MAJOR DEPRESSION PATIENTS TREATED WITH CITALOPRAM

Z. Uçkun1, B. Başkak2, H. Özdemir3, E. Tuba Özel Kızıl4, H. Özgüven2, H. S. Süzen4
1 Departmant of Toxicology, Faculty of Pharmacy, University of Ankara, Ankara, Turkey
2 Departmant of Psychiatry, Faculty of Medicine, University of Ankara, Ankara, Turkey
3 Departmant of Psychiatry, Faculty of Medicine, University of Kırıkkale, Kırıkkale,Turkey
4Department of Toxicology, Faculty of Pharmacy, University of Ankara, Ankara, Turkey

INTRODUCTION
Citalopram (CIT) is an efficient and safe selective serotonin reuptake inhibitor (SSRI) antidepressant and is widely prescribed SSRI in treatment of major depression (MD) [1]. CIT is metabolized to desmethylcitalopram (DCIT) and didesmethylcitalopram (DDCIT) by oxidative phase-I metabolic processes. The hepatic clearance is mediated primarily by cytochrome P450 (CYP) 3A4, CYP2C19 (CIT to DCIT), and CYP2D6 (DCIT to DDCIT) [2]. Main enzyme in metabolism of CIT is CYP2C19. Polymorphisms in CYP2C19 gene can affect CYP2C19 activity, and thus affecting CIT metabolism and plasma concentrations of CIT and its major metabolite DCIT. There has been much recent interest in the CYP2C19 genetic polymorphisms. The objective of this study was to determine CYP2C19*17 genetic polymorphisms in a healthy subjects among Turkish population and to investigate effects of CYP2C19*17 polymorphism on citalopram metabolism in patients with major depression.

MATERIALS AND METHODS
The present investigation was conducted in Turkish patients at the Departments of Psychiatry, Faculties of Medicine, Ankara University and Kırıkkale University, Turkey. Fifty patients with major depression were determined according to DSM-IV criteria by psychiatrists. CYP2C19*17 polymorphism analysis (209 healthy individuals and 50 patients for CIT metabolism) were carried out using polymerase chain reaction-restriction fragment length polymorphism techniques. Plasma concentrations of CIT and DCIT were measured by using high performance liquid chromatography.

RESULTS AND DISCUSSION
In this study, the allele frequencies of CYP2C19*1, *17 for patient group and control group were found to be 71.0%, 18.0% and 81.1%, 18.9% respectively. There was no difference between the two groups (p>0.05). These results were in good accordance with the expected genotype distributions, calculated using the Hardy–Weinberg equation (P=0.488). For the control group, we had a comparison of our population and other populations in terms of frequencies of CYP2C19*1 allele and observed that the allele frequency of CYP2C19*17 in the Turkish population (18.9%) was not significantly different from that seen in other European ethnic groups; a similar distribution was found in Swedish (20%), French (18.8%), Danish (20.1%), Greek (19.6%), and Norwegian (22%) populations (p>0.05). In Asian groups (Chinese, Japanese, and Koreans), the CYP2C19*1 allele frequencies (range: 0.3% -1.3%) were significantly lower than seen in Turkish population (p<0.05). For patients, it was identified as 32 (64%) subjects for CYP2C19*1/*1 (wild type) and 18 (36%) subjects for CYP2C19*1/*17 (heterozygous mutant) but not homozygous mutant subjects. In order to be able to assess effeects of CYP2C19*17 polymorphism, we compared mean plasma concentrations of CIT and DCIT, CIT-to-DCIT ratios (metabolic ratio, MR), C/D (concentration-over-dose ratio) between subjects with CYP2C19*1/*1 and those with CYP2C19*1/*17. The results obtained, for subjects with wild type were determined 60.99 ± 32.55 ng/ml, 17.85 ± 10.43 ng/ml, 3.69 ± 1.32 and 2.68 ± 1.42 ng/ml for CIT, DCIT, CIT/DCIT, C/D, respectively. Similarly, for subjects with heterozygous mutant were determined 57.24 ± 26.11 ng/ml, 16.54 ± 9.13 ng/ml, 3.69 ± 0.97 and 2.41± 0.88 ng/ml for CIT, DCIT, CIT/DCIT, C/D, respectively. No differences were statistically significant found between CYP2C19*1/*1 subjects and CYP2C19*1/*17 subjects (p>0.05).

CONCLUSIONS
The allele frequency of CYP2C19*17 in the Turkish healthy population was similar to those of European ethnic groups compared to those of Asian populations. Furthermore, in view of the fact that the above results
are taken into consideration, it was shown that there are no effects of CYP2C19*17 polymorphism on citalopram metabolism.

ACKNOWLEDGMENTS
This study was supported by The Scientific and Technological Research Council of Turkey, (Project No: 109S147).

REFERENCES

P-408: ISOLATION OF LACTIC ACID BACTERIA AND LACTIC ACID BACTERIA PHAGES FROM DIFFERENT SOURCES
A. Gumustas¹, E. Kakabadze, I. Janashia, A. Akin¹, N. Chanishvili²

¹Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, TURKEY
²George Eliava Institute of Bacteriophages, Microbiology and Virology, R&D Department, Tbilisi, GEORGIA

INTRODUCTION
It is well known that lactic acid bacteria (LAB) are participating in various fermentation processes. However, the majority of them is lysogenic (carries phages). This may result in a spontaneous lysis of the product which is associated with great material losses. Therefore, for manufacturing purposes isolation of the phage-free LAB strains or selection of the phage resistant cultures is very important. The aim of this study is isolation of lactic acid bacteria and corresponding phages from different environmental sources and dairy products, investigation of phage-host interactions using these isolates, examination of colony morphologies and study of the phage life cycles.

MATERIALS AND METHODS
In this research, 59 different lactic acid bacteria strains have been used; 18 out of these cultures were isolated from different homemade matsoni, raw milk and cheese samples, 41 strains were kindly provided by Eliava Institute of Bacteriophage, Microbiology and Virology (IBMV) culture collection. The waste water, whey, matsoni (Georgian yoghurt) and raw milk samples were used for isolation of phages. M17 (HiMedia Lab., Mumbai, India) and Lactobacillus MRS (HiMedia Lab., Mumbai, India) media were used for isolation of lactic Streptococci and Lactobacilli respectively. Bacteriophages were isolated from different environmentsl sources according to Clokie et. al. [1]. Mitomycin C test [1] was applied for isolation of phage via induction of lysogens. Spot test [1] was performed to prove lytic activity of phages and study of their host ranges. Double layer agar method [2] was performed for enumeration of phages (titration) in 1 mL of stock.

RESULTS AND DISCUSSION
The phages isolated from matsoni and raw milk samples were named as 2C, 3C, MR3 and SR3. The phages: 2C and 3C phages were active to the following hosts: Streptococcus 6AX and Streptococcus 2B (both were isolated from raw milk), respectively. The titer of 2C phage was 1x10^5 pfu/mL. The results of MC test demonstrated that the straubs Lactobacillus 59, Lactobacillus 53S, Lactobacillus 58B isolated from matsoni were carrying prophages. Due to MC induction two Lactobacillus phages: MR3 and SR3 were isolated using Lactobacillus 58B and 53S strains as host cultures, while Lactobacillus phage MC 59 appeared to be active against Lactobacillus Kd3 strain.

Preliminary characterization of the phages demonstrated that all of them appeared to be strictly corresponding to their host i.e. having extremely narrow host ranges. Further analysis are to be done to ensure that the lysogenic i.e. phage carrier LAB strains may be used for commercial purposes. If necessary, the isolated phages may be used for selection of the phage-resistant mutanst strains.

ACKNOWLEDGMENTS
I would like to thank the George Eliava Institute of Bacteriophages, Microbiology and Virology, (R&D Department), Tbilisi, Georgia for supporting our research.

REFERENCES

P-409: ANTIBACTERIAL EFFECTS OF CINNAMON OIL AGAINST CARBAPENEM-RESISTANT NOSOCOMIAL ACINETOBACTER BAUMANNII ISOLATES
B. Kaskatepe¹, M. E. Kiymaci², S. Suzuk², S. Aslan Erdem³, S. Cesur⁴, S. Yildiz³

¹ Ankara University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, 06100 Ankara/Turkey
INTRODUCTION
There has been an increasing interest to essential oils during recent years because of the need of new therapies against multi drug resistance pathogens. The aim of this study was to determine the antibacterial activity of cinnamon oil against carbapenem-resistant nosocomial Acinetobacter baumannii isolates.

MATERIALS AND METHODS
GC and GC/MS Analysis of cinnamon oil: Cinnamon oil purchased from herbalists was analysed by GC and GC-MS. GC analysis of the essential oils was performed on an Agilent 6890N Network GC system, using a HP Innowax Capillary column with dimensions 60.0 m × 0.25 mm × 0.25 μm. The oil was also analyzed by GC–MS using the Agilent 6890N Network GC system combined with an Agilent 5973 Network mass selective detector. Identity of the individual components in the oils was assigned by comparison of their retention times and mass spectra with previously published data (Adams, 2001) and by comparison of their mass spectra in the Wiley and NIST libraries. Percentage of the components was calculated from the GC peak area using the normalization method.

Antimicrobial activity test: 111 A. baumannii isolates identified as causative agents of nosocomial infections in various hospitals in Turkey between January 2011 and December 2011, were used in the study. Second strains isolated from same patients were excluded. Meropenem and imipenem susceptibilities of these strains were evaluated using the E-test (AB Biodisk, Solna, Sweden) according to CLSI criteria. Antibacterial activity of the essential oils was determined using the disk diffusion assay.

RESULTS AND DISCUSSION
The content of cinnamon oil was determined as 99.54% cinnamaldehyde and benzaldehyde 0.24% by GC and GC-MS analyses. The antimicrobial activity results of cinnamon oil against carbapenem resistant nosocomial A. baumannii isolates are given in table 1.

Table 1. Zone diameters of A.baumannii isolates.

<table>
<thead>
<tr>
<th>Zone diameter (mm)</th>
<th>Acinetobacter baumannii (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-18 mm</td>
<td>2</td>
</tr>
<tr>
<td>19-21 mm</td>
<td>4</td>
</tr>
<tr>
<td>&gt;22 mm</td>
<td>105</td>
</tr>
<tr>
<td>Total (n)</td>
<td>111</td>
</tr>
</tbody>
</table>

The observed zone diameters for cinnamon oil were interpreted based on the antibiotic sensitivity limits of the carbapenem group of antibiotics given in the CLSI standards (CLSI, 2014). Sensitivity limits of meropenem, imipenem and doripenem for A. baumannii isolates are given in Table 2.

Table 2. Sensitivity limits (mm) of carbapenems for A.baumannii according to CLSI standards.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>≥ 18</td>
<td>17-15</td>
<td>≤ 14</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥ 22</td>
<td>21-19</td>
<td>≤ 18</td>
</tr>
<tr>
<td>Doripenem</td>
<td>≥ 18</td>
<td>17-15</td>
<td>≤ 14</td>
</tr>
</tbody>
</table>

CONCLUSIONS
Cinnamon oil shows strong antibacterial activity against carbapenem resistant nosocomial A. baumannii isolates, and these results indicate that this oil is a potential candidate for the development of alternative bioactive agents.

REFERENCES

P-410: IN VITRO ANTIMICROBIAL EFFECTS OF MONOTERPENE ALCOHOLS AGAINST PROPIONIBACTERIUM ACNES AND STAPHYLOCOCCUS EPIDERMIDIS

C. Elmacı1,2, G. İşcan3,4.

1 Anadolu University, Institute of Science, Department of Advanced Technologies, 26470, Eskişehir, TURKEY.
2 Osmangazi University, Institute of Science, Department of Biochemistry, 26480, Eskişehir, TURKEY.
3 Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskişehir, TURKEY.
4 Anadolu University, Yunus Emre Vocational School, 26470, Eskişehir, TURKEY.

INTRODUCTION
Monoterpenes are mostly biologically active and naturally occurring secondary metabolites found in essential oils [1]. They comprise of two isoprene units (C₆H₁₀) and they can occur as volatile hydrocarbons, alcohols, aldehydes, ketones or esters. The biological precursors to the isoprenoids are isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). Monoterpenes are widely used in agriculture, cosmetic and pharmaceutical industries for their flavour and fragrance properties and they are also attracted attention due to their antibacterial,
antifungal, antioxidant, analgesic, anaesthetic, anticancer, anti-spasmodic, hypotensive, and vasorelaxant effects [2].

MATERIALS AND METHODS
In the present study, 22 monoterpene alcohols (aromatic, cyclic and acyclic) were evaluated partly modified The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) methods M11-A8 (Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria) and M7-A7 (Methods For Dilution Antimicrobial Susceptibility Tests For Bacteria that Grow Aerobically) in comparison with ampicillin, amoxicillin, clarithromycin and tetracycline as standard agents [3, 4].

RESULTS AND DISCUSSION
Antibacterial effects of 22 different monoterpene alcohols were examined against Staphylococcus epidermidis and Propionibacter acnes strains that are widely suspected to contribute to the development of acne vulgaris (Fig. 1). Aromatic alcohols Carvacrol and thymol were found as the most effective compounds having a MIC (Minimum Inhibitory Concentration) value of 0,5 mg/mL.

CONCLUSIONS
These results are suggesting that another different monoterpenes such as hydrocarbons, aldehydes, ketones or esters could be tried against the human skin pathogens in combination with unrefined fixed oils. We continue to evaluate combined effects of different type of monoterpenes with checker board method which is performed by the robotic pipetter and automated liquid dispensing system used in high throughput screenings (HTS).

ACKNOWLEDGMENTS
The authors acknowledge Anadolu University Research Fund BAP1501S131 for financial support.

REFERENCES


P-411: IN VITRO ANTIMICROBIAL EFFICACY OF FOUR CONTACT LENS CARE SOLUTIONS

M. Eryilmaz, B. Kaskatepe, M. E. Kiymaci, D. Simsek, H. B. Erol, A. Gumustas
Ankara University, Faculty of Pharmacy
Department of Pharmaceutical Microbiology
Ankara, TURKEY

INTRODUCTION
Contact lenses (CLs) are optical medical devices that used directly on the cornea of the eye. They are primarily used to correct refractive errors as myopia, hyperopia, astigmatism and presbyopia. Although they can be used for therapeutically and cosmetically purposes. Recurrent erosion, metaherpetic ulcers, epithelial defects, keratitis sicca are some of clinical situations that contact lenses used therapeutically. The main cosmetically usage aim of CLs is changing the apparent color of the eye. CLs have significant importance because of transferring microorganisms on the ocular surface. Also commensal microorganisms found on lid margins-conjunctiva and potential pathogens that found transiently on the ocular surface can inoculate CLs. In the presence of reduced tissue resistance, these resident microorganisms or transient pathogens can invade and colonize the cornea or conjunctiva and they can cause serious eye infections. CL-related infections are often associated with imperfect CL hygiene practices, because of that reason; CL care products should be able to sufficiently minimize the amount of pathogen microorganisms. In this study, invitro efficacy of four frequently used contact lens care solutions (CLCSs) were investigated against five bacteria and a yeast, according to the EN ISO 14729 (2001) [1, 2].

MATERIALS AND METHODS
Contact lens care solutions: The CLCSs listed in Table 1 were used. They were assessed before their stated expiration dates and were taken from their original packages.

Test Microorganisms: Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300, Staphylococcus epidermidis ATCC 12228, Staphylococcus epidermidis ATCC 35948 and Candida albicans ATCC 10231 were used in this study.
Table 1. Contact lens care solutions

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Active ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>ReNu® MultiPlus (Bausch+Lomb, USA)</td>
<td>HYDRANATE® (hydroxyalkylphosphonate) % 0.03, boric acid, sodium edetate, % 1 poloxamine, sodium borate, sodium chloride, % 0.0001 DYMEDTM (polymethopropyl biguanide)</td>
</tr>
<tr>
<td>Opti-Free® Express (Alcon, USA)</td>
<td>Sodium chloride, sorbitol, edetate disodium, boric acid, aminomethyl propanol, citrate, TETRONIC®1304, % 0.001 POLYQUAD® (polidronium chloride), % 0.0005 ALDOX® (myristamidopropyl dimethylamine)</td>
</tr>
<tr>
<td>All In One Light (Sauflon, UK)</td>
<td>% 0.0001 polyhexanide, % 0.128 disodium EDTA, % 0.7 sodium chloride, % 0.8 disodium phosphate dodecylhydrate, % 1.0 poloxamer</td>
</tr>
<tr>
<td>Biotrue® Multi Purpose Solution (Bausch+Lomb, USA)</td>
<td>hyaluronan, sulfobetaine, poloxamin, boric acid, sodium borate, edetate disodium, sodium chloride, %0.00013 polyaminopropyl biguanide, %0.0001 poliquaternium</td>
</tr>
</tbody>
</table>

Neutralization / Recovery System: Neutralizer efficacy is important for accurate determination of the efficacy of an antiseptic or disinfectant. Dey-Engley Neutralizing Broth (Sigma-Aldrich, USA) was previously tested to determine whether it was appropriate to inactivate the active ingredients of the contact lens solutions [3].

Quantitative Suspension Test Method: The quantitative suspension test was performed in accordance with EN ISO 14729 (2001) [4, 5].

RESULTS AND DISCUSSION

According to the mean log reduction values of CLCSs for 6 h contact time, Opti-Free® and Biotrue® TM were found the most effective CLCSs that achieved the required 3.0 log reduction for all the microorganisms tested. ReNu® and All In One Light were found effective against all tested microorganisms, except P. aeruginosa ATCC 9027 and C. albicans ATCC 10231.

CONCLUSIONS

In conclusion, Opti-Free® (contains poldronium chloride and myristamidopropylidimethylamine) and Biotrue® TM (contains polyaminopropyl biguanide and poliquaternium) were found the most effective CLCSs against the microorganisms that were tested. Although there are various types of CLCSs in markets, the efficacy of the product depends on the ingredients and concentration of the antimicrobials included. CL wearers should comply with the manufacturer’s labelled instructions for the proper and safe usage of CLCSs.

REFERENCES


P-412: THE EFFECT OF BIO-ACTIVE COMPONENTS CONTAINING ADHESIVE SYSTEMS ON BACTERIAL MICROLEAKAGE: IN-VITRO STUDY

G. Demirel1, G. Gür1,, M. Eryılmaz2, N.Altanlar2

1Ankara University, Faculty of Dentistry, Department of Restorative Dentistry, Ankara, TURKEY
2Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology Ankara, TURKEY

INTRODUCTION

A major reason for dental composite restoration replacement is related to secondary caries promoted by microleakage of cariogenic bacteria such as Streptococcus mutans [1]. While the dental adhesives on the market exhibit a reasonable clinical performance, it is also proposed that bio-active adhesive materials contribute to a better prognosis of restorative treatments. Quaternary ammonium compounds based resin monomers, silver and calcium phosphate nanoparticles, ion-releasing glass fillers and growth factors such as 4-META/MM are added into the resin materials to provide the bioactive properties such as antibacterial, matrix metalloproteinase inhibitory, remineralization and anti-plaque effects [2]. However, only one quaternary ammonium compound, 12-methacryloyloxydodecylpyridinium bromide (MDPB), and one ion-releasing glass filler, surface pre-reacted glass ionomer (S-PRG), containing adhesives are available on the market. Other bio-active components are still in the experimental phase. Thus, the present study aims to assess and compare the effectivity of MDPB and S-
PRG containing bio-active and non-bio-active self-etch adhesive systems on bacterial microleakage (BML) of Class II cavities, in-vitro.

MATERIALS AND METHODS
Forty extracted human molars were used in this experiment. Two proximal box cavities were prepared for each tooth. After the preparations were completed, the teeth were randomly divided into four groups. The restorative materials and the protocols used for the restorations are described in Table 1. All specimens were sterilized by using gamma radiation from a $^{137}$Cs source for 6 hr. The teeth were stored in a dextrose broth culture of $S$. mutans (ATCC 25175) adjusted to a McFarlane Turbidity standard $N=0.5$ $(1.5 \times 10^8 \text{ cfu/ml})$ and incubated anaerobically for 24 hours at $37^\circ$C. After the incubation, the teeth were fixed in a $10\%$ neutral-buffered formal saline solution for 48 hours. All of the specimens were decalcified in $17\%$ formic acid and processed through using the double-embedding method. Serial sections were prepared and four sections were randomly selected from each paraffin block. Bacterial staining was done with modified Gram staining method. The BML on the gingival wall of the restorations was qualitatively evaluated under a light microscope at different magnifications according to a scoring system: 0-no BML, 1-BML up to half of the cavity floor, 2-BML along the entire cavity floor, 3-BML throughout the entire cavity floor, axial wall and dentin tubules. The highest score of the four sections on each restoration was selected for statistical analysis to represent the bacterial microleakage score for the restoration. Obtained data was analyzed using a two-way mixed ANOVA. Bonferroni correction was used for multiple comparisons.

RESULTS AND DISCUSSION
BML through microgaps and the scoring results are shown in Figures 1 and 2 respectively. The statistical analysis indicates that there is no significant difference between each group listed under the Groups 1-3 $(p>0.05)$, or the Groups 2-4 $(p>0.05)$. However, a significant difference is present between the Groups 1-3 and the Groups 2-4 $(p<0.05)$. The incorporation of antibacterial components into an adhesive system such as fluoride and MDPB has been reported to exhibit antibacterial effects against $S$. mutans [3]. But, this fact could not be verified in the present study. The results of the study show that bacteria were present in all of the samples regardless of the materials’ antibacterial property. The most extensive BML was observed in the samples restored with FL Bond II and Clearfil S3 adhesive systems. The antibacterial effect of MDPB after being polymerized is dependent upon contact with the bacteria and FL II Bond adhesive system release fluoride to prevent bacterial microleakage. However, both adhesive systems failed to present superior antibacterial properties probably due to the exposed area being limited to a thin line at the tooth-restoration interface.

### Table 1: The adhesive materials and the protocols used for the restorations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Adhesive System</th>
<th>Material Type</th>
<th>Contenu</th>
<th>Application Procedure</th>
<th>Restorative Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (bio-active self-etch)</td>
<td>Clearfil Protect Bond, Kuraray Medical Inc., Okayama, Japan</td>
<td>Two-component self-etch adhesive</td>
<td>Primer: MDP, HEMA, Hydrophilic dimethacrylate, Water</td>
<td>Primer applied to the entire cavity walls after 20 seconds, the visible ingredients were evaporated with a mild stream.Bond applied to the entire surface of the cavity a gentle stream of air was used. Light-cured for 10 seconds.</td>
<td>Filtek Ultimate composite resins, Shade A2, 3M ESPE, DSL, USA</td>
</tr>
<tr>
<td>Group 2 (bio-active self-etch)</td>
<td>FL Bond II, Shofu Inc.,Kyoto, Japan</td>
<td>Two-component self-etch adhesive</td>
<td>Primer: Water, Ethanol, Carboxylic acid monomer, Photopolymer acid monomer and Initiator</td>
<td>Primer applied to the entire cavity walls after 10 seconds, the visible ingredients were evaporated with a mild stream.Bond applied to the entire surface of the cavity. Light-cured for 10 seconds.</td>
<td>Filtek Ultimate composite resins, Shade A2, 3M ESPE, DSL, USA</td>
</tr>
<tr>
<td>Group 3 (non-bio-active self-etch)</td>
<td>Clearfil SR Bond, Kuraray Medical Inc., Okayama, Japan</td>
<td>Two-component self-etch adhesive</td>
<td>Primer: MDP, HEMA, Hydrophilic dimethacrylate, Hydrophilic methacrylate, Initiator</td>
<td>Primer applied to the entire cavity walls after 20 seconds, the visible ingredients were evaporated with a mild stream.Bond applied to the entire surface of the cavity. Light-cured for 10 seconds.</td>
<td>Filtek Ultimate composite resins, Shade A2, 3M ESPE, DSL, USA</td>
</tr>
<tr>
<td>Group 4 (non-bio-active self-etch)</td>
<td>Clearfil S3 Bond, Kuraray Medical Inc., Okayama, Japan</td>
<td>One-component self-etch adhesive</td>
<td>Primer: MDP, HEMA, BiGMA, Hydrophilic dimethacrylate, Hydrophilic methacrylate, Colloidal silica, Carboxymethyl cellulose, Accelerator, Initiator, Water</td>
<td>Bonding applied to the entire cavity walls after 20 seconds, the visible ingredients were evaporated with a mild stream. Light-cured for 10 seconds.</td>
<td>Filtek Ultimate composite resins, Shade A2, 3M ESPE, DSL, USA</td>
</tr>
</tbody>
</table>

Fig 2: a-Group 1, $S$. mutans are in the cross-sectioned dentin tubules (black arrow), $S$. mutans chains (white arrows) in the adhesive-dentin and adhesive-composite interface. BML score 3, b-Group 2 adhesive-dentin interface, $S$. mutans chains, BML score 2, c-Group 3, $S$. mutans are in the longitudinal-sectioned dentine tubules, BML score 1, d-Group 4, $S$. mutans in the cross-sectioned dentin tubules. K-composite, A-adhesive, D-dentin.)
Fig 3: BML scores on the gingival wall of the restorations.

CONCLUSIONS
Within the limitations of this in-vitro study, it could not be demonstrated that there is an advantage of using antibacterial components containing bio-active adhesives instead of non-bio-active adhesives in preventing BML.

REFERENCES

P-413: EFFECT OF CAPSAICIN ON TRANSCRIPTION FACTORS IN 3T3-L1 CELL LINE

G. Bora¹, M. Berkoz², M. Yıldırım³, O. Turkmen⁴, O. Allahverdiyev⁵

Yuzuncu Yıl University, Faculty of Pharmacy
¹Department of Pharmaceutical Microbiology, ²Department of Pharmaceutical Biotechnology, ³Department of Pharmaceutical Technology, ⁴Department of Pharmacology, Van, TURKEY
⁵Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, TURKEY

INTRODUCTION
Capsaicin is a spicy ingredient of Capsicum annuum and a lipophilic, crystalline, odorless and colorless alkaloid. Although the effect of capsaicin on adipocyte differentiation is well-known, the role of capsaicin on transcription factors while adipocyte differentiation is not clear. The aim of this study is thus to identify and characterize the transcription factors in the process of adipocyte differentiation after the capsaicin treatment.

MATERIALS AND METHODS
In this study, concentration of 0, 50, 100, 150, 200 and 250 µM capsaicin were treated to 3T3-L1 pre-adipocytes in cell culture. MTT cell cytotoxicity, cell viability with trypan blue staining, Lactate Dehydrogenase (LDH) enzyme assay, triglyceride content assay, Glycerol-3-Phosphate Dehydrogenase (GPDH) activity, Oil Red O staining and transcription factors (PPARγ, C/EBPα and SREBP-1c) mRNA levels were investigated in capsaicin induced 3T3-L1 preadipocyte cell line.

RESULTS AND DISCUSSION
Capsaicin treatment decreased cell population growth of 3T3-L1 preadipocytes, assessed with trypan blue staining, MTT test and rising of LDH release proportion. Capsaicin inhibited GPDH activity and intracellular triglyceride content in 3T3-L1 adipocytes in all treated groups in a dose-dependent manner. Oil Red O staining indicated that capsaicin inhibited adipocyte differentiation in 3T3-L1 adipocytes in all treatment groups. In this study, it was revealed that exposing 3T3-L1 preadipocytes and differentiating postconfluentpreadipocytes to different doses of capsaicin decreased PPARγ, C/EBPα and SREBP-1c mRNA levels as compared with their controls without treatment in dose dependent manner. Although, reduction of PPARγ mRNA level was statistical significant, this decrease was not significant in C/EBPα and SREBP-1c mRNA levels. This study demonstrated that capsaicin treatment inhibited the adipogenesis through the down-regulation of transcription factors, especially PPARγ. Alternative mechanisms may involve cell cycle arrest and the induction of apoptosis.

CONCLUSIONS
Since capsaicin is the main component found in hot pepper, the consumption of hot pepper may contribute to the maintenance of body weight and prevent the development of obesity.

REFERENCES
P-414: APOPTOTIC EFFECTS OF TWO ETODOLAC DERIVATIVES, SGK-205 AND SGK-216, ON K562 LEUKEMIA CELL LINE

O. Orun¹, S. Averbek¹, P. Mega Tiber¹, P. Çikla-Süzgün², Ş.G. Küçükgülzel²

Marmara University, ¹ School of Medicine, Department of Biophysics, ² Faculty of Pharmacy, Department of Pharmaceutical Chemistry; İstanbul, TURKEY

INTRODUCTION
More than a decade, non-steroidal anti-inflammatory drugs (NSAID) are accepted as molecular targets for cancer therapy, especially through their inhibitory effects in COX-2 pathway. Etodolac is a NSAID which acts through COX-2 inhibition and shows anti-cancer effects such as prevention of tumor development and metastasis. In this study, we studied apoptotic effects of two etodolac hydrazide derivatives (SGK-205 and SGK-216) on leukemia cancer cell line K562.

MATERIALS AND METHODS
Apoptotic effects of etodolac derivatives were evaluated by applying TUNEL assay 24 and 48 hour after addition of derivatives. K562 cell line was maintained in RPMI, supplemented with 10% FBS, 1% glutamine and penicillin/streptomycin. Drugs were added in increasing concentrations of 5, 10, 15, 25 and 40 μM and apoptosis levels were determined for each concentration at two time points (24 and 48th hours) according to the instructions of the supplier (Apoptag Plus Peroxidase In Situ Apoptosis Detection Kit, Milipore).

RESULTS AND DISCUSSION
Etodolac derivatives [1] SGK-205 and SGK-216 were tested for their apoptotic effects on K562 cells. The base substance, etodolac, was used as a control. Antiproliferative effects of these compounds were previously determined with MTT assay (IC₅₀ values for SGK-205 21.3 and 17.7 μM at the end of 24 and 48 hour incubations, and SGK-216 were 28 and 14.9 μM respectively). Here we investigated apoptotic effects of the same compounds by TUNEL assay. TUNEL assay has indicated that the number of apoptotic cells increased with increasing concentrations of drugs in a dose-dependent manner for both derivatives, SGK-205 and SGK-216 (Table 1 and 2). The cells showed clear morphological indications of necrosis and cell death after 75 and 100 μM concentrations, therefore TUNEL assay was only applied up to 40 μM concentration.

Number of apoptotic cells increased regularly as the concentrations were increased. Apoptotic effects were doubled at the end of 48 h compared to 24 h in small concentrations (5 to 10 μM), but this difference reduced when higher concentrations were added. Both compounds had very similar apoptotic effects on K562 cells.

Table 1: Number of apoptotic cells 24 and 48 hours after the application of SGK 205 compound at different concentrations.

<table>
<thead>
<tr>
<th>Concentrations (μM)</th>
<th>Apoptotic Cells (%) 24 h</th>
<th>Apoptotic Cells (%) 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>54</td>
</tr>
<tr>
<td>15</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>25</td>
<td>61</td>
<td>72</td>
</tr>
<tr>
<td>40</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2: Number of apoptotic cells 24 and 48 hours after the application of SGK 216 compound at different concentrations.

<table>
<thead>
<tr>
<th>Concentrations (μM)</th>
<th>Apoptotic Cells (%) 24 h</th>
<th>Apoptotic Cells (%) 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>58</td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td>66</td>
</tr>
<tr>
<td>25</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>40</td>
<td>80</td>
<td>73</td>
</tr>
</tbody>
</table>

CONCLUSIONS
TUNEL assays indicate that etodolac derivatives SGK-205 and SGK-216 both have apoptotic effects in K562 cells in a dose dependent manner and they show highly toxic effects after 75-100 μM. These results, together with MTT assays, imply that both compounds have dramatic effects in proliferation and apoptosis even at small doses.

ACKNOWLEDGMENTS
This study was supported by Marmara University Research Fund SAG-C-YLP-090414-0082.

REFERENCES
P-415: SYNTHESIS OF SOME NOVEL THIADIAZOLE DERIVATIVES AND EVALUATION OF THEIR ANTIDEPRESSANT-LIKE EFFECTS IN MICE

O. D. Can¹, Y. Özkay², D. N. Yıldız¹

Anadolu University, Faculty of Pharmacy, ¹Department of Pharmacology, ²Department of Pharmaceutical Chemistry Eskişehir, TURKEY

INTRODUCTION
Thiadiazole ring is a 5-membered diunsaturated structure containing 1 sulphur and 2 nitrogen atoms. Among the thiadiazole ring bearing compounds, especially 1,3,4 thiadiazoles have received a great deal of attention in terms of centrally related effects such as antidepressant, anxiolytic, anticonvulsant and analgesic activities [1,2]. In the present work, based on the notable CNS-related activity potential of these compounds, we synthesized some novel thiadiazol derivatives and investigated their antidepressant-like effects.

MATERIALS AND METHODS
For the synthesis of 2-chloro-N-5-substituted-1,3,4-thiadiazole derivatives (1a-1f), appropriate 5-substituted-2aminothiadiazole derivative (50 mmol) was dissolved in tetrahydrofuran (100 mL) and triethylamine (60 mmol) was added. The mixture was cooled in an ice bath and chloroacetyl chloride (60 mmol) was added dropwise with stirring. After addition of chloroacetyl chloride the reaction mixture was stirred for additional 1h at room temperature. The solvent was evaporated under reduced pressure, product was washed with water, dried and recrystallized from ethanol.

For the synthesis of 2-[5-Chlorobenzothiazol-2-ylsulfanyl]-N-5-substituted-1,3,4-thiadiazole derivatives (2a-2f), the compounds 1a-1f (10 mmol) were dissolved in acetone (30 mL) and 5-chlorobenzothiazol-2-thiol (10 mmol) was added and the mixture was refluxed for 8h. After TCL control, the mixture was cooled down, excess of solvent was removed, and resulting precipitate was washed with water, dried and recrystallized from ethanol.

Male Balb-c mice were used for the tests. Control solution, fluoxetine (10 mg/kg), and the test compounds (30 mg/kg) were injected (i.p) for 3 times; 24, 5 and 0.5 h before testing [3]. Antidepressant-like activities of the test compounds was evaluated by modified forced swimming tests (MFST), as described earlier [3]. Spontaneous locomotor activities were monitored for 4 min, in an activity cage apparatus [3].

RESULTS AND DISCUSSION
The structural elucidations of the target compounds were performed by spectroscopic methods and elemental analyses. All compounds gave expected IR, 1H-NMR and MS spectral data and satisfactory elemental analysis results.

In the MFST, administration of the compounds 2a, 2b, 2d, 2e and 2f significantly decreased the immobility times of mice with respect to the control values [4]. The same compounds increased the swimming time of mice in the MFST without any change in the climbing durations. Shortened immobility and prolonged swimming duration, without any change in the climbing time, indicated that, similar to the reference drug fluoxetine (10 mg/kg), antidepressant-like effects of these test compounds may be related to serotonergic, rather than noradrenergic mechanisms in the CNS.

Activity cage tests showed no specific difference between the groups. Absence of test compounds induced-alteration in the locomotor activity values pointed out that the observed antidepressant-like activities are specific.

CONCLUSIONS
This study supports the previous papers reporting the antidepressant-like activities of 1,3,4 thiadiazole derivatives [1,2]. In addition, exhibited antidepressant-like activities seem to be related with the serotonergic system rather than the noradrenergic system. However, involvement of the serotonergic system in the observed antidepressant activity needs to be confirmed with further detailed studies.

REFERENCES
P-416: EVALUATION OF IN VITRO ANTIMICROBIAL EFFICACY OF SOME NEW GENERATION DISINFECTANTS

M. Eryilmaz, B. Kaskatepe, M. E. Kiymaci, H. B. Erol, D. Simsek, A. GümUSTas
Ankara University, Faculty of Pharmacy
Department of Pharmaceutical Microbiology, Ankara, TURKEY

INTRODUCTION
Disinfectants are agents, which are extensively used in hospitals and other health care settings, to inhibit or to destroy microorganisms and consequently to prevent infections. They are also used in many industrial areas. A wide variety of chemical agents like alcohols, phenols, iodine, chlorine compounds have been used for centuries. Although most of them demonstrate broad-spectrum antimicrobial activity at higher concentrations, they have serious side effects for human and also can cause environmental problems at these concentrations. Therefore, safer and better compounds are urgently needed. For this reason researchers try to improve new substances with lack of these disadvantages [1, 2]. New generation disinfectants are defined as products that are completely broken down in nature and don’t leave any harmful residues for the environment. They are also defined as non-carcinogenic products for users. This study was conducted to determine the efficacy of three commercially available new generation disinfectants against some bacteria and yeast [3].

MATERIALS AND METHODS
Disinfectants: Ar-Dez Sniper® (0.2 % chlorine dioxide), Zns-Cloridityoxy® (0.3 % chlorine dioxide) and Pulirex-Oxy® (50 % hydrogen peroxide-stabilized by colloidal silver) were used in this study as new generation disinfectants. Sodium hypochlorite (4.5 %) (45000 ppm) was used as positive control. Sterile distilled water was used as diluent. The disinfectants were stored in the dark at room temperature.

Test Microorganisms: Escherichia coli ATCC 25922, Klebsiella pneumoniae RSKK 574, Pseudomonas aeruginosa ATCC 9027, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300, Staphylococcus epidermidis ATCC 12228, Staphylococcus epidermidis ATCC 35948 and Candida albicans ATCC 10231 were used in this study.

Neutralization / Recovery System: Firstly, Dey-Engley Neutralizing Broth (Sigma-Aldrich, USA) was tested to determine if it was appropriate to inactivate each of the disinfectant [4].

Quantitative Suspension Test Method: The quantitative suspension test was used to determine the efficacy of the disinfectants. Bacterial suspensions in Tryptic Soy Broth (Difco, USA) were adjusted to the McFarland 0.5 standard. Then, 100 μl of bacterial suspension was added to 900 μl of the disinfectant solution at room temperature for contact times of 1, 3 and 5 minutes. At the end of the each contact time 10 μl was removed to 990 μl of the neutralization system and serially diluted to $10^{-1}$ to $10^{-3}$. 100 μl of each dilution was placed onto Tryptic Soy Agar (Difco, USA) plates in duplicate by the spread-plate technique and incubated at 37 °C for 24 hours. Then surviving colonies were enumerated and expressed as cfu per milliliter. Before this procedure, pre-disinfection counts were calculated with using sterile distilled water. The reduction rate was calculated as the expression of the disinfectant efficacy, according to the following formula:

$$\text{Log10 reduction} = \text{Log10 pre-disinfection count} - \text{Log10 disinfection count}.$$ 

Log10 reductions of 5 or more were taken as an indication of satisfactory microbicidal activity [5].

RESULTS AND DISCUSSION
All of the new generation disinfectants were found effective against all tested microorganisms for all contact times. Both chlorine dioxide and hydrogen peroxide are powerful oxidizing agents. Their degradation products are safe for environment but they may cause serious problems in case of the skin contact.

CONCLUSIONS
In conclusion, tested new generation disinfectants showed perfect antimicrobial activity against all tested microorganisms for all contact times. Users should follow with manufacturer’s labelled instructions for the correct and safe usage of these products.

REFERENCES
INTRODUCTION
The purpose of this study was to evaluate the accumulation level of Streptococcus mutans colonization during the four weeks of use of the toothbrush by 187 nursery-kindergarten age children aged between 24-72 months with considering the level of parental education and income and to evaluate the response to disinfectant chlorhexidine gluconate solution.

MATERIALS AND METHODS
187 children (96 in the control group and 91 in the experiment group) age between 24-72 months, who chosen randomly from 600 kindergarten children included this study. In this study, 187 children (96 control, 91 chlorhexidine gluconate group) was selected from 600 nursery-kindergarten children age between 24-72 months. The selected children did not have any dental treatment and antibiotics, antimycotic in the last 3 months. This random distribution of the children were carried out. First, a survey was conducted with parents about their education, employment and income and then the children was examined and their number of dental caries was determined and thier first microbiological cultures were taken. Standart toothbrushes, toothpaste and solutions; distilled water (control group) or%0.12 chlorhexidine gluconate solution (experiment group) which was obtained by dilution of %2 Klorhexidin Irrigation Solution (Drogsan, Türkiye) in spray bottle given for four weeks. Tooth brushes were collect at the end of each week and MS colonization evaluation was done [1].

RESULTS AND DISCUSSION
The decrease in the dmft-T index according to the differences in education and occupation status of the parents were found to be statistically significant (p<0,05), but income and dmft-T relation was found to be statistically insignificant (p>0,05). Reduction of colony count at experimental group was found to be statistically significant (p<0,05).

CONCLUSIONS
Due to the moist environment even in the first brusing the bacteria that accumulated in the surface of toothbrush and will find opportunities to groth very quickly. The finding from this study shows that in order to prevent re-infection and contamination of oral flora by bacterial the toothbrush disinfection is important in terms of preventive medicine and family-child health.

REFERENCES

P-418: EFFECT OF CINNAMALDEHYDE ON BIOFILM FORMATION BY STAPHYLOCOCCUS EPIDERMIDIS
M.E. Kiymaci1, N. Altanlar1, A.Akin1
Ankara University, Faculty of Pharmacy, 1Department of Pharmaceutical Microbiology, Ankara, TURKEY

The aim of this study was to investigate the effect of cinnamaldehyde on biofilm formation by Staphylococcus epidermidis ATCC 35984 at subminimum inhibitory concentration of commercial cinnamon essential oil.

INTRODUCTION
Biofilms are defined as an extracellular matrix which full of adherent microorganism communities. They develop on living or non-living surfaces as a process that includes adhesion, growth, motility and extracellular polysaccharide production. Biofilms act as a physical barrier to protect microorganism from antibiotics, hosts immune system etc. and can cause significant problems in many different areas. Natural products have a widespread usage as traditional medicines. They can be effective as an antimicrobial alternative for the control of microorganisms according to their biological activities [1].

MATERIALS AND METHODS
Essential oil: In this study, commercial cinnamon essential oil which contains 99.54% cinnamaldehyde as a main component, was taken from herbalist and used for determination of inhibitory effect on biofilm formation.

Antimicrobial activity and biofilm assay: The minimum and sub-minimum inhibitory concentrations were determined by broth dilution method. Tween 80 was used at concentration of 0.1% to enhance essential oil solubility in medium. Serial dilutions of cinnamon oil were prepared with Mueller Hinton broth and gather with Staphylococcus epidermidis ATCC 35984, incubated at 37°C for 24 h. The MIC was defined as the lowest concentration of the cinnamon oil inhibiting the growth of bacterial strain.
For determination of biofilm experiment crystal violet (CV) assay was applied; round-bottomed polystyrene 96-well microplate wells were inoculated with 200µl of Mc Farland 0.5 turbidity suspension of Staphylococcus epidermidis ATCC 35984 (these wells were also evaluated as positive control). Staphylococcus epidermidis ATCC 35984 at sub-minimum inhibitory concentration of cinnamon oil, negative control wells were inoculated with Staphylococcus epidermidis ATCC 12228 (biofilm negative strain) and incubated at 37°C for 24 h. Wells that contained sterile medium were used for determine negative strain) and incubated at 37°C for 24 h. Wells for 30 minutes. After CV solution removed, the plate was washed three times by sterile water and air-dried. 200µl of 95% ethanol was added into wells for 30 minutes at room temperature and the absorbance was measured at 540nm [2].

RESULTS AND DISCUSSION
Depending on results, cinnamon oil that contains cinnamaldehyde 99.54% had an antimicrobial activity against Staphylococcus epidermidis strain and very effective on its biofilm formation. According to the CV assay, Staphylococcus epidermidis ATCC 35984 (biofilm producer) was classified as moderately adherent at the end of the incubation period. Furthermore under the sub-MIC concentration of cinnamon oil, biofilm formation of proposed strain was reduced and classified as weakly adherent [3]. All tests were performed four times and the precision of this method was shown as relative standard deviation (2.27%).

CONCLUSIONS
In conclusion, commercial cinnamon oil shown to be effective on biofilm culture of Staphylococcus epidermidis ATCC 35984. In addition to this results, cinnamon oil may use as a natural alternative resource for the development of new anti-biofilm agent, depending on its content.

REFERENCES

P-419: ANTIMICROBIAL PROPERTIES OF THYME AND SAGE OILS AGAINST SOME PATHOGENS
B. Kaskatepe1, M.E. Klymaci1, S.Suzuk2, A.N. Yazgan3, S. Aslan-Erdem1, S. Cesur4

1. Ankara University Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara/Turkey
2. Public Health Institution of Turkey, National Antimicrobial Resistance Laboratory, Ankara/Turkey
3. Ankara University Faculty of Pharmacy, Department of Pharmacognosy, Ankara/Turkey
4. Ankara Training Hospital, Department of Infection Diseases, Ankara/Turkey

The aim of this study was to evaluate the effect of thyme and sage essential oils against some pathogen microorganisms and investigate the essential oil profile by GC/MS.

INTRODUCTION
Due to drug resistant pathogens, new and alternative antimicrobial sources are sought for successful treatment of infectious diseases. Essential oils produced by plants and their rich biologically active components are widely used in different areas like folk medicine, perfumery and food industry, etc. due to their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties.

MATERIALS AND METHODS
Bacterial strains: Eleven collection strains, Escherichia coli ATCC 25922, Escherichia coli ATCC 35218, Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 43300, Staphylococcus epidermidis ATCC 35984, Staphylococcus epidermidis ATCC 12228, Candida albicans ATCC 10231, Candida albicans ATCC 033, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa ATCC 3027, Bacillus subtilis ATCC 6633 obtained from American Type Culture Collection were used in this study. Stock cultures of all strains were maintained on Tryptic Soy Broth (Merck, Germany) at 4°C.

Essential oils: The essential oils (obtained from two different species of thyme and two different species of sage) investigated in this study were purchased from two different companies that produce commercial essential oils. Before the analysis, samples were coded as Thyme oil 1, 2 and Sage oil 1, 2. Composition of the oils were analyzed by GC and GC/MS. GC analyses of the essential oils were performed on an Agilent 6890N Network GC system, under the following conditions: column, HP Innowax Capillary;
60.0 m x 0.25 mm x 0.25 μm; injector temperature: 250°C; detector (FID) temperature: 250°C; carrier gas: He; split ratio: 60:1; and injected volume: 1.0 μL. The oil was also analyzed by GC–MS using Agilent 6890N Network GC system combined with Agilent 5973 Network mass selective detector.

The GC conditions were given in following order. Column: HP Innowax Capillary (60.0 m x 0.25 mm x 0.25 μm); injector temperature, 250°C; carrier gas, helium; column flow, 1.2 mL/min; split ratio, 60:1; injected volume, 1.0 μL. MS conditions were regulated as follows; ionization energy: 70 eV and mass range: 34–450 atomic mass units.

Identification of the components in the essential oils was assigned by comparison of their retention times and mass spectra with corresponding data [1] and by comparison of their mass spectra with Wiley and Nist essential oil libraries. Percentages of the components were calculated from GC peak areas using the normalization method.

**Antimicrobial and antifungal activity:** Antimicrobial and antifungal activity of four different commercial oils (two thyme and two sage oil) were determined by disk diffusion method [2]. For each oil, a volume of 15μl was impregnated into sterile antimicrobial blank paper disks (6mm diameter) and placed onto Mueller-Hinton agar plate which was inoculated with 100μl of Mc Farland 0.5 turbidity suspension of each microorganism. The plate was incubated at 37°C for 24 h and zone of inhibition measured by vernier caliper. Each assay was performed in duplicate.

**RESULTS AND DISCUSSION**

According to GC-MS data; carvacrol (25.49%) was the main component of Thyme oil 1 (Talya), followed by p-cymene, γ-terpinene and β-bisabolene while carvacrol (51.39%) was the main component of Thyme oil 2 (Tabya), followed by linalool and p-cymene. α-Pinene (82.08%) was the main component of Sage oil 1 (Talya) and α-thujone (28.33%) was the main component of Sage oil 2 (Tabya), followed by eucalyptol, β-pinene and caryophyllene. The sensitivity to the present oils was classified as follows [2, 3]:

- Resistant (−) for total diameter smaller than 8 mm
- Sensitive (+) for total diameter 9–14 mm
- Very sensitive (+++) for total diameter 15–19 mm
- Extremely sensitive (++++) for total diameter larger than 20 mm

### Table 1. Zone of inhibition diameters of microorganisms for each essential oil (in mm)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Thyme oil 1</th>
<th>Thyme oil 2</th>
<th>Sage oil 1</th>
<th>Sage oil 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli ATCC 25922</td>
<td>30</td>
<td>40</td>
<td>No zone</td>
<td>16</td>
</tr>
<tr>
<td>E.coli ATCC 35218</td>
<td>30</td>
<td>40</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>S.aureus ATCC 29213</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>S.aureus ATCC 43300</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>S.epidermidis ATCC 35584</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>S.epidermidis ATCC 12228</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>P.aeruginosa ATCC 27853</td>
<td>20</td>
<td>24</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>P.aeruginosa ATCC 9027</td>
<td>19</td>
<td>30</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>B. subtilis ATCC 6633</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>C.albicans ATCC 10231</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>C.albicans ATCC 033</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

**REFERENCES**


**P-420: ANTIMICROBIAL ACTIVITIES OF ACTINOBACTERIA ISOLATED FROM ALGERIAN SAHARA SOILS**

M. Harir1, M. Bellahcene1, Z Fortas1

1 Es-Senia University, Faculty of Sciences, Department of Biotechnology, Oran, ALGERIA

**INTRODUCTION**

Actinomycetes naturally inhabit soils and have a great importance in biotechnological processes due to their ability to produce a large number of antibiotics and other bioactive secondary metabolites. The search for such substances of microbial origin is largely based on the isolation, from diverse sources, of different strains. In addition, one of the strategies for enhancing the probability of obtaining particular isolates and secondary metabolites is to analyse ecosystems.
subjected to extreme conditions and consider genera of actinomycetes poorly studied in the past. Several researchers found that extreme environments were inhabited by micro-organisms especially adapted to these ecological niches. So, a lot of these micro-organisms may represent new taxa and, thus, can provide a valuable resource for their use in future biotechnological processes [1]. In the last years, the discovery of new actinomycetes and new metabolites found in microbiologically poorly explored areas worldwide suggests that a careful exploration of new habitats might continue to be useful for the above-mentioned purposes [2]. In the present work, we describe the isolation of actinomycetes strains from Sahara soil samples, their antimicrobial activity against pathogenic microorganisms.

MATERIALS AND METHODS
Thirty-two actinomycetes were isolated from 6 soil samples collected from Algerian Sahara soils in searching for new antimicrobial secondary metabolites producers. All the isolates were further subjected to antimicrobial screening against pathogenic bacteria, yeast and fungi. After that the 0) active isolates named (C; Ms1and 10) were identified by using morphological, biochemical and physiological methods. In addition, the production of antimicrobial secondary metabolites from the selected isolates (i.e. C, MS1 and 10) was investigated.

RESULTS AND DISCUSSION
The obtained results indicated that three of the isolates (named C, MS1 and 10) showed antimicrobial activity against most of the pathogenic bacteria tested, the yeasts and the phytopathogenic fungi Fusarium culmorum, Fusarium oxysporum f sp albidinis and Verticillium dahliae. Consequently, the three promising isolates were identified as Streptomycyes by morphological, biochemical and physiological methods. Most antimicrobial tests showed that the three isolates had antimicrobial activity against different Gram-positive and Gram-negative pathogenic bacteria and three phytopathogenic fungi. After fermentation in ISP2 medium and extraction the crude extract of isolate C was tested by the well-diffusion method on Muller Hinton medium (MHA) and results revealed that the maximum zone of inhibition was recorded against C. albicans (17 mm) followed by M. luteus and K. pneumoniae (15 mm). For the strain MS1, its crude extract was also active against bacteria and fungi but much less active than strain C. The strain 10 exhibited an antimicrobial activity higher than the strains C and Ms1, especially against P. fluorescens (36 mm). These differences can be attributed to their different chemical structures, their disintegration during the extraction process and environmental factors (temperature and pH of the crude extract). It is worthy to mention that the crude extracts showed antimicrobial activity against Gram-negative bacteria since; in general, they are more resistant to antimicrobial compounds than the Gram-positive bacteria [3].

CONCLUSIONS
The obtained results highlight the use of actinomycetes, in particular extremophile actinomycetes, as a source of novel antimicrobial compounds which could be likely used as new antibiotics after proper pharmacological evaluation. Future research will be required to indentify the produced antimicrobial compounds, which will involve their purification and the use of different chemical analysis such as HPLC, spectroscopy and other sophisticated techniques. This will be pursued in the next future in our laboratory.

REFERENCES

P-421: ANTIBACTERIAL EFFECT OF CORIANDER (CORIANDRUM SATIVUM L.) SEED EXTRACT AND SYNERGISTIC INTERACTION WITH CEFOTAXIN

N. ildiz1, A. Baldemir2, Y. Konca3, H. Özbilge4

1 Erciyes University, 2 Faculty of Pharmacy, Department of Pharmaceutical Microbiology  
3 Faculty of Agriculture Department of Animal Science,  
4 Faculty of Medicine Department of Microbiology, Kayseri, TURKEY

INTRODUCTION
Coriander (Coriandrum sativum L.) is a well-known umbelliferous annual plant of parsley family. The seed of coriander in the form of whole, ground or extract, are used primarily as a flavoring agent in the food industry or as spice in the kitchen [1]. Recent research demonstrated that herbal extract from some plant can prevent pathogenic microbial growth in poultry intestine [2]. In this study, we hypothesized that coriander extract would have antimicrobial effects on intestinal flora of hens resulting from its effects in vitro. In addition, in order to test this hypothesis that in vitro and in vivo bacterial counts can be influenced by coriander seed extract.
**MATERIALS AND METHODS**

Coriander (*Coriandrum sativum* L.) seeds (Aslanbey breed) were harvested from plants grown in Faculty of Agriculture at Erciyes University in 2012.

**Test organisms:** *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* NJ-1 ATCC and three clinical strains methicillin resistant *S. aureus* (MRSA), extended spectrum beta lactamase producing *E. coli* and *Klebsiella pneumoniae* were used to evaluate antibacterial effects of coriander seed extract in *vitro* condition using disc diffusion method.

**Extract Preparation:** The extraction of seed was performed using methanol (ME) and petroleum ether (PE) as solvents. The yield of coriander seed was determined to be as 3.0 and 3.3% for ME and PE, respectively.

**Animal experiments:** Ninety laying hens (Hyline-5 White, 58 weeks old) were randomly assigned into 5 treatment groups with 6 replicates of 3 layer hens each (18 laying hens per group) and fed diets supplemented with 0, 1, 2.5, 5 or 10 % coriander seeds for 10 weeks. Immediately a section of ileum was removed and transported in Thioglycollate broth (BD, USA) medium.

**Antibacterial activity assay:** Extracts were dissolved in DMSO and 312.5, 625, 1250, 2500, and 5000µg/ml concentrations were prepared and disc diffusion test was performed according to CLSI standards. All concentrations of extracts were replicated three times and average values were taken. Ileal samples were inoculated into different media.

**RESULTS**

The PE extract had a higher antibacterial activity against *E. coli* and *S. aureus* standard strains compared to methanol extract (p<0.01). However, the ME extracts affected all standard strains. No inhibition zone was detected in the lowest concentration (312.5 µg/ml) of all extracts of coriander seed against *S. aureus* and *E. faecium*. The ME, PE and their combinations with cefoxitin were also studied on clinically resistant strain of MRSA, *E. coli*, *K. pneumoniae* strains by the disc diffusion method. The inhibition zones were detected in only 1250 µg/ml for ME and PE extracts, no inhibition was detected in 625µg/ml. The combination of cefoxitin+1250 µg/ml PE showed significantly higher inhibition zone than other groups for *K. pneumoniae* isolate. The microorganism counts were significantly decreased in the ileum by coriander seed. This decrease was correlated with an increase of proportion of coriander seeds in the diet (p<0.01).

**DISCUSSION**

According to literature; there are very few *in vitro* antimicrobial studies showed the effectiveness of coriander essential oil [3]. In this study, it was found that a synergistic effect, when coriander extracts and cefoxitin used together. This synergistic behavior was determined more visible in clinically resistant *K. pneumoniae* strain. Recently, herbal extracts are utilized widely, and significant effects are obtained to prevent growth of harmful microorganism in intestine of poultry [2].

**CONCLUSION**

This is the first study where coriander seeds have been found effective *in vivo* and *in vitro* against three different clinically resistant strains. Extracts of *C. sativum* revealed antibacterial activity against both Gram positive and negative bacteria *in vitro* and *in vivo* conditions. Also, cefoxitin effectiveness increased with coriander extracts. Therefore, coriander seed extracts can be suggested to use as antimicrobial agent against pathogenic and standard strains of Gram positive and negative bacteria.

**ACKNOWLEDGEMENTS**

We would like to thank Erciyes University Agricultural Research, Pharmacy Faculty and Application Center (ERUTAM) for support and animal care. We would also like to thank Mr. Ufuk Ince and Mrs. Selen Ilgun for their technical assistance in the laboratory and Dr. Md. Mahmodul Hasan Sohel for editing.

**REFERENCES**


**P-422: MICROBIOLOGICAL QUALITY CONTROL OF THE VETERINARY INJECTABLE VITAMIN PREPARATIONS MANUFACTURED IN TURKEY**

Ş. Öztürk, S. Yıldız

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ankara, TURKEY

**INTRODUCTION**

To achieve convenient qualified of human and veterinary injectable preparations; GMP, Quality Control, Quality Management and Quality Assurance elements are considered as a whole and must be implemented in harmony [1]. In the framework of
these rules sterility conditions are needed for veterinary injectable drugs. Regarding these requirements, in this study it is intended to be made microbiological quality control of injectable vitamin preparation supplied by the companies which produce veterinary drugs in Turkey.

MATERIALS AND METHODS
50 veterinary injectable preparations have been studied which 23 of them are fat-soluble, 27 of them are water-soluble that used in the market. Sterility control of these preparations are performed by membrane filtration method. Sterility control was carried out under aseptic conditions using a closed filtration system. (Vacuum Filter Holders Sterility Testing System) For this purpose, Fluid Thioglycollate Medium (FTM) was used to the growth of anaerobic bacteria and Triptic Soy Broth (TSB) medium was used according with the development of aerobic bacteria and fungi. The presence of microbial growth have been identified by waiting media in the oven for 14 days. Firstly, bacteria isolated from media that observed microbial growth was primarily examinedGram characteristic by made Gram staining and identification were performed using conventional bacteriological methods.

RESULTS AND DISCUSSION
In our study 5 injectable preparations was observed growth from 50 of them that was identified as Bacillus polymyx, Bacillus brevis and Staphylococcus aureus that cause contamination in pharmaceutical. It is intended these bacteria have been caused by from the air, from the production site, from equipment and personnel during the production. In our study; it was concluded that is not sufficient necessary technical use and is not shown the necessary attention during production of veterinary injectable vitamin preparations produced in Turkey that should be sterile.

CONCLUSIONS
In this study; it is considered that microbial contamination exists at an important level in veterinary parenteral vitamin preparations by the filtration method. It is probable that animal losses after applying veterinary drugs which does not meet the sterility conditions and has microbial contamination. Creating serious losses in economic terms has brought to focus with precision on this issue. Due to the measures to be taken according to the results obtained from this study, potential risk factors modification may be done according to drug delivery. In this regard, pharmaceutical production centers are responsible for validate their conditions and methods and it also necessary to thicken audits and controls by relevant government agencies about this subject.

REFERENCES

P-423: PLANT EXTRACTS AS A CONTROL AGENT OF ASPERGILLOSIS IN HUMAN AND AFLATOXIN FORMATION IN FOOD
Y. Alptekin
Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection, 46100, Avşar, Kahramanmaraş

The genus Aspergillus comprises about 250 species possessing various secondary metabolites that can affect our daily life negatively. Contamination of certain crops and our indoor and outdoor environment with the toxic and carcinogenic aflatoxins is a serious concern for agriculture and animal and human health. The predominant species associated with this aflatoxin contamination of crops is Aspergillusflavus. Aflatoxins produced by this fungus are often responsible for invasive aspergillosis, cutaneous aspergillosis and aspergilloma in humans. Once is ingested, aflatoxins may cause a mycotoxicosis that can result in an acute or chronic disease such as reduced growth and development, immunosuppression, and cancer are chronic conditions. Certain plant extracts and essential oils have been demonstrated to show antifungal effect against Aspergillusflavus.

INTRODUCTION
Aspergillus is a genus of mold found Worldwide, commonly in soil, decaying vegetation, and indoor environments ([1].Aspergillus consisted of about 250 described species in nine distinct telemorph[2]. Some species in the genus Aspergillus is plant and/or animal pathogens. For example, all animal diseases caused by the growth of any member of the genus called ‘Aspergillosis’ [3]. After the first report of Aspergillosis in human by Bennett in Edinburg in 1842, approximately 20 species have been reported as causative agents of opportunistic infections in humans. Aspergillus species can also cause allergic reactions, and toxicoses in humans. Among the opportunistic pathogens, Aspergillusflavus followed by Aspergillusflavus and Aspergillusniger are the most often isolated species. The other less commonly isolated species are Aspergillusclavatus, Aspergillusglaucaus group, Aspergillusnidulans, Aspergillusoryzae, Aspergillusterreus, Aspergillusstutus, and Aspergillusversicolor. Several species belong to Aspergillus section flaviproduce aflatoxin and aflatoxin B1 is the most
MATERIALS AND METHODS
The materials of this study are published papers in periodicals in ScienceDirect and Google Scholar search engine between year 2000 and 2014. The articles related to this study have been read carefully summarized and some were cited.

RESULTS AND DISCUSSION
As a result of this review, 150 articles were read that has keywords such as Aspergillus, aflatoxin, aspergillosis, carcinogenic, plant extracts and essential oils. The results show that plant extracts and essential oils are attracting an increasing attention due to their potential as a natural antifungal agent in human and animal diseases and in controlling aflatoxin formation in food and feed.

CONCLUSION
Contamination of food and our environment with Aspergillus spp. carry a risk for humans and animals health due to its potential to produce aflatoxin that can cause various acute and chronic diseases. Nowadays, studies suggested that certain plant extracts and essential oils can be safely used as an antifungal agent.

REFERENCES
statistically no significant difference was determined in terms of susceptibility test results ($p = 0.230>0.05$). According to the comparison of test results of amoxicillin (87.5%) and amikacin (33.3%) a statistically significant difference was determined ($p = 0.000$). The sensitivity of E.coli to imipenem (100%) and amikacin (87.5%) were subjected to the test, as a result the difference between the imipenem and amikacin was found to be statistically significant ($p = 0.017$).

There was no difference between the sensitivity of E.coli against aztreonam (44.7%) ceftazidime (44.7%) ($p = 0.818$). Likewise statistically no difference was detected between cefuroxime (38.5%) and cefazolin (38%) antibiotics ($p = 0.968$). The obtained results from sensitivity test of Klebsiella pneumoniae bacteria were ranged as follows: Ampicillin/Sulbactam(%13.04) antibiyotiği ile Cefazolin (%26), ($p=0.258>0.05$); Cefepime (%30),($p=0.292>0.05$); Levofoksasin (%26),($p=0.281>0.05$); Trimethoprim/Sulfamethoxazol (%30),($p=0.144>0.05$); Ceftazidime (%30),($p=0.144>0.05$) ve Gentamicin(%35), ($p=0.074$) When antibiotic types were separately subjected to comparison test, statistically there was no difference between the ratios. However, when the exhibited sensitivity to Ampicillin/Sulbactam (%13.04) antibiotic compared with the that of Amikacin (%96), Imipenem (%61), Meropenem (%61) statistically a significant difference was detected ($p=0.00<0.05$). Statistically there was found to be no significant difference between the Amikacin (%96.5) and Cefepime (%30) ($p=0.000$). Statistically there was a significant difference between the sensitivity level of Amikacin (%87.5) and Trimethoprim/Sulfamethoxazol (%30) ($p=0.000$). Whereas, when we compared the percentage of sensitivity of Imipenem (%61) and Meropenem (%61), statistically no significant difference was observed ($p=1.000$). According to the comparison of sensitivity ratio of Acinetobacter Baumanibacteria against Colistin-to which it is most sensitive with rate of 100%-between Amikacin(%6 Ampicillin/Sulbactam (%6), Cefoperazone/Sulbactam (%6), Ceftazidime (%6), Ciprofloxacın (%6 Gentamicin (%6), Levofloxacın (%6 Piperacillin/Tazobactam(%6), statistically significant difference was found ($p<0.05$). Moreove according to the ratio test which was carried on between sensitivity ratio of Acinetobacter baumann to Colistin and all the other antibiotics, statistically significant difference was determined ($p<0.05$). Furthermore, again between the sensitivity of Acinetobacter baumanii bacteria to Amikacin(%5.4 and Ampicillin/Sulbactam(%5.9) and in the same we to Ciprofloxacın(%5.9) and Gentamicin(%5.4) antibiotics, statistically no significant difference was found ($p=1.000$). In spite of this, when the sensitivity ratio of amikacin and ceftriaxone (0%) was compared, statistically there was a significant difference between two ratios ($p=0.303$).

**CONCLUSIONS**

According to the results of this study, it can be clearly seen that development of resistance against Ampicillin/Sulbactam is more than that developed against Cefoperazone/Sulbactam, Cefazolin, Cefepime, Levofloxsasin, Trimethoprim/Sulfamethoxazol, Ceftazidime ve Gentamicin antibiotics.

The developed resistance to amoxicillin is more than that developed with amikacin. Ceasing or controlled restriction of the use of antibiotics particularly such as Ampicillin / Sulbactam to which a high resistance has been developed by bacteria may constitute a measure against the elevation of resistance level.

Avoiding the prescription of antibiotics without performing antibiogram test in healthcare organizations among the measures that can be taken against this issue. Determining a health policy by taking the antibiotic resistance percentages into consideration for antibiotic use in all patients especially in those whose immun system suppressed by various factors would reduce the rate of resistance development against antibiotics and prevent the useless effort to perform the unconscious treatment with antibiotics to which resistance has been developed.

**Keywords:** immunosuppressive, bacterial resistance, antibiotic susceptibility

**REFERENCES**


**P-425: STUDIES ON THE SYNTHESIS OF SOME NEW BENZAMIDE COMPOUNDS AS ANTICANCER AGENTS AND HDAC INHIBITORS**

O. Bozdağ-Dundar, B. Torun

Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, TURKEY

**INTRODUCTION**

Histone deacetylases (HDACs) are a class of enzymes with a predominant role in regulating gene expression through a chemical modification to DNA-associated
proteins known as histones. Inhibition of HDAC enzymes has been identified as one of the promising approaches for cancer treatment [1]. Entinostat is a selective HDAC inhibitor that has been well-tolerated in clinical trials to date, belongs to benzamide class among HDAC inhibitors [2]. There is growing interest in using naturally occurring compounds as potential cancer chemopreventive agents in human populations. Resveratrol, trans-3,5,4’-trihydroxy-trans-stilbene is a phytoalexin produced by plants, resveratrol which affects the processes underlying all three stages of carcinogenesis; namely, tumor initiation, promotion and progression. It has also been shown to suppress angiogenesis and metastasis. Extensive data in human cell cultures indicate that resveratrol can modulate multiple pathways involved in cell growth, apoptosis, and inflammation [3].

MATERIALS AND METHODS
To a suspension of 1,1’-CDI in THF was added stilbene methanol in THF at 10°C and the mixture stirred for 2 h at room temperature. The resulting solution was added to a suspension of 4-(aminomethyl)benzoic acid, DBU and TEA in THF. After stirring for 5h at room temperature, the mixture was evaporated to remove THF and then dissolved in water. The solution was acidified with HCl (pH=5) to precipitate a white solid which was collected by filtration, washed with water and methanol, respectively, and dried to give stilbene carboxylic acid. To a suspension of stilbene carboxylic acid in toluene was added DMF and oxalyl chloride and the mixture stirred for 4 h at room temperature. The precipitate was collected by filtration, washed with diethylether and dried. The precipitate was dissolved in THF and was added imidazole in THF. The mixture stirred for 1 h at room temperature. The precipitate was filtrated. The filtrate was added o-substitutedphenylamine and trifluoroacetic acid. The mixture was stirred for 15 h at room temperature. The crude product was purified by column chromatography

RESULTS AND DISCUSSION
In this study, in view of the anticancer property of these pharmacophores, in order to increase the activity of stilbene ring, a new series stilbene derivatives containing benzamide structure have been synthesized as seen in below Formula. The structural evaluation of the synthesized compounds was based on the ^1H NMR, Mass and elementary analysis data. We are expecting that compounds can bind the enzymes active site and make the inhibition of HDAC.

CONCLUSIONS
Their docking studies are shown that they have enough interactions for the enzyme inhibition. The synthesized compounds are going to be investigated for their anticancer and HDAC inhibitory activities.

ACKNOWLEDGMENTS
This work was supported by The Scientific and Technological Council of Turkey (TUBITAK), Turkey (Project No: 213S097).

REFERENCES
1-Kalyaanamoorthy, S.; Chen, Y.P. P., Biochimica et Biophysica Acta 2013, 1834, 317–328
AUTHOR INDEX
Behret, L. OP-4, P-354
Bektas, N. P-123, P-126, P-127
Bektas, S. P-54
Bellduz, A. O. OP-11
Belhattab, R. P-320
Bekassam, A. P-331, P-332
Bellahcene, M. P-142, P-420
Belveren, S. P-187, P-226
Benaiissa, O. P-372
Bendaoud, A. O. OP-11
Belhattab, R. P-320
Belkassam, A. P-331, P-332
Bellahcene, M. P-142, P-420
Belveren, S. P-187, P-226
Benaissa, O. P-372
Benayache, F. P-372
Benayache, S. P-372
Bendjeddou, D. P-367
Benmeddour, T. P-308
Bensabri, Z. P-367
Bentamen, A. P-372
Berk, B. P-249
Berkoz, M. P-141, P-150, P-151, P-413
Berk, B. P-249
Biber, A. P-168
Bicha, S. P-372
Bildirici, I. P-201
Bilgen, S. OP-20, P-402
Bilgen Sokmen, B. P-157, P-158
Bilka, F. P-167
Bindo, S. P-374
Bingol Oztakin, O. P-195
Bingol Ozakpinar, O. P-214, P-279, P-333
Birman, H. P-181
Birtokos Tan, S. P-279, P-333
Bisits, L. P-279, P-306, P-333
Biver, T. P-48
Biyik, B. P-347, P-348
Boga, M. P-18, P-276, P-277, P-311, P-312, P-334
Boluk, A. P-66
Bora, G. P-115, P-141, P-413, P-424
Bora, Y. P-424
Borchard, G. PL-16
Borlinghaus, J. PL-26
Borotna, A. PL-18
Bortzel, J. P-371
Bosgelmez Tınak, G. P-233
Boukoula, A. P-25
Boyaci, I. H. P-55
Boylug, Z. E. OP-35, P-199
Boynurgri, S. P-34
Bozal Palabiyik, B. P-7
Bozdag Dundar, O. P-208, P-217, P-230, P-240
Bozdöveci, A. P-227, P-330
Bozkir, A. P-67, P-83
Bozok, A. P-152, P-153
Bozok, D. E. OP-11
Budak, E. P-67
Buken, J. P-302, P-303
Buhraraliloglu, C. K. P-122
Buler, E. P-47
Bulus, D. P-403
Bulut, F. OP-4, P-356
Burdur, O. T. P-131
Burgaz, S. P-394
Butcher, R. J. P-209
Buyuk, A. S. P-114
Buyukbingol, E. OP-14
Buyukbingol, Z. OP-14
Buyukkilic, B. P-345

C
Caglayan, G. P-5
Caglayan, M. G. OP-13, P-11, P-31
Calak Haberdar, B. P-293
Cakir, K. P-109
Cakir, O. P-25
Cakmak, N. P-118
Calgin, Z. OP-25, P-255, P-256
Calis, I. P-299, P-300
Caliskan, C. OP-22, P-72, P-73, P-75, P-76
Camca, G. R. P-83, P-101
Can, E. P-11
Can, N. O. P-33, P-36, P-50
Can, O. D. P-415
Canançakanat, N. P-154
Canayakın, D. P-149
Can Eke, B. P-399
Cankaya, Z. A. P-67
Canturk, Z. OP-204
Canturk Talmaz, R. Y. P-43
Catak, A. P-246
Cavojsky, T. P-167
Cayci, M. K. P-373
Caylar, M. P-29, P-30
Cebe, D. B. P-88
Cecen, P. P-122
Ceken Toptanci, B. P-175
Celebi, M. P-58
Celebier, M. P-34, P-35
Celenk, S. P-417
Cep, E. P-290, P-291, P-321
Cep, E. P-417
Celik, B. OP-17
Celik, H. OP-23, P-32
Celik, M. P-74
Celik, S. P-406
Celik, T. P-391, P-392, P-400
Celikker, A. P-129, P-130
Cemiloglu Ulker, O. OP-34
Cesur, S. P-419, P-409
Cete, S. P-160, P-161, P-162, P-163
Cetin, A. P-201
Cetin, A. P-374, P-375
Cetin, D. P-5
Cetin, K. P-91
Cetin, M. P-61, P-70, P-78, P-105
Cetin, N. M. P-85
Cevher, E. P-82
Ceyhan, M. S. OP-33, P-377, P-404
Ceylan, M. P-170, P-171, P-172, P-173, P-174
Ceylan Isik, A. F. OP-30
Ceylan Unlusoy, M. P-208
Chankvetadze, B. PL-8, OP-9, P-20, P-26, P-56
Chankvetadze, L. P-20
Chessrasag, M. P-290, P-291
Ciccek, K. P-202
Ciccek, M. P-121
Ciccek Polat, D. P-346
Ciftio, G. A. P-144
Ciftio, O. P-375
Ciftio, U. P-393
Cikla Suzgun, P. P-414
Cimok, S. P-215, P-216
Cirdakli, D. P-186
Cizmeciolgu, M. P-237
Clark, P. M. OP-26, P-131
Coban, O. P-104
Coban, T. P-239, P-405
Cok, I. P-393, P-394
Coksal, G. P-405
Colak, M. P-88
Col Ayvaz, M. P-283
Comeleoglu, U. P-150
Comert Onder, F. P-206
Conk Dalay, M. OP-15
Coplu, M. P-406
Coskun, G. P-195
Coskun, I. P-233
Coskun, M. P-346
Coskun, N. P-194
Culhaoglu, B. P-181, P-285, P-334

D
Dadas, Y. P-195
Dal, A. G. P-1, P-51
Dal, T. P-417
Dall'Acqua, S. P-319
Darcan, S. P-373
Degim, I. T. OP-21, P-104, P-107
Degim, Z. P-104
Demir, A. OP-11
Demir, B. P-6
Demir, G. M. P-104, P-149
Demir, O. P-100
Demirayak, S. P-249
Demirbugen, M. P-397
Demirci, B. P-298, P-303, P-317, P-351
Demirci, F. P-234, P-302, P-303, P-317
Demirci, M. A. P-357
Demirci, S. P-200, P-311, P-360
Demirel, G. P-412
Demirel, M. P-81, P-90
Demirel, Z. OP-15
Demirez, L. O. P-314
Demirhan, B. P-17
Demirhan, B. E. P-17
Demirsoy Kaya, F. F. P-191
Demirtas, I. OP-4, P-176, P-354, P-355, P-356, P-357
Denizli, A. P-91
Derdovski, G. P-358
Demirci, S. P-47
Devillers, J. PL-24
Devrim, B. P-69, P-83
Devrimoglu Ozgunen, H. P-397
Diker, N. Y. P-352

470
Hacimuftuoglu, A. P-61, P-78
Hacisevki, A. P-136
Hadjipavlou Litina, D. P-304
Halici, Z. P-149
Hamamci, B. P-115
Hamamcioglu, AC. P-133
Hamurcu, F. P-244
Hancer, F. OP-20
Handali, S. P-77, P-108
Harir, M. P-142, P-420
Harpur, U. S. P-342
Hasanoglu Ozkan, E. P-138, P-139, P-155, P-156
Hascicek, C. P-71
Hashemitabar, M. P-108
Hatipoglu, H. S. P-390
Hatipoglu, S. D. P-181
Haznedaroglu, M. Z. P-359
Hermann, M. B. PL-1
Heydari, H. P-305
Hiemke, C. PL-30
Hokelek, T. P-244
Homan Gokce E. P-97, P-98
Hortolomei, M. P-92
Hosgoren, H. P-88
Hunt, A. O. P-151
Hurkul, M. M. P-349, P-353
Husnugil, H. P-168
Ickstadt, K. PL-1
Ilcim, A. P-327, P-329
Idug, T. P-336
Ilbasmis Tamer, S. OP-21, P-55, P-107
Ilcim, A. P-327, P-329
Ilbasmis Tamer, S. OP-21, P-55, P-107
Ilcim, A. P-327, P-329
Ilem Ozdemir, D. P-72, P-73, P-75, P-76, P-80, P-93, P-94
Ilgun, S. P-248
Ilgun, S. P-376
Ilter, A. Z. P-309, P-310
Inal, E. K. P-44
Inal, O. P-102, P-109
Ince, E. P-383
Inceyay, T. OP-21
Injac, T. P-152, P-153
Isan, G. P-410
Iscan, M. OP-20, P-402
Istik, C. P-250
Istik, S. OP-23, P-322
Isilk, A. B. P-223
Istil, F. OP-6, P-209
Izbirak, A. P-168
Izgi, H. P-118
Izol, E. P-23
Izzettilin, F. V. P-306
J
Janashia, I. P-408
Johannes Greten, H. P-371
Jovanovic, M. P-132
K
Kadioglu, O. OP-5, OP-27, P-124, P-371
Kadioglu, S. B. P-131
Kadioglu Duman M. P-120, P-128
Kahriman, N. P-227
Kakabadze, E. P-408
Kakava, R. OP-9, P-56
Kalfa, O. M. P-44
Kanit, L. OP-12
Kapche, W. F. G. D. P-355
Kaplanlci, Z. A. P-179, P-180, P-204, P-234, P-245, P-248
Karaslan, C. P-383
Karaslan, M. P-390
Karaslan, M. G. P-335
Karababa, G. P-393
Karabay, A. Z. OP-14
Karabiber, E. P-253
Karaca, N. P-302, P-317
Karacam, H. P-54
Karacaoglan, V. P-406
Karacaoglu, E. P-398
Karadas Bakirhan, N. P-39
Karagoz Genc, Z. P-241, P-242
Karakaya, A. OP-34, P-379, P-396
Karakaya, G. P-213
Karakaya, S. P-363
Karakucuk Iyidogan A. P-176, P-185
Karakus, G. P-85
Karakus, S. P-232
Karaoglu, K. P-165
Karaomerlioglu, I. P-119
Karasali, O. P-27
Karasulu, E. PL-21
Karasulu, H. Y. PL-21
Karat, D. P-186
Kart, S. OP-4, P-27
Kartal, M. P-268, P-322
Karteri, I. P-29, P-30
Kasap, E. P-5
Kasap, Z. P-207, P-253
Kaskatepe, B. OP-32, P-409, P-411, P-416, P-419
Kaskatepe, B. OP-32, P-409, P-411, P-416, P-419
Kaskatepe, B. OP-32, P-409, P-411, P-416, P-419
Koldas, S. OP-4, P-354
Kolsal, M. P-32
Kolsal Oguz, R. OP-8
Kolsal Oguz, R. OP-8
Kolsal Oguz, R. OP-8
Kolak, U. P-21, P-41, P-275, P-312, P-285
Koldas, S. OP-4, P-354
Koljii, R. PL-20
Konca, Y. P-421
Kongul, E. P-236
Konyar, D. P-217, P-230
Kooi, J. PL-12
Korkmaz, B. P-154
Korkmaz, D. B. P-185
Korkmaz, O. T. P-8, P-10
Koroglu, A. OP-28, P-353, P-280, P-349
Kortch, L. P-369
Kosar, M. P-344
Kose, Y. B. P-350
Koseoglu Yilmaz, P-41
Kose Ozkan, C. OP-16, P-103
Kostekci, S. P-364
Koyuncu, H. P-11
Koyuncu Zeybek D. P-6, P-27
Koyuturk, S. P-1, P-51
Koz, G. P-194
Koz, O. P-318
Kozanli, M. P-33
Kozanoglu, A. S. OP-17
Kucukboyaci, N. P-298, P-299
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Kucukoz, I. P-214
Kucukoz, S. G. P-195, P-233, P-414
Q
Queneau, Y. P-233

R
Rabti, A. OP-2
Ramezani, M. P-77
Ramezani, Z. P-108
Raoufi, N. OP-2
Ravalli, A. PL-17
Rebbas, K. P-331
Rebbas, K. P-332
Recher, T. OP-8, P-57
Reis, A. P-326
Renda, G. P-283, P-301, P-316, P-326
Rivas, L. P-46
Rouf, M. A. P-95

S
Sabanoglu, S. P-323
Sabuncuoglu, S. P-286
Sac, D. P-220
Saeed, M. OP-27, P-370, P-371
Safak, C. OP-23, P-405
Salva, E. P-113
Sancak, B. PL-29
Sancar, M. P-306
Sandalli, C. OP-11
Sar, S. OP-24, P-256, P-257, P-266, P-270, P-274
Sara, Y. P-223
Saracoğlu, I. OP-15, P-341, P-343
Sarac Tarhan S. P-229
Saremy, S. P-108
Sari, A. N. P-122
Sari, E. P-220
Sari, N. P-138, P-139, P-155, P-156, P-188
Sarialioğlu, G. P-339
Sarigöl, Z. P-395
Sarimahmut, M. P-299
Sarıoğlu, Y. OP-6, P-209
Sariternur, B. P-32
Saso, L. PL-13
Satana Kara H. E. P-17
Satrioglu, M. H. P-393
Savaser, A. OP-16, P-103
Sayar, G. P-196
Saygılı, N. P-178, P-198
Saygılı, R. P-44
Schumacher, U. P-124
Schwerdtle, T. PL-19
Secen, H. P-340
Sekic, E. P-32
Sekin, G. P-339
Seferoglu, Z. P-241, P-242
Segker Karatoprak, G. P-344
Sellittepe, E. P-229
Selmanoglu, G. P-398
Selvi, E. K. OP-11
Sen, A. P-279, P-306
Sen, O. OP-4
Sen, T. P-109
Sener, E. P-8, P-10, P-49
Sener, G. P-303, P-361
Sener, S. O. P-164, P-272, P-273, P-337, P-338, P-340
Sengel Türk, C. T. P-71, P-110
Sengezer, T. P-394
Sengul Koseoglu, M. P-292
Senkardes, S. P-233
Senkardesler, A. P-333
Senol, F. S. P-301
Senol, S. P-122
Sensoy, M. OP-15
Senturk, K. P-22, P-24
Senturk, O. S. P-244
Senturk, Z. PL-23, P-42, P-58, P-59
Serinyigit, Z. A. OP-22, P-72, P-73, P-75, P-76
Serbest, K. P-165
Serin, M. S. P-187, P-226
Server, B. P-70
Sever, B. OP-10
Seyler Yilmaz, B. OP-31, P-281
Sevgi, S. P-316, P-326
Sevin, G. P-72, P-73
Sevinc Ozakar, R. P-105
Seza, E. G. P-117
Sezen Karaoglan, E. P-292
Sezgin, Z. P-112
Sезик, E. P-294, P-295
Shirzad, M. M. P-189
Sicak, Y. P-185, P-246
Silku, S. N. P-274
Simsek, D. P-411, P-416
Simsek, R. OP-23, P-182, P-209, P-223
Sınan, G. P-82

Skaltsa, H. P-304
Slovak, L. P-167
Slusarenko, A. J. PL-26
Smaili, T. P-331, P-332
Smejkal, K. P-319
Sogut, O. K. P-131
Sohretoglu, D. P-286, P-342
Söltesova Prnova, M. P-404
Somay Dogan, T. P-168
Somsen, G. PL-12
Son, C. D. OP-33
Sonmez, E. P-350
Sonmez, M. P-28
Souleire, L. P-233
Soyagir, A. P-166
Soydas, E. OP-20
Soyer, Z. P-237, P-254
Soykut, B. P-392
Sozen Sahne, B. OP-24, P-255, P-256, P-266
Spac, A. P-63
Stefanache, A. P-63, P-92
Stefek, M. OP-33, P-404, P-377
Subak, H. P-19
Sucu, N. P-154
Sugimoto, Y. OP-27, P-370
Suleyman, H. P-149
Suleymanoglu, E. P-190, P-191, P-192
Suludere, Z. P-5
Sumbul, E. P-126, P-127
Sunguroglu, A. P-205, P-228
Sunnetcioglu, A. P-424
Sunnetcioglu, M. P-424
Suntar, I. P-325
Susu, B. P-261
Suzen, H. S. P-396, P-397, P-407
Suzen, S. P-383
Suzuk, S. P-409, P-419
Suzubet, E. P-96

T
Takim, K. P-147
Taktak, F. P-210, P-211, P-212, P-224, P-225
Talay Pınar, P. P-42
Tamer, L. P-122, P-154
Tamer, U. P-5, P-55, P-394
Tamer, Z. B. P-53, P-54
Tanaka, C. P-328
Tarhan, A. O. P-166
Tarhan, N. P-270
Tarimci, N. P-86, P-109, P-110
Tarkan, F. P-258
Tasci, M. P-219
Tascioglu, A. P-383
Tasdemir, N. P-406
Tasdemir, S. P-389
Taskin, T. P-333
Taskin Tok T. P-206
Yilmaz, S. P-381, P-382
Yilmaz, S. P-405
Yilmaz, S. P-55
Yorulmaz, N. P-193
Yuce, Y. Y. OP-30
Yukse, N. P-101, P-112
Yumitkan, N. P-318
Yurdasiper, A. OP-7
Yurdasiper, A. P-98
Yurdas Kirimlioglu, G. P-87
Yurttas, L. P-144, P-145, P-179, P-249
Yuvali Celik, G. P-287

Z
Zama, D. P-372
Zaza, A. PL-33
Zellagui, A. P-331, P-332
Zengin, F. P-250, P-251, P-252
Zeybek, B. P-4, P-6, P-44
Zilddar, F. P-347, P-348, P-349, P-353, P-381, P-382, P-386, P-387
Zuerguine, K. P-367
LIST OF PARTICIPANTS
<table>
<thead>
<tr>
<th>Name-Surname</th>
<th>e-mail</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Nur Onar</td>
<td><a href="mailto:nonar@omu.edu.tr">nonar@omu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Abdelouahab Belkassam</td>
<td><a href="mailto:belkassamabdou@yahoo.fr">belkassamabdou@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Abdulselem Ertaş</td>
<td><a href="mailto:abdulselemertas@hotmail.com">abdulselemertas@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Afife Büşra Ugur</td>
<td><a href="mailto:melcetin@hotmail.com">melcetin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmed Serdar Kozanoğlu</td>
<td><a href="mailto:aserdar.kozanoglu@gmail.com">aserdar.kozanoglu@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Akıcı</td>
<td><a href="mailto:ahakici@yahoo.com">ahakici@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Alper Öztürk</td>
<td><a href="mailto:aaozturk@anadolu.edu.tr">aaozturk@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Başaran</td>
<td><a href="mailto:abasaran@hacettepe.edu.tr">abasaran@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Doğan Ergin</td>
<td><a href="mailto:adergin@ankara.edu.tr">adergin@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Emir</td>
<td><a href="mailto:ahmet.emir@ege.edu.tr">ahmet.emir@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Oğul Araman</td>
<td><a href="mailto:ecza_dekan@istanbul.edu.tr">ecza_dekan@istanbul.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Oğuz Ada</td>
<td><a href="mailto:ada@pharmacy.ankara.edu.tr">ada@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ahmet Özdemir</td>
<td><a href="mailto:ahmeto@anadolu.edu.tr">ahmeto@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Akgül Yeşilada</td>
<td><a href="mailto:info@kemerburgaz.edu.tr">info@kemerburgaz.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Alan Sluseranko</td>
<td><a href="mailto:alan.slusarenko@bio3.rwth-aachen.de">alan.slusarenko@bio3.rwth-aachen.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Aleksandra Klisic</td>
<td><a href="mailto:aleksandranklisic@gmail.com">aleksandranklisic@gmail.com</a></td>
<td>Montenegro</td>
</tr>
<tr>
<td>Alev Tascioglu</td>
<td><a href="mailto:alev.tascioglu@gmail.com">alev.tascioglu@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Alev Tosun</td>
<td><a href="mailto:pharmacogalev@gmail.com">pharmacogalev@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ali Erkan Aşçı</td>
<td><a href="mailto:erkanali32@gmail.com">erkanali32@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ali Şen</td>
<td><a href="mailto:ali.sen@marmara.edu.tr">ali.sen@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Alina Stefanache</td>
<td><a href="mailto:stef.alina@yahoo.com">stef.alina@yahoo.com</a></td>
<td>Romania</td>
</tr>
<tr>
<td>Alper Gökbulut</td>
<td><a href="mailto:gokbulut@pharmacy.ankara.edu.tr">gokbulut@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Amal Rabti</td>
<td><a href="mailto:amal.rabti@gmail.com">amal.rabti@gmail.com</a></td>
<td>Tunis</td>
</tr>
<tr>
<td>Anake Kijjoa</td>
<td><a href="mailto:ankijjoa@icbas.up.pt">ankijjoa@icbas.up.pt</a></td>
<td>Portugal</td>
</tr>
<tr>
<td>Antonio Zaza</td>
<td><a href="mailto:antonio.zaza@unimib.it">antonio.zaza@unimib.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Arben Merkoci</td>
<td><a href="mailto:arben.merkoci@icn.cat">arben.merkoci@icn.cat</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Arın Güll Dal</td>
<td><a href="mailto:agdal@anadolu.edu.tr">agdal@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Arzu Beşikçi</td>
<td><a href="mailto:abesikci@ankara.edu.tr">abesikci@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Arzu Z. Karabay</td>
<td><a href="mailto:zeynepkarabay@yahoo.com">zeynepkarabay@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Asim B. Abdel-Mageed</td>
<td><a href="mailto:amageed@tulane.edu">amageed@tulane.edu</a></td>
<td>USA</td>
</tr>
<tr>
<td>Aslı Can Ağıca</td>
<td><a href="mailto:aslican.agca@titck.gov.tr">aslican.agca@titck.gov.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aslı Çetin</td>
<td><a href="mailto:aslicetin1@yahoo.com">aslicetin1@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aslı Demirci</td>
<td><a href="mailto:asli.demirci@marmara.edu.tr">asli.demirci@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aslı F. Ceylan-Isık</td>
<td><a href="mailto:asliceylan@kku.edu.tr">asliceylan@kku.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aslıhan Hilal Algan</td>
<td><a href="mailto:kurtoglu@pharmacy.ankara.edu.tr">kurtoglu@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Asuman Bozkir</td>
<td><a href="mailto:bozkir@pharmacy.ankara.edu.tr">bozkir@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Asuman Karakaya</td>
<td><a href="mailto:karakaya@pharmacy.ankara.edu.tr">karakaya@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayça Altay</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayfer Yalçın</td>
<td><a href="mailto:info@biruni.edu.tr">info@biruni.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aylin Üstündag</td>
<td><a href="mailto:dur@pharmacy.ankara.edu.tr">dur@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aymelek Gonenç</td>
<td><a href="mailto:aymelek@gazi.edu.tr">aymelek@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aysu Selçuk</td>
<td><a href="mailto:aysu.selcuk@ankara.edu.tr">aysu.selcuk@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Aysu Yurdasiper</td>
<td><a href="mailto:aysuyurdasiper@hotmail.com">aysuyurdasiper@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Aysun Hacısevki</td>
<td><a href="mailto:abozkir@gazi.edu.tr">abozkir@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Baldemir</td>
<td><a href="mailto:aysebaldemir@erciyes.edu.tr">aysebaldemir@erciyes.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Ceylan Hamamcıoğlu</td>
<td><a href="mailto:achamamcioglu@beun.edu.tr">achamamcioglu@beun.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Eken</td>
<td><a href="mailto:eken.ayse@gmail.com">eken.ayse@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Filiz Öner</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Göksu Kaya Özsan</td>
<td><a href="mailto:goksuk@gmail.com">goksuk@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Mine Gençler Özkan</td>
<td><a href="mailto:gencler_65@yahoo.com">gencler_65@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Nur Yazgan</td>
<td><a href="mailto:anyazgan@hotmail.com">anyazgan@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Şahin Yağlıoğlu</td>
<td><a href="mailto:aysesahin1@gmail.com">aysesahin1@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşe Şeyma Büyük</td>
<td><a href="mailto:aseymabuyuk@gmail.com">aseymabuyuk@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşegül Doğan</td>
<td><a href="mailto:ayseguld@hacettepe.edu.tr">ayseguld@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşegül Karataş</td>
<td><a href="mailto:akaratas@pharmacy.ankara.edu.tr">akaratas@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşegül Köroğlu</td>
<td><a href="mailto:Aysegul.Koroglu@ankara.edu.tr">Aysegul.Koroglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ayşen Gümüştaş</td>
<td><a href="mailto:aysengumustas@gmail.com">aysengumustas@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Azime Berna Özçelik</td>
<td><a href="mailto:brndemirci@yahoo.com">brndemirci@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bahar Akyüz</td>
<td><a href="mailto:bahar.akyuz@omu.edu.tr">bahar.akyuz@omu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Banu Kaskatepe</td>
<td><a href="mailto:bkaskatepe@ankara.edu.tr">bkaskatepe@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Banu Sancak</td>
<td><a href="mailto:banusancak@yahoo.com">banusancak@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Başak Özlem Perk</td>
<td><a href="mailto:perk@ankara.edu.tr">perk@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bedriye Seda Kurşun Akar</td>
<td><a href="mailto:sedakursun@windowslive.com">sedakursun@windowslive.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Begüm Nurpelin Sağlık</td>
<td><a href="mailto:bnsaglik@anadolu.edu.tr">bnsaglik@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Behice Yavuz Erdoğan</td>
<td><a href="mailto:behicey@omu.edu.tr">behicey@omu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Belgin Sever</td>
<td><a href="mailto:belginsever@anadolu.edu.tr">belginsever@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Belma Güven</td>
<td><a href="mailto:bguven@ankara.edu.tr">bguven@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Belma Turan</td>
<td><a href="mailto:Belma.Turan@medicine.ankara.edu.tr">Belma.Turan@medicine.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Benaiissa Ouahiba</td>
<td><a href="mailto:benaiissaouahiba@yahoo.com">benaiissaouahiba@yahoo.com</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Benay Can Eke</td>
<td><a href="mailto:eke@pharmacy.ankara.edu.tr">eke@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bendjeddou Dalila</td>
<td><a href="mailto:dalila.bendjeddou@yahoo.fr">dalila.bendjeddou@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Bengi Uslu</td>
<td><a href="mailto:buslu@pharmacy.ankara.edu.tr">buslu@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Beril Kadioğlu</td>
<td><a href="mailto:kadiogluberil@gmail.com">kadiogluberil@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Berna Sürekli</td>
<td><a href="mailto:belma.surekli@gmail.com">belma.surekli@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Berrin Kucukturkmen</td>
<td><a href="mailto:bbasaran@pharmacy.ankara.edu.tr">bbasaran@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Bozlar Pınarolu</td>
<td><a href="mailto:betul.bozlar@hotmail.com">betul.bozlar@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Büyükkılıç</td>
<td><a href="mailto:betulbuyukkilic@gmail.com">betulbuyukkilic@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Erdoğan</td>
<td><a href="mailto:betul-r-erdogan@hotmail.com">betul-r-erdogan@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Eymur</td>
<td><a href="mailto:betuleymur@gmail.com">betuleymur@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Kaya</td>
<td><a href="mailto:betulkaya@anadolu.edu.tr">betulkaya@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Sever Yılmaz</td>
<td><a href="mailto:sever@pharmacy.ankara.edu.tr">sever@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Taşkoparan</td>
<td><a href="mailto:b.taskoparan@gmail.com">b.taskoparan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Betül Tekiner Gülbüş</td>
<td><a href="mailto:btekiner@pharmacy.ankara.edu.tr">btekiner@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Beyza Filiz</td>
<td><a href="mailto:tenzilbeyzafiliz@gmail.com">tenzilbeyzafiliz@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Bezhan Chankvetadze</td>
<td><a href="mailto:jpba_bezhan@yahoo.com">jpba_bezhan@yahoo.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Bilge Kılıçarslan</td>
<td><a href="mailto:bilgekilicasl@gmail.com">bilgekilicasl@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bilge Sözen Şayne</td>
<td><a href="mailto:bilgesozen@yahoo.com">bilgesozen@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bilge Şener</td>
<td><a href="mailto:bilgesen@gazi.edu.tr">bilgesen@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bilgehan Doğru</td>
<td><a href="mailto:bdogru@ankara.edu.tr">bdogru@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bircan Çeken Toptancı</td>
<td><a href="mailto:bircan@dicle.edu.tr">bircan@dicle.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Birgül Yiğitcan</td>
<td><a href="mailto:berenberil44@hotmail.com">berenberil44@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Buket Aksu</td>
<td><a href="mailto:baksu@santafarma.com.tr">baksu@santafarma.com.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burak Barut</td>
<td><a href="mailto:burakbarut@ktu.edu.tr">burakbarut@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burak Biyik</td>
<td><a href="mailto:burakins@hotmail.com">burakins@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burak Çelik</td>
<td><a href="mailto:abozkir@gazi.edu.tr">abozkir@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Culhaoğlu</td>
<td>burcuculhao@<a href="mailto:gy@gmail.com">gy@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Demir</td>
<td><a href="mailto:brc.demir@windowslive.com">brc.demir@windowslive.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Devrim</td>
<td><a href="mailto:bdevrim@pharmacy.ankara.edu.tr">bdevrim@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Doğan-Topal</td>
<td><a href="mailto:doganb@ankara.edu.tr">doganb@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Nahi Palabiyik</td>
<td><a href="mailto:burcunbozal@hotmail.com">burcunbozal@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Burcu Ergene Öz</td>
<td><a href="mailto:burcinerogene@gmail.com">burcinerogene@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bülent Gümüşay</td>
<td><a href="mailto:eczdekan@hacettepe.edu.tr">eczdekan@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bülent Kiran</td>
<td><a href="mailto:kiran.bulent@gmail.com">kiran.bulent@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Bülent Zeybek</td>
<td><a href="mailto:bzybek43@hotmail.com">bzybek43@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Büyük Server</td>
<td><a href="mailto:melcetin@hotmail.com">melcetin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Büyük Yayıli</td>
<td><a href="mailto:busra.yayili@hotmail.com">busra.yayili@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Çağlar Macit</td>
<td><a href="mailto:cmean@yeditepe.edu.tr">cmean@yeditepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Can Özgür Yalçın</td>
<td><a href="mailto:coyalcin@ankara.edu.tr">coyalcin@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Canan Hasçiček</td>
<td><a href="mailto:cogan@pharmacy.ankara.edu.tr">cogan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Canan Kuş</td>
<td><a href="mailto:kus@pharmacy.ankara.edu.tr">kus@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Caner Günaydın</td>
<td><a href="mailto:cnrgunaydin@hotmail.com">cnrgunaydin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Cansel Köse Özkan</td>
<td><a href="mailto:ckozkan@gata.edu.tr">ckozkan@gata.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Cem Erkmen</td>
<td><a href="mailto:cmrkmin@gmail.com">cmrkmin@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Cem Yamali</td>
<td><a href="mailto:c.yamali@yahoo.com">c.yamali@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ceren Elmacı</td>
<td><a href="mailto:ceren_elmaci@hotmail.com">ceren_elmaci@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ceren Emir</td>
<td><a href="mailto:ceren.acir@ege.edu.tr">ceren.acir@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ceren Ertekin</td>
<td><a href="mailto:certekin@ankara.edu.tr">certekin@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ceyda Sibel Kılıç</td>
<td><a href="mailto:erdurak@pharmacy.ankara.edu.tr">erdurak@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ceyda Tuba Sengel-Turk</td>
<td><a href="mailto:ctsengel@pharmacy.ankara.edu.tr">ctsengel@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Cherif Abdennour</td>
<td><a href="mailto:cherifabdeenour@yahoo.fr">cherifabdeenour@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Christoph Hiemke</td>
<td><a href="mailto:hiemke@uni-mainz.de">hiemke@uni-mainz.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Cihat Şafak</td>
<td><a href="mailto:rsimsek@hacettepe.edu.tr">rsimsek@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Çağrı Çalışkan</td>
<td><a href="mailto:cagri.caliskan@gmail.com">cagri.caliskan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Çağrı Urfali</td>
<td>cagriur@<a href="mailto:l@gmail.com">l@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Cığdem Karaaslan</td>
<td><a href="mailto:karaaslan@pharmacy.ankara.edu.tr">karaaslan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Çiğdem Saral</td>
<td><a href="mailto:cigdem.saral@tubitak.gov.tr">cigdem.saral@tubitak.gov.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Demet Begüm Korkmaz</td>
<td><a href="mailto:korkmaz.demet@hotmail.com">korkmaz.demet@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Demet Cetin</td>
<td><a href="mailto:utamer@gazi.edu.tr">utamer@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Demet Dincel</td>
<td><a href="mailto:dinceldmt@hotmail.com">dinceldmt@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Derya Çiçek Polat</td>
<td><a href="mailto:polatd@ankara.edu.tr">polatd@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Derya İlem-Özdemir</td>
<td><a href="mailto:deryailem@gmail.com">deryailem@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Derya Koyuncu Zeybek</td>
<td><a href="mailto:derya.kzeybek@dpu.edu.tr">derya.kzeybek@dpu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Didem Şöhretoğlu</td>
<td><a href="mailto:didems@hacettepe.edu.tr">didems@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Didem Yılmaz</td>
<td><a href="mailto:didemyilmaz144@gmail.com">didemyilmaz144@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilan Konyar</td>
<td><a href="mailto:konyar@pharmacy.ankara.edu.tr">konyar@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilara Çırdaklı</td>
<td><a href="mailto:dilaracirdakli@hotmail.com">dilaracirdakli@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilara Eren</td>
<td><a href="mailto:dr.ern@gmail.com">dr.ern@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilara Nemutlu</td>
<td><a href="mailto:dilaranemutlu@gmail.com">dilaranemutlu@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilek Ak</td>
<td><a href="mailto:dak@anadolu.edu.tr">dak@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilek Kul</td>
<td><a href="mailto:dilekk@ktu.edu.tr">dilekk@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilek Şahin</td>
<td><a href="mailto:dilek.sahin@tubitak.gov.tr">dilek.sahin@tubitak.gov.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilem Doğan</td>
<td><a href="mailto:dogandilem@erciyes.edu.tr">dogandilem@erciyes.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Dilşad Onbaslı</td>
<td><a href="mailto:odilsad@gmail.com">odilsad@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Doğu Nebioglu</td>
<td><a href="mailto:dnebioglu@ankara.edu.tr">dnebioglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Duygu Ergen</td>
<td><a href="mailto:duyguersen@yahoo.com">duyguersen@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Duygu Paslı</td>
<td><a href="mailto:duygupasli@gmail.com">duygupasli@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Duygu Saç</td>
<td><a href="mailto:duygusac@gmail.com">duygusac@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Duygu Simsek</td>
<td><a href="mailto:dsimsek@ankara.edu.tr">dsimsek@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ebru Arioglu Inan</td>
<td><a href="mailto:arioglu@ankara.edu.tr">arioglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ebru Başaran</td>
<td><a href="mailto:ebcengiz@anadolu.edu.tr">ebcengiz@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ebru Türk Özcaşar</td>
<td><a href="mailto:ebruturkozacar@gmail.com">ebruturkozacar@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ece Özcan</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ecem Kaya</td>
<td><a href="mailto:ecemkaya1989@gmail.com">ecemkaya1989@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Eda Aydin</td>
<td><a href="mailto:aydn_edai@yahoo.com">aydn_edai@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ede Gökbülüt</td>
<td><a href="mailto:edayigit@hotmail.com">edayigit@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Eğemen Foto</td>
<td><a href="mailto:egemenfoto@yahoo.com">egemenfoto@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ekin Kurtul</td>
<td><a href="mailto:ekurtul@ankara.edu.tr">ekurtul@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ekrem Karabiber</td>
<td><a href="mailto:ekremkarabiber@yahoo.com">ekremkarabiber@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elene Kakabdze</td>
<td><a href="mailto:elene.kakabadze@gmail.com">elene.kakabadze@gmail.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Elif Aksun</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Aynacı Koyuncu</td>
<td><a href="mailto:elaynaci@gmail.com">elaynaci@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Batum</td>
<td><a href="mailto:eelfbtm@gmail.com">eelfbtm@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Gizem Taşkiran</td>
<td><a href="mailto:elifgizemtaskiran@gmail.com">elifgizemtaskiran@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif İnce</td>
<td><a href="mailto:ecz.elifince@gmail.com">ecz.elifince@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Kiymaz</td>
<td><a href="mailto:naz1990@gmail.com">naz1990@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Müderrisoğlu</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elif Nur Gazioğlu</td>
<td><a href="mailto:elifgazioglu@ktu.edu.tr">elifgazioglu@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Elif Taşlıdere</td>
<td><a href="mailto:eliftaslidere@hotmail.com">eliftaslidere@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Elvan Hasanoğlu Özkan</td>
<td><a href="mailto:elvanelnvan_06@hotmail.com">elvanelnvan_06@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emel Önder Fırat</td>
<td><a href="mailto:emel.onder@tubitak.gov.tr">emel.onder@tubitak.gov.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emel Sönmez</td>
<td><a href="mailto:emls222224@gmail.com">emls222224@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emine Akyuz Turumtay</td>
<td><a href="mailto:emine.turumtay@gmail.com">emine.turumtay@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emine Şalva</td>
<td><a href="mailto:emine_salva@yahoo.com">emine_salva@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emirhan Nemutlu</td>
<td><a href="mailto:enemutlu@hacettepe.edu.tr">enemutlu@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Emrah Özakar</td>
<td><a href="mailto:melcetin@hotmail.com">melcetin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Engin Celep</td>
<td><a href="mailto:enginecelep@gmail.com">enginecelep@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Engin Er</td>
<td><a href="mailto:eer@ankara.edu.tr">eer@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Engin Şarar</td>
<td><a href="mailto:esarer@ankara.edu.tr">esarer@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erdal Bedir</td>
<td><a href="mailto:erdalbedir@gmail.com">erdalbedir@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erdal Dinç</td>
<td><a href="mailto:dinc@ankara.edu.tr">dinc@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erdem Büyükbingöl</td>
<td><a href="mailto:erdem@pharmacy.ankara.edu.tr">erdem@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erdoğlu Kablan</td>
<td><a href="mailto:nozaltin@hacettepe.edu.tr">nozaltin@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erhan Palaska</td>
<td><a href="mailto:epalaska@hacettepe.edu.tr">epalaska@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Erhan Süleymanoğlu</td>
<td><a href="mailto:erhans@mail.ru">erhans@mail.ru</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ernest Szubert</td>
<td><a href="mailto:ernest.szubert@gmail.com">ernest.szubert@gmail.com</a></td>
<td>Poland</td>
</tr>
<tr>
<td>Erol Eroğlu</td>
<td>eeroグル@akdeniz.edu.tr</td>
<td>Turkey</td>
</tr>
<tr>
<td>Erol Şener</td>
<td><a href="mailto:erolsener@anadolu.edu.tr">erolsener@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Esat Can</td>
<td><a href="mailto:esat0624@hotmail.com">esat0624@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Esen Sezen Karaoğlan</td>
<td><a href="mailto:esen.karaoqlan@atauni.edu.tr">esen.karaoqlan@atauni.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Esin Aki Yalçın</td>
<td><a href="mailto:esinaki@ankara.edu.tr">esinaki@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Esin Erçin Aslan</td>
<td><a href="mailto:esin_ercin@yahoo.com">esin_ercin@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Eskandar Moghimipour</td>
<td><a href="mailto:moghimipour@yahoo.com">moghimipour@yahoo.com</a></td>
<td>Iran</td>
</tr>
<tr>
<td>Etil Güzelmeric</td>
<td><a href="mailto:etil.ariburnu@yeditepe.edu.tr">etil.ariburnu@yeditepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Evren Gundogdu</td>
<td><a href="mailto:evren.gundogdu@ege.edu.tr">evren.gundogdu@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ezgi Öner</td>
<td><a href="mailto:ezgioner@windowslive.com">ezgioner@windowslive.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ezgi Turunc Bayrakdar</td>
<td><a href="mailto:ezgi.turunc.bayrakdar@ege.edu.tr">ezgi.turunc.bayrakdar@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ezgi Uluğ</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatemeh Bahadori</td>
<td><a href="mailto:fatemehbahadori@hotmail.com">fatemehbahadori@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatih Göğer</td>
<td><a href="mailto:fatihgoger@gmail.com">fatihgoger@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatih Turhan</td>
<td><a href="mailto:fturhan@ankara.edu.tr">fturhan@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Doğanç</td>
<td><a href="mailto:doganc@ankara.edu.tr">doganc@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Ağın</td>
<td><a href="mailto:fagin@ktu.edu.tr">fagin@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Ayaz</td>
<td><a href="mailto:nurgunkucukboyaci@gmail.com">nurgunkucukboyaci@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Kazdal</td>
<td><a href="mailto:fatmakazdal@hotmail.com">fatmakazdal@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Pınar Türkmenoğlu</td>
<td><a href="mailto:fpt@hacettepe.edu.tr">fpt@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatma Zilidfar</td>
<td><a href="mailto:fatmazlf@gmail.com">fatmazlf@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fatmanur Tuğcu Demiröz</td>
<td><a href="mailto:fatmanur@gazi.edu.tr">fatmanur@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fawaz Nasser Shekh Al Heibsy</td>
<td><a href="mailto:fnssaa.aden@gmail.com">fnssaa.aden@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>--------------------</td>
<td>-------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Fernando Goglia</td>
<td><a href="mailto:goglia@unisannio.it">goglia@unisannio.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Feyyaz Mihoğlugil</td>
<td><a href="mailto:feyyazmihoglugil@gmail.com">feyyazmihoglugil@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Feyyaz Onur</td>
<td><a href="mailto:onur@pharmacy.ankara.edu.tr">onur@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Feyza Oke-Altuntas</td>
<td><a href="mailto:feyzaoke@gazi.edu.tr">feyzaoke@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Filiz Bakar</td>
<td><a href="mailto:fizbakar@yahoo.com">fizbakar@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Funda Nuray Yalçın</td>
<td><a href="mailto:funyal@hacettepe.edu.tr">funyal@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Fügen Aktan</td>
<td><a href="mailto:fugenaktan@hotmail.com">fugenaktan@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Füsun Acartürk</td>
<td><a href="mailto:acarturk@gazi.edu.tr">acarturk@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gamze Ergin</td>
<td><a href="mailto:gmz.ergin@gmail.com">gmz.ergin@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gamze Göğer</td>
<td><a href="mailto:gamzecayirdere@gmail.com">gamzecayirdere@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gamze Koz</td>
<td><a href="mailto:gamzedkoz@gmail.com">gamzedkoz@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Genada Sinani</td>
<td><a href="mailto:genada.sinani@kemerburgaz.edu.tr">genada.sinani@kemerburgaz.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gerrit Borchard</td>
<td><a href="mailto:Gerrit.Borchard@unige.ch">Gerrit.Borchard@unige.ch</a></td>
<td>Switzerland</td>
</tr>
<tr>
<td>Giovanna Marazza</td>
<td><a href="mailto:giovanna.marrazza@unifi.it">giovanna.marrazza@unifi.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Gizem Gülpinar</td>
<td><a href="mailto:gaykac@gmail.com">gaykac@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gizem Kayki Mutlu</td>
<td><a href="mailto:gKayki@ankara.edu.tr">gKayki@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gizem Rüya Çamca</td>
<td><a href="mailto:ruyacamca@gmail.com">ruyacamca@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gökçe Öztürk</td>
<td><a href="mailto:nasa.gokce@hotmail.com">nasa.gokce@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Göknil Pelin Coşkun</td>
<td><a href="mailto:gkucukguzel@marmara.edu.tr">gkucukguzel@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gökşu Özçelikay</td>
<td><a href="mailto:ozcelikayg@ankara.edu.tr">ozcelikayg@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Görkem Şener</td>
<td><a href="mailto:gorkemsener41@gmail.com">gorkemsener41@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gözde Aydoğdu</td>
<td><a href="mailto:gaydogdu@science.ankara.edu.tr">gaydogdu@science.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gözde Sayar</td>
<td><a href="mailto:gozde.sayar@ege.edu.tr">gozde.sayar@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gözde Ünsoy</td>
<td><a href="mailto:gozdeunsoy@hotmail.com">gozdeunsoy@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülalçı Topçu</td>
<td><a href="mailto:ecza@bezmialem.edu.tr">ecza@bezmialem.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülberk Uçar</td>
<td><a href="mailto:gulberk.ucar@gmail.com">gulberk.ucar@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülbike Demirel</td>
<td><a href="mailto:gdemirel@ankara.edu.tr">gdemirel@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülbin Özçelikay</td>
<td><a href="mailto:gozcelikayg@ankara.edu.tr">gozcelikayg@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>GÜlce Taşkor</td>
<td><a href="mailto:gulcet@hacettepe.edu.tr">gulcet@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülçin Saltan</td>
<td><a href="mailto:gulcin.saltan@pharmacy.ankara.edu.tr">gulcin.saltan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülden Z. Omurtag</td>
<td><a href="mailto:pharmacy@marmara.edu.tr">pharmacy@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülderen Karakuş</td>
<td><a href="mailto:gulderenkarakus@gmail.com">gulderenkarakus@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülderen Yılmaz</td>
<td><a href="mailto:gyilmaz@ankara.edu.tr">gyilmaz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülenn Melike Demir</td>
<td><a href="mailto:gulennmelikedemir@gmail.com">gulennmelikedemir@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülgün Kilciğil</td>
<td>kilciğ<a href="mailto:il@pharmacy.ankara.edu.tr">il@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülgün Ozansoy</td>
<td><a href="mailto:ozansoy@ankara.edu.tr">ozansoy@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülhan Bora</td>
<td><a href="mailto:gulhanarvas@yahoo.com">gulhanarvas@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülın Amasya</td>
<td><a href="mailto:gamasya@pharmacy.ankara.edu.tr">gamasya@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülin Güvendik</td>
<td><a href="mailto:guvendik@pharmacy.ankara.edu.tr">guvendik@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülin Renda</td>
<td><a href="mailto:gulingurhan@yahoo.com">gulingurhan@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülnur Eksi</td>
<td><a href="mailto:gulnur_eksi@yahoo.com">gulnur_eksi@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülru Gürdemir</td>
<td><a href="mailto:gulru@hacettepe.edu.tr">gulru@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Gülsel Yurtdaş Kırımlıoğlu</td>
<td><a href="mailto:gyurtdas@anadolu.edu.tr">gyurtdas@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülsen Kendir</td>
<td><a href="mailto:aguven@ankara.edu.tr">aguven@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülsen Sönmez</td>
<td><a href="mailto:gulsensonmez93@gmail.com">gulsensonmez93@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülsen Akalin Çiftçi</td>
<td><a href="mailto:gakalin@anadolu.edu.tr">gakalin@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Gülsen Göney</td>
<td><a href="mailto:gulsengoney@gmail.com">gulsengoney@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Güneş Açıkgöz</td>
<td><a href="mailto:gunesani@hotmail.com">gunesani@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hajar Heydari</td>
<td><a href="mailto:phch85b@gmail.com">phch85b@gmail.com</a></td>
<td>Iran</td>
</tr>
<tr>
<td>Hakan Göker</td>
<td><a href="mailto:goker@ankara.edu.tr">goker@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Halide Edip Temel</td>
<td><a href="mailto:heincedal@anadolu.edu.tr">heincedal@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Halil Aksoy</td>
<td><a href="mailto:aksoyhalil@yahoo.com">aksoyhalil@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Halil Bal</td>
<td><a href="mailto:halilibal@ankara.edu.tr">halilibal@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Halil Hoşgören</td>
<td><a href="mailto:hosgoren@dicle.edu.tr">hosgoren@dicle.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Haluk Dedda</td>
<td><a href="mailto:halukdeda@hotmail.com">halukdeda@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hamdi Temel</td>
<td><a href="mailto:htemelh@hotmail.com">htemelh@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hamdi Temel</td>
<td><a href="mailto:gensek@dicle.edu.tr">gensek@dicle.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Harir Mohamed</td>
<td><a href="mailto:mohamedharir57@yahoo.fr">mohamedharir57@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Hasan Erdinç Sellitepe</td>
<td><a href="mailto:esellitepe@hotmail.com">esellitepe@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hasret Subak</td>
<td><a href="mailto:Ecz.hasrets@gmail.com">Ecz.hasrets@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hatice Gül Göktaş</td>
<td><a href="mailto:haticegulgoktas@gmail.com">haticegulgoktas@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hayati Celik</td>
<td><a href="mailto:hayaticelik@gmail.com">hayaticelik@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hayriye Eda Şatana Kara</td>
<td><a href="mailto:eda@gazi.edu.tr">eda@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hazel Sipahi</td>
<td><a href="mailto:HASI@lundbeck.com">HASI@lundbeck.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Henk Lingeman</td>
<td><a href="mailto:h.lingeman@vu.nl">h.lingeman@vu.nl</a></td>
<td>Netherlands</td>
</tr>
<tr>
<td>Hilal Bardakci</td>
<td><a href="mailto:hilalbardakci@hotmail.com">hilalbardakci@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hilal Başak Erol</td>
<td><a href="mailto:hcuhadaroglu@ankara.edu.tr">hcuhadaroglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hocine Laouer</td>
<td><a href="mailto:hocine_laouer@yahoo.fr">hocine_laouer@yahoo.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Hülüya Akgün</td>
<td><a href="mailto:pharmacy@yeditepe.edu.tr">pharmacy@yeditepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Hülüya Unan</td>
<td><a href="mailto:tekinan@buffalo.edu">tekinan@buffalo.edu</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ilham Eroz Poyraz</td>
<td><a href="mailto:ieroz@anadolu.edu.tr">ieroz@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ilker Ates</td>
<td><a href="mailto:ilkerates976@gmail.com">ilkerates976@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Isil Ozakca</td>
<td><a href="mailto:ozakca@ankara.edu.tr">ozakca@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Istvan Toth</td>
<td><a href="mailto:i.toth@uq.edu.au">i.toth@uq.edu.au</a></td>
<td>Australia</td>
</tr>
<tr>
<td>Işık Aşar</td>
<td><a href="mailto:isik.asar@tubitak.gov.tr">isik.asar@tubitak.gov.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Işık Özgüney</td>
<td><a href="mailto:isik.ozguney@ege.edu.tr">isik.ozguney@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İşil Gazioğlu</td>
<td><a href="mailto:igazioglu@bezmialem.edu.tr">igazioglu@bezmialem.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İşil Yıldırım</td>
<td><a href="mailto:isilyild@hotmail.com">isilyild@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İzka Matarashvili</td>
<td><a href="mailto:iza_matarashvili@mail.ru">iza_matarashvili@mail.ru</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>İbrahim Demirtaş</td>
<td><a href="mailto:ibdemirtas@gmail.com">ibdemirtas@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İkay Yıldız</td>
<td><a href="mailto:iyildiz@pharmacy.ankara.edu.tr">iyildiz@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İmer Okar</td>
<td><a href="mailto:tanitim@yeniuyuzil.edu.tr">tanitim@yeniuyuzil.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İnci Selin Doğan</td>
<td><a href="mailto:selinci@gmail.com">selinci@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İrem Atay</td>
<td><a href="mailto:irematay@yahoo.com">irematay@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
<td>-----------------</td>
</tr>
<tr>
<td>İrem Karaömerlioğlu</td>
<td><a href="mailto:ikaraomerlioglu@ankara.edu.tr">ikaraomerlioglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İrem Ulusoy</td>
<td>e-mail</td>
<td>Turkey</td>
</tr>
<tr>
<td>İshak Bildirici</td>
<td><a href="mailto:ishakbildirici@gmail.com">ishakbildirici@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmail Murat Palabiyik</td>
<td><a href="mailto:mpala@pharmacy.ankara.edu.tr">mpala@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmail Özdemir</td>
<td><a href="mailto:eczacilik@inonu.edu.tr">eczacilik@inonu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmail Tuncer Değim</td>
<td><a href="mailto:ecza@gazi.edu.tr">ecza@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmail Yalçın</td>
<td><a href="mailto:yalcin@pharmacy.ankara.edu.tr">yalcin@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmail Yener</td>
<td><a href="mailto:ismail.yener@dicle.edu.tr">ismail.yener@dicle.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmet Çok</td>
<td><a href="mailto:ismetcok@gmail.com">ismetcok@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>İsmet Rezani Toptancı</td>
<td><a href="mailto:ismettoptanci@gmail.com">ismettoptanci@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>James Devillers</td>
<td><a href="mailto:j.devillers@ctis.fr">j.devillers@ctis.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Jean Michel Kaufmann</td>
<td><a href="mailto:jmkauf@ulb.ac.be">jmkauf@ulb.ac.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Juan Bueno</td>
<td><a href="mailto:juangbueno@gmail.com">juangbueno@gmail.com</a></td>
<td>Colombia</td>
</tr>
<tr>
<td>Juan Miguel Feliu Martinez</td>
<td><a href="mailto:juan.feliu@ua.es">juan.feliu@ua.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Kadir Özden Yerdelen</td>
<td><a href="mailto:dadasozden@gmail.com">dadasozden@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Kadir Yazıcı</td>
<td><a href="mailto:kadrigulec@gmail.com">kadrigulec@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Katerina Goracinova</td>
<td><a href="mailto:kago@ff.ukim.edu.mk">kago@ff.ukim.edu.mk</a></td>
<td>Macedonia</td>
</tr>
<tr>
<td>Kayhan Boletti</td>
<td><a href="mailto:boletti@pharmacy.ankara.edu.tr">boletti@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Kemal Çetin</td>
<td><a href="mailto:kemalcetin@hacettepe.edu.tr">kemalcetin@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Keykavous Parang</td>
<td><a href="mailto:parang@chapman.edu">parang@chapman.edu</a></td>
<td>USA</td>
</tr>
<tr>
<td>Khatuna Gogaladze</td>
<td><a href="mailto:khat78@gmail.com">khat78@gmail.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Kübra Çiçek</td>
<td><a href="mailto:kubracicek1905@gmail.com">kubracicek1905@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Kübra Gizem Yıldıztekin</td>
<td><a href="mailto:kubragizem89@hotmail.com">kubragizem89@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Kübra Ünal</td>
<td><a href="mailto:kubra.unal.k@gmail.com">kubra.unal.k@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Lacramioara Ochiuz</td>
<td><a href="mailto:ochiuzz@yahoo.com">ochiuzz@yahoo.com</a></td>
<td>Romania</td>
</tr>
<tr>
<td>Laïfa El-Adoui</td>
<td><a href="mailto:laifaell@gmail.com">laifaell@gmail.com</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Lalaou Korrichi</td>
<td><a href="mailto:lalaouiko@yahoo.fr">lalaouiko@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Lale Gözcü</td>
<td><a href="mailto:lgozcu@atauni.edu.tr">lgozcu@atauni.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Levent Altun</td>
<td><a href="mailto:altun@pharmacy.ankara.edu.tr">altun@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Leyla Yurtdaş</td>
<td><a href="mailto:lyurtdas@anadolu.edu.tr">lyurtdas@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Luciano SASO</td>
<td><a href="mailto:luciano.saso@uniroma1.it">luciano.saso@uniroma1.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Lütfi Genç</td>
<td><a href="mailto:lgenc@anadolu.edu.tr">lgenc@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>M. Çelebier</td>
<td><a href="mailto:merve.ecz@gmail.com">merve.ecz@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Maksut Coşkun</td>
<td><a href="mailto:mcoskun@ankara.edu.tr">mcoskun@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Marek Lachmann</td>
<td><a href="mailto:marek.lachmann@gmail.com">marek.lachmann@gmail.com</a></td>
<td>Turkish Republic of Northern Cyprus</td>
</tr>
<tr>
<td>Marie-Aleth Lacaille-Dubois</td>
<td><a href="mailto:malacd@u-bourgogne.fr">malacd@u-bourgogne.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Mecit Özdemir</td>
<td><a href="mailto:mecitozdemir@kilis.edu.tr">mecitozdemir@kilis.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehlika Dilek Altıntop</td>
<td><a href="mailto:mdaltintop@anadolu.edu.tr">mdaltintop@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Alp</td>
<td><a href="mailto:malp@pharmacy.ankara.edu.tr">malp@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Mehmet Ay</td>
<td><a href="mailto:mehmetay06@comu.edu.tr">mehmetay06@comu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Barlas Uzun</td>
<td><a href="mailto:mehmetbarlasuzun@gmail.com">mehmetbarlasuzun@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Berköz</td>
<td><a href="mailto:mehmet_berkoz@yahoo.com">mehmet_berkoz@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Boğa</td>
<td><a href="mailto:mehmetboga1980@gmail.com">mehmetboga1980@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Gökhan Çağlayan</td>
<td><a href="mailto:caglayangokhan@gmail.com">caglayangokhan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Gümüスタート</td>
<td><a href="mailto:mgumustas@hotmail.com">mgumustas@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Koca</td>
<td><a href="mailto:dadasozden@gmail.com">dadasozden@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mehmet Zeki Haznedaroglu</td>
<td><a href="mailto:zeki.haznedaroglu@gmail.com">zeki.haznedaroglu@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Melek Karacaoglu</td>
<td><a href="mailto:mkaracaoglu@ankara.edu.tr">mkaracaoglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Melek Sirin Baymak</td>
<td><a href="mailto:msbaymak@marmara.edu.tr">msbaymak@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Melih Altan</td>
<td><a href="mailto:maltan@ankara.edu.tr">maltan@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Melih Ekinci</td>
<td><a href="mailto:melihia.ekinci@ege.edu.tr">melihia.ekinci@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Meltem Ceylan Ünüsoy</td>
<td><a href="mailto:munlusoy@pharmacy.ankara.edu.tr">munlusoy@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Meltem Macit</td>
<td><a href="mailto:meltem.macit@yeditepe.edu.tr">meltem.macit@yeditepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Meral Tunçbilek</td>
<td><a href="mailto:tuncbile@pharmacy.ankara.edu.tr">tuncbile@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Merve Demirbugen</td>
<td><a href="mailto:demirbugen@ankara.edu.tr">demirbugen@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Merve Eylül Kıymaci</td>
<td><a href="mailto:pharmaeyill@gmail.com">pharmaeyill@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Merve Nenni</td>
<td><a href="mailto:merve.ecz@gmail.com">merve.ecz@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Merve Şensoy</td>
<td><a href="mailto:opium.msensoy@gmail.com">opium.msensoy@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Meryem Temiz</td>
<td><a href="mailto:meryemtemiz88@gmail.com">meryemtemiz88@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mesud Hürkul</td>
<td><a href="mailto:mhrkul@ankara.edu.tr">mhrkul@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mesut Çiçek</td>
<td><a href="mailto:mcicek@ankara.edu.tr">mcicek@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Metin Emrah Kartal</td>
<td><a href="mailto:eczemertinkartal@gmail.com">eczemertinkartal@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Miray Görgöz</td>
<td><a href="mailto:mgorgoz@ankara.edu.tr">mgorgoz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Miray Arslan</td>
<td><a href="mailto:eczmiraarslan@gmail.com">eczmiraarslan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Miyase Gözde Gündüz</td>
<td><a href="mailto:rsimsek@hacettepe.edu.tr">rsimsek@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mohamed Saeed</td>
<td><a href="mailto:saeedm@uni-mainz.de">saeedm@uni-mainz.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Mohammed Fathi Abdallah</td>
<td><a href="mailto:mohamed.fathi@hacettepe.edu.tr">mohamed.fathi@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Murat Kartal</td>
<td><a href="mailto:pharmmurat@gmail.com">pharmmurat@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Murat Kozanlı</td>
<td><a href="mailto:mkozanli@anadolu.edu.tr">mkozanli@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Murat Oral</td>
<td><a href="mailto:ecz.murat.oral@gmail.com">ecz.murat.oral@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Murat Yıldız</td>
<td><a href="mailto:murat.yildiz@usak.edu.tr">murat.yildiz@usak.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mustafa Çelebier</td>
<td><a href="mailto:mcelebier@gmail.com">mcelebier@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mustafa Fethi Şahin</td>
<td><a href="mailto:pharmacy@emu.edu.tr">pharmacy@emu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mutlu Diliz Aytemir</td>
<td><a href="mailto:mutlud@hacettepe.edu.tr">mutlud@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Müberra Koşar</td>
<td><a href="mailto:pharmacy@erciyes.edu.tr">pharmacy@erciyes.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Müge Kiliçarslan</td>
<td><a href="mailto:Muge.Kilicarslan@pharmacy.ankara.edu.tr">Muge.Kilicarslan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Müjde Eryılmaz</td>
<td><a href="mailto:meryilmaz@ankara.edu.tr">meryilmaz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mümtaz İşcan</td>
<td><a href="mailto:iscan@pharmacy.ankara.edu.tr">iscan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nadire Özenver</td>
<td><a href="mailto:nadire@hacettepe.edu.tr">nadire@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nafiz Öncü Can</td>
<td><a href="mailto:nafizoc@anadolu.edu.tr">nafizoc@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nasrullah Abdullaev</td>
<td><a href="mailto:n_abdullaev@rambler.ru">n_abdullaev@rambler.ru</a></td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Nebojsa Kavaric</td>
<td><a href="mailto:aleksandranklisic@gmail.com">aleksandranklisic@gmail.com</a></td>
<td>Montenegro</td>
</tr>
<tr>
<td>Necla Kulaş</td>
<td><a href="mailto:necla.kulbas@marmara.edu.tr">necla.kulbas@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Necmiye Canacankatan</td>
<td><a href="mailto:ncanacankatan@gmail.com">ncanacankatan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Neslihan Genişel</td>
<td><a href="mailto:neslihan.genisel@dicle.edu.tr">neslihan.genisel@dicle.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Neslihan Üstünç Okur</td>
<td><a href="mailto:neslihanustundag@yahoo.com">neslihanustundag@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Net Evcimen</td>
<td><a href="mailto:nevcimen@ankara.edu.tr">nevcimen@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nevin Erk</td>
<td><a href="mailto:erk@pharmacy.ankara.edu.tr">erk@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nibal Y.A. Abunahlah</td>
<td><a href="mailto:nibal.abunuhlal@kemerburgaz.edu.tr">nibal.abunuhlal@kemerburgaz.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nilanjana Maulik</td>
<td><a href="mailto:nmaulik@neuron.uchc.edu">nmaulik@neuron.uchc.edu</a></td>
<td>USA</td>
</tr>
<tr>
<td>Nilay Ildiz</td>
<td><a href="mailto:nilaygucluer@erciyes.edu.tr">nilaygucluer@erciyes.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nilay Tarhan</td>
<td><a href="mailto:nilaytar@yahoo.com.tr">nilaytar@yahoo.com.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nilgün G. Göğer</td>
<td><a href="mailto:ngoger@gazi.edu.tr">ngoger@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nilüfer Tanrımcı</td>
<td><a href="mailto:ntarimci@pharmacy.ankara.edu.tr">ntarimci@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nilüfer Yüksel</td>
<td><a href="mailto:nyuksel@pharmacy.ankara.edu.tr">nyuksel@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nina Chanishvili</td>
<td><a href="mailto:nina.chanishvili@gmail.com">nina.chanishvili@gmail.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Nuerbiye Aoubulai kem</td>
<td><a href="mailto:nuerbiyeg@gmail.com">nuerbiyeg@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nuraniye Eruygur</td>
<td><a href="mailto:nuraniye58@gmail.com">nuraniye58@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nuray Ari</td>
<td><a href="mailto:ari@ankara.edu.tr">ari@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nuray Ulusoy Güzeldemirci</td>
<td><a href="mailto:nulusoy@istanbul.edu.tr">nulusoy@istanbul.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurcan Bektas</td>
<td><a href="mailto:nurcanbektas@anadolu.edu.tr">nurcanbektas@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurdan Kurnaz Yetim</td>
<td><a href="mailto:nudankurnaz81@gmail.com">nudankurnaz81@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurdan Yazıcı</td>
<td><a href="mailto:ecz.nurdayazici@gmail.com">ecz.nurdayazici@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurettin Yaylı</td>
<td><a href="mailto:ecz@ktu.edu.tr">ecz@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurgul Karadas-Bakirhan</td>
<td><a href="mailto:nurgulk44@gmail.com">nurgulk44@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurhan Gümrükçüoğlu</td>
<td><a href="mailto:ngumrukcugolgluku@ktu.edu.tr">ngumrukcugolgluku@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nursenem Karaca</td>
<td><a href="mailto:nursesenemyetimoglu@hotmail.com">nursesenemyetimoglu@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nursen Başaran</td>
<td><a href="mailto:nbasaran@hacettepe.edu.tr">nbasaran@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurten Altnar</td>
<td><a href="mailto:altnlar@ankara.edu.tr">altnlar@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Nurten Özdemir</td>
<td><a href="mailto:nozdemir@pharmacy.ankara.edu.tr">nozdemir@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Onat Kadioğlu</td>
<td><a href="mailto:kadioglu@uni-mainz.de">kadioglu@uni-mainz.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Ongun Saka</td>
<td><a href="mailto:omsaka@gmail.com">omsaka@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Onur Altınbaşak</td>
<td><a href="mailto:oaltinbasak@gmail.com">oaltinbasak@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Orhan Yılmaz</td>
<td><a href="mailto:eczfak@yyu.edu.tr">eczfak@yyu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Oruc Allahverdiyev</td>
<td><a href="mailto:orucfarm@yahoo.com">orucfarm@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Oya Bozdağ Dündar</td>
<td><a href="mailto:bozdag@pharmacy.ankara.edu.tr">bozdag@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Oya Orun</td>
<td><a href="mailto:oyaorun@yahoo.com">oyaorun@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Oya Unsal Tan</td>
<td><a href="mailto:oyaunsal@hacettepe.edu.tr">oyaunsal@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özge Inal</td>
<td><a href="mailto:inal@pharmacy.ankara.edu.tr">inal@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Temiz Arpacı</td>
<td><a href="mailto:temiz@pharmacy.ankara.edu.tr">temiz@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ömer Koz</td>
<td><a href="mailto:omer.koz@btu.edu.tr">omer.koz@btu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ömür Demirezer</td>
<td><a href="mailto:omurd@hacettepe.edu.tr">omurd@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Önder Demir</td>
<td><a href="mailto:ondemir601@hotmail.com">ondemir601@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Özen Erol</td>
<td><a href="mailto:nonar@omu.edu.tr">nonar@omu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özge Gün</td>
<td><a href="mailto:gun@ankara.edu.tr">gun@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özge Karasallı</td>
<td><a href="mailto:ozgekarasalli43@gmail.com">ozgekarasalli43@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özge Köse</td>
<td><a href="mailto:ozge.kse@gmail.com">ozge.kse@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özge Ülker</td>
<td><a href="mailto:oulkan@pharmacy.ankara.edu.tr">oulkan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özgen Özer</td>
<td><a href="mailto:ozgen.oz@ege.edu.tr">ozgen.oz@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özgür Eşim</td>
<td><a href="mailto:oesim@gata.edu.tr">oesim@gata.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özgür Güler</td>
<td><a href="mailto:ozgur-guler@msn.com">ozgur-guler@msn.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özgür Üstündag</td>
<td><a href="mailto:ustundag@pharmacy.ankara.edu.tr">ustundag@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Alptekin</td>
<td><a href="mailto:oalptekin@cu.edu.tr">oalptekin@cu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Bahadır Acıkar</td>
<td><a href="mailto:bahadir-ozlem@hotmail.com">bahadir-ozlem@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Coban</td>
<td><a href="mailto:o.coban88@gmail.com">o.coban88@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Güven</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özlem Nazan Erdoğan</td>
<td><a href="mailto:nazan.erdogan@istanbul.edu.tr">nazan.erdogan@istanbul.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Öznur Özkan</td>
<td><a href="mailto:oznurozkn@gmail.com">oznurozkn@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Özüm Öztürk</td>
<td><a href="mailto:ozozturk@ankara.edu.tr">ozozturk@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Patrick J. Walsh</td>
<td><a href="mailto:pwalsh@sas.upenn.edu">pwalsh@sas.upenn.edu</a></td>
<td>USA</td>
</tr>
<tr>
<td>Pelin Köseoğlu Yılmaz</td>
<td><a href="mailto:koseoglupelin@gmail.com">koseoglupelin@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Pınar Aslaner</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Pınar Egemen</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Pınar Özmert</td>
<td><a href="mailto:reyhan.tascioglu@novartis.com">reyhan.tascioglu@novartis.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Pınar Talay Pinar</td>
<td><a href="mailto:ptalay@gmail.com">ptalay@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rachid Belhattab</td>
<td><a href="mailto:rbelhat@yahoo.fr">rbelhat@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Rahime Şimşek</td>
<td><a href="mailto:rsimsek@hacettepe.edu.tr">rsimsek@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rahman Başaran</td>
<td><a href="mailto:rahman.basaran@gmail.com">rahman.basaran@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rahmiye Ertan</td>
<td><a href="mailto:ertan@pharmacy.ankara.edu.tr">ertan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rana Arslan</td>
<td><a href="mailto:rbeis@anadolu.edu.tr">rbeis@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Resul Şahin</td>
<td><a href="mailto:resull_3536@hotmail.com">resull_3536@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rezzan Alyazıcıoğlu</td>
<td><a href="mailto:rezzanaoglu@mynet.com">rezzanaoglu@mynet.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rukiye S.</td>
<td><a href="mailto:rukiyeso@ata.uni.edu.tr">rukiyeso@ata.uni.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rukiye Saygılı</td>
<td><a href="mailto:rukiyesaygili@dpdu.edu.tr">rukiyesaygili@dpdu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Rusudan Kakava</td>
<td><a href="mailto:rusudan.kakava@gmail.com">rusudan.kakava@gmail.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Saadet Dermiş</td>
<td><a href="mailto:dermis@pharmacy.ankara.edu.tr">dermis@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sabriye Aydınoglu</td>
<td><a href="mailto:saydinoglu@cu.edu.tr">saydinoglu@cu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sakine Atila Karaca</td>
<td><a href="mailto:sakineatila@anadolu.edu.tr">sakineatila@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Salvatore Fanali</td>
<td><a href="mailto:salvatore.fanali@cnr.it">salvatore.fanali@cnr.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Samet Poyraz</td>
<td><a href="mailto:s.poyraz88@gmail.com">s.poyraz88@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sami Türkoğlu</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Saniye Özcan</td>
<td><a href="mailto:snyozcan1@gmail.com">snyozcan1@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Seçkin Engin</td>
<td><a href="mailto:seckinengin@ktu.edu.tr">seckinengin@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Seda Fandaklı</td>
<td><a href="mailto:sedaf_84@hotmail.com">sedaf_84@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Seda Nur Topkaya</td>
<td><a href="mailto:sedanur6@gmail.com">sedanur6@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Seda Nurdağ Silkü</td>
<td><a href="mailto:snnurdag@hotmail.com">snnurdag@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Seda Toprak</td>
<td><a href="mailto:neriman.ozhan@sandoz.com">neriman.ozhan@sandoz.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sedef Kir</td>
<td><a href="mailto:sekir@hacettepe.edu.tr">sekir@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Segueni Narimane</td>
<td><a href="mailto:segueninarimane@yahoo.fr">segueninarimane@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Selen Gurkan-Alp</td>
<td><a href="mailto:sglurn@pharmacy.ankara.edu.tr">sglurn@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Selin Akbaş</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Selin Işık</td>
<td><a href="mailto:selin_nocyprus@hotmail.com">selin_nocyprus@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Selman Şabanoğlu</td>
<td><a href="mailto:selmansabanoglu@hotmail.com">selmansabanoglu@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sema Arisoy</td>
<td><a href="mailto:Sema.Arisoy@ankara.edu.tr">Sema.Arisoy@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sema Koyutürk</td>
<td><a href="mailto:skoyturk@anadolu.edu.tr">skoyturk@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sena Atal</td>
<td><a href="mailto:senatal@hotmail.com">senatal@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sencar Tepe</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serap Gür</td>
<td><a href="mailto:serapgur@ankara.edu.tr">serapgur@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serap Yalçın</td>
<td><a href="mailto:ankaraserap@yahoo.com">ankaraserap@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serap Yalın</td>
<td><a href="mailto:pharmacy@mersin.edu.tr">pharmacy@mersin.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serap Yılmaz</td>
<td><a href="mailto:eczserapyilma@gmail.com">eczserapyilma@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sercan Yıldırım</td>
<td><a href="mailto:sercanyildirim@ktu.edu.tr">sercanyildirim@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serdar Bilgen</td>
<td><a href="mailto:serdarbilgen79@hotmail.com">serdarbilgen79@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serdar Tort</td>
<td><a href="mailto:serdartort@gmail.com">serdartort@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serhat Sevgi</td>
<td><a href="mailto:serhat_svg@hotmail.com">serhat_svg@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serkan Levent</td>
<td><a href="mailto:serkanlevent@anadolu.edu.tr">serkanlevent@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serkan Özbingin</td>
<td><a href="mailto:serkan_ozbingin@hotmail.com">serkan_ozbingin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serpil Demirci</td>
<td><a href="mailto:haziranserpil@gmail.com">haziranserpil@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serpil Nebioğlu</td>
<td><a href="mailto:snebioglu@ankara.edu.tr">snebioglu@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Servet Çete</td>
<td><a href="mailto:scete@gazi.edu.tr">scete@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevda Güzel</td>
<td><a href="mailto:sevdacanguzel@yahoo.com">sevdacanguzel@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevda Türk</td>
<td><a href="mailto:sevdaturk61@gmail.com">sevdaturk61@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevgi Şar</td>
<td><a href="mailto:sevgisar98@gmail.com">sevgisar98@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevil Şenkardeş</td>
<td><a href="mailto:sevil.aydin@marmara.edu.tr">sevil.aydin@marmara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevilay Erdoğan</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevim Alan</td>
<td><a href="mailto:salan@anadolu.edu.tr">salan@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevinc Ozakar</td>
<td><a href="mailto:melcetin@hotmail.com">melcetin@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevinç Kurbanoğlu</td>
<td><a href="mailto:skurbanoglu@gmail.com">skurbanoglu@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sevtem Gökbulut</td>
<td><a href="mailto:sevtem91@gmail.com">sevtem91@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Seyed Moien Moghimi</td>
<td><a href="mailto:moien.moghimi@sund.ku.dk">moien.moghimi@sund.ku.dk</a></td>
<td>Denmark</td>
</tr>
<tr>
<td>Sezen Yılmaz</td>
<td><a href="mailto:sezen.yilmaz@ankara.edu.tr">sezen.yilmaz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sibel A. Özkan</td>
<td><a href="mailto:ozkan@pharmacy.ankara.edu.tr">ozkan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sibel Avunduk</td>
<td><a href="mailto:sibelavunduk@mu.edu.tr">sibelavunduk@mu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sibel Gürpinar</td>
<td><a href="mailto:sbl.gurpinar@gmail.com">sbl.gurpinar@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sibel Ilbasmis-Tamer</td>
<td><a href="mailto:ilbasm@yahoo.com">ilbasm@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sibel Süzen</td>
<td><a href="mailto:sibel@pharmacy.ankara.edu.tr">sibel@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sinan Süzen</td>
<td><a href="mailto:suzen@pharmacy.ankara.edu.tr">suzen@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Sinem Altintop</td>
<td><a href="mailto:sinem.altintop@pfizer.com">sinem.altintop@pfizer.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sinem Aslan Erdem</td>
<td><a href="mailto:sinemaslanerdem@yahoo.com">sinemaslanerdem@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sinem Göktürk</td>
<td><a href="mailto:sinemgokturk@gmail.com">sinemgokturk@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Somayeh Handali</td>
<td><a href="mailto:handali_s81@yahoo.com">handali_s81@yahoo.com</a></td>
<td>Iran</td>
</tr>
<tr>
<td>Songül Karakaya</td>
<td><a href="mailto:karakayas@ankara.edu.tr">karakayas@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sreeparna Banerjee</td>
<td><a href="mailto:banerjee@metu.edu.tr">banerjee@metu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Sulhiye Yıldız</td>
<td><a href="mailto:suyildiz@ankara.edu.tr">suyildiz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Süleyman Kayan</td>
<td><a href="mailto:eczkayan@hotmail.com">eczkayan@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Süfnay Parlar</td>
<td><a href="mailto:sulunay.parlar@ege.edu.tr">sulunay.parlar@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şahin Yıldırım</td>
<td><a href="mailto:eczacilik@cumhuriyet.edu.tr">eczacilik@cumhuriyet.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şebnem Yılmaz</td>
<td><a href="mailto:sebnem2003@hotmail.com">sebnem2003@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şerif Samim</td>
<td><a href="mailto:seniz.yy@gmail.com">seniz.yy@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şerif Demirayak</td>
<td><a href="mailto:bilgi@medipol.edu.tr">bilgi@medipol.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Serife Selma Uras Güngör</td>
<td><a href="mailto:urasselma@hotmail.com">urasselma@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şeyda Akkaya</td>
<td><a href="mailto:seydaakkaya@ktu.edu.tr">seydaakkaya@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şeyda Akkuş Arslan</td>
<td><a href="mailto:seydaakkus@gmail.com">seydaakkus@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şirin Uysal</td>
<td><a href="mailto:sirin.uysal@ege.edu.tr">sirin.uysal@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Şükran Öztürk</td>
<td><a href="mailto:sukranozturk79@gmail.com">sukranozturk79@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tahar Smaili</td>
<td><a href="mailto:smaili_tahar@yahoo.fr">smaili_tahar@yahoo.fr</a></td>
<td>Algeria</td>
</tr>
<tr>
<td>Talha Zahid Yeşiloğlu</td>
<td><a href="mailto:talhazahidyesioglu@windowslive.com">talhazahidyesioglu@windowslive.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tamar Khatiashvili</td>
<td><a href="mailto:khatiashvilitako@gmail.com">khatiashvilitako@gmail.com</a></td>
<td>Georgia</td>
</tr>
<tr>
<td>Tangül Şen</td>
<td><a href="mailto:kilinc@pharmacy.ankara.edu.tr">kilinc@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tanja Schwerdtle</td>
<td><a href="mailto:tanja.schwerdtle@uni-potsdam.de">tanja.schwerdtle@uni-potsdam.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Tanju Özelikay</td>
<td><a href="mailto:ozcelikay@ankara.edu.tr">ozcelikay@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tansel Çomoğlu</td>
<td><a href="mailto:comoglu@pharmacy.ankara.edu.tr">comoglu@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tansu Erkan</td>
<td><a href="mailto:tansu.erkan@gmail.com">tansu.erkan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tayfun Ersöz</td>
<td><a href="mailto:ersoz@hacettepe.edu.tr">ersoz@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Taylan Turan</td>
<td><a href="mailto:tylanturan35@gmail.com">tylanturan35@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Telal Yanık</td>
<td><a href="mailto:eczacilik@agri.edu.tr">eczacilik@agri.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tomas Cavoysky</td>
<td><a href="mailto:tomascavoysky@gmail.com">tomascavoysky@gmail.com</a></td>
<td>Slovakia</td>
</tr>
<tr>
<td>Tuba İncecayır</td>
<td><a href="mailto:tincecayir@gazi.edu.tr">tincecayir@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tuba Kuşman</td>
<td><a href="mailto:kusmantuba@gmail.com">kusmantuba@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tuba Reçber</td>
<td><a href="mailto:tuba.recber@hacettepe.edu.tr">tuba.recber@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tügba Idug</td>
<td><a href="mailto:tidug@medipol.edu.tr">tidug@medipol.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tügba Bolelli</td>
<td><a href="mailto:tertan@pharmacy.ankara.edu.tr">tertan@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tügba Eren</td>
<td><a href="mailto:erent@ankara.edu.tr">erent@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tügba Somay Doğan</td>
<td><a href="mailto:canozen@metu.edu.tr">canozen@metu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tügbagül Çal</td>
<td><a href="mailto:tgc89@gmail.com">tgc89@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tuğrul Mert Serim</td>
<td><a href="mailto:serim@pharmacy.ankara.edu.tr">serim@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tunca Gul Altuntas</td>
<td><a href="mailto:altuntas@pharmacy.ankara.edu.tr">altuntas@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Turan Arabacı</td>
<td><a href="mailto:turan.arabaci@inonu.edu.tr">turan.arabaci@inonu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Tülay Çoban</td>
<td><a href="mailto:coban@pharmacy.ankara.edu.tr">coban@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Name-Surname</td>
<td>e-mail</td>
<td>Country</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Ufuk Özgen</td>
<td><a href="mailto:uozgen@ktu.edu.tr">uozgen@ktu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ulvi Zeybek</td>
<td><a href="mailto:ecz.dekanlik@mail.ege.edu.tr">ecz.dekanlik@mail.ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Uluviye Acar</td>
<td><a href="mailto:uacar@anadolu.edu.tr">uacar@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ulya Badilli</td>
<td><a href="mailto:unuman@pharmacy.ankara.edu.tr">unuman@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Umut Can Öz</td>
<td><a href="mailto:umutcanoz@ankara.edu.tr">umutcanoz@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Unzile Yaman</td>
<td><a href="mailto:unzileyaman@gmail.com">unzileyaman@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ümmühana Özdemir Özmen</td>
<td><a href="mailto:ummuhan@gazi.edu.tr">ummuhan@gazi.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Vahap Murat Kutluay</td>
<td><a href="mailto:kutluay88@gmail.com">kutluay88@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Volkan Karacaoglan</td>
<td><a href="mailto:volkan78@gmail.com">volkan78@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yalcin Duydu</td>
<td><a href="mailto:duydu@pharmacy.ankara.edu.tr">duydu@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yalcin Ozkan</td>
<td><a href="mailto:yozkangata@gmail.com">yozkangata@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yasin Genç</td>
<td><a href="mailto:ygncyasin@gmail.com">ygncyasin@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yaşar Alptekin</td>
<td><a href="mailto:alptekin69@ksu.edu.tr">alptekin69@ksu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yavuz Yardim</td>
<td><a href="mailto:yavuzyardim2002@yahoo.com">yavuzyardim2002@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yeşim Cantürk Talman</td>
<td><a href="mailto:ytalman@yahoo.com">ytalman@yahoo.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yeşim Karasulu</td>
<td><a href="mailto:yesim.karasulu@ege.edu.tr">yesim.karasulu@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yıldız Bora</td>
<td><a href="mailto:boramat65@hotmail.com">boramat65@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yıldız Özalp</td>
<td><a href="mailto:yildiz.ozalp@neu.edu.tr">yildiz.ozalp@neu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yusuf Özay</td>
<td><a href="mailto:yusufozay33@hotmail.com">yusufozay33@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yusuf Öztürk</td>
<td><a href="mailto:eczfak@anadolu.edu.tr">eczfak@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yusuf Sicak</td>
<td><a href="mailto:ysicak@gmail.com">ysicak@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yücel Kadioğlu</td>
<td><a href="mailto:ecz_fak@erzincan.edu.tr">ecz_fak@erzincan.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Yüksel Bayrak</td>
<td><a href="mailto:eczfak@trakya.edu.tr">eczfak@trakya.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zafer Asım Kaplancıklı</td>
<td><a href="mailto:zakaplan@anadolu.edu.tr">zakaplan@anadolu.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zafer Şahin</td>
<td><a href="mailto:zshahin@medipol.edu.tr">zshahin@medipol.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zekiye Ceren Artutuluk</td>
<td><a href="mailto:cerenarituluk@hotmail.com">cerenarituluk@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeliha Büyükbingöl</td>
<td><a href="mailto:zbuyukbingol@ankara.edu.tr">zbuyukbingol@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zerrin Sezgin Bayındır</td>
<td><a href="mailto:zsezgin@pharmacy.ankara.edu.tr">zsezgin@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Adigüzel</td>
<td><a href="mailto:neriman.ozhan@sandoz.com">neriman.ozhan@sandoz.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Ates-Alagoz</td>
<td><a href="mailto:zates@pharmacy.ankara.edu.tr">zates@pharmacy.ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Bayramoğlu</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Berna Tamer</td>
<td><a href="mailto:zbernatamer@gmail.com">zbernatamer@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Burcu Akkuş</td>
<td><a href="mailto:zcalgan@gmail.com">zcalgan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Çalış</td>
<td><a href="mailto:zeynepocalcan@gmail.com">zeynepocalcan@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Doğan</td>
<td><a href="mailto:zeynep.ocak@hacettepe.edu.tr">zeynep.ocak@hacettepe.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Kasap</td>
<td><a href="mailto:dadasozden@gmail.com">dadasozden@gmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zeynep Soyer</td>
<td><a href="mailto:zeynep.soyer@ege.edu.tr">zeynep.soyer@ege.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Ziad El Rassi</td>
<td><a href="mailto:ziad.el_rassi@okstate.edu">ziad.el_rassi@okstate.edu</a></td>
<td>USA</td>
</tr>
<tr>
<td>Zuhal Güvenalp</td>
<td><a href="mailto:ecza@atauni.edu.tr">ecza@atauni.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zuhal Uğcun</td>
<td><a href="mailto:uckland@usta.edu.tr">uckland@usta.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zühal Kılıç Kurt</td>
<td><a href="mailto:zkurt@ankara.edu.tr">zkurt@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zühre Şentürk</td>
<td><a href="mailto:zuhresenturk@hotmail.com">zuhresenturk@hotmail.com</a></td>
<td>Turkey</td>
</tr>
<tr>
<td>Zümrə Kara</td>
<td><a href="mailto:zkara@ankara.edu.tr">zkara@ankara.edu.tr</a></td>
<td>Turkey</td>
</tr>
</tbody>
</table>
Naturmed İlaç Kimya ve Kozmetik San. Tic. Ltd. Şti
www.naturmed.com.tr

Bilimin Doğayla Buluştuğu Nokta
1975 YILINDAN BERİ
İNSAN SAĞLIĞINA DUYDUĞUMUZ
SAYGI
İLE HİZMET EDİYORUZ...
Hayat en doğal halyle AFYE'de!

- **Pronezya**: Propoli, Anı çubu, Ekstremsa Ekstrakti, Vitamin E
- **Royal Jelly**: Anı sıvı)
- **Omega-3**: (DHA, EPA, Vitamin E)
- **Propolis**: Propolis

Neden takviye edici gıda

Hayat En Doğal Halile

AFYE TÜRKİYE
Deva Destek Hizmetleri Kim. Şirk. İhs. İhr. Ltd. Şti. / Ankara
Tel: 0312 397 18 78

Ücretsiz Danışma Hattı: 0850 455 23 93
www.afye.com.tr

Sadece Eczanelerde
Hidroten
Su Bazlı / Yağ Bazlı Cilt Bakım Kremi

Gerçekten nem ister

CILDİNİZ İPEKSI HİS VERİR, JOJOBA YAĞI İÇERİR

SU BAZLI

YAĞ BAZLI

NOBEL

Referanslar: 
1. 
2. 
3. 
4. 
5. 
6. 
7. 
8. 
9. 
10. 
11. 
12. 
13. 
14. 
15. 
16. 
17. 
18. 
19. 
20. 
21. 
22. 
23. 
24. 
25. 
26. 
27. 
28. 
29. 
30. 
31. 
32. 
33. 
34. 
35. 
36. 
37. 
38. 
39. 
40. 
41. 
42. 
43. 
44. 
45. 
46. 
47. 
48. 
49. 
50. 
51. 
52. 
53. 
54. 
55. 
56. 
57. 
58. 
59. 
60. 
61. 
62. 
63. 
64. 
65. 
66. 
67. 
68. 
69. 
70. 
71. 
72. 
73. 
74. 
75. 
76. 
77. 
78. 
79. 
80. 
81. 
82. 
83. 
84. 
85. 
86. 
87. 
88. 
89. 
90. 
91. 
92. 
93. 
94. 
95. 
96. 
97. 
98. 
99. 
100. 

Cildiniz ipeksi his verir, jojoba yağı içerir.
90 yıldır sağlık için bilimin yolunda
DÜNYANIN EN ZORLU
SAĞLIK SORUNLARININ
ÇÖZÜMÜ İÇİN
BİRLİKTE ÇALIŞIYORUZ

Glob al bir biyofarma şirketi olarak, işbirliği içinde olduğumuz paydaşlarımızla hastaların hayatlarında iz bırakmak için çalışıyor, onlara daha sağlıklı yaşamalar sunmak için olasılıkları gerçeğe dönüştürüyoruz.

İNSANLAR,
DAHA İYİSİNİ YAPMA TUTKUMUZ.
OLASILIKLAR.

www.abb vie.com.tr
Hayatı Seviyoruz...

Dünyada sadece beyin hastalıklarının tedavisi için adanmış ve tamamen odaklanmış tek ilaç firması olarak, Lundbeck, alkol bağımlılığı, depresyon ve anksiyete, psikoz, epilepsi, Huntington, Alzheimer ve Parkinson hastalıklarının tedavisi için onde gelen ilaçları geliştirmek pazara sunmuştur.

Geçtiğimiz yüzyılda, milyonlarca insan bu terapiler ile tedavi edilmiştir. Beyin hastalıkları için ileri tedavileri geliştirmek karmaşık ve zorlu olsa da odağımızı koruyoruz: Beyin hastalığı ile yaşayan insanlara daha iyi bir yaşam sağlamak için, gelecek 100 yılda başarımımız gereken hala çok şey var.
Her çeşit laboratuar kimyasalları ve sarf malzemeleri firma portföyümüzdə mevcut olup, proje alımlarınızda teknik bilgi ve malzeme desteği sağlamaktan memnuniyet duyarız.

NOVAGENTEK LABORATUAR ÜRÜNLERİ VE TEKNOLOJİLERİ TİC. LTD. ŞTİ.
Atatürk Blvd. Atayurt İşhani, 169/45-46 Kavaklidere 06680 Ankara
Tel: 0312 418 56 56 (pbx) • Faks: 0312 417 26 26
www.novagentek.com.tr • info@novagentek.com.tr
Daha iyi ilaç, daha iyi yaşam

Koçak Farma, Türkiye ve Avrupa Birliği GMP uygunluk sertifikasına sahip tesislerinde yüzde yüz yerli sermaye ile 44 yılı aşkın Türk Tibbinin hizmetindedir. Çerkezköy Organize Sanayi Bölgesinde 140.000 m² alan üzerinde kurulu 70.000 m² kapalı alana sahip tesislerinde en son teknoloji ve yüksek kaliteyle GMP kurallarına uygun olarak üretim yapmaktadır.

Koçak Farma ilaç ve ilaç hammaddelerinin üretiminde, özellikle onkoloji, biyoteknoloji ve hormon alanlarında, dünyada sınırlı sayıda ilaç üreticisinin sahip olduğu gelişmiş teknolojileri ülkemizde kullanıma sunarak ve Ar-Ge çalışmalarını sonucu gerçekleştirildiği buluşların patentini alarak yerli ilaç sanayiinin gelişmesine öncülük etmektedir.