ANKARA UNIVERSITY
FACULTY OF PHARMACY

ISOPS 12th International SYMPOSIUM ON PHARMACEUTICAL SCIENCES

PROCEEDING BOOK

JUNE 26-29, 2018
ANKARA, TURKEY

Ankara University Faculty of Pharmacy Publication No:xxxxxxxx

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Dear Participants and Guests,

I would like to thank all the participants of 12th International Symposium on Pharmaceutical Sciences for their valuable contributions. You had spent your days in a charming country that shelters the oldest and the greatiest 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 bianually until 1997 and then every three years. The previous 11 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
4th International Symposium on Pharmaceutical Sciences	27-30 June 1995
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
11th International Symposium on Pharmaceutical Sciences	9-12 June 2015

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 6904 graduates since the established and the current number of students is 967.

Present educational and scientific resources allow a total of 138 faculty members, 49 professors, 13 associate professors, 16 assistant professors, 60 research assistants in our faculty. Moreover, 66 administrative staff members and other personnel are working at different offices.

The mission of 12th International Symposium of Pharmaceutical Sciences was to perform a broad scientific perspective by the invitation of distinguished scientists having national / international reputation in their areas, so most recent advances were discussed interactively and empower the knowledge-based drug research development and multidiciplinary collaborations. It was our intention to make this symposium a memorable event, both scientifically and socially for the attendees.

We are pleased to announce that around 800 scientists were registered to ISOPS-12 in which 664 oral/poster presentations participated as well as 41 distinguished lecturers invited from several countries.

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

The topic of the Panel was “The road to the strong Turkish Pharmaceutical Industry”. The lecturers were: Dr Hakkı Gürsöz, Prof.Dr.Nurten Özdemir, Ali Alkan, Turgut Tokgöz, Kemalettin Akalın, Ümit Dereli, Fatma Taman.

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

Prof. Dr. Gülbin ÖZÇELİKAY

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PL-34: TOWARDS ADVANCED ELECTROANALYTICAL NANOSENSORS: A PROMISING TOOL FOR THE ANALYSIS OF PHARMACEUTICALS

Ozkan, SA.

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Introduction:

In recent times Nanotechnology has become very popular, in the sensor fields. It is thought that the utilization of such technologies, as well as the use of Nano-sized materials, could well have beneficial effects for the performance of sensors. Nanomaterials have an impact in each and every sphere of human lives, from cosmetics to drug research. The small size, amazing nature, and unique optical absorption properties of nanomaterials make them quite useful for therapeutic applications in the pharmaceutical drug development, selective destruction of cancer cells and their selective assay. Nanoscience deals with the objects whose smallest dimensions range from several nanometers up to 100 nanometers (Figure 1).

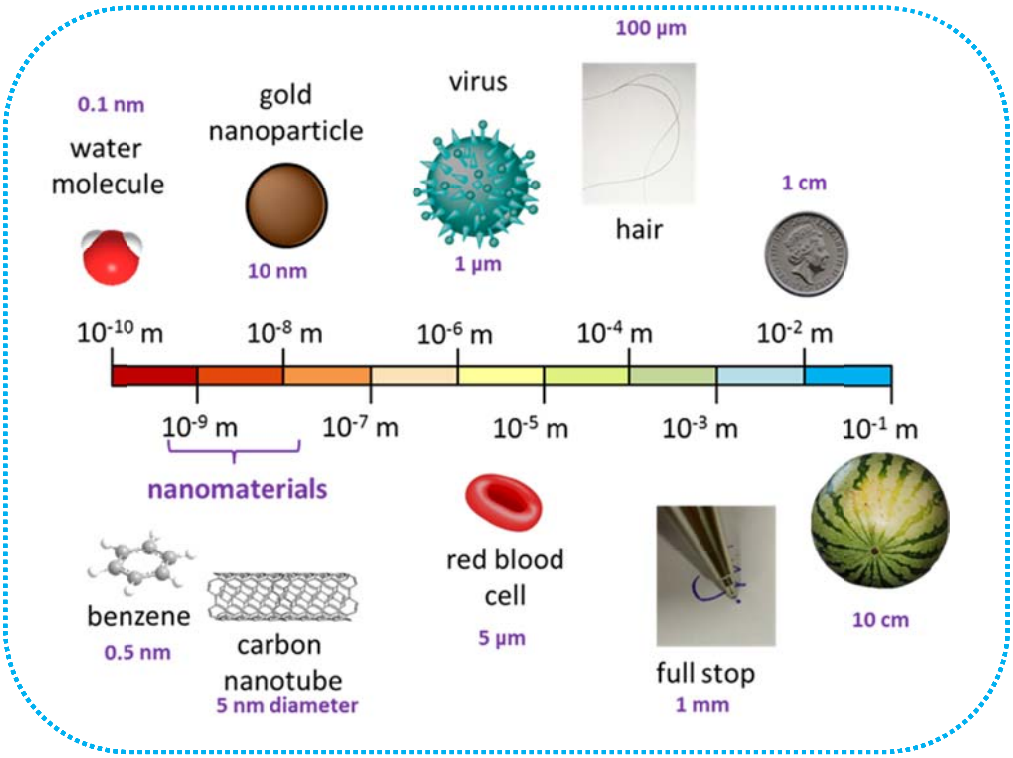

Figure 1. Nanomaterials in nanoscience scale diagram

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Materials and Methods:

Nanomaterials exhibit properties that are quite different from those of materials at large scales. The objects under study in nanotechnology are the nanomaterials, also called as nanostructured materials. All materials are composed of grains, which in turn are made of molecules and atoms. Nanomaterials are those having grain sizes in the range of nanometers.

Results:

The unique properties of these different types of nanomaterials have novel electrical, catalytic, magnetic, mechanical, thermal, or imaging characteristics. Hence, they are highly desirable for applications such as in medical, military, and environmental sectors. The currently used nanomaterials could be classified into four types as; carbon based materials, metal based materials, dendrimers, and composites. Carbon based nanomaterials are common forms with hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanomaterials are referred to as fullerenes, and the cylindrical ones are called as nanotubes. These particles have many potential applications in electroanalytical drug assay (1, 2). Carbon nanotubes (CNTs) represent an increasingly important group of nanomaterials with unique geometric, mechanical, electronic, and chemical properties. Because of their significantly larger dimensions, carbon nanofibers can overcome aggregation without functionalization, coating or decoration (3). The growing interest in microelectrodes and carbon-reinforced structural materials has led to a widespread use of carbon fibers in electroanalytical chemistry.

Electroanalytical methods have certain advantages over other analytical methods:

- ✓ Allows for the determination of different oxidation states of an element in a solution, not just the total concentration of the element.
- ✓ Electroanalytical techniques can be used for the analyzing of drug active compounds with a very low detection limits.
- ✓ It includes the determination of electrode mechanisms. Redox properties of organic molecules can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity.

Conclusions:

Using sensitive pulse methods, the electroanalytical studies are more regularly used on industrial, environmental applications and on the drug analysis in their dosage forms and especially in biological samples (Figure 2). The field of micro-electromechanical systems coupled with biosensors and nanosensors will be everywhere in the near future. This studies

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provides an overview of some of the important and recent developments brought about by the application of electroanalytical nanosensors based nanostructures to nanotechnology for both chemical and biological sensor development and their application examples on pharmaceutical and biomedical area. Even though electrochemistry finds widespread use in analytical chemistry, and there exist still an ever-increasing number of researches dealing with the application of electrochemistry, it has not been conventional method for routine analysis (4). Trends over the past decades go through biosensor, integration, and miniaturization. After Agilent introduced the first commercial lab-on-a-chip (LOC) system in 1999, various LOC products on glass chips exist in the literature such as capillary electrophoresis, micro-reactors, micro-mixers. drug screening, Point of care (POC) testing, DNA analysis, ensuring safety of air, food and water.

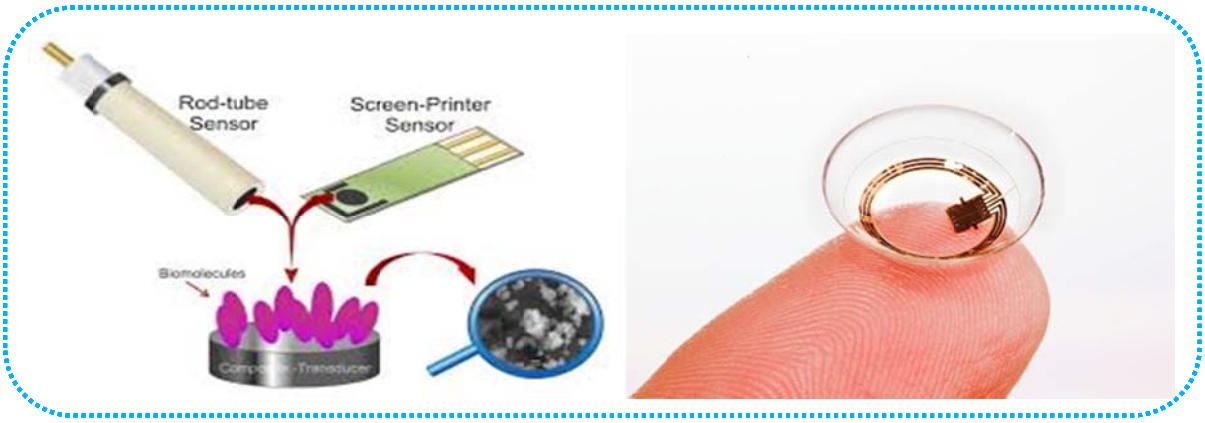

Figure 2. Schematic representation about electrochemical applications of nanomaterials

The future of biosensors will depend on lab-on-a-chip systems in order to miniaturize biochemical analysis systems and to do-skill biochemical analysis. Since the need for science to be smaller, cleaner, cheaper, more reproducible and faster, LOC systems meet with these requirements. This system has positives such as small sample volumes, faster diffusion of reactants, reduction of background noise, easier to fill and manipulate, cheap and portable. The sensitivity is sufficiently high and can be increased more by modifications of classical voltammetric techniques (modified microelectrodes and ultra-microelectrodes) that enhance significantly sensitivity and selectivity of the method (5). The field of micro-electromechanical systems coupled with biosensors and nanosensors will be everywhere in the near future.

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OP-001: ALBUMIN-BASED NANOPARTICULATE DRUG DELIVERY SYSTEMS

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Introduction:

Nanoparticles have emerged as versatile carrier systems for the specific delivery of drugs to organs and tissues (1). In general, nanoparticles may enhance drug absorption by facilitating diffusion through the epithelium, modify pharmacokinetic and drug tissue distribution profile, protect a drug from degradation and improve intracellular distribution (2). Among the available potential colloidal drug carrier systems, protein-based nanoparticles play an important role (3). The ideal properties such as being non-toxic, non-antigenic, having active targeting ability makes human serum albumin (HSA) an ideal natural substance for protein-based nanoparticulate drug delivery systems (4). Basically, emulsion formation, desolvation, or coacervation methods have been described for protein-based nanoparticulate preparation. Since disadvantage of the need for applying organic solvents during particle preparation in emulsion methods, as an alternative, a desolvation process was developed. The objective of the present study is the optimization of a desolvation procedure for the preparation of HSA-based nanoparticles which can be used as a potential radiopharmaceutical drug delivery system.

Materials and Methods:

Preparation of HSA Nanoparticles

HSA nanoparticles were prepared by small modification on the desolvation technique. In principle, 150 mg HSA in 2.0 ml of purified water or 10 mM NaCl solution, respectively, both titrated to pH 7 and 9. By using a flow regulator 8.0 ml of desolvating agent ethanol was added at a defined rate (1.0 $mL.min^{-1}$) drop wisely under constant stirring (550 rpm) at room temperature. Both 35 µl and 177 µl of 8% glutaraldehyde solution were added to induce particle cross-linking. The crosslinking process was performed under stirring (550 rpm) of the suspension over a time period of 24 h. 24h after incubation for crosslinking process, the resulting nanoparticles were purified by two cycles of centrifugation (10000 G, 10 min) and redispersion of the pellet with 10 mM NaCl to the original volume at pH value of 9. HSA nanoparticles particle size and zeta potential were measured by a Zetasizer Nano ZS (Malvern Instruments GmbH, Herrenberg, Germany). The samples were diluted 1:400 with purified water and measured at a temperature of 25 °C and a scattering angle of 173°.

The stability studies for the prepared formulations were made for 7 and 30 days. Stability studies are ongoing. Formulation stability has been put into the stability cabinet at 25 and 40°C.

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Atomic force microscopy (AFM) analyzes were performed with the specified optimum formulations. Particle sizes measured at AFM were compared with Malvern results.

Results:

Purified water or 10 mM NaCl solution was used as a solvent type. The sizes of nanoparticles prepared using pure water were found to be smaller than those of 10 mM NaCl solution. HSA nanoparticles were prepared at pH 7 or pH 9. However, in the case of size and zeta measurements, the size of samples prepared at pH 9 is obviously smaller than pH 7. To examine the glutaraldehyde amount effect on the preparation of HSA nanoparticles, 35 µl and 177 µl were added. The better results for particle size were obtained with 177 µl formulations. According to zeta potential values, there was no significant difference between 35 µl and 177 µl of glutaraldehyde addition formulation. For HSA concentration effect for preparation of nanoparticle, 50 and 75 mg/mL were tried. The results demonstrate that size and zeta potential results were better in formulations prepared at 50 mg/mL concentration.

The samples were found to be stable for 7, 30 days at 25°C and 40°C, and the stability studies will be continued.

We compared the results of AFM and Malvern measurement and similar results were obtained for both of them. In addition, the shape and size distribution of nanoparticles were found to be spherical and narrow respectively.

Conclusions:

HSA nanoparticles were prepared for use in the new radiopharmaceuticals to be developed. The developed formulations were selected as the most ideal by examining parameters such as particle size, zeta potential, formation efficiency and stability. This ideal formulation we choose will reduce the side effect of the new radiopharmaceutical to be prepared and increase the targeting to the desired tissue. The ideal formulation for stability, size and yield: 100 mg HSA in 2.0 ml of purified water, pH 9. By using a flow regulator 8.0 ml of desolvating agent ethanol was added at a defined rate (1.0 $mL.min^{-1}$) drop wisely under constant stirring (550 rpm) at room temperature. Also, 177 µl of 8% glutaraldehyde solution was added to induce particle cross-linking. The crosslinking process was performed under stirring (550 rpm) of the suspension over a time period of 24 h. After preparation, the ideal formulation, active substance loading and radiolabeling studies will be done.

The preparation of new HSA-based nanoparticles was performed with the particle size between 100 and 300 nm in combination with a narrow size distribution. In accordance with the performed study, several factors of the preparation process, such as the rate of addition of particle cross-linking agent, the pH value, solvent types and the protein

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concentration were evaluated. The pH value of the HSA solution before the desolvation procedure was identified as the major factor determining particle size.

Acknowledgements

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OP-003: CLINICAL PHARMACY SERVICES IN INTERNAL MEDICINE UNIT

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Introduction:

Clinical pharmacy is a health specialty, which describes the services of the clinical pharmacist to provide the rational use of medicinal products and devices (1). Clinical pharmacists evaluate patient's health problems and medications. Clinical pharmacy practice is not limited with hospitals, clinical pharmacists can perform in community pharmacies, nursing homes, home-based care services, clinics and any other settings where medicines are prescribed and used. In Hacettepe University Hospitals, clinical pharmacy postgraduate program students participate in clinical ward rounds with physicians and other health care professionals to provide clinical pharmacy services. The treatments of the patients are reviewed by clinical pharmacists in order to provide rational drug use and to identify drug related problems. The aim of this study is to explore the role of clinical pharmacist in the process of pharmaceutical care of patients in internal medicine service.

Material and Methods:

Hospitalized patients were followed between 22 December 2017 and 23 February 2018 in the internal medicine ward of Hacettepe University Hospitals. The data were collected from patient file, hospital information system database (Nucleus®) and via verbal communication with patients and physicians. Three postgraduate students of clinical pharmacy program were participated in clinical ward rounds. They collected data, evaluated patients and provided interventions about problems related to drug therapy.

Results:

In this study, 39 (46.4%) women, a total of 84 patients with the median age of 58 years (18-97 years) were evaluated. The most observed admission reason was dyspnea associated with pneumonia, asthma, cystic fibrosis, chronic obstructive pulmonary disease. The most common reasons of admission are shown in Table 1 (other reasons: acute renal failure, leg edema, nausea and vomiting). Median number of drugs per patient was 6 and the maximum drug number for per patient was 21. Pharmacists made total of 15 recommendations, of those 14 (93.3%) were accepted and 12 (80.0%) were applied (Table 2). One recommendation was not accepted because of the misinformation obtained from the patient file. Two recommendations were accepted but not applied, because the patients were discharged. The recommendations were provided mainly on drug-drug interactions (n=7). Besides that, with the request of physicians, the clinical pharmacists provided information about steroid side effects, usage and interactions of enoxaparin and warfarin, penetration profiles of antibiotics into the bile and interactions between clopidogrel and proton pump inhibitors.

Conclusion:

Clinical pharmacy services can improve the patient care, prevent the drug related problems and reduce the workload of physicians. Rational drug use can be maintained with the involved of clinical pharmacist.

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Table 1. The most common reasons for hospital admissions of the patients.

Reason for admission	n (%)
Dyspnea	20 (23.8)
➢ Pneumonia	7 (8.3)
➢ Asthma	2 (2.4)
➢ Cystic Fibrosis	2 (2.4)
➢ Chronic Obstructive Pulmonary Disease	2 (2.4)
➢ Other	7 (8.3)
Complications of Diabetes	10 (11.9)
Pain	5 (6.0)
Dizziness	4 (4.7)
Gastrointestinal Bleeding	3 (3.6)
Other	42 (50.0)
Total	84 (100)

Table 2. Clinical pharmacists' recommendations and physicians' acceptance and application ratio.

Recommendations	n (%)	Accepted, n (%)	Applied, n (%)
Drug-Drug Interaction	7 (46.66)	7 (50)	5 (41.68)
Switching Drugs	3 (20.0)	3 (21.44)	3 (25.0)
Dose Adjustment	2 (13.33)	2 (14.28)	2 (16.66)
Drug-Food Interaction	1 (6.67)	1 (7.14)	1 (8.33)
Non-treated Indication	1 (6.67)	0 (0)	0 (0)
Discontinuation of the Drug	1 (6.67)	1 (7.14)	1 (8.33)
Total	15 (100)	14 (93.33)	12 (80.0)

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Acknowledgement: This study was not supported by any sponsor.

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OP-007: CORRELATION BETWEEN METABOLOMIC PROFILING AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS FROM 8 CULTIVATED MEDICINAL PLANTS

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[2]Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey

Introduction:

Free radicals and reactive oxygen species play a important role on many diseases such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis [1,2]. Many of plant extracts or single metabolite have antioxidant activity. It is very important to know which single metabolite or full metabolomic profile is effective on antioxidant activity.in this study,we aimed to investigate the antioxidant activity and to evaluate correlation between metabolomic profiling and antioxidant activity.

Materials and Methods:

In this study methanolic extracts of 8 cultivated plant material (*Achillea millefolium, A. Filipendulina, Mentha piperita, Nigella damascena, N. Sativa, Salvia officinalis, Silybum marianum, Echinacea purpurea*) is used. Differant assays (ABTS, DPPH, NO, FRAP, CUPRAC) were choosed to determine antioxidant activity of eight cultivated plants on different antioxidant mechanisms . GC-MS analysis has been performed for metabolomic profile studies (Instrument: GCMS-QP2010 Ultra, Mass range: 50-650 dalton). Fiehn RTL Library is used to identify metabolites. Finally univariate, multivariate and correlations analysis were applied to evaluate correlation between metabolomic profiling and antioxidant activity.

Results:

Antioxidant activity results, at 100μg/mL concentration are:

	A. Filipendulina	A. Millefolium	E. Purpurea	M. Piperita	N. Damascena	N. sativa	S. Officinalis	S. marianum
DPPH	24,12	1,34	0	91,5	0	0	58,89	0
ABTS	64,29	42,52	17,18	79,76	4,93	11,22	84,01	7,99
NO	22,98	20,19	26,09	31,99	16,46	19,57	25,47	13,35
FRAP (KEAK)	223,81	188,09	141,05	605,9	83,33	92,86	417,24	150,57
CUPRAC (GAEAK)	150,39	118,2	59,68	485,39	31,46	13,96	389,59	72,91

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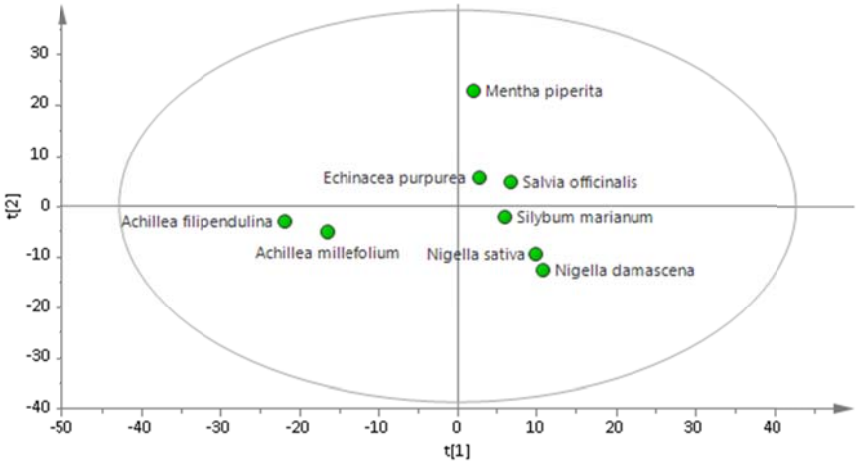
PCA (Principal Component Analysis) is performed and the similarity of the metabolic profiles of eight plant has been determined as a schema:



Also the metabolomics analysis of eight cultivated plant has been performed by using gas chromatography-mass spectrometry (GC-MS). After deconvolution and aligned of the chromatograms, 279 mass spectral features have been detected and 129 of them were annotated using retention index libraries.

Conclusions:

It is a fact that synergy and antagonism play a important role of the whole metabolite instead of single metabolite. Therefore, we compared the whole metabolomic profile of different species and antioxidant activity in order to find metabolites that have negative positive activity. We found 12 metabolites have negative correlation between metabolites levels and antioxidant activity, while 69 positive correlation ($r > 0.70$). 15 metabolites are common for positive and no metabolite is common for negative correlation of 8 plants.

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Also correlation between antioxidant activities and metabolomic (identified) profiles are determined as:

ASSAY	NUMBER OF POSITIVE CORRELATED METABOLITES	NUMBER OF NEGATIVE CORRELATED METABOLITES
FRAP-CUPRAC	16	1
CUPRAC-NO	12	0
CUPRAC-ABTS	8	0
CUPRAC-DPPH	15	1
FRAP-NO	14	0
FRAP-ABTS	8	0
FRAP-DPPH	19	1
NO-ABTS	6	0
NO-DPPH	14	0
ABTS-DPPH	7	0

Acknowledgements:

This study was supported by grants from Hacettepe University Scientific Research Projects (Project No: THD-2016-9171)

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OP-018: MODES OF ACTION OF CYTOTOXICITY OF ALOE-EMODIN ON LEUKEMIA CELLS

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Introduction:

Rumex acetosella has a long tradition in folk medicine for the treatment of cancer (1). *Rumex* species were reported to have anthraquinone-rich phytochemical contents in previous studies (2,3). The main anthraquinone aglycones (emodin, aloe-emodin, physcion, rhein), reported to be present in *R. acetosella* previously (1,4,5). Structural similarities of anthraquinone aglycones to anthracyclines allow to speculate on their possible anticancer activities.

Materials and Methods:

In this study, main anthraquinone aglycones and isolated compounds were tested for their cytotoxicities by resazurin reduction and protease viability marker assays. Detection of inducement of reactive oxygen species (ROS), apoptosis and necrosis, DNA damage as well as cell cycle analysis were performed.

Results:

Aloe-emodin as the most cytotoxic compound revealed IC_{50} values from 9.872 to 22.3 µM in drug-sensitive wild-type cell lines and from 11.19 to 33.76 µM in drug-resistant sublines (Figure 1), was selected to investigate its mechanism against cancer. Aloe-emodin induced S phase arrest, ROS generation, DNA damage and apoptosis. Microarray hybridization revealed a profile of deregulated genes in aloe-emodin-treated CCRF-CEM cells with diverse functions such as cell death and survival, cellular growth and proliferation, cellular development, gene expression, cellular function and maintenance, which was validated by qPCR analysis (Table 1 and Figure 2). COMPARE and hierarchical cluster analyses of transcriptome-wide mRNA expression (Table 2) were examined to predict sensitive or resistant cells in 60 tumor cell lines of DTP (Developmental Therapeutics Programme) (NCI, USA) to aloe-emodin.

Conclusions:

Aloe-emodin as well as *R. acetosella* deserve further investigations as possible antineoplastic drug candidates.

Acknowledgments

This study was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) 2214-A scholarship programme.

The short version of this text is also published in the ISOPS-12 abstract book

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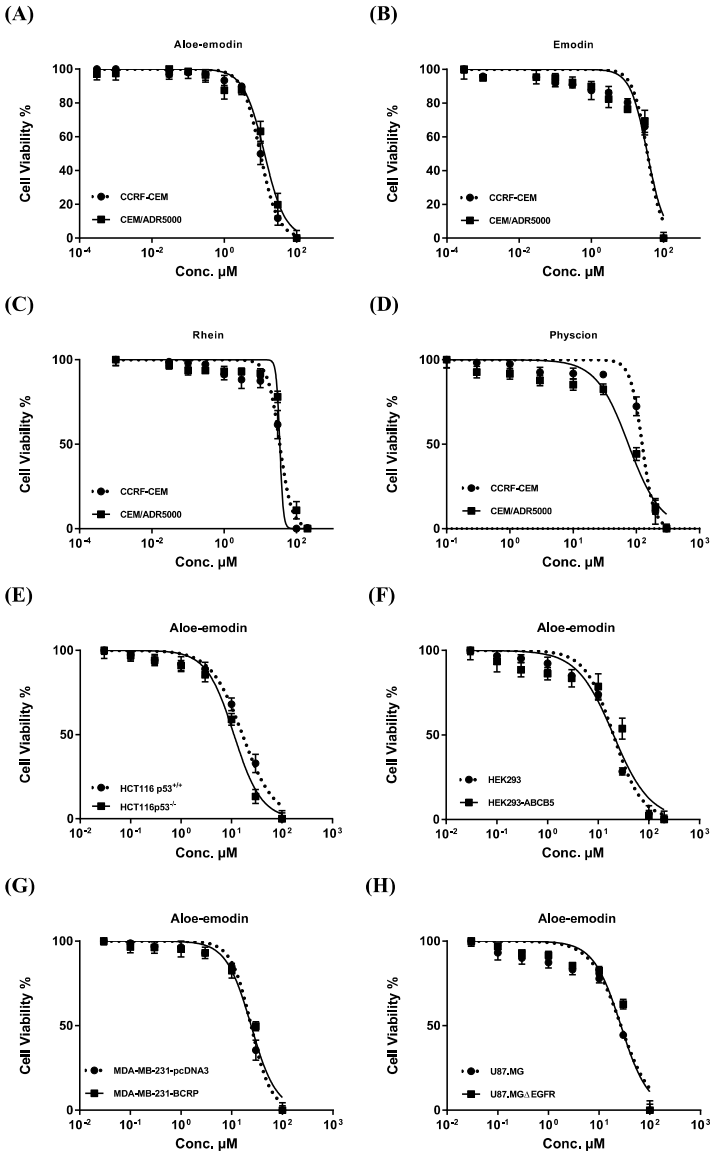

Figure 1. Cytotoxicity of Aloe-emodin (**A**), emodin (**B**), rhein (**C**) and physcion (**D**) towards sensitive CCRF-CEM and multidrug-resistant P-glycoprotein-expressing CEM/ADR5000 acute lymphoblastic cells and of Aloe-emodin as the most cytotoxic compound towards HCT116 ($p53^{+/+}$) colon cancer cells and its knockout clone HCT116 ($p53^{-/-}$) (**E**), HEK293 human embryonic kidney cells and its resistant counterpart HEK293/ ABCB5 transfected with a cDNA of *ABCB5* (**F**), MDA-MB-231-pcDNA3 breast cancer cells and its resistant subline MDA-MB-231-BCRP clone 23 (**G**), and U87.MG glioblastoma cells and its transfected subline U87.MGΔEGFR, respectively (**H**). Mean values ± SD of three independent experiments are shown.

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Table 1. Validation of microarray-based gene expressions by real-time reverse transcription-PCR.

Gene name	Microarray data (FC)*	qPCR data (FC)
DUSP6	2.403	1.40
HHEX	2.321	2.23
MCMDC2	-2.227	-2.47
CRCP	-2.219	-2.63

R value = 0.989 (Correlation coefficient of mRNA expression values between microarray and qPCR was determined by Pearson correlation test).

*FC: Fold change.

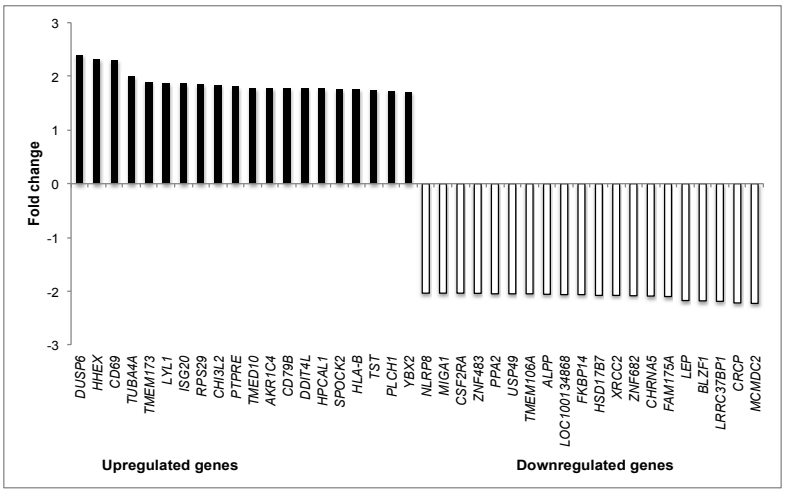

Figure 2. Top up- and downregulated genes in CCRF-CEM leukemia cells upon treatment with Aloe-emodin.

Table 2. Separation of clusters of NCI cell line panel obtained by hierarchical cluster analysis in comparison to drug sensitivity[a].

	Partition	Cluster 1	Cluster 2	Cluster 3
Sensitive	< -4.35 M	0	8	12
Resistant	> -4.35 M	16	5	0
chi-square test: $p= 5.90 \times 10^{-7}$				

[a]The median $\log_{10}IC_{50}$ value (−4.35 M) for each compound was used as a cutoff to separate tumor cell lines as being “sensitive” or “resistant”.

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OP-020: RETROSPECTIVE ANALYSIS OF HACETTEPE DRUG AND POISON INFORMATION UNIT –TERATOGENICITY -CONSULTANCY SERVICES (HIZBIB-TDS)' DATA ABOUT DRUG USE IN PREGNANTS

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Introduction:

A multinational study found that >80% of pregnant women use a drug at some point during their pregnancies [1]. Exposure to drugs and some other other environmental agents such as chemicals, radiation, viruses etc in pregnancy can lead to notorious consequences on the fetus. Historically the most prominent cases in humans have been reported during "thalidomide disaster" in late 1950s and early 1960s and those alarming case reports caused anxiety in both patients and physicians about fetus and drug relationship. Since, resources available to health care providers individually regarding pregnancy exposures were often inaccessible, inadequate, difficult to interpret, and outdated, Teratogenicity Information / Consultation Centers have been established throughout the world since 1980s to provide information especially for physicians [2]. In 1979, the U.S. Food and Drug Administration (FDA) instituted a risk classification system of five categories (A, B, C, D, X) based on their teratogenic potential and a risk/benefit assessment and an Australian classification system was established in 1989 (A, B1, B2, B3, C, D, X) also.

The aim of those centers, are to contribute to health professionals and public on rational decisions about the prognosis of maternal and neonatal health, through increasing awareness of dangerous factors and risks for fetuses as well as pregnants, to decrease preventable birth defects and unnecessary terminations, and to give reassurance regarding exposures that are not known to be of risk through the scope of the most updated and unbiased information resources.

On the other hand, the drug and / or poison information centers have been in service throughout the world almost seventy years with the aims of providing current, unbiased and rapid information to promote rational drug use and decreasing the mortality, morbidity and cost in poisoning cases for both health professionals and public. Hacettepe Drug and Poison Information Unit (HIZBIB) (formerly Center), being the first one in an academic environment in Turkey, has been established for working hours in 1992 under the auspieces of Dean's Office.of Hacettepe University Faculty of Pharmacy. HIZBIB started a project in 2015 for providing consultancies to obstetricians in Hacettepe University (HU) Faculty of Medicine Department of Gynecology and Obstetrics about xenobiotics, radiation etc. exposures during pregnancy, In this connection, after the interviews with the patients referred to HIZBIB, the evaluation reports about the determinants affecting the prognosis of pregnancy were submitted to the patients to pass it their obstetricians.

This study was undertaken in an attempt to analyze and evaluate the extent and characteristics of consultancy services provided by HIZBIB-TDS and to draw attention to the magnitude of the need of such services.

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Materials and Methods:

This is a retrospective descriptive study. Within the period of late October 15, 2015 and March 30, 2018, 79 patients referred to HIZBIB-TDS from HU Faculty of Medicine, Department of Obstetrics and Gynecology were recorded One month after the interview, the pregnants were called by telephone and inquired about the current situation and they were called again in the expected delivery dates. The descriptive statistics were assessed retrospectively and analyzed through IBM SPSS® Statistics v. 23.0.

Results:

The range of the ages of pregnants was 19-41, mean age was 31,54 years. Seventy three pregnants had ultrasonography outcome with a range of 4 - 23 weeks and an average of 8.78 weeks. In addition to drugs, other co-agents were smoking (12 patients), herbs (10 patients), radiation (8 patients) , kin marriages (4 patients), alcohol (3 patients) and illicit substances (2 patients). The distribution of medicines showed that antidepressants ranked first with 22,78 % and antibiotics followed them with 21,51%. The distribution of recommendations of HIZBIB are as follows: Risky pregnancy 36,7 %, careful follow up 34, 8, normal follow up 15,19, therapeutic abortus 8,86. When distributed of the rates of **healthy fetuses** after 1 month by recommendation types of HIZBIB, it was recorded that 100,0% of normal follow ups, 92,59 of careful follow ups, 57,14% of risky pregnancies, and 28,58% of therapeutic abortus recommended ones were healthy. The rates of **healthy babies** in expected delivery time by recommendation types of HIZBIB are as follows also: 70,0% of normal follow ups, 70,0% of careful follow ups, 42,1% of risky pregnancies, and 16,7% of therapeutic abortus recommended ones were healhy. The results obtained showed that the the recommendations provided by HIZBIB have been taken into consideration by the physicians.

Conclusion:

In every pregnancy, a woman starts out with a 3-5% an inherent chance of having a baby with malformation [3]. Many medications provided for treatment of medical comorbidities in pregnancy have known teratogenic potential. Therefore, physicians should be very careful while prescribing during pregnancy and pregnants should also be aware of the probable factors endangering the course of their pregnancies. Being overly cautious or underestimating the situation may lead to negative consequences. In a study conducted by Bakkeba and colleagues,showed that the recommendations regarding teratogenicity potential of drugs provided by a drug information center had a strong impact in increasing the knowledge and confidence of physicians in prescribing for pregnants [4].

HIZBIB provides a substantial contribution through patient-specific advice to the physicians seeking guidance for prescribing and / or evaluating pregnant women by keeping them as updated. Although the analysis of requests from a drug and/or poison information center like HIZBIB is not a real measure of the picture in a given setting, this report still enlightens the magnitude of the problem of the need of updated and unbiased information about drug use in pregnancy. It also emphasizes the importance of the information services given by such centers with the aim of preventing of serious problems' creation as well as almost unbearable cost burden for individuals and the country, besides contributing in better and more appropriate drug selection to treat acute or chronic diseases for physicicans.

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Acknowledgement:

We thank to Gülru Gürdemir and Derya Burtul for their valuable contributions in recording and retrieval of data.

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OP-022: DRUG INTERACTION OF TACROLIMUS AND CYCLOSPORINE IN RENAL TRANSPLANT PATIENTS

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Introduction:

It has been known that many adult patients with end-stage renal disease (ESRD) are considered for a dialysis initially than the transplantation during their treatment, unfortunately 70% mortality at 5 years was observed as a result of this preference. However, improvements in the patient's quality of life and increased survival rate indicated the success of early transplantation which made a renal transplantation as a cost-effective option in the treatment of patients with end-stage renal disease (1).

A graft rejection is a common complication of the transplantation. Although the use of immunosuppressive drugs has reduced the rate of acute rejection about 15-50% recently (2), they have the risk of adverse drug reactions (ADRs), particularly the drug-drug interactions which cause an increased risk of morbidity and mortality following transplantation. The study showed that approximately 10% of hospital admissions of renal transplant patients are related to probable ADRs, of those 29% are clinically significant. Renal transplant patients are at greater risk of drug-drug interactions due to existence of several comorbid conditions due to administration of many drugs concurrently (average of 5.6 interactions per patient) (3).

Calcineurin inhibitors, such as tacrolimus and cyclosporine, are the most commonly used immunosuppressive drugs for renal transplant patients to prevent the rejection. These immunosuppressive drugs have a narrow therapeutic range and drug elimination is mainly maintained by the cytochrome P450 (CYP3A4 and CYP3A5) enzymes and the efflux pump P-glycoprotein which also increases the risk of drug interactions in this particular patient population (4) where nephrotoxicity, neurotoxicity or graft rejection can be observed as a consequence. Therefore, an assessment of drug interactions is crucial; drug-drug interactions should be routinely screened for all transplant patients initially or whenever a new drug is added to pre-existing immunosuppressive treatment.

To our knowledge, there are not many studies in Turkey investigated the potential drug interactions with immunosuppressive drugs in renal transplant patients (5). Thefore, this

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study aimed to assess possible and clinically relevant drug interactions of tacrolimus and cyclosporine individually in patients with renal transplantation.

Materials and Methods:

A prospective, observational study was conducted in nephrology outpatient clinic at the Hacettepe University Hospitals between November 2017-February 2018. The patients older than 18 years, treated with tacrolimus or cyclosporine at least one month and gave a consent were included in the study. A clinical pharmacist attended the clinic visits with the physician and reviewed patient's demographics, drug treatments, comorbid conditions and laboratory results and then evaluated drug interactions. An evaluation of drug interactions was performed using Micromedex© information source and classified as minor, moderate, major and contraindicated. Identified drug interactions were then crosschecked with the patients' clinical status and the Drug Interaction Probability Scale (DIPS) criteria was used for causal evaluation between an observed event and the administration of drugs once the drug interaction is confirmed. DIPS (range of score: 0-12?) was used to determine drug-drug interactions that thought to be responsible for the change in serum concentration. A probability of drug interaction was categorised as doubtful (<2), possible (2-4), probable (5-8), or highly probable (>8). The study was approved by the Hacettepe University Clinical Trials Ethics Committee (KA-180008).

Results: A total of 93 patients (67 in tacrolimus; 26 in cyclosporine) were included in the study; 52.69% were male and average (±standard deviation) age was 40.1±1.27 years. The median (range) daily drug dose of patients was calculated as 3 mg (1-10) for tacrolimus and 100 mg (50-150) for cyclosporine. In total, 135 potential drug interactions (86 with cyclosporine; 49 with tacrolimus) were identified by a clinical pharmacist; of those 90 were moderate, 41 were major and 4 were contraindicated (Table 1.). The most common drug interaction of cyclosporine and tacrolimus was with prednisolone (n=23; 25.56%) and lansoprazole (n=18;36.73%) respectively. According to the DIPS evaluation; cyclosporine-prednisolone (n= 6), tacrolimus-lansoprazole (n=1), cyclosporine-lercanidipine (n=1), cyclosporine-amlodipine (n=1), cyclosporine-prednisolone-allopurinol (n=1) and cyclosporine-colchicine (n=1) interactions are classified as 'possible'. Although daily doses of immunosuppressive drugs were not too high, the serum concentration of these drugs have been increased. As a result, in order to maintain normal therapeutic range of serum concentrations, dose reduction or drug change was applied where appropriate.

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Conclusions:

The monitoring of possible drug interactions in the treatment process of transplant patients by a multidisciplinary healthcare team that includes a clinical pharmacist, will help to identify and prevent potential drug interactions and to ensure desired outcomes.

Acknowledgements

The authors would like to thank all participating patients and hospital staff at the outpatient clinic.

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OP-025: ENANTIOSEPARATION OF KETOCONAZOLE ANTIFUNGAL DRUG USING CAPILLARY ELECTROPHORESIS

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Introduction:

Chiral imidazole and triazole derivatives are widely used human and veterinary drugs and also agrochemicals (fungicides), Ketoconazole,1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl-methyl)-1,3- dioxolan-4-yl]methoxy] phenyl] piperazine], (pK a =2.95 and 6.54) is known as a potent, orally active, broad-spectrum antifungal agent [1], which is marketed as a racemic mixture of the cis-(2S,4R) and -(2R,4S) enantiomers. Ketoconazole as an important category of antifungal agents, inhibit the synthesis of ergosterol, the essential composition, on the cell membrane to prohibit the growth of fungus [2]. The chiral resolution of enantiomers by liquid chromatography and capillary electrophoresis is one of the emerging areas. Liquid chromatographic separations of racemic pharmaceuticals and agrochemicals have achieved a great reputation in separation science. Many racemates have been resolved on various chiral stationary phases (CSPs).The enantiomeric resolutions of some antifungal agents have been reported on cyclodextrins and polysaccharide [3].

In this work, the analytical methods used for chiral separations of ketoconazole include several methods, CE has attracted greatly increasing interest for chiral separations [4].

Materials and Methods:

the separation of enantiomers of some chiral antifungals was studied by CE. In this study, Ketonazole was kindly provided from bilim ilaç sanayi ve ticaret A.S (Istanbul, Turkey).the separation was achieved using three cyclodextrins (2-Hydroxypropyl)-β-CD, (2-Hydroxypropyl)-α-CD and heptakis (2, 3, 6-tri-O-methyl)-β-cyclodextrin (TM-β-CD) were purchased from Sigma Aldrich (Milwaukee, WI, USA).

The CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector and a temperature control device maintained at 29°C. Acquisition and data treatment software were supplied by the manufacturer (HP ChemStation B.04.02-SP1). The separation was conducted in an uncoated fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40 cm total length (8.5 cm effective length x 50 µm I. D. x 363 µm O. D.).

The influence of TM-β-CD concentration, phosphate buffer concentration, buffer pH, Temperature and applied voltage were investigated. The optimum conditions for chiral separation of ketoconazole was achieved using 50 mM phosphate buffer at pH 2.5 containing 1-100 mM TM-β-CD with an applied voltage of 30 kV at 15°C with a 3-s injection time (hydrodynamic injection).

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Results:

The separation was achieved using different CD, with (2-Hydroxypropyl)-α -CD we can see that no separation, we can see the enantioseparation of Ketoconazole as a function of Cyclodextrin concentration of (2-Hydroxypropyl)-β-CD, the good resolution observed with 25mM with (R= 1.69, α= 1.04), but with Heptakıs (2, 3, 6 trıs-O-methyl)-β-CD, it was successfully resolved. Under described conditions, the increase of concentration leads to an increase of resolution for this we chose 50 mM of Heptakıs (2, 3, 6 trıs-O-methyl)-β-CD for this study, it gave the highest resolution for ketoconazole (R= 3.65, α= 1.05) for the first time within 7 min fig.1.

Conclusions:

In this present work the CE method is more selective and rapid to achieve a fast and very efficient separations of variety of antifungals. The resolution of ketoconazole depends on multiple variables such as cyclodextrins, concentration of buffer and Temperature, pH, the type of cyclodextrins play significant role for resolution of enantiomers. Finally, CE is much more environmentally friendly method.

In conclusion, the major goal of this research is not only the separate all enantiomers with the related techniques, but We will investigate the EEO with understanding chiral recognition mechanisms, then the determination and validation using CE and analysis of Ketoconazole from biological matrices such as rat urine

Acknowledgements

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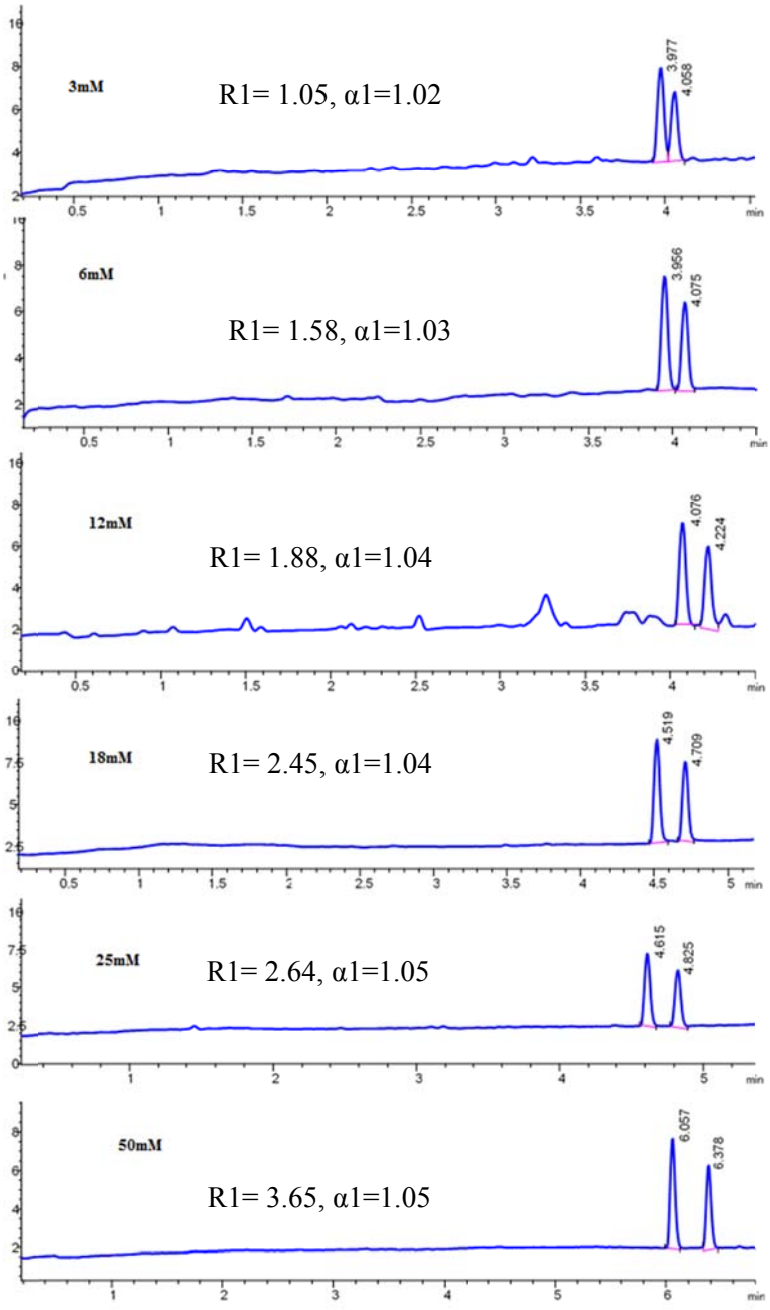

Fig.1. Electropherograms of enantioseparation of ketoconazole. Separation conditions: 50mM phosphoric acid/NaOH, pH: 2.5, 15°C, 50mb/3sinj: 3-50mM TM-β-CD +30kV

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OP-026: EVALUATION OF GENOTOXICITY IN TURKISH WELDERS BY COMET ASSAY

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Introduction:

Worldwide, an estimated 11 million workers have a job title of welder, and around 110 million additional workers probably incur welding related exposures. Exposure to welding fumes can induce free radical activity in the body and free radicals, can cause DNA damage, which has been suggested as a possible mechanism for cancer development induced by exposure to welding fumes.The carcinogenicity of welding fumes was assessed by International Agency for Research on Cancer (IARC) in 1989 and classified as “possibly carcinogenic to humans” (Group 2B), based on ‘‘limited evidence in human beings’’ and ‘‘inadequate evidence’’ in experimental animals. Substantial new evidence has since been accumulated from observational and experimental studies. In the recent evaluation of IARC in 2017, welding fumes and UV radiation from welding were classifed as “carcinogenic to humans” (Group 1) (1). The single cell electrophoresis (COMET) assay has been found to be a very sensitive, rapid, reliable and fairly inexpensive way of measuring DNA damage (2). The aim of this study was to investigate the possible genotoxic effects associated with occupational exposure in Turkish welders using COMET assay in lymphocytes and whole blood cells.

Materials and Methods:

This study was approved by Hacettepe University Clinical Research Ethical Committee (Date and Number: 04/02/2015, GO15/86-05). The study group consisted of 48 male welders. Male office workers (n=48) without any occupational exposure comparable for age and smoking habits to the workers were selected as the control group. Approximately 3 ml whole blood samples were taken and lymphocytes were isolated by Ficoll-Hypaque density gradient procedure (3). The basic alkaline COMET assay of Singh et al. (4), as further described by Collins et al. (5), was performed. Analysis of data was done using the computer program SPSS 20.0 for Windows.

Results:

The general characteristics of the study subjects are shown in Table 1. Age, smoking and alcohol habits were similar between the groups. All of the workers claimed to use safety gloves. 93.75 % and 97.92 % of the workers used masks and safety googles, respectively. But it was observed that the protective equipments were not suitable for a proper protection. DNA damage in the lymphocytes and the whole blood cells of the workers were found to be significantly higher than the control group ($p<0.05$) (Figure 1 and 2) .

Conclusions: The findings of increased DNA damage in peripheral lymphocytes and whole blood of welding workers may demonstrate the possibility of genotoxic risk. The workers in this study must be examined in detail to avoid the development of a carcinogenic process. Also further epidemiological studies with large groups and using different genotoxicity assays are needed to confirm the genotoxic risk due to the exposure of welding fume.

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Acknowledgements:
This work was supported by Hacettepe University (Grant number: THD-2015-7282).

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Table I. Demographic characteristics of the study population

Factors	**Controls (n=48)**	**Workers (n=48)**
Age (years)*	39.83 ± 9.166	36.47 ± 8.94
18-34	19 (39.6 %)	19 (39.6 %)
≥35	29 (60.4 %)	29 (60.4 %)
Smokers	14 (29.2 %)	31 (64.6 %)
Non-smokers	34 (70.8 %)	17 (35.4 %)
Alcohol consumption		
Yes	0 (0 %)	8 (16.7 %)
No	48 (100 %)	40 (83.3 %)
Duration of Exposure (years)		
1-16		30 (62.5 %)
17-32		18 (37.5 %)

* Expressed as Mean ± SD, SD: standard deviation.

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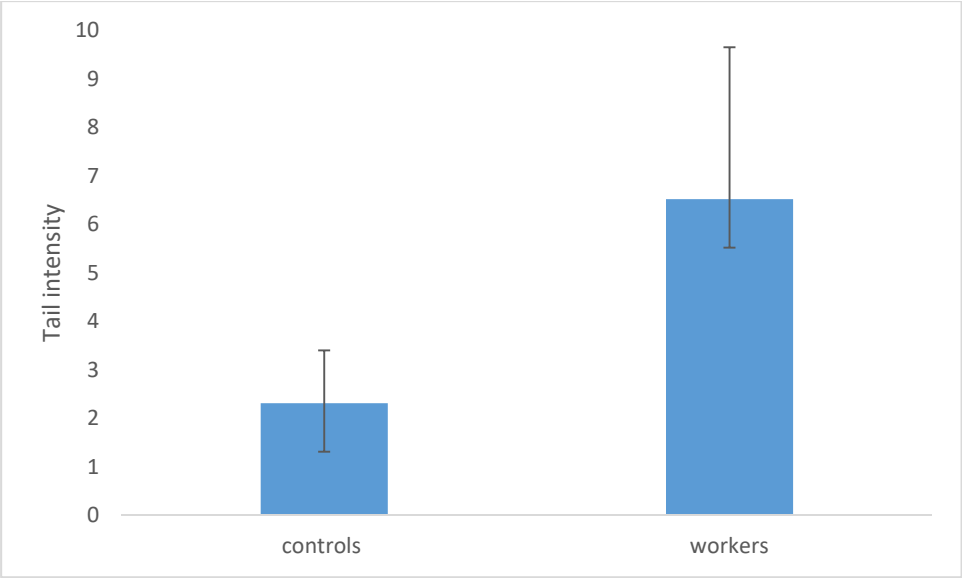

Figure 1. DNA damage in lymphocytes among workers and control group

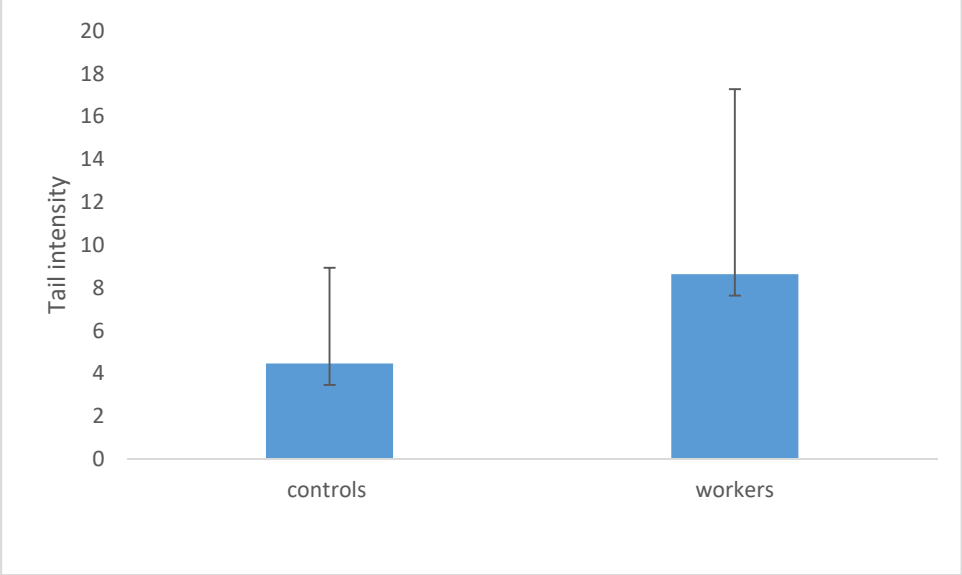

Figure 2. DNA damage in whole blood among workers and control group

OP-047: MEDICINAL PLANTS USED FOR THE TREATMENT OF DIABETES IN ELMADAG (TURKEY)

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Introduction:

Elmadağ is a very old settlement, located on the northeast skirts of Elmadağ, 41 km east of Ankara in the Yukarı Sakarya part of the Central Anatolia Region. It is located between Elmadağ mount in the southwest and İdris mound in the north. The elevation from the sea level is 1135 meters. It has a very defective topography and the western part is flat. Hills and ridges are seen in the district. In some parts of the district, there are white colored crystallized limestones. It is under the influence of cold and hard terrestrial climate. There are big differences between night and day and summer-winter temperatures due to its height and being surrounded by mountains (1, 2). Steppe vegetation is dominant. The flora is usually concentrated in creek beds. Elmadağ steppes are among the richest habitats in Ankara because of its endemic or rare plants and high biodiversity (1, 3). It consists of 30 neighborhoods. The significant decrease in the population of the mountain and in-forest regions and the significant increase in road and industrial enterprises have been observed. Urbanization in Elmadağ has increased in proportion to the development of industry (1). Diabetes mellitus is an endocrinological disorder resulting from insulin deficiency or due to ineffectiveness of the insulin produced by the body. According to the International Diabetes Federation (IDF) there are 415 million people in the world suffer from diabetes, and it is estimated that it will be increased to 642 million by 2040 (4, 5). Plants for the treatment of diabetes have been used since ancient times. Oral antidiabetic drugs used for the treatment of type II diabetes cause severe toxicity to the liver and kidneys. There is no alternative to insulin in the treatment of type I diabetes. For these reasons, studies on the discovery of antidiabetic drugs from plants have been increased (6). The aim of this study was to determine the plants used by the people in the treatment of diabetes in Elmadağ.

Materials and Methods:

The visits were carried out to the district center and neighborhoods between 2015 to 2018. The interviews were conducted face to face with local people to collect ethnobotanical data. The plants were collected and voucher specimens deposited in ANK Herbarium (Ankara University, Science Faculty, Biology Department). During these interviews, local names,

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utilized parts and preparation methods of the medicinal plants were recorded. The medicinal plants used for antidiabetic purposes were identified.

Results:

The plants used by local people for diabetes in direction of the findings from interviews were determined. During the field works, 92 informants were interviewed (30 male, 62 female). Their age ranged from 26 to 88 years (the mean age is over 50 years). The use of 11 plants for diabetes was recorded. Findings from our study were evaluated in table 1.

Our results	Local name	Previous ethnobotanical studies in Ankara	Previous ethnobotanical studies in Turkey	Antidiabetic activity studies
Astragalus microcephalus Willd. (Fabaceae)	Geven	-	-	-
Berberis crataegina DC. (Berberidaceae)	Karamuk	-	+ (7, 8)	-
Crataegus orientalis Pall. ex M.Bieb. (Rosaceae)	Alıç	+ (9)	+ (9)	-
Malva neglecta Wallr. (Malvaceae)	Ebegümeci	+ (9)	+ (7)	+ (10)
Paliurus spina-christi Mill. (Rhamnaceae)	Karaçalı	-	+ (7, 8)	+ (11)
Portulaca oleraceae L. (Portulacaceae)	Semizotu	+ (9, 12)	+ (7, 8)	+ (13)
Prunus divaricata Ledeb. (Rosaceae)	Dağ eriği	-	+ (7, 8)	+ (14)
Rosa canina L. (Rosaceae)	Kuşburnu	+ (15, 16, 9, 17, 12)	+ (7, 8)	+ (18)
Teucrium polium L. (Lamiaceae)	Yavşan	+ (12)	+ (7, 8)	+ (19)
Tribulus terrestris L. (Zygophyllaceae)	Çoban çökerten	-	+ (7)	+ (20)
Urtica dioica L. (Urticacaee)	Isırgan	+ (12, 15, 16, 17, 21, 22)	+ (7, 8)	+ (23)

Table 1. Evaluating of obtained the findings from Elmadağ (Ankara) in Turkey.

Conclusions:

The findings firstly were compared with ethnobotanical studies conducted in Ankara district and later ethnobotanical studies conducted in Turkey. When the studies of folk medicine conducted in the districts of Ankara are examined, it was determined that *Astragalus microcephalus*, *Prunus divaricata*, *Paliurus spina-christi*, *Berberis crataegina* and *Tribulus terrestris* have been used against diabetes in Elmadağ, unlike these districts. It has not been reported a finding with antidiabetic using of *Astragalus microcephalus* in performed ethnobotanical studies in Turkey. The presence of the antidiabetic effect of the designated plants was supported by *in vitro* and *in vivo* biological activity studies, previously published. Biological activity studies on antidiabetic effects of *Astragalus microcephalus*, *Berberis crataegina* and *Crataegus orientalis* have not also been determined.

Acknowledgements

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OP-051: CHEMICAL CONSTITUENTS OF *PRANGOS UECHTRITZII* BOISS&HAUSKN ROOTS

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Introduction:

Prangos sp. is an Iran-Turan element, consist of 17 species, is a member of Apiaceae family (1) There are 30 species around the world. The aerial parts of the genus is used as stimulant and carminative whereas roots of the plant is used as aphrodisiac and wound healing agent in Anatolian folk medicine. It is detected that roots of *Prangos sp.* are riches of coumarin, furanocoumarin and its derivatives by the previous pythochemical studies. Osthol, imperatorin, oxypeucedanin, heraclenol etc. are major components of the plant (2-4). *Prangos uechtritzii* Boiss&Hauskn is a perennial herb and endemic species which is distributed in Central, East and Southeast Anatolia and the plant is known as ‘’Deli çakşır’’ (1). The species epithet of Prangos uechtritzii Boiss&Hausknn were dedicated to German botanist and entomologist Rudolf Karl Friedrich von Uechtritz. There are studies related to essential oil of fruits of the plant (5, 6). However, the pyhtochemistry of the plant has not been investigated previously.

The aim of this study is to isolate and elucidate the secondary metabolites from *P.uechtritzii*.

Experimental

Plant material:

The plant material was collected from Taskent/Konya,1350 m. on June 3, 2016. and identified by Sura Baykan. Voucher specimens have been deposited in the Ege University Herbarium, Faculty of Pharmacy, Izmir, Turkey. (IZEF no: 6050) (www.izef.ege.edu.tr).

Extraction and Isolation and Structual Analysis:

Air dried roots were extracted with hexane, chloroform and methanol (3*3lt ,for each), sequentally using ultrasonic water bath for 24 h. Hexane, chlorofom and methanol extracts were obtained with different polarity, then filtered. The combined extracts were evaporated with a rotary evaporator to dryness at 40 ˚C.

After TLC assays of these extracts, it was decided to start isolation process with chlorofom extract because of its rich content. Fractionation and isolation studies were carried out with column chromatography, preparative TLC and precipitation.

Hexane/Ethyl acetate and Chloroform/Acetone were mostly preferred as mobile systems. Silica gel, RP C18 and Sephadex were used as adsorbents.

Structural elucidation of the compounds was based on both spectroscopic evidence (1D, 2D NMR(400 Mhz) and MS) and reference data comparison.

Results:

7 molecules have been isolated which 3 of them are coumarins and 4 are furanocoumarin derivatives. Isolated compounds were identified as suberosin, psoralen, oxypeucedanin, peucedanol, imperatorin, prantschimgin and 7-demethyl suberosin.

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Table 1 ^{1}H NMR assignments of 1,2,3,4,5,6 and 7 in CD3OD. 400 Mhz.

	1	2	3	4	5	6	7
3	6.21 d(9.4)	6.22 d(9.4)	6.22 d(9.5)	6.38 d(9.6)	6.35 d(9.6)	6.20 d(9.5)	6.29 d(9.8)
4	7.60 d(9.4)	7.66 d(9.4)	7.57 d(9.5)	7.80 d(9.6)	7.75 d(9.6)	7.58 d(9.5)	8.18 dd(9.8, 0.6)
5	7.17 s	7.19 s	7.17 s	7.68 s	7.35 s	7.20 s	
6							
7							
8	6.76 s	7.08 s	6.78 s	7.48 d(0.4)		6.73 s	7.17 m
9							
10							
1’						3.22 m	
2’				7.69 d(2.3)	6.80 d(2.2)	5.13 m	7.60 d (2.4)
3’				6.83dd(2.3,1)	7.68 d(2.2)		6.94 dd (2.4, 1)
4’						1.53 s	
5’						1.59 s	
1’’	3.30 d (7.3)	3.36 d(7.3)	2.83 dd(16.6, 5.9)		5 d(7.2)	3.36 s	4.59, 4.42 dd (10.9, 4.3) dd(10.9, 6.5)
2’’	5.27 m	5.32 td(7.3, 1.3)	3.11 dd(16.6, 5.9)		5.61 m	5.55 m	3.22 dd(6.5, 4.3)
3’’							
4’’	1.76 s	1.77 s	1.39 s		1.73 s	1.84 s	1.32 s
5’’	1.70 s	1.72 s	1.37 s		1.71 s	2.09 s	1.40 s
OCH3	3.89 s		3.87 t(5)				
OH		8.21 Broad s					

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1.Suberosin 2. 7-demethyl Suberosin 3. Peucedanol 4. Psoralen 5.Imperatorin 6.Prantschimgin 7. Oxypeucedanin.

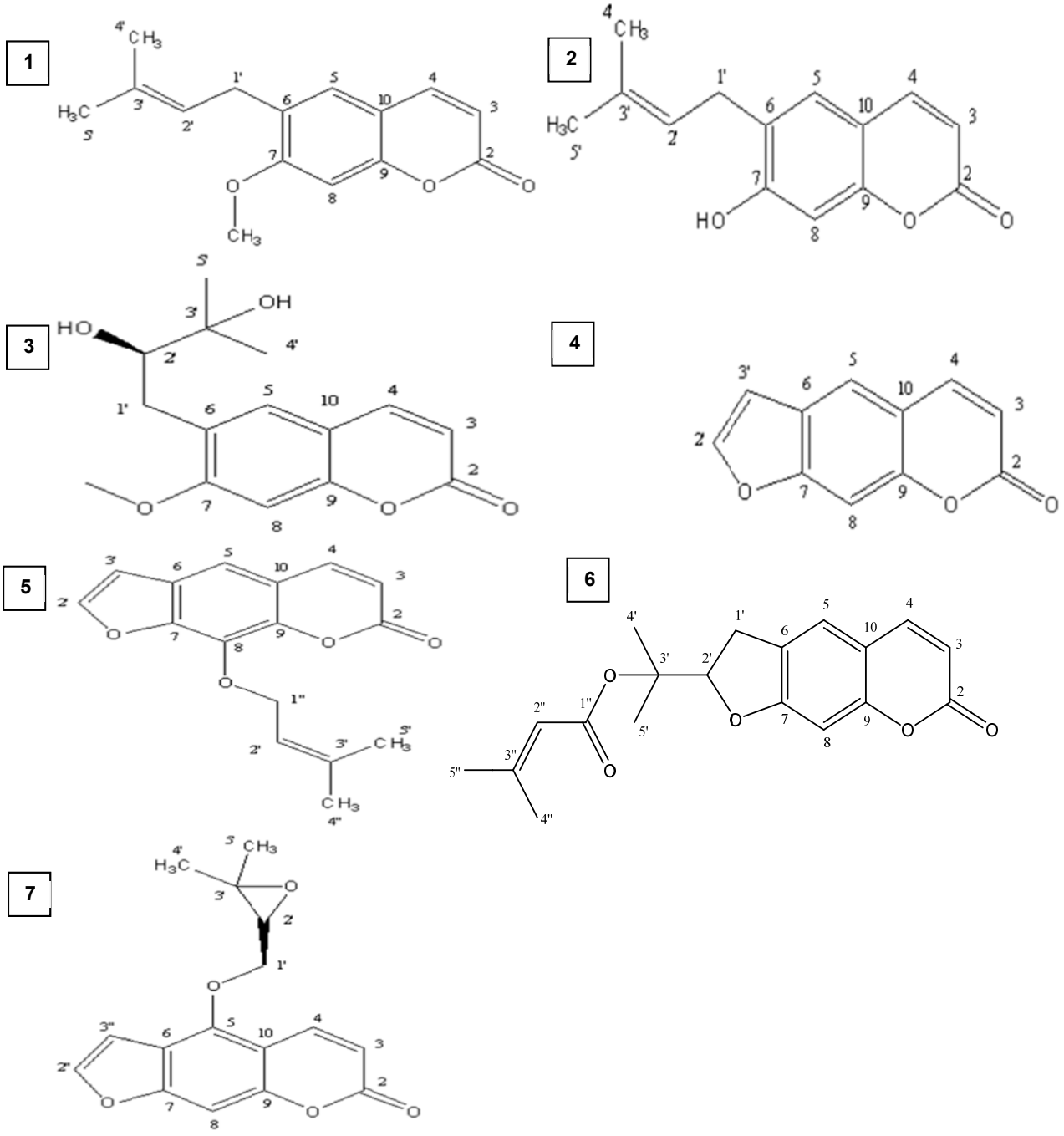

Fig. 1. Structures of compounds. 1-7.

Conclusions:
Through our ongoing study on *Prangos uechtritzii*, 7 molecules have been isolated and identified so far. Pyhtochemical experiments are going on with chloroform, hexane and methanol extract of *Prangos uechtritzii*.

Acknowledgements

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OP-053: PROTECTIVE EFFECT OF mTOR INHIBITION ON LPS-INDUCED SYSTEMIC INFLAMMATION AND TISSUE INJURY: CONTRIBUTION OF mTOR/IκB-α/NF-κB/HIF-1α SIGNALING PATHWAY AND NADPH OXIDASE SYSTEM ACTIVITY.

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Introduction:

Sepsis, the leading cause of death in intensive care units, reflects a detrimental systemic inflammatory host response to infection (1). Lipopolysaccharide (LPS), is found in the outer membrane of Gram-negative bacteria which is a potent inducer of systemic inflammatory response (2). The transcription factor, nuclear factor (NF)-κB, regulates the innate immune response but recently, it has become clear that the hypoxia-inducible transcription factor (HIF) pathway plays a key role in the regulation of immunity and inflammation (3). However, to define the relationship between NF-κB and HIF-1α is proved elusive (4,5). Rapamycin (RAPA), is a potent immunosuppressant agent that effects cell cycle, growth, autophagy, and protein synthesis through the selective inhibition of mammalian target of rapamycin (mTOR) (6). As the continuation of our previous studies, we hypothesized that mTOR inhibition may prevent LPS-induced hypotension, inflammation, and oxidative stress via modulation of mTOR/IκB-α/NF-κB/HIF-1α signaling pathway.

Materials and Methods:

All procedures were carried out in male Wistar rats (n = 24) according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Mersin University Experimental Animals Local Ethics Committee. Animals were housed under controlled environmental conditions of a 12 h light/dark cycle and fed a standard chow. Rats were randomly allocated into four groups: control (n = 6), LPS (n = 6), RAPA (n = 6) and LPS+RAPA (n = 6). Control and RAPA groups received saline (4 ml/kg, i.p.) at time zero. In the LPS and LPS+RAPA groups, LPS (10 mg/kg, i.p.) and/or RAPA (1 mg/kg, i.p.) were injected to rats at the same time. Mean arterial pressure (MAP) and heart rate (HR) were measured by using a tail-cuff device. Rats were sacrificed 4 h after LPS challenge. Blood, kidneys, heart, and lungs were harvested for the measurement of expression and/or phosphorylation of rpS6, IκB-α, NF-κB p65, HIF-1α, iNOS, TNF-α, IL-1β, $gp91^{phox}$, $p47^{phox}$, and β-actin as well as nitrite levels in the tissues and/or sera.

Results:

Administration of LPS to rats decreased MAP and increased HR. LPS caused an increase in expression and/or phosphorylation of rpS6, IκB-α, NF-κB p65 and HIF-1α, TNF-α, IL-1β,

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iNOS, and oxidative stress markers (gp91^{phox} and p47^{phox}) with an increase in nitrite levels while a decrease in IκB-α expression in the tissues and/or sera. These changes caused by LPS were prevented by RAPA. RAPA alone had no effect on the parameters measured.

Conclusions:

These findings suggest that mTOR activation enhances the expression of proinflammatory mediators that contribute to the development of tissue injury via stimulation of IκB-α/NF-κB/HIF-1α signaling pathway and NADPH oxidase system. Moreover, our study shows the importance of mTOR inhibition for the treatment of the inflammatory conditions associated with oxidative stress and should provide the original contribution to preclinical and clinical studies for the development of selective mTOR inhibitors.

Acknowledgments

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OP-056: ELECTROCHEMICAL DETECTION OF NASAL DECONGESTANT DRUG OXYMETAZOLINE BY -COOH FUNCTIONALIZED MWCNTs AND TITANIA NANOPARTICLES MODIFIED ELECTRODE

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Introduction:

Oxymetazoline (OMZ), a nasal vasoconstrictor drug which belongs to imidazoline group, has been used as nasal decongestant for more than forty years (1). In the literature, there are flow injection analysis (2) and liquid chromatography (3, 4) studies. No electrochemical study has previously been reported on OMZ with bare or modified electrodes. Carbon nanotubes (CNTs) are one of the most preferred nanomaterials for electrode modification. They have electrocatalytic effect, provide wide potential window; moreover they generate large surface area and flexible surface (5). Metallic nanoparticles (NPs) are also popular for electrochemical studies due to their useful physical and chemical characteristics. In this study, TiO_2 NPs were preferred because of their chemical stability, cheapness, large surface area and potential window, and reproducible response (6, 7). Here, a differential pulse anodic stripping voltammetric (DPASV) method was suggested and validated for OMZ detection and determination using –COOH functionalized multi-walled carbon nanotubes (*f*MWCNTs) and TiO_2 NPs modified glassy carbon electrode (GCE).

Materials and Methods:

AUTOLAB-PGSTAT302 (Eco Chemie, Utrecht, The Netherlands) with General Purpose Electrochemical Software (GPES) 4.9 was used for DPASV and cyclic voltammetric (CV) measurements. AUTOLAB-PGSTAT100N (EcoChemie, Utrecht, The Netherlands) electrochemical analyzer controlled by NOVA 2.1 software was used for electrochemical impedance spectroscopy (EIS) measurements. Three electrode system in one compartment containing working electrode (GCE), reference electrode (Ag/AgCl) and counter electrode (Pt wire) was used. EIS and CV were used for the characterization of the developed sensor. All chemicals and solvents were of analytical grade for preparing buffer solutions (acetate, phosphate and borate) and other required solutions. OMZ stock solution was prepared in methanol and in working solutions 20 % methanol ratio was kept constant. Nasal spray solution of OMZ (Iliadin®) was also prepared in the same way. The studied pH range was 2.0 – 10.0. *f*MWCNTs and TiO_2 NPs suspensions were prepared as the concentration of 0.5 mg mL^{-1} and 0.5 %, respectively. The optimum amount for dropping on to the clean GCE surface was 5 µL for both nanomaterial and the electrode was dried in a vacuum oven at 40^{o}C.

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Results:

In the first stage of this study optimum electrode surface was prepared. When the bare and modified GCE results were compared, it was seen that peak current increased with modification. After that the buffer solution was selected according to OMZ peak shape and peak current. Best results were obtained in pH 7.0 phosphate buffer solution. EIS and CV studies were realized using 5 mM $K_3[Fe(CN)_6]$ solution in 0.1 M KCl and it can be said that fabrication of electrode surface using nanomaterials were successful. Scan rate studies were performed using CV and logarithm of peak current vs logarithm of scan rate plot had 0.9414 slope value for peak 1. This value showed that OMZ process was adsorption controlled. For the adsorption controlled process accumulation potential and accumulation time were optimized and obtained as 0 V and 180 s, respectively. In Figure 1, two peaks of OMZ can be seen in the optimized conditions but for analytical purposes peak 1 was selected. DPASV responses of OMZ was determined linear between the concentration ranges of 0.05 µM to 1.5 µM in pH 7.0 phosphate buffer solution (Figure 1). Regression data for this calibration were summarized in Table 1.

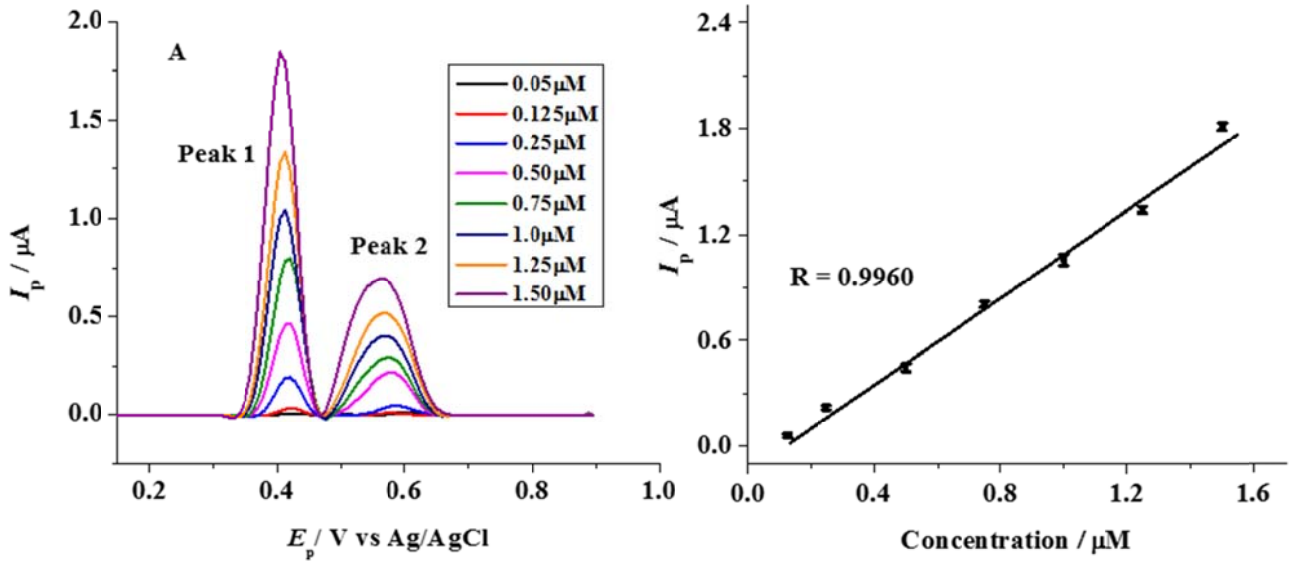

Figure 1. (A) ASDP voltammograms and (B) calibration plot of OMZ.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated by these equation LOD: 3s/m and LOQ: 10s/m where m is the slope of calibration plot and s is the standard deviation of blank solution (five runs). Repeatability and reproducibility studies were obtained five repeated within day and between days measurements for 0.75 µM OMZ.

Application of developed nanosensor and technique was shown by using nasal spray preparation of OMZ. The recovery studies were also performed and all results were summarized in Table 2.

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Table 1. Regression data of the calibration of OMZ peak 1 in the optimum conditions

	Peak 1
Slope (μA μM^{-1})	1.111
LOD (nM)	4.40
LOQ (nM)	14.68
Repeatability of peak current (RSD%)	0.465
Repeatability of peak potential (RSD%)	0.516
Reproducibility of peak current (RSD%)	1.009
Reproducibility of peak potential (RSD%)	0.632

Conclusions:

The nanomolar concentration of OMZ drug was successfully determined by the current developed nanosensor and DPASV technique. The validation of present technique was assessed and the amount of OMZ in pharmaceutical nasal spray was successfully determined. Recovery results proved the accuracy of the developed technique.

Table 2. Results obtained for the determination and recovery experiments in nasal spray of OMZ.

	Peak 1
Labeled claim (mg)	5.00
Amount found (mg)	5.05
RSD %	0.89
Bias %	-1.00
Added (μM)	0.25
Found (μM)	0.249
Average recovered %	99.80
RSD % of recovery	0.88

Acknowledgements

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OP-057: APPLICATION, CHARACTERIZATION AND COMPARATIVE ANTIMICROBIAL ACTIVITY OF *HYPERICUM AUCHERI* JAUB. & SPACH VE *HYPERICUM PERFORATUM* L. *EXTRACTS* CONJUGATED HYBRID NANOFLOWERS

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In recent years, increasing interests of plants, leads to performing more researches about alternative treatments, plants which have antimicrobial effect against infections. *Hypericum* species are one of the important plants which grow up naturally in our country. *Hypericum* L. genus (Hypericaceae) are represented a hundered species in Turkey. In public these plants known as appetizer, sedative, antispazmolitic, antilaxative, antiparasitic. (1). In this study, the upper parts of the flowering soil of *Hypericum perforatum* (Balikesir-Edremit) and *H. aucheri* species were collected from Balıkesir-Kazdağları Sarıkız hill and diagnosed (ERCH 5112, 5113). Methanol extracts of the plants were prepared from the underground parts of H. perforatum and H. aucheri by maceration method. Methanol extracts of the plants were prepared and the total phenol contents, the scavenging activity of DPPH radicals, MIC and MBC were calculated.

Methanol extracts of the plants were prepared from the underground parts of *H. perforatum* and *H. aucheri* by maceration method. The total phenol contains of the extracts were calculated using the Folin-Ciocalteu method as the gallic acid equivalent. The scavenging activity of DPPH radicals of the extracts were also studied. In addition, for the first time the synthesis, characterization and antimicrobial activities of extract+Cu^{2+} hybrid nanoflowers (NFs) from both methanol extracts were investigated according to CLSI standards at 7,825 – 500 µg/ml concentrations. Synthesized nanoflower structures were identified by SEM, EDX and FTIR.

Extracts	Yield [%]	Total phenol contents (mg/g)
H.aucheri	26.5	292.33±1.16
H.perforatum	18.75	316.13±0.07

Extracts and standards(1mg/ml)	Inhibition %
HP	74,1±0,2
HA	74,1±0,1
BHA	78,0±0,2
BHT	79,0±0,3
RA	78,7±0,25

Table 1: Total phenol contents of the extracts and the scavenging activity of DPPH radicals of the extracts (HP: methanol extract of *H. perforatum*; HA: methanol extract of *H. aucheri*; BHA: butylated hydroxi anisole; BHT: butylated hydroxi toluen; RA: rosmarinic acid)

Synthesis of HA,HP extracts and hypericin based-Cu^{2+} hybrid nanoflowers:

Typically, extracts with concentrations of 0.1and 0.5 mgmL−1 were separately added into the mixture containing 50 mL of 10 mM PBS(pH7.4) and 0.8 mM Cu2+ ion. The each mixture was vortexed for 30 s, and then was incubated at 4 °C for 3 days without disturbing. The precipitates occurred at the bottom of reaction tubes were collected and washed with water

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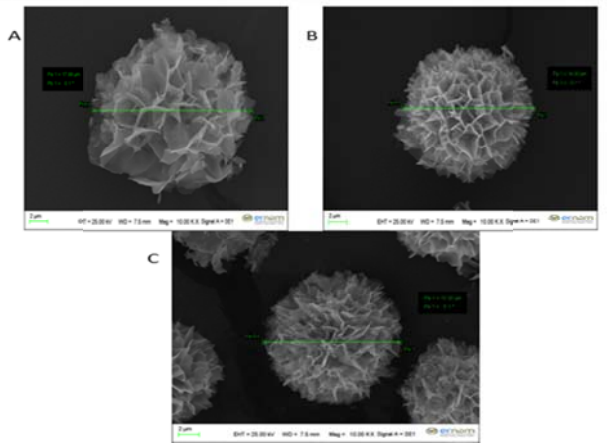
using centrifugation at 10,000 rpm for 15 min. The washing process was repeated 3 times and the final products were dried at 50 °C for futher characterization and use.

Characterization of extracts based-Cu 2+ hybrid nanoflowers:

The nanoflowers were characterized using Scanning Electron Microscopy (SEM, ZEISS EVO LS10), Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer 400 FT-IR Spectrometer Spotlight 400 Imaging System), Energy Dispersive X-Ray (ZEISS EVO LS10) and X-Ray Diffraction (XRD, Bruker AXS D8 Advance Model) analysis.



Picture 1: SEM images of A)Hypericin NF B)H.aucheri NF C) H. perforatum NF

Evaluation of antimicrobial activity:

The Gram negative (E. coli ATCC 35218, P. aeruginosa ATCC 27853,A. baumannii ATCC 19606) Gram positive (E. faecium ATCC 8459, B. subtilis ATCC 6633, S. aureus ATCC 29213) bacterial strains and fungus (C. albicans ATCC 90028) were obtained from Inonu University Medical Microbiology Laboratory collection. Extracts and the nanoflowers were tested to determine their antimicrobial activities based on Clinical Laboratory Standards Institute (CLSI) guidelines by using broth microdilution method. The MIC and MFC values were determined by the broth microdilution method which was carried out in 96-well microtitre plates in triplicate at a dilution of the tested samples from 1000 to 3,9125 µg/mL.

The bacterial cultures were sub-cultured in Mueller Hinton broth (MHB, HIMEDIA, India) and incubated at 37 °C for 14 h. Then, the bacteria cultures measured at 0.5 Mc Farland .The MIC values were determined by the broth microdilution method which was carried out in 96-well microtitre plates in triplicate at a dilution of the tested samples from 1000 to 3,9125 µg/mL. Negative control formed MHB and the positive control was appropriate antibiotics for bacteria and MHB + bacterial suspension only. Then MHB, bacterial suspension and samples were added in each well. After incubation at 37 °C for 18–24 h, the plates were visually examined for bacterial growth. The well containing the lowest concentration of the compound with no visible bacterial growth was taken as the MIC value. The experiments were conducted in triplicates. Theinvitro antifungalsusceptibility wasperformedby broth microdilution methods recommended by the CLSI documents. L-Glutamine, sodium bicarbonate-free RPMI 1640 broth added with 0.2% glucose was used as a media to determine MICs. The MICs were obtained with the final concentration ranged from 0.5 × 103 to 2.5 × 103 cells/mL after incubation at 35 °C during 72 h. Broth, each compounds and fungus added to well. After 24–48 h, plates were determined by visually. Amphotericin B was used as a positive control and dimethyl sulfoxide (DMSO) was used as a negative control. Each experiment was performed in duplicate and repeated three times. Results were evaluated visually.

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Once the MIC values are determined in the microplates, 10 µl is taken and quantitatively added to mueller hinton agar for bacteria and saboraud dextrose agar plates for fungi. The concentration inhibiting 99.9% of the microorganisms is evaluated as MBC / MFC.

	Hypericum aucheri		*Hypericum perforatum*	
	MIC	MBC	MIC	MBC
E. faecium ATCC 8459	125	250	31,25	62,5
S. aureus ATCC 29213	15,625	31,25	250	500
B. subtilis ATCC 6633	7,825	15,625	250	500
E. coli ATCC 35218	31,25	62,5	15,625	31,25
A. baumanni ATCC 19606	7,825	15,625	125	250
P. aeruginosa ATCC 27853	7,825	15,625	250	500
C. albicans ATCC 90028	62,5	125	250	500

	Hypericum aucheri (nanoflower)		*Hypericum perforatum* (nanoflower)	
	MIC	MBC	MIC	MBC
E. faecium ATCC 8459	62,5	125	15,625	31,25
S. aureus ATCC 29213	3,9125	7,825	125	250
B. subtilis ATCC 6633	3,9125	7,825	62,5	250
E. coli ATCC 35218	15,625	31,25	7,825	250
A. baumanni ATCC 19606	3,9125	7,825	62,5	125
P. aeruginosa ATCC 27853	3,9125	7,825	125	250
C. albicans ATCC 90028	15,625	62,5	125	250

Table 2: Antimicrobial activities of the methanol extracts of *H. aucheri* and *H. perforatum* and antimicrobial activities of the nanoflowers of *H. aucheri* and *H. perforatum.*

Also antimicrobial effect of hypericin was tested. There were no effect of hypericin and hypericin NF on tested microorganisms at tested concentrations. NFs were more effective on tested microorganisms than extracts in low concentration, especially Gram negatives.In addition, among the nosocomial infections, A. baumannii and P. aeruginosa, which have high resistance to treatment, appear to have high antimicrobial activity at low dose. Both extracts were evaluated for DPPH radical scavenging effects as percent inhibition and were found to be equally effective when compared to the standard materials used.

H. perforatum has been evaluated with important biological and chemical perspectives and has been documented for its use in the treatment of infectious diseases in ethnobotanical reports. The literature shows that it has a higher antibacterial activity against Gram-positive than Gram-negative bacteria and that alcohol extracts (methanol / ethanol) have a more pronounced activity than water extract. Novoimanine and imanine (antibacterial preparations from H. perforatum) produced in Russia have been tested against Staphylococcus aureus infections in vivo and in vitro and found to be more effective than sulfanilamides. According to our results, both extract and hybrid nanoflower of H. aucheri has higher antimicrobial activity against S. aureus than H. perforatum, and the results are consistent with the literature.

According to the results of the study, H. aucheri species is richer in phenolic content than H. perforatum and has antimicrobial activity about four times higher than H. perforatum on other tested microorganisms other than E. coli and E. faecium in antimicrobial activity experiments. In addition, among the nosocomial infections, A. baumannii and P. aeruginosa, which have high resistance to treatment, appear to have high susceptibility at low doses of nanoflowers.

We designed and reported, for the first time, the synthesis of HA, HP extract based Cu2+ hybrid nanostructures and their effective antimicrobial properties compared to extracts. We proposed that the presence of Cu-O bonds in the nanoflowers could be indication of the molecule of extract-Cu 2+ complexes. The nanoflowers also were utilized as antimicrobial agent against several bacterial and fungal pathogens. We claim that our findings are quite promising to effectively and rationally design and use plant extracts in a variety of technical and scientific fields including textile, biosensor and biomedicine.

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OP-058: NOSE TO BRAIN DELIVERY OF ELETRIPTAN HYDROBROMIDE PLGA NANOPARTICLES

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Introduction:

Over the past few years there has been a great interest to patient-friendly and compliant dosage forms. Nano and micro sized particles are examples of these dosage forms and can be used as sustained release preparations which provide constant and prolonged action with reduced gastrointestinal toxic effects. In addition, these systems allow drug applications other than oral route. Taken into account the possibility to enhance the drug action and reduced side effects, Eletriptan Hydrobromide micro and nano-sized drugs have been developed as described in the literature aiming to apply the drug to various administration types.

The objective of this study was to prepare Eletriptan Hydrobromide loaded PLGA nanoparticles and provide brain targeting, and sustained release of Eletriptan Hydrobromide with-in the brain. This benefit would help to improve its clinical utility in migraine, reduce side effects and improve therapeutic efficacy.

Materials and Methods:

Preparation of Nanoparticles

PLGA nanoparticles were prepared using the W/ O/W emulsion technique. Briefly, different amount of Eletriptan Hydrobromide and PVA was dissolved into 1 ml of distilled water (internal aqueous phase) and then added to 3 ml of dichloromethane containing PLGA (oil phase). The primary W /O emulsion was prepared by a probe sonicator (Bandelin, Germany) at 30 W for 45 s. The primary emulsion was re-emulsified with the external aqueous phase containing PVA and 10 mL distilled water (external aqueous phase), using the probe sonicator at 50 W for 60 s. Afterwards, the organic solvent was evaporated using rotary evaporator (Buchi, Switzerland).

Characterization of Nanoparticles

Release of Eletriptan Hydrobromide from the nanoparticles was studied at 37°C using a dialysis bag diffusion technique. The released drug in the buffer was collected at predetermined time intervals and frozen for further quantitative analysis using HPLC (1). To investigate the cytotoxicity of Eletriptan Hydrobromide with or without encapsulation into PLGA, CaCo2 cells. The intracellular Eletriptan Hydrobromide accumulation was examined as described (2).

In vivo studies

Wistar albino female rats (weighing between 250 and 300 g) were selected for the study. All animal experiments were approved by University of Health Sciences Ethical Committee (Case Number 2017-001) Turkey. Nanoparticulate formulation and drug solution equivalent to 75 µg of EH were administered intranasally or intravenously. Nanoparticulate formulation was prepared by dispersing NP in PBS (pH 7.4). Drug solution was injected intravenously into the tail vein of the rats or pluming the drug to nose with the help of an insulin injector attached to low density polyethylene tubing with internal diameter 0.1 mm for intranasal

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application. Subsequently, animals were sacrificed at different time intervals (n= 3 for each time point), and their blood and brains were collected. Brain samples were homogenized using high speed homogenizator (IKA, Staufen, Germany) in PBS (pH 7.4). Same amount of each sample homogenate was centrifuged at 10000 rpm for 15 min. at 4 °C. The supernatant of brain homogenates (200µL) and plasma samples were prepared for quantification using the same procedure. Samples were mixed with 400 µL acetonitrile, 200 µL methanol and 200 µL distilled water and kept in a refrigerator for 1 h [32]. The mixture was then centrifuged at 10000 rpm for 15 min, and supernatant was taken for analysis by LC-MS.

Results:

Effect of formulation variables on nanoparticle size, PDI, EE % and zeta potentials are shown in Table 1. It can be concluded that formulation variables effects particle size, PDI, EE% and zeta potential (Figure 1).

Formulation	PLGA (mg)	EH (mg)	Inner PVA (%)	Outer PVA (%)	Water pH	Prt Size(nm)	PDI	EE (%)	Zeta Pot. (mV)
F1	90	1	1	1	7	244,3±28,1	0,110±0,050	43,89±7,34	-4,57±0,4
F2	60	1	1	1	7	211.2±26.0	0.140±0.021	46,12±5,42	-4,14±0,6
F3	90	5	3	3	7	167.6±18.1	0.120±0.013	45,40±6,11	-5,30±0,7
F4	90	1	3	3	7	168.4±11.4	0.061±0.008	43,24±5,19	-0,42±0,3
F5	60	1	3	1	7	178.2±12.5	0.166±0.010	46,84±7,49	-1,90±0,6
F6	90	1	1	3	7	201.5±13.6	0.014±0.004	47,02±6,28	-0,54±0,2

Table 1. Effect of formulation variables on nanoparticle properties

The difference in the particle sizes, zeta potentials, PDI and EE% of the nanoparticles prepared with W/O/W emulsification method that the water pH plays a key role on the resultant nanoparticle encapsulation efficieny (Figure 2).

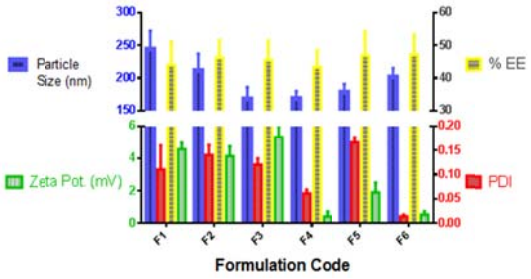

Figure 1. Effect of formulation variables on nanoparticles

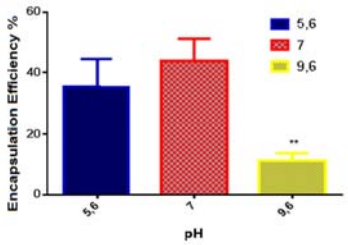

Figure 2. Effect of water pH on encapsulation efficiency of nanoparticles.

It is shown that nanoparticles was not cytotoxic at application dose (Figure 3). As shown in Figure 4, highest blood drug concentration was obtained at intravenous drug solution and highest brain drug concentration was obtained after intranasal drug nanoparticle administration.

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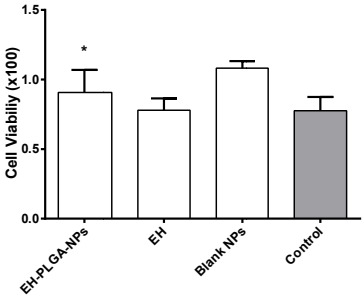
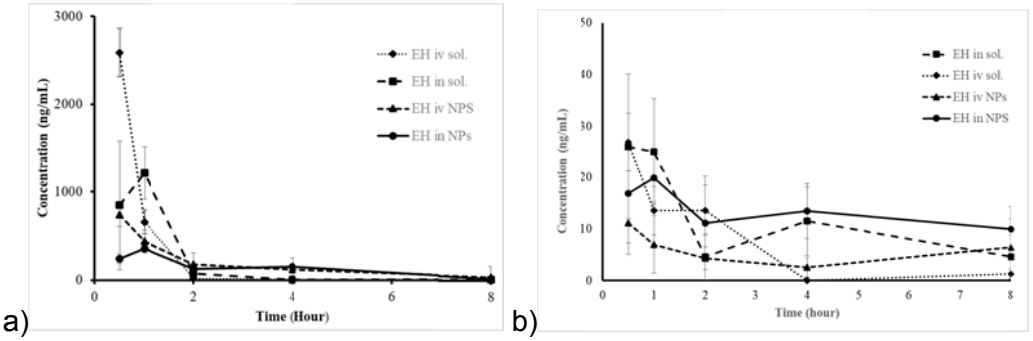


Figure 3. Cytotoxicity of formulations



a) b)

Figure 4. (a) Blood and (b) brain EH concentrations.

Conclusions:

It can be concluded from study that it is possible to prepare Eletriptan Hydrobromide−PLGA nanoparticles. PLGA NP showed no significant toxicity on CaCo2 cells, Drug efflux studies showed that PLGA nanoparticles can inhibit the function of P-gp and increase. From the results of in vivo studies, one can conclude that the NP provided a higher brain concentration of drug after IN administration compared with IN- and IV-administered drug solution. Thus, it can be concluded that drug-loaded PLGA NP are capable of providing direct nose-to-brain delivery, thereby enhancing drug concentration in the brain.

Acknowledgements

This study was supported by a grant of University of Health Sciences (2017/006)

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OP-059: ESTIMATION AND PREPARATION OF DRY POWDER INHALER FORMULATIONS THAT CONSISTING OF CIPROFLOXACIN HCL LOADED NANO AND MICROCOMPOSITE PARTICLES FOR PULMONARY ADMINISTRATION

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Introduction:

Compared to many delivery routes, pulmonary route has some advantages for delivery of antibiotics for treatment of lung infections. Higher drug concentrations in pulmonary tissue could be obtained by inhalation (1). However, conventional inhalation therapy causes in a short duration of drug action at the target site. Because of these reasons, it would be useful to design the sustained release formulations to localize the drug action in the lungs (2, 3). The most important advantage of nanoparticles is their small size and ability to cross various barriers, increase the contact surface with the tissue. However high cohesive properties due to the large surface area is a problem in the preparation. Preparation of low-density microparticles provide controlling of nanoparticle sizes and surface charges, and agglomerates. They are called nanocomposite microparticles, they will be easily separated into nanoparticles in aqueous media in the lungs and aerosolized. In this study, ciprofloxacin(CIP)-loaded nanoparticles and nanocomposite microparticles for pulmonary delivery were developed and evaluated in-vitro. The nanoparticles were prepared in order to encapsulate the hydrophilic CIP. Nanocomposite microparticles were prepared using nanoparticle to obtain micron size required for pulmonary delivery.

Materials and Methods:

CIP was kindly gift from Zentiva-Turkey. Dichloromethane, poly-€-caprolactone (PCL), PLGA-50/50 (MA=40000-75000/MA=24000-38000), polyvinyl alcohol (Mw=30000–70000) were obtained from Sigma (Germany). Chitosan-HCl was purchased from NovaMatrix (Norway).

Preparation of polymeric nanoparticles:

Nanoparticles containing CIP were prepared with solid-in-oil-in-water (s/o/w) emulsion-solvent evaporation method. In brief, the organic phase was prepared by dissolving polymers and PEG 4000 in dichloromethane. Ciprofloxacin was poured into polymer-PEG 4000 solution and sonicated. The resulting suspension was injected into the external aqueous phase consisting of 40mL of a PVA solution and sonicated for 5 min to form the emulsion. To coat the nanoparticles with chitosan, chitosan was added to aqueous PVA solution. The

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obtained emulsion was stirred with a mechanical stirrer to evaporate organic solvent and subsequent particle hardening at room temperature. Finally, the nanoparticles were collected by centrifugation and then lyophilized. Composition of nanoparticles was given Table-1.

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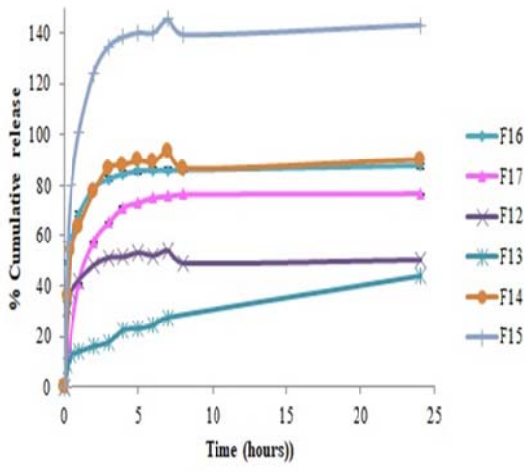
Table 1. Composition of ciprofloxacin-loaded nanoparticles

Formulation code	Organic solvent	External phase	Polymer type and amount (mg)	PEG 4000 (mg)	Drug (mg)
F1	DCM	%2 PVA (w/v)	PCL:100	10	5
F2	DCM	%2 PVA (w/v)	PCL:100	10	10
F3	DCM	%2 PVA (w/v)	PCL:100	10	20
F4	AC:DCM (1:1)	%2 PVA (w/v)	PCL:100	10	10
F5	EA:DCM (1:1)	%2 PVA (w/v)	PCL:100	10	10
F6	AC	%2 PVA (w/v)	PCL:100	10	10
F7	DCM	%2 PVA (w/v)	PCL:150	10	10
F8	DCM	%2 PVA (w/v)	PCL: 200	10	10
F9	DCM	%2 PVA (w/v)	PCL: 300	10	10
F10	DCM	%2 PVA (w/v)	PCL (Mw. 70000-90000):200	10	10
F11	DCM	%2 PVA (w/v)	PCL (Mw. 14000):PCL (Mw. 70000) (1:1):200	10	10
F12	DCM	%2 PVA (w/v)	50:50PLGA(Mw. 40000-75000):PCL(1:1):200	10	10
F13	DCM	%2 PVA (w/v)	75:25 PLGA(Mw. 66000-107000):PCL(1:1):200	10	10
F14	DCM	%2 PVA (w/v)	50:50 PLGA (Mw. 24000-38000):PCL (1:1):200	10	10
F15	DCM	%2 PVA (w/v)	PCL:Lecithin (200:20)	10	10
F16	DCM	%2 PVA (w/v)+%1 chitosan (w/v)	PCL: 200	10	10
F17	DCM	%2 PVA (w/v)+%2 chitosan (w/v)	PCL: 200	10	10

DCM: dichloromethane; EC: ethyl acetate; AC: acetone; PVA: polyvinyl alcohol; PCL: polycaprolactone; PLGA: poly-lactide-co-glycolic acide.

Physicochemical Characterization of Nanoparticles

The effects of type of the organic solvent, polymer type and ratios, quantity of active substance, amount of the mucoadhesive polymer used to modify surface properties, on particle size, encapsulation efficiencies, zeta potentials, drug release profiles were investigated. The particle size and polydispersity index (PDI) of the nanoparticles were determined by dynamic light scattering (DLS) (5). For encapsulation efficiency, lyophilized nanoparticles were dispersed in 5 ml DCM and the 5 ml pure water was added to these solution. Then DCM was evaporated and the aqueous solution was filtered determined spectrophotometrically at a wavelength of 270 nm. The results were shown in Table-2. In vitro release from nanoparticle were studided in pH 7.4 PBS containing 0.05% (w/v) Tween 80. A quantity of CIP-loaded nanoparticles were suspended in 500 µL PBS buffer. The samples were incubated at 37°±0.5°C in a shaking water bath at 100 rpm. The samples were taken at predetermined intervals. Fresh PBS was added in place of taken samples. Samples were filtered by 0.45 µm filter and determined spectrophotometrically at a wavelength of 270 nm (Figure-1). The shape and morphology of nanoparticles were observed using transmission electron microscopy (TEM) (Figure-2).



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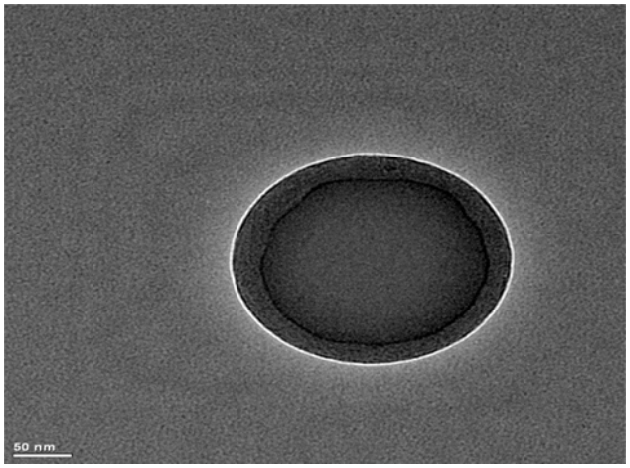

Figure 2. TEM image of nanoparticle

Table 2. Physicochemical characterization of nanoparticles (n=3)

Formulation code	Particle size (nm) ±SD	PI **	Zeta potential (mV)±SD*	Encapsulation efficiency (%) ± SD*
F1	200,98±1,45	0,13±0,02	-11,33±1,75	1,05±0,01
F2	228,77±2,81	0,17±0,04	-24,28±1,22	2,87±0,01
F3	202,10±1,55	0,09±0,06	-12,07±1,56	1,46±0,05
F4	143,92±0,71	0,08±0,02	-2,98±2,26	3,44±005
F5	195,65±45,33	0,18±0,05	-4,68±2,43	3,39±0,11
F6	269,50±2,02	0,21±0,01	-17,73±3,28	1,10±0,01
F7	235,23±10,40	0,25±0,01	-12,3±0,65	2,68±0,01
F8	212,47±1,69	0,09±0,01	-14,25±1,79	2,95±0,01
F9	263,68±2,25	0,22±0,02	-20,93±2,05	2,5±0,00
F10	197,87±1,69	0,21±0,02	-20,83±1,42	2,59±0,01
F11	181,90±1,97	0,10±0,01	-20,73±4,75	1,63±0,01
F12	205,75±2,27	0,07±0,01	-16,02±1,02	5,35±0,01
F13	213,30±2,69	0,09±0,01	-19,68±0,84	3,84±0,01
F14	205,83±1,36	0,13±0,03	-16,45±1,39	6,71±0,02
F15	298,00±4,51	0,24±0,01	-4,44±0,91	4,20±0,01
F16	384,1±4,14	0,24±0,00	56,3±2,02	8,02±0,02
F17	489,00±7,32	0,22±0,03	62,00±0,93	8,26±0,02

*Standart deviation
**Polydispersity index

Tapped density and MMADts results were found 0,17±0,00 and 0,81 respectively and results showed nanocomposite microparticles have suitable aerodynamic properties for pulmonary administration. Antimicrobial activity test indicated CIP-encapsulated PCL-nanoparticles and nanocomposite microparticles inhibited growth of bacteria.

Conclusions:

Surface modification of nanoparticles with chitosan caused an increase in encapsulation efficiency. Antibacterial activity test indicated, ciprofloxacin retained its antimicrobial efficacy when incorporated in the particular system. Considering nanoparticles 0^{th} and 6^{th} month activities, the particular system was stabile. As a result, nanocomposite microparticles containing CIP-loaded nanoparticles can be used for pulmonary delivery.

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OP-061: DETERMINATION OF ANTI-INFLAMMATORY AND ANTIDIABETIC ACTIVITIES OF 14 *BALLOTA* TAXA GROWING IN TURKEY.

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Introduction:

Ballota (L.) is represented by 12 species and 16 taxa in Turkey. Eleven of 16 taxa are endemic to Turkey. *Ballota* species belong to Lamiaceae family commonly distributed in the mild climate condition locations of the world and have been used in folk medicine as antiulcer, antispasmodic, diuretic, choleretic, antihemorrhoidal and sedative agents. The main components of the *Ballota* species are flavonoids, terpenoids, and phenylpropanoids (1). Inflammation is a protective mechanism of living organisms in response to an abnormal stimulation caused by a physical, chemical, or biological agent. In general, generation of cytokines is accepted to play a major role inducing inflammatory process and free radicals can propagate inflammation by stimulating the release of pro-inflammatory cytokines such as interleukin-1β, interleukin-6 and tumor necrosis factor-α. Drugs that are currently used for the treatment of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. NSAIDs drugs inhibit prostaglandins and thromboxane inflammatory mediators synthesize by deactivating of cyclooxygenase (COX), COX-1 and COX-2 enzymes. Some of these drugs such as aspirin, diclofenac, ketorolac, naproxen, and piroxicam have toxic effects such as the risk of gastrointestinal bleeding (2, 3). Diabetes mellitus is a major endocrine disorder and is characterized by abnormalities in carbohydrate, lipid and lipoprotein metabolisms, which not only lead to hyperglycemia but also cause many complications such as hyperlipidemia, hyperinsulinemia, hypertension and atherosclerosis (4). Plants secondary metabolites have extensively different bioactivity properties. Anti-inflammatory agents that derived from natural sources play a significant role in the prevention and treatment of infectious diseases. The aim of this study was to examine 14 *Ballota* taxa from Turkey for their antidiabetic and anti-inflammatory activities.

Materials and Methods:

The plant material is Fourteen *Ballota* species which were collected from different locations of Turkey (Table). From the air-dried and powdered aerial parts of 14 *Ballota* species, 2 different extracts were prepared by using ethanol (75%) and water in this study. The extracts were prepared by maceration 50 g of each plant powder in 300 ml of ethanol or water for 8 hours, for 3 days. The macerates obtained with ethanol were evaporated until dryness, the macerates obtained with water were also lyophilized.

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Table. 14 *Ballota* taxa which collected from different locations of Turkey

1	*Ballota acetabulosa* (L.) Benth.	B1 Izmir: Yenifoça, 10 m, AFF 21602
2	*Ballota antalyense* F. Tezcan & H. Duman	C3 Antalya: Turunçova, 150 m
3	*Ballota cristata* P.H. Davis*	C3 Isparta: Egridir, 910 m, AEF 19899
4	*Ballota glandulosissima* Hub.-Mor & Patzak*	C3 Antalya: Kumluca, 500 m, AEF 19900
5	*Ballota inaequidens* Hub.-Mor & Patzak*	C3 Antalya: Alanya, 200 m, AEF 19901
6	*Ballota larendana* Boiss. & Heldr.*	A4 Ankara: Kızılcahamam, 830 m, AEF 21604
7	*Ballota latibracteolata* P.H. Davis & Doroszenko*	C3 Antalya: Gazipasa, 425 m, AEF 19902
8	*Ballota macrodonta* Boiss. & Bal.*	B5 Kavseri: Yahyalı, 1150 m, AEF 19907
9	*Ballota nigra* L. subsp. *anatolica* P.H. Davis*	B4 Ankara: Gölbası, 800 m, AEF 21601
10	*Ballota nigra* L. subsp. *uncinata* (Fiori & Beg.) Patzak	B1 Izmir: Gökçealan, 250 m, AEF 21607
11	*Ballota pseudodictamnus* (L.) Benth. subsp. *lycia* Hub.-Mor.*	C2 Mugla: Fethiye, 20 m, AEF 21603
12	*Ballota rotundifolia* C. Koch*	A8 Erzurum: Tortum Lake, 1200 m, AEF 21606
13	*Ballota saxatilis* Sieber ex. J & C. Presl subsp. *brachyodonta* (Boiss.) P.H. Davis & Doroszenko*	C4 Içel: Silifke, 1400 m, AEF 21505
14	*Ballota saxatilis* Sieber ex. J & C. Presl subsp. *saxatilis*	C4 Içel: Anamur, 1530 m, AEF 19904

The aqueous and alcoholic extracts of 14 *Ballota* taxa were examined for their anti-inflammatory activities by using membrane stabilization method as explained below (3). Fresh whole human blood was collected from healthy human volunteers and centrifuged. The packed cells were washed three times with equal volume of isosaline (0.85 %, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline. Membrane stabilizing activity of the extracts was assessed using heat-induced human erythrocyte hemolysis. The reaction mixture consisted of equal volume of the test sample and 10% RBCs suspension. The solvents were used as control. Acetylsalicylic acid was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath then the tubes were cooled with water. The reaction mixture was centrifuged. Then the absorbance of the supernatant was measured. The experiment was performed in triplicates for all the test samples. The percentage of hemolysis and protection was calculated according to the formula:

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Hemolysis % = (Absorbance of test sample/Absorbance of control) ×100

Protection (Stabilization) % = 100-[(Absorbance of test sample/ Absorbance of control) ×100]

The aqueous and alcoholic extracts of 14 *Ballota* taxa were examined for their anti-diabetic activities by using α-glucosidase method as explained below (5). The substrate solution pNPG was prepared with 0.2 M of Na-phosphate buffer (pH=6.8). The reaction mixture contained 10 µL of 0.02 U/µL α-glucosidase solution in 0.2 M Na-phosphate buffer (pH=6.8), 10 µL of a sample, 50 µL of Na-phosphate buffer (pH=6.8), which were mixed and incubated at 37°C for 20 min. Then, 50 µL of 0.02 M pNPG was added, and the mixture was incubated at 37°C for another 30 min. Finally, the reaction was stopped by the addition of 100 µL 0.2 M Na2CO3 solutions. Acarbose was used as a standard drug. Amount of the p-nitrophenol released from PNP-glycoside was quantified on a 96 microplate spectrophotometer at 405 nm. The inhibitory activity of sample on α-glucosidase was calculated by the following formula and the results were expressed as IC_{50}.

Inhibition(%)=[($Absorbance_{control}$-$Absorbance_{test\ sample}$)/$Absorbance_{control}$)] x 100

Results: Aqueous extracts of *B. nigra* subsp. *anatolica* (IC_{50}=3.18 mg/ml) exhibited the maximum anti-inflammatory effect following by ethanolic extracts of *B. acetabulosa* and *B. glandulosissima*, respectively (IC_{50}=4.31-4.59 mg/ml). And for antidiabetic activity, aqueous and ethanol extracts of *B. glandulosissima* (IC_{50}=2.18 and 2.30 µg/ml, respectively) exhibited the maximum α-glucosidase inhibitory activity.

Conclusions:

The ethanolic extracts showed higher membrane stabilization profile than aqueous extracts generally. In contrast, the aqueous extracts showed higher α-glucosidase inhibitory activity than the ethanol extracts. It is concluded that the solvent type could affect the profile of biologically active components. Biologically active phytoconstituents such as flavonoids and phenolics could be responsible compounds contributing antidiabetic activity of the extracts.

Acknowledgements

There is no acknowledgement to be declared in this study.

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OP-062: DEVELOPMENT AND EVALUATION OF ETOPOSIDE LOADED POLYMERIC TUBULAR NANOSTRUCTURES

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Introduction:

Nanoparticulate drug carriers have become key tools for carrying drugs, in particular, for chemotherapeutic drugs, because of their very small size which helps them to increase drug solubility and prolong drug half-life in the bloodstream (1). Up-to-date studies have demonstrated that, besides nanocarrier size, especially shape of the nanocarrier is an important parameter which has an effect on circulation time and tumor accumulation. Researchers have shown that cylindrical nanostructures, depending on their unique hydrodynamic behavior, circulate longer than spherical counterparts with the same diameter. This status provides them more time to reach target tissue and deliver the loaded drug more efficiently (2). Template synthesis which provides a better of control of size and shape of materials, has become one of the most promising ways of fabrication of polymer tubular nanostructures. Anodized aluminum oxide (AAO) membrane that contains highly ordered cylindrical nanopores with high aspect ratios, is one of the most popular templates for nanorod/nanotube fabrication by template wetting method (3). Etoposide (ETP), the drug, is a chemotherapeutic agent with poor aqueous solubility and short half-life (4). The aim of the study was to produce ETP loaded biodegradable polymeric tubular nanostructures by template wetting of nanoporous membranes.

Materials and Methods:

Commercial AAO (Anodisc 47, Whatman™, GE Healthcare) with an average pore diameter of 200 nm used as template membrane. Poly-ε-caprolactone (PCL), Poly-d,l-lactide-co-glycolide (PLGA), poly(l-lactic acid-co-caprolactone-co-glycolic acid) (PLCG) were purchased from Sigma-Aldrich. ETP was kindly supplied by Kocak Farma Ilac ve Kimya Sanayi A.S. (Turkey)

Preparation of etoposide loaded polymeric tubular nanostructures

Polymer and ETP were dissolved in a mixture of dichloromethane (DCM) and dimethyl formamide (DMF). For wetting AAO membrane template, immersion technique was applied in ETP/polymer solution for a predetermined time in tightly close glass containers. Drug/polymer embedded AAO membranes were dried at room temperature in order to remove all residual solvent. AAO template etching was performed with aqueous phosphoric acid to dissolve AAO completely. After etching of the template membranes, liberated tubular

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nanostructures were collected by vacuum filtration, washed with pure water, and they were freeze dried.

Surface morphologies

Imaging is a very important approach to understand the morphology of template synthesized nanostructures. In the case of particle shape, the success of the production method could really be analyzed by a screening method. In our study the morphology of the produced polymeric tubular nanostructures was examined by scanning electron microscopy (SEM).

Results:

Characterization of drug-loaded tubular nanostructures

In the study ETP was analyzed by HPLC which was described in our previous work (5). Entrapment efficiency (EE%) and drug loading (DL%) were calculated using the following equations and on the basis of three formulations the results were represented in Table 1. Maximum EE% and DL% were achieved as 18% and 3%, respectively for the formulation F2, produced with PLGA polymer.

$$\mathrm{EE}(\%) = \frac{\text{amount of ETP entrapped in nanoparticles}}{\text{total amount of ETP in formulation}} \times 100$$

$$\mathrm{DL}(\%) = \frac{\text{amount of ETP entrapped in nanoparticles}}{\text{amount of nanoparticles}} \times 100$$

Table1. Evaluation of formulations

Code	Polymers	Solvent system	Polymer/ETP ratio	EE (%)	DL (%)
F1	PCL	DCM:DMF	10:2	10.25	1.71
F2	PLGA	DCM:DMF	10:2	18.21	3.04
F3	P(LCG)	DCM:DMF	10:2	12.08	2.01

Surface morphologies

The morphology of the polymeric tubular nanostructures was examined by scanning electron microscopy (SEM). As seen in Fig. 1, SEM images showed that ETP loaded nanostructures, regardless of the type of polymer, were obtained successfully in nano dimensions as per diameter. Smooth surfaced tubular nanostructures with structural integrity were achieved by only PCL and PLGA polymers (Fig 1a and Fig 1b). As a result of structural acceptability and high drug loading capacity F2 formulation was found to be advantageous for further studies.

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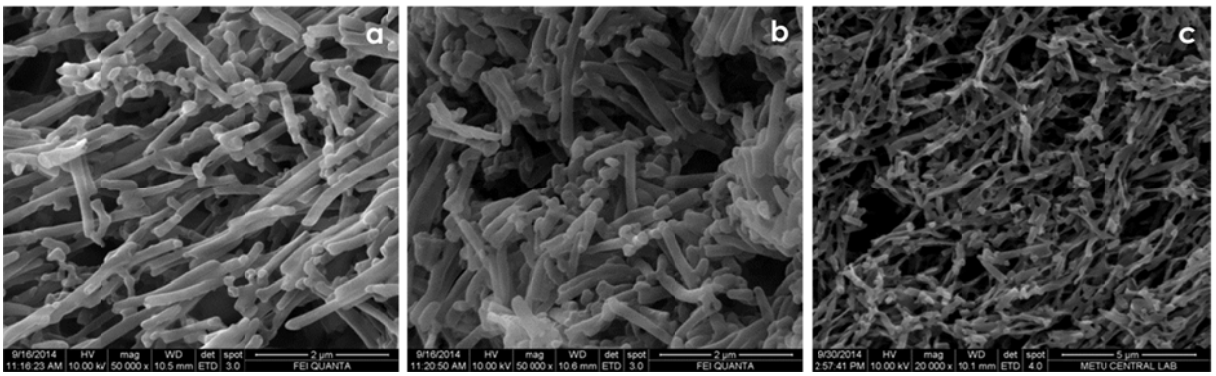

Figure 1. SEM image of a) F1 formulation produced by PCL. b) F2 formulation produced by PLGA. c) F2 formulation produced by (PLCG).

Conclusions:

Consequently, template wetting method which produces monodisperse particles of specific in size and shape, is one of the promising approaches for fabrication new nanoparticulate drug delivery systems. In the study, etoposide loaded polymeric tubular nanostructures were produced using biodegradable polymers. In the case of F2 formulation, produced by PLGA polymer, the developed polymeric tubular nanostructures were found promising and improvable for the delivery of chemotherapeutic agents.

Acknowledgements:

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OP-063: PREPARATION AND *IN VITRO* CHARACTERIZATION OF DEXAMETHASONE LOADED ETHOSOME FORMULATIONS

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Introduction:

Atopic dermatitis (AD), also referred to as atopic eczema, is one of the most common chronic inflammatory skin diseases affecting approximately 2-5% of the world's population [1]. It is characterized by dry erythematous lesions and intense pruritus. Trigger factors of AD are dry skin, irritants, emotional stress, allergens, heat and sweating and infections. The first-line management of AD is the use of topical corticosteroids. Dexamethasone (DEX) is a synthetic adrenal glucocorticoid with a relevant clinical use mainly due to its potent anti-inflammatory and immunosuppressive effects. The mechanism of action of DEX is as a Corticosteroid Hormone Receptor Agonist. DEX has a wide range of indications such as atopic dermatitis, erythema multiform, alcohol withdrawal syndrome, cerebral edema, congenital adrenal hyperplasia, nausea and vomiting, high altitude disorder, cerebral malaria, opportunistic mycobacterial infections, respiratory disorders, rheumatism, meningitis, early mild carpal tunnel syndrome and diagnostic agent in Cushing's syndromecerebral edema. The indication list is very long. However, DEX have serious systemic side effects such as hypertension, weight gain, hydroelectrolytic disorders, hyperglycemia, peptic ulcers, glycosuria restrict, emotional irritability and edema. To avoid complications of systemic steroid treatment, DEX given topically is a more suitable alternative. Many inflammatory skin diseases respond to topical corticosteroids like dexamethasone. But the clinical outcome of the treatment depends highly on the treatment duration and drug formulation. Thus, controlled administration of corticosteroids to the target site in an appropriate vehicle may significantly improve their treatment outcome. Ethosomes are liposomal formulations containing high amount of ethanol. This system is composed mainly of phospholipids, ethanol (up to 50%) and water. High ethanol content of ethosomes results in being much smaller and more flexible than liposomes and enhances solubility of more lipophilic drugs. Besides these, disruption of intercellular lipid structure of stratum corneum by the phospholipids improves drugs permeation. Ethosomal formulations offer several benefits over the conventional methods. These systems have biocompatible composition. Delivery of large molecules is possible. It contains non-toxic raw material in formulation. Ethosomes enhance permeation of the drug through skin transdermal and dermal delivery, low risk profile and high patient compliance. The purpose of this study was to prepare and characterize ethosomal formulations containing DEX for topical treatment of AD.

Methods:

Ethosomal formulations were prepared according to the method reported by Tanrıverdi et. al., 2013 [2]. Lipoid S100 and DEX were dissolved in ethanol. Distilled water was added slowly to the lipid mixture with constant stirring. The system was kept at 30 °C during preparation procedure. The resulting vesicle suspension was homogenized with using probe sonicator and small ethosomal vesicles were obtained. The formulations were prepared with two different ratio of phospholipid. The compositions of the formulations were given in this Table 1. The prepared ethosomes were characterized pH, particle size (PS), polydispersite index (PI), zeta potential (ZP), encapsulation efficiency (EE), drug loading (DL), morphological analysis and *in vitro* drug release. The pH values of the formulations were determined by using a pH meter. The ZP, PS and PI were measured using a Nano-ZS Zetasizer (Malvern Instruments, Malvern, UK) by light scattering spectroscopy at room

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temperature. For EE study, ethosomal formulations were ultracentrifuged, the supernatant was used for DEX analysis by validated HPLC method and the quantity of free drug was determined. The DL and amount of drug were determined by dissolving the ethosome suspensions in methanol. DEX concentration was determined by HPLC method and the percentage of drug loading was calculated. Morphological analysis was performed by transmission electron microscopy (TEM). The dialysis bag diffusion technique was used to study for *in vitro* drug release. 2 mL of ethosome suspensions was placed in the dialysis bag, hermetically sealed and immersed into pH 5.5 sorenson buffer at 32±0.5 ºC during 24 h. Samples were withdrawn from the receptor compartment at predetermined time intervals. The amount of drug released was determined by HPLC. The dissolution data were fit to Peppas equation, and best-fit parameters were calculated to determine the release mechanism of formulations.

Table 2. The compositions of the formulations

Formulation Code	DEX	Lipoid S100	Ethyl alcohol	Distilled Water
F1	-	2 g	30 g	68 g
F2	-	1 g	30 g	69 g
F3	0.1 g	2 g	30 g	67.9 g
F4	0.1 g	1 g	30 g	68.9 g

Results:

Ethosomal formulations were successfully prepared using modified ethanol injection method. The pH values of the formulations were found to be 7.59, 6.91, 7.68 and 7.53, respectively (Table 2). Neutral pH values indicated the formulation could be used as topical delivery with low risk skin irritation. PS, PI and ZP of the ethosomes were given in Table 2. The reduction of vesicle size in ethosome formulation could be explained by high ethanol concentration. The PI of all formulations was found less than 0.5, indicating a narrow and homogenous size and distribution. The ZP values of formulations were found similar. ZP were showed that the high ethanol concentration make the positive charge on vesicles surface. The EE% of DEX from the ethosomal formulations was as high as 84.119%–77.254%. The EE also increased with an increase in concentration of lipoid. The amount of drug was found 95.099 and 95.133%, respectively. Also, DL capacity was found 5.039% and 8.648%, respectively. The ethosomes were scanned using TEM and spherical structures were confirmed (Figure 1). As you seen, Figure 2 showed the *in vitro* drug release profile of formulations was observed similar and controlled drug release by the fact that the drug molecules are entrapped in the lipid matrix. However, the dissolution rate of F3 formulation is higher than F4 formulation. According to Peppas equation, the values of n fell within the range of 0.671-0.756, indicating that the drug release from the ethosomes is non-Fickian. Non-Fickian release refers to the combination of both diffusion and erosion mechanisms of controlled release.

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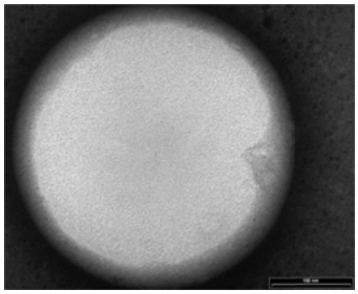
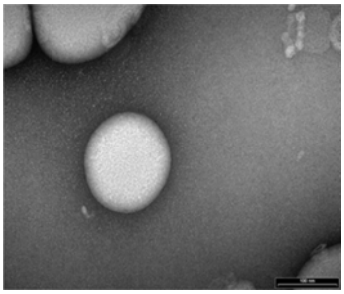
Table 3. Characterization of formulations

Formulation Code	pH	PS (nm)±SD	PI±SD	ZP (mV)±SD
F1	7.59	311.3±2.12	0.454±0.076	0.92±0.07
F2	6.91	249.3±2.54	0.513±0.022	1.74±0.06
F3	7.68	997.4±2.75	0.290±0.033	0.96±0.06
F4	7.53	930.3±2.37	0.443±0.011	1.54±0.09



Microphotographs of F3 by TEM (scale bar= 100 nm)



Microphotographs of F4 by TEM (scale bar= 100 nm)

Figure 2. TEM images of DEX loaded ethosomes

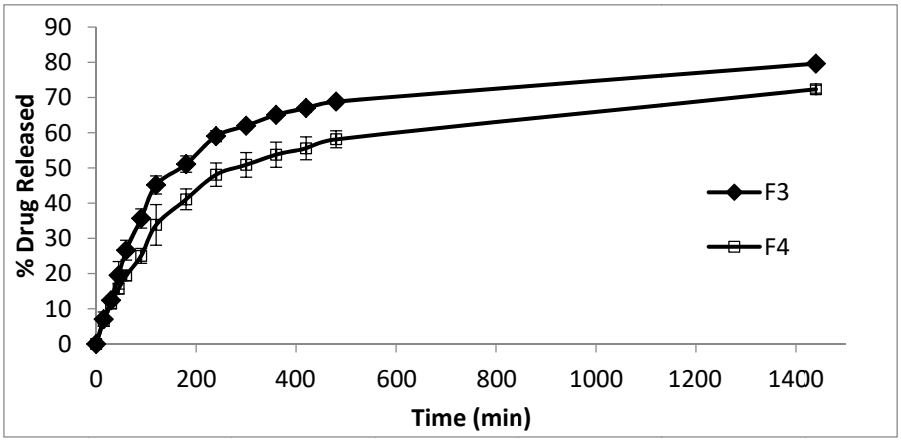


Figure 3. *In vitro* drug release of ethosomes

Conclusion:

In conclusion, ethosomal formulations were successfully prepared using modified ethanol injection method and characterized for topical treatment of AD. It was planned to work on *ex vivo* permeation studies and *in vivo* anti-inflammatory activity studies.

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OP-064: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF SECONDARY METABOLITES FROM VAGINAL *LACTOBACILLUS* SPECIES

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Introduction:

An increasing number of new multi-resistant microorganisms have been reported from different parts of the world and the infections caused by these microorganisms have emerged as a major source of morbidity and mortality. Inefficiency in treating the infections have brought about the necessity for new compounds with antimicrobial activity (1). *Lactobacillus* genus, grouped among lactic acid bacteria, is microorganisms producing lactic acid as the major metabolic product end of carbohydrate fermentation. In addition to forming lactic acid as the major fermentation product from glucose, some groups of them are also known to produce various amounts compounds such as acetic acid, ethyl alcohol and carbon dioxide (2). The genus *Lactobacillus* has recently been focal point of researchers due to their ability to produce the secondary metabolites with antimicrobial effects (3). The aim of this study was to determine the quantities of lactic acid, acetic acid and hydrogen peroxide in secondary metabolites from vaginal *Lactobacillus* species by High Performance Liquid Chromatography (HPLC).

Materials and Methods:

Vaginal isolates were identified by analyzing 16S rRNA gene sequence. *Lactobacillus* isolates were inoculated into tubes containing 5 ml of De Man-Rogosa Sharpe Broth (pH 6,5) at 37 ºC, under anaerobic conditions for 72 hours. After incubation, *Lactobacillus* sp. cells were removed by centrifugation (12000 g, 10 min, 4 °C). Cell free supernatants were filter-sterilized (4). HPLC analysis of secondary metabolites of *Lactobacillus* species was carried out on Agilent 1100 series. Separation was carried out on Inertsil ODS-3 (150 length x 4.6 mm i.d., 5 µm). The calibration curves were established the ranging from $1.96 \times 10^{-2} – 6.35 \times 10^{-4}$ mg/µL for hydrogen peroxide,$1.047 \times 10^{-2} – 2.09 \times 10^{-3}$ mg/µL for acetic acid, $2.13 \times 10^{-3} – 1.06 \times 10^{-4}$ mg/µL for lactic acid. Solution was injected three times and the areas of peaks, as measured at 210 nm, were integrated. Regression coefficients are 0.99, 0.98 and 0.99 for hydrogen peroxide, acetic acid and lactic acid, respectively. Mean recoveries and relative standard deviations are 97.79% and 0.79%, 95.62% and 1.52% and 104.31% and 1.91% for hydrogen peroxide, acetic acid and lactic acid, respectively. Elution was performed with a mobile phase as 0.025% phosphoric acid solution. Mobile phase were filtered through 0.45 µm nylon membrane filter paper and degassed in ultrasonic bath prior to use. The flow rate

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was set at 0.75 mL/min, injection volume was 5 µL and temperature of the column was maintained at 25°C. Compounds were detected at a wavelength of 210 nm.

Results:

In this study, of the 53 vaginal isolates identified by analyzing 16S rRNA gene sequence, 18 were established to be *L. crispatus*, 17 *L. gasseri*, 5 *L. jensenii*, 4 *L. vaginalis*, 3 *L. fermentum*, 2 *L. coleohominis*, 1 *L. saerimneri*, 1 *L. reuteri*, 1 *L. johnsonii* and 1 *L. helveticus* species. According to the HPLC analysis results, secondary metabolites of all tested isolates contain hydrogen peroxide between 0,007306 and 0,00033 mg/µL range. It was found that the secondary metabolites of some isolates contained both acetic and lactic acid, while some of them contained either acetic or lactic acid at various concentrations.

Conclusions:

Decrease or absence of lactobacilli destroys the microbial balance of the vaginal flora, which can lead to development of Gram negative bacteria and consequently, bacterial vaginosis (5). Lactic acid, acetic acid and hydrogen peroxide may be responsible for their antimicrobial activity. When considering the conditions of different women such as pregnancy, sexual activity, menstruation, smoking, age, hygiene habits and antimicrobial agent usage, it is known that they may have different vaginal environment. The different environmental conditions have effect on the content of secondary metabolites. In this study, *Lactobacillus* species are different from each other in terms of secondary metabolites production quantities. Based on this difference, the antimicrobial activity of these species may change.

Acknowledgements

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OP-068: ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* ISOLATED FROM VARIOUS CLINICAL SAMPLES.

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Introduction:

Increased resistance to antimicrobials is a major problem in the treatment of infections caused by resistant microorganisms in worldwide. Patients with infections caused by drug-resistant bacteria are at increased risk of worse clinical outcomes and death, and consume more health-care resources than patients infected with non-resistant strains of the same bacteria. *Escherichia coli* is a Gram-negative, rod-shaped bacterium that is the most important specie of Escherichia genus. *E. coli* is intensely present in the gastrointestinal tract and is an opportunistic pathogen that causes gastrointestinal and extraintestinal diseases. And it's one of the most frequent cause of many common bacterial infections, including bacteremia, cholecystitis, cholangitis, urinary tract infection, and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia (1, 2, 3). This study was conducted to determine the antimicrobial susceptibility patterns of 80 *E. coli* strains isolated from various clinical samples.

Materials and Methods:

A total of 80 *E. coli* isolates (59 urine, 9 sputum, 4 blood, 3 tissue, 2 drain, 2 vagina, 1 peritoneum) were obtained from Ankara University, Faculty of Medicine, Cebeci Hospital Central Laboratory between February and April 2018. Isolates were identified both by the conventional methods and MALDI-TOF MS. Antimicrobial susceptibility testing was conducted on Mueller-Hinton Agar plates (Merck, Germany) using disc diffusion method in accordance with EUCAST (The European Committee on Antimicrobial Susceptibility Testing) recommendations. Isolates were tested against 17 antimicrobial drugs: ampicillin (10 mcg), trimethoprim/sulphamethoxazole (1.25/23.75 mcg), ciprofloxacin (5mcg), levofloxacin (5 mcg), amoxicillin clavulanic acid (20/10 mcg), cephalothin (30 mcg), cefotaxime (5 mcg), cefepime (30 mcg), ceftazidime (10 mcg), fosfomycin (200 mcg), cefoxitin (30 mcg), piperacillin/tazobactam (30/6 mcg), gentamicin (10 mcg), ertapenem (10 mcg), imipenem (10 mcg), amikacin (30 mcg), nitrofurantoin (100 mcg). Results were expressed as susceptible or resistant according to the criteria recommended by EUCAST (4, 5).

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Results:

The resistance rates detected were 71.25 % to ampicillin, 42.5 % to amoxicillin clavulanic acid, 11.25 % to piperacillin/tazobactam, 42.5 % to cephalothin, 36.25 % to cefotaxime, 32.5 % to ceftazidime, 32.5 % to cefepime, 13.75 % to cefoxitin, 33.75 % to fosfomycin, 7.5 % to gentamicin, 1.25 % to amikacin, 8.75 % to ertapenem, 3.75 % to imipenem, 47.5 % to ciprofloxacin, 46.25 % to levofloxacin, 57.5 % to trimethoprim/sulphamethoxazole and 1.25 % to nitrofurantoin.

Conclusions:

Antimicrobial resistance of bacteria is a global problem. There is a relationship between the misuse of antibiotics and resistance. Empirical therapy should be based on local antimicrobial resistance monitoring in order to prevent increase in resistance to drugs used in the treatment. When the high resistance rates against beta lactam and sulfonamide antibiotics are taken into consideration,empirical therapies should be based on the datas of these types of studies.

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OP-073: EFFECT OF ANTICOAGULANTS ON ETHINYL ESTRADIOL AND LEVONORGESTREL ANALYSIS IN PLASMA USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Introduction: Ethinyl estradiol and levonorgestrel are commonly used combination of low-dose contraceptives (1). These low-dose combinations will have a result of low drugs concentration in plasma. The maximum concentration (Cmax) of ethinyl estradiol in plasma is 102.4 pg / mL and levonorgestrel is 14.6 ng / mL (2,3). Therefore a selective and sensitive method for analysis is to determine ethinyl estradiol and levonorgestrel in plasma.There are many kinds of anticoagulants often used to obtain plasma from whole blood. The most commonly used anticoagulants are ethylenediamintetraacetic acid (EDTA), heparin, and citrate. Different types of anticoagulants may cause suppression or enhancement ionization efficiency of analytes or metabolites (4,5,6). This research investigated the effect of the various anticoagulants on the recovery, peak area, stability, and matrix effects of ethynil estradiol and levonorgestrel analysis in plasma using LC-MS/MS.

Materials and Methods:
Plasma with citrate anticoagulant was obtained from Indonesia Red Cross, while heparin and EDTA plasma with anticoagulant Li heparine and K_3EDTA were obtained from blood sample separation of six healthy subjects. Analysis was performed on Waters Xevo TQD using positive electrospray ionization with Acquity UPLC BEH Waters C_{18} (2,1 × 100 mm; 1,7 µm) column; mobile phase consisted of 0.1% (v/v) formic acid in water and acetonitrile in gradient elution; flow rate of 0.3 ml/min; and prednisone as internal standard. Sample preparation used protein precipitation techniques and followed by liquid-liquid extraction. Detection of the mass was performed for ethinyl estradiol, levonorgestrel and prednisone with m/z value : 530.1596 > 171.0781; 313.1596 > 245.1044; 359.0957 > 147.0364, respectively.

Results:

This method was linear in the concentration range of 5-500 pg/ml for ethinyl estradiol and 100-1000 pg/ml for levonorgestrel with r value > 0,999. The result indicated that ethinyl estradiol was stable at -20°C for at least 10 days, while levonorgestrel was stable at -20°C for at least 15 days for plasma with citrate, heparine, and EDTA anticoagulants. The matrix effect test on the analytes and internal standard fulfilled the criteria of % CV value for both internal standard normalized matrix factor and matrix factor, not exceeding ±15%. There was no significant difference matrix effect occurred on each plasma, but EDTA had the highest ion suppression.

Comparisons of some parameter analysis indicated that there were no statistically significant differences ($p > 0,05$; ANOVA) in citrate, heparin, and EDTA of ethinyl estradiol and levonorgestrel analysis in human plasma for recovery and stability. On the other hand, peak area ratio for the three plasma showed a significant difference ($p < 0,05$;Kruskal Wallis) for all concentrations. The results can be seen in table 1 and 2.

Table 1. Comparison of Citrate, Heparin, and EDTA as Anticoagulant on Ethinyl Estradiol Analysis

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Parameter Analysis	Plasma Type			P value	Differences
	Citrate	Heparin	EDTA		
Peak Area Ratio (±SD)					
1. LLOQ	2.49 ± 0.15	1.04 ± 0.15	1.07 ± 0.02	p > 0.05	No significant difference
2. QCL	7.13 ± 0.89	3.02 ± 0.19	2.48 ± 0.16	P < 0.05	Significant difference
3. QCM	99.60 ± 7.61	38.22 ± 0.53	33.42 ± 1.62	P < 0.05	Significant difference
4. QCH	189.52 ± 14.72	77.28 ± 2.55	66.17 ± 2.00	P < 0.05	Significant difference
Recovery (% ± SD)					
1. QCL	68.03 ±1.74	65.23 ± 4.41	68.64 ± 2.13	p > 0.05	No significant difference
2. QCM	84.74 ± 4.97	85.08 ± 6.58	73.12 ± 4.07	P > 0.05	No significant difference
3. QCH	77.76 ± 5.72	80.59 ± 7.36	81.69 ± 0.85	P > 0.05	No significant difference
Matrix effect					

Table 2. Comparison of Citrate, Heparin, and EDTA as Anticoagulant on Levonorgestrel Analysis

Parameter Analysis	Plasma Type			P value	Differences
	Citrate	Heparin	EDTA		
Peak Area Ratio (±SD)					
1. LLOQ	0.22 ± 0.01	0.13 ± 0.02	0.10 ± 0.01	P < 0.05	Significant difference
2. QCL	0.70 ± 0.09	0.38 ± 0.03	0.26 ± 0.01	P < 0.05	Significant difference
3. QCM	9.50 ± 0.79	4.14 ± 0.10	3.38 ± 0.30	P < 0.05	Significant difference
4. QCH	20.82 ± 0.47	8.35 ± 0.20	6.96 ± 0.41	P < 0.05	Significant difference
Recovery (% ± SD)					
1. QCL	88.83 ± 7.92	86.16 ± 2.97	77.01 ± 5.41	p > 0.05	No significant difference
2. QCM	87.81 ± 4.76	93.90 ± 4.28	89.38 ± 4.53	P > 0.05	No significant difference
3. QCH	89.71 ± 2.65	83.99 ± 7.58	84.77 ± 2.36	P > 0.05	No significant difference
Matrix effect					

Conclusions:

There was no statistically significant differences in plasma with citrate, heparin, and EDTA anticoagulants on recovery and stability, but for peak area ratio of the three plasma showed a significant difference.

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OP-075: A NEW LC METHOD FOR QUANTITATIVE ESTIMATION OF AVANAFIL IN COMBINATION TABLETS

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Introduction:

Avanafil (AVA), which was approved by US Food and Drug Administration in 2012 and by European Medicines Agency in 2013, is a pyrimidine–derivative phosphodiesterase-5 inhibitor (PDE5) and one of the most effective drugs prescribed for erectile dysfunction [1]. AVA is more frequently preferred because of its rapid action and low side effects among other PDE5 inhibitors such as sildenafil, vardenafil, tadalafil and udenafil, which are also commercialized in ED treatment [2].

Liquid chromatography has become widespread in recent years as it has many advantages for fast and efficient quantitative and qualitative analysis [3, 4]. According to the structure of the analyzed material, this technique can be enriched by various detectors (such as LC-DAD, LCMS, LCMSMS), and more advanced analysis methods can be developed. Advantages and disadvantages can be seen for each of the methods: Some of them are simple and cost-effective, while they do not reveal much information; on the other hand, some others acquire more data about the analyte, while they need expensive and high-quality instruments [5]. In addition, use of novel sorbents can also increase throughput and analytical value of the methods. Monolithic silica columns were invented and patented by Holloway in 2001 [6] and since their invention, they have been regenerated and developed continuously. Regarding the higher permeability and higher efficiency for less retained solutes; monolithic silica columns permitted to high-speed separations at low pressure, especially in instruments with very small extra column effects [7]. Besides, pressure problems are effortlessly defeated by using the columns packed with monolithic sorbents without reducing efficiency, thanks to a unique bimodal structure [8, 9].

Few analytical methods are available for the determination of AVA in the literature. These may be divided into three main groups: 1. Single compound HPLC analysis of AVA in blood samples [2] and pharmaceutical dosage forms [10, 11]; 2. Analysis of AVA with dapoxetine in binary mixtures using UV-spectrophotometry [12] and HPLC [13]; 3. LC-MS/MS analysis of AVA in human plasma [7] and besides other acetildenafils and sildenafils in binary-mixtures [14]. In this study, a new LC method which is applicable for both MS/MS and photodiode array detector (PDA) was developed for determination of AVA in dapoxetine (DPX)/AVA

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combination tablets; separation of the compounds was achieved by using a C_{18}-bonded monolithic silica column.

Materials and Methods:

Studies were performed using a Nexera XR series liquid chromatograph, which was composed of a DGU-20A3R on-line degasser, 2 × LC-20AD gradient pumps, a SIL-20AC autosampler, a CTO-10ASVP column oven, a FCV20AH$_6$ high-pressure flow line selection valve, and a CBM-20A communications bus module; two different detectors, LCMS-8040 triple quadrupole mass spectrometric detector for mass-based detections and SPD-M20A photodiode array detector for absorbance-based detections (all from Shimadzu, Kyoto, Japan). Also, Shimadzu LC LabSolutions 3.43 SP1 data integration software was used for instrumental control and data processing.

The separation of AVA and DPX was carried out using a second generation C_{18}-bonded monolithic silica column (Chromolith® High Resolution RP-18e, 100×4.6 mm, Merck KGaA) as stationary phase, and a solution consisted of 0.1% (*v/v*) formic acid in water and 0.1% (*v/v*) formic acid in acetonitrile (75: 25 (*v/v*), pH=2.6) as mobile phase. The flow rate was 0.5 mL/min, and the column temperature was maintained at 40.0 °C. Sample injection volume was 1 µL for LC-PDA and 0.3 µL for LC–MS/MS analyses. In PDA analyzes, the quantification was performed by the absorbance of the avanafil at 247 nm, while in the MS analyzes Multiple Reaction Monitoring (MRM) was developed. The avanafil with m/z value of 484 was used to generate daughter ions with different parameters (**Table 1**) and the MRM optimization was performed by the instrument.

Table 1. MRM conditions of avanafil in LC-MS/MS analyses.

Compound	Precursor Ion	Product Ion	Q1 Pre-Bias (V)	CE (V)	Q3 Pre-Bias (V)
Avanafil	483.95	155.05	-22.0	-47.0	-27.0
		375.10	-22.0	-28.0	-25.0
		233.10	-22.0	-36.0	-23.0

The developed method was validated according to ICH Q2(R1); moreover, the specificity and robustness of the method, as well as stability of AVA solutions were studied.

Results:

The proposed method offers high flexibility due to use of very similar chromatographic conditions for MS/MS and PDA detection; reliability and easiness in method transfer and application are some other remarkable features. Some of the validation data are given in Table 2; acceptable regression coefficients, repeatability and accuracy were obtained in both

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methods. The limit of quantitation was 217 ng/mL for PDA detection, while it was 3.55 ng/mL for MS/MS.

Table 2. Some validation results for LC-PDA and LC-MS/MS studies.

Parameters	**LC-PDA**	**LC-MS/MS**
Linearity range (n=15)	0.5-20 µg/mL	150-6000 ng/mL
Equation (n=15)	y = 10294x + 1883	y=8583.6x + 1034238.931
Regression coefficient (n=15)	0.9991	0.9973
Limit of Quantitation	0.217 µg/mL	3.55 ng/mL
Limit of Detection	0.072 µg/mL	1.17 ng/mL
Repeatability (intraday, RSD%, n=10)	0.57	0.77
Accuracy (n=3, k=3) (Mean rec.± SD (%))	95.42 ± 3.56	102.09 ± 0.36
Injection precision (retention time) (min)	0.229	0.401

Assay results of combination tablets containing DPX and AVA (TOP-AVANA® from Sunrise Remedies, India) are 47.93 ± 3.77 mg for LC-PDA and 49.52 ± 3.06 mg for LC-MS/MS.

Conclusions:

In this study, quantitative analysis of AVA in combination tablets was successfully carried out and validated using the same analytical conditions on LC-DAD and LC-MS/MS instruments. Being applicable on two different types of instruments makes the method utilizable for possible cross-checking of the results. Also, it is thought that each of the methods should make a significant contribution to the field of pharmaceutical analysis and clinical and bioanalytical research with the advantages of saving labor, time and money.

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OP-077: DETERMINATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN HUMAN MILK BY DLLME-HPLC

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Introduction:

Since the introduction of dispersive liquid-liquid microextraction (DLLME) in 2006 by Assadi Y. et al. [1], this method has gained a large reputation among other microextraction techniques due to its simplicity, cost-efficiency, rapidity and low consumption of organic solvents. DLLME is mainly used to provide high preconcentration and/or sample clean up. In this study, DLLME with a back-extraction (BE) step was used prior to high-performance liquid chromatography (HPLC) for the extraction and determination of four non-steroidal anti-inflammatory drugs (NSAIDs) [i.e., ketoprofen (KET), etodolac (ET), flurbiprofen (FBP) and ibuprofen (IBU)] in mother milk.

Materials and Methods:

The analytes were separated using a 1200 Series Agilent Technologies Gradient HPLC instrument combined with a diode-array detector (DAD), reversed-phase column [i.e., Grom-Sil 80 Octyl-4 FE, 4.6 mm ID x 250 mm (3 µm)], a mobile phase consisting of ACN:1.0% TFA, 40:60 (%, v/v) at pH* 1.4, a flow rate of 0.8 mL min^{-1}, column temperature of 40 °C and an injection volume of 20 µL.

Results and Discussion:

Optimum extraction conditions were achieved using 200 µL of chloroform (as an extraction solvent) and 2.5 mL acetonitrile (ACN, as a disperser solvent). The mixture was completed to 11.0 mL with 3.0% (w/v) sodium chloride (NaCl) at an extraction time of 1 min. Back-extraction of NSAIDs into 100 µL of (30% ACN: 70% 1.0 M NaOH) solution within 1 min, facilitated the direct injection of the analytes into the reversed-phase column. Optimum extraction conditions are summarized in **Table 4**.

Table 4: Optimum DLLME and BE conditions

DLLME	Extraction solvent	Chloroform
	Volume of extraction solvent	200 µL
	Disperser solvent	Acetonitrile, 2.5 mL
	Salt addition	NaCl, 3.0% (w/v)
	Extraction time	1 min
BE	Back-extraction solution	30% ACN: 70% 1.0 M NaOH
	Volume of back-extraction solution	100 µL
	Back-extraction time	1 min

Limits of detection (LOD), calculated based on $3S_b/m$, where 'S_b' is the standard deviation of the blank and 'm' is the slope of the regression equation, were found in the range of 0.2-0.9 mg L^{-1}, whereas, limits of quantitation (LOQ), calculated based on $10S_b/m$, were in the range of 0.7-3.0 mg L^{-1}. Good linearity of the calibration graphs over the linear dynamic range (LDR) from the

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corresponding LOQ to 25.0 mg L^{-1}, was obtained with coefficient of determination (R^2) in the range of 0.9951-0.9989 (**Table 5**).

Table 5: Analytical performance parameters of DLLME-HPLC.

Method	Sample	Analyte	Regression equation[a]	R^2	LOD[b] (mg L^{-1})	LOQ[c] (mg L^{-1})	LDR[d] (mg L^{-1})
Conventional HPLC	Aq.	KET	$y = 96.5(\pm0.3)x - 80.3(\pm23.3)$	0.9998	0.6	2.0	2.0 – 150
		ETO	$y = 175.7(\pm1.7)x + 525.6(\pm19.8)$	0.9978	2.0	6.7	6.7 – 150
		FBP	$y = 118.6(\pm0.5)x - 82.6(\pm38.9)$	0.9998	1.2	4.0	4.0 – 150
		IBU	$y = 32.7(\pm0.1)x + 6.5(\pm7.0)$	0.9999	0.8	2.7	2.7 – 150
DLLME-HPLC	12 –month milk	KET	$y = 947.6(\pm9.6)x - 285.8(\pm134.8)$	0.9980	0.3	1.0	1.0 – 25
		ETO	$y = 1265.1(\pm11.5)x + 246.9(\pm144.8)$	0.9981	0.3	1.0	1.0 – 25
		FBP	$y = 1058.0(\pm13.8)x - 91.2(\pm171.1)$	0.9971	0.6	2.0	2.0 – 25
		IBU	$y = 311.5(\pm4.6)x - 53.2(\pm64.9)$	0.9974	0.7	2.3	2.2 – 25
	6-month milk	KET	$y = 893.3(\pm10.8)x - 270.9(\pm151.4)$	0.9972	0.4	1.3	1.3 – 25
		ETO	$y = 1344.5(\pm8.7)x - 19.3(\pm107.8)$	0.9989	0.2	0.7	0.7 – 25
		FBP	$y = 1038.7(\pm18.2)x - 293.4(\pm225.5)$	0.9951	0.8	2.7	2.7 – 25
		IBU	$y = 284.1(\pm5.3)x - 145.4(\pm74.7)$	0.9958	0.9	3.0	3.0 – 25
	2-month milk	KET	$y = 963.1(\pm11.8)x + 307.1(\pm165.2)$	0.9972	0.4	1.3	1.3 – 25
		ETO	$y = 1328.6(\pm13.3)x + 409.3(\pm165.0)$	0.9975	0.3	1.0	1.0 – 25
		FBP	$y = 1058.6(\pm16.4)x - 35.6(\pm202.1)$	0.9959	0.7	2.3	2.3 – 25
		IBU	$y = 277.1(\pm4.9)x + 110.3(\pm68.1)$	0.9963	0.8	2.7	2.7 – 25

[a] $Peak\ area = slope(\pm SD) \times concentration(mg\ L^{-1}) + intercept(\pm SD)$.

[b] Limit of detection.

[c] Limit of quantitation.

[d] Linear dynamic range.

Three mother milk samples with different age of breastfeeding (i.e., 2, 6 and 12 months) were collected from three healthy volunteers and studied in order to estimate the effect of different matrices on the extraction efficiency. Similar slopes of DLLME-HPLC for each analyte in the different milk samples indicated negligible matrix effect (**Table 5**). Enrichment factors, calculated as the ratio of calibration slope with DLLME–HPLC to that with external aqueous calibration (conventional HPLC), fell in the range of 7.2-10.0 (**Table 6**).

Finally, the proposed method was applied to determine FBP in genuine milk samples (with breastfeeding of 12 months) at different time intervals (0.5-3.5 h). It was found that the highest concentration in the milk was reached after 2 h.

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Table 6: Enrichment factors, percentage relative recoveries and reproducibility.

Age of breastfeeding / month	Analyte	EF[(a)]	%RR	%RSD[(b)]	
				Intraday	Interday
12	KET	9.8	99.2	4.0	10.7
	ETO	7.2	101.4	5.0	10.7
	FBP	8.9	100.3	5.1	10.9
	IBU	9.5	96.7	5.2	10.0
6	KET	9.3	103.5	3.0	6.8
	ETO	7.7	102.7	2.6	3.6
	FBP	8.8	101.5	3.7	7.8
	IBU	8.7	97.9	4.4	9.2
2	KET	10.0	104.6	3.5	5.4
	ETO	7.6	102.3	5.0	10.5
	FBP	8.9	93.1	5.3	9.7
	IBU	8.5	100.5	5.1	13.5

[a] Enrichment factor: Ratio of calibration slope with DLLME–HPLC to that with external aqueous calibration (conventional HPLC).
[b] Percentage relative standard deviation ($n = 3$).

Conclusions:

DLLME-HPLC was demonstrated to be a simple and rapid method for the determination of NSAIDs in mother milk with high percentage relative recoveries (%RR) in the range of 93.1-104.6%. Intraday and interday precision of the method, as indicated by percentage relative standard deviation (%RSD), was found to be less than 5.3 and 13.5%, respectively, indicating good reproducibility of the proposed method (**Table 6**).

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OP-081: EXPERIMENTAL DESIGN APPROACH TO OPTIMIZE HPLC SEPARATION OF ACTIVE INGREDIENTS, PRESERVATIVES AND COLORANTS IN SYRUP FORMULATION

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Introduction:

Food additives such as preservatives, colorants, sweeteners and aromatic substances are commonly used in pharmaceutical formulations, especially syrups and oral suspensions, to improve their consumer characteristics and apperance. However, these substances can cause various adverse reactions in sensitive individuals. There is a risk of exposure to preservatives and food colorants likely to induce urticaria, asthma or pseudoallergic reactions for all individuals taking commercial drug products (1, 2). Therefore, determination of synthetic preservatives and colorants in pharmaceutical products is an important issue in order to control the amount of use permitted, to ensure quality control and consumer health (3).

In recent years, chemometric tools have been increasingly utilized in the optimization of analytical methods, because of their advantages such as a reduction in the number of experiments, in turn having lower reagent consumption and considerably time saving. Moreover, experimental designs used in the optimization process enable the development of mathematical models that allow evaluation of the convenience and reveal both significance of the factors effects and the interaction effects between the factors (4).

In this study, a simple, accurate, precise and rapid HPLC method was developed for the simultaneous analysis of pseudoephedrine HCl (PSE) and guaifenesin (GU), along with synthetic preservatives, methyl paraben (MP) and propyl paraben (PP), and colorants, ponceau 4R (PO) and sunset yellow (SY) in syrup sample. Optimum conditions of HPLC separation were performed with Box-Behnken experimental design.

Materials and Methods:

Analytical standards of the active and additive compunds were supplied from a local pharmaceutical company. Chromatographic separation was achieved using reversed phase C18 column (4.6 mm x 250 mm x 5 µm particle size) along with phosphate buffer (0.025 M, pH 6.3) and acetonitrile based on gradient elution. The eluents were monitored via a diode array detector. Injection volumes were 20 µL and peak area was used to quantify each of the analyte.

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Box-Behnken experimental design (BBD) was employed for the optimization of chromatographic separation conditions. A total of 27 different combinations of random order were performed according to a BBD configuration for four factors (3). These factors were pH (A; 6.0, 6.5, and 7.0) and flow rate of the mobile phase (B; 2.0, 2.2 and 2.4 mL/min) and mobile phase ratios for the first and second gradient elutions in terms of phosphate buffer percent (C; 75, 80, and 85% for Gradient 1 and D; 50, 55, and 60% for Gradient 2, respectively). Resolution between adjacent peaks was used for the response of the factors.

Results:

According to the results of variance analysis at the end of experimental design step, C, D, interaction of C by D and quadratic contribution of C are the most significant terms on resolution, besides, all of them show positive effect. For other factors and interactions are not considered significant in the studied range. The best resolution values can be obtained when C and D are adjusted to their high levels.

By taking into consideration these results, optimum chromatographic conditions were found to be pH, 6.3; flow rate, 2.4 mL/min; mobile phase ratios for gradient 1 and 2, 85 and 60%, respectively. Additionally, mobile phase ratio of the third step in gradient elution which effects only analysis time is 30%.

Coclusions:

Under optimum HPLC conditions, all analytes were eluted within 10 min (retention times of 2.5, 4.5, 6, 7.5, 9 and 9.8 min for PSE, PO, SY, GU, MP and PP, respectively). Finally, after the developed method was validated in accordance with ICH guidelines, this method was successfully applied for the simultaneous determination of PSE, GU, MP, PP, PO and SY in a commercial syrup sample. It can be said that the developed method without any pretreatment is suitable in order to quantify simultaneously these active pharmaceuticals and additive compounds in similar syrup formulations.

Acknowledgements

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OP-087: EVALUATION OF RATIONAL ANTIBIOTIC USE IN A CHILDREN'S HOSPITAL.

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Introduction:

Medication error is defined as 'a failure in the treatment process that leads to or has the potential to lead to harm to the patient' [1]. These errors occur during prescribing, preparing and administering processes of medications and are frequently seen and increase the likelihood of undesirable effects in patients [2]. Pediatric patients are exposed to 3 times more medication errors than adults [3]. In pediatric patients, most frequent medication errors are dosing errors which are commonly seen in antibiotic usage [4]. Upon introduction into medicine in the 1940s, antibiotics have been centered in modern healthcare. Nowadays, they are the most commonly used drug group throughout the world. Unfortunately in terms of antibiotics consumption, Turkey leads Europe. In this study, it was aimed to evaluate antibiotic related medication errors in pediatric inpatients' service.

Material and methods:

This study was carried on at Hacettepe University Ihsan Dogramacı Children's Hospital. It is a tertiary care hospital with 250 acute-care beds and 215,000 admissions per year. It includes a bone-marrow transplantation unit, newborn intensive care unit, pediatric intensive care unit and cardiovascular surgery intensive care unit. On November 16, 2016, hospitalized patients' orders were evaluated regarding to rational antibiotic use by point prevalence method by two clinical pharmacists and three pediatric infectious diseases physicians. Antibiotic related drug- drug interactions, dose accuracy, and administration time were evaluated. Drug indications were evaluated by 3 pediatric infectious diseases physicians whereas drug doses, administration times, and drug-drug interactions were evaluated by 2 clinical pharmacists through the electronic database system, drug orders and nurse forms. Physicians were informed about drug related problems identified by clinical pharmacists. Appropriateness of doses was evaluated according to the indications by using The Harriet Lane Handbook (20th Edition, 2015, Elsevier-Saunders) as a reference. Updated literature data was also used for the evaluation of doses if necessary from Uptodate® and

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Nelson Textbook of Medicine 20th Edition. For potential drug interactions, 'Drug interactions' section of the IBM Micromedex Solutions® database was used. In this database interactions are rated as contraindicated, major, moderate and minor. Drug package inserts were also used as a reference for drug interactions.

Results:

At the time of the study 89 inpatients were using antibiotics. Median age was 42 months (range 1 to 226 months) and 40 (44.9%) patients were female. Twenty-one (23.6%) of the patients were in surgical units and, 68 (76.4 %) were in pediatric medicine units. Two of the patients (2.2%) were using antibiotics for prophylaxis and all others were on the treatment protocol. Thirty-two patients (36%) were hospitalized for suspected bacterial infection and 17 patients (19.2%) for bacterial pneumonia according to their primary hospitalization indication. The evaluation of antimicrobial drug use showed that, 64% of the patients were using at least one antimicrobial drug. The median number of antimicrobial drugs used in surgical services was 2 (range: 1-3) and in pediatric services was 2 (range: 1-8). There were no statistically significant differences between the surgical and pediatric services in terms of the number of antimicrobial and antibacterial drugs ($p>0.05$). Thirty-two (36%) patients were on 1, 25 (28.1%) patients were on 2 and 15 (16.9%) patients were on 3 antimicrobial drugs, and the number of antimicrobial drugs ranges from 1 to 8. The most commonly used three antibiotic groups were broad spectrum penicillins (14.9%), glycopeptides (13.4%) and carbapenems (13.4%). In surgical services, totally 21 patients indications of antimicrobials in 10 of them (47.6%) were not appropriate and even though 1 of them (4.8%) regarded as acceptable based on personal experiences of pediatric infectious disease specialists. In non-surgical pediatric services 9 (13.2%) patients' antimicrobial indications were not appropriate and 9 (13.2%) patients' indications were considered as acceptable out of 68 patients. The percentage of the inappropriate indications in surgical services was significantly higher ($p=0.006$). Twenty nine patients' orders had medication errors. Twelve patients' antibiotic orders had antibiotic related errors, and total of 17 inappropriate treatments (2 drug-drug interactions, 3 administration time errors, 12 dose errors) were observed.

Conclusions:

In this study, drug orders of pediatric inpatients who were on antibacterial drugs were evaluated by point prevalence method in terms of medication errors. In a point prevalence study by Grohskopf et al., 54.4% of the patients in pediatric wards and intensive care unit were using at least one antimicrobial [5]. In a study conducted by Gerber et al., it was found that 60% of pediatric inpatients were using antimicrobial drugs [6]. Compared with the literature, antimicrobial drug usage rate was found slightly higher (%64.4) in our study because only the patients using antibacterial drugs were included in the study. In a study of Thiruthopu et al. 53% of the prescribed drugs were found to be 'most appropriate' and the rest were 'eligible' [7]. Compared to study of Thiruthopu et al. appropriateness of antibiotic indication was higher (78.7% vs 53%) in our study. Ceyhan et al., evaluated the appropriateness of indications for antimicrobials in a point prevalence study including 12 pediatric hospitals and they were found that 54.6% of 1302 patients had at least one antimicrobial usage. In 46.7% of patients using antibiotics, an inappropriate indication was detected. The percentage of inappropriate indication in surgical services was found to be higher (80.2%) compared to other services [8]. Compared with the study by Ceyhan et al., the incidence of inappropriate indication was lower in our study in surgical services however it was higher in the surgical services compared to other pediatric services in both studies. Although only antibacterial prescribed patients were evaluated in our study, at least one medication error was detected in 40% of the patients. Forty-three percent of the errors were related to antimicrobials. The number of medication errors in surgical services was found to be higher than other pediatric services. Antimicrobial drug error rates were similar to those of previous studies [9,10]. In other studies, medication errors were detected mostly in pediatric

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intensive care units, but in our study, it was seen mostly in surgical services [11]. Evaluation of antibiotic use in pediatric inpatients' service by a clinical pharmacist in terms of drug related problems such as drug interactions, side effects and prescribing errors will improve treatment-related outcomes of the patients. Clinical pharmacists' involvement in the multidisciplinary team will alleviate physicians' increased workload.

Acknowledgement:

The authors would like to thank all patients and their parents.

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OP-091: EVALUATION OF DRUG-DRUG INTERACTIONS OF ANTIHYPERTENSIVE DRUGS

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Introduction:

Antihypertensives are one of the most commonly used drugs. Most patients using these drugs have multiple medical conditions, therefore polypharmacy is common in these patients. Patients with polypharmacy are at risk of drug-drug interactions (DDIs). A drug interaction is defined as the qualitative or quantitative modification of the effect of a drug by the simultaneous or successive administration of a different one. This may result in the alteration of therapeutic effect and safety of either or both drugs (1). One review reported that DDIs were held responsible for 0.054% of the emergency visits, 0.57% of the hospital admissions, and 0.12% of the re-hospitalizations. In elderly population, DDIs were held responsible for 4.8% of the admissions (2). The World Health Organization emphasizes that adverse drug reactions and its impact can be significantly minimized by implementing careful attention to the population at risk of DDIs (3). Therefore, early recognition of potential DDIs is important as well as the prevention and management of them. Potential DDIs can be evaluated with different online databases and the results of these databases may vary from each other (4,5). The aim of this study is to demonstrate and analyze the difference between three different online databases. This study also aims to display the drug-drug interaction pattern in the internal medicine ward of the Hacettepe University Hospitals.

Material and Methods:

Patients were followed up between 25 December 2017 and 15 March 2018 in general medicine inpatient service at Hacettepe University Hospitals. Only the patients who were using antihypertensive drugs for any reason were included in this study. All the drugs used by these patients were evaluated with 3 different online databases; Micromedex®(6), Medscape®(7) and Drugs.com®(8).

Results:

The mean age of the patients was 65.3±17.97 years and total of 42 patients [24 (57.1%) females)] were included in the study. Thirty of the patients (71.4%) were suffering from hypertension, the remaining patients were taking antihypertensive drugs due to different problems. Patients had a median 4 (1-9) different disease (including hypertension). The most used antihypertensive drug class was beta blockers (n=22, 52.4%), followed by angiotensin receptor blockers/angiotensin-converting enzyme inhibitors (n=21, 50%), calcium channel blockers (n=18, 42.9%), diuretics (n=17, 40.5%), and alpha blockers (n=5, 11,9%). Patients were using median 2 (1-5) different antihypertensive drugs and 9 (1-18) non-antihypertensive drugs. According to Micromedex®, Medscape® and Drugs.com® databases, 29 (59%), 37 (88.1%) and 36 (85.3%) patients had potential DDIs, respectively. Majority of the patients (n=26, 62%) had at least one potential drug-drug interaction according to the all three

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databases. The severity ratings and potential drug-drug interaction detection of each database are given in Table 1. Different then others, Drugs.com has no category defined as contraindicated.

Table 1: Severity ratings and potential DDIs detection of each databases.

Micromedex®		**Medscape®**		**Drugs.com®**	
Contraindicated	0 (0,0%)	Contraindicated	0 (0,0%)	-	-
Major	19 (22,9%)	Serious – Use Alternative	8 (4,0%)	Major	9 (3,7%)
Moderate	64 (77,1%)	Significant – Monitor Closely	166 (82,6%)	Moderate	202 (83,1%)
Minor	0 (0,0%)	Minor	27 (13,4%)	Minor	32 (13,2%)
Total	83 (100,0%)	Total	201(100,0%)	Total	243(100,0%)

Conclusions:

Patients using antihypertensive drugs are relatively older and have numerous co-morbidities and co-medications. More than half of the patients have a potential to have DDIs. The rate of determination of DDIs and the degrees of DDIs differ between online databases. Generally, Micromedex detects less potential drug-drug interactions compared to Medscape and Drugs.com databases. In all of the three databases the highest number of potential DDIs were seen in the “moderate” category. While evaluating DDIs, clinicians need to be consider using different databases and monitoring the patients for the management of DDIs.

Acknowledgements

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OP-095: PATIENTS' ATTITUDES ON SAFE HANDLING OF ORAL CHEMOTHERAPEUTICS

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Introduction:

Safe handling and appropriate storage of oral chemotherapeutics are crucial for maintaining the success of chemotherapy. It is also important for patients and their caregivers to avoid unexpected and hazardous exposures during treatment. Although guidelines are available for aseptic drug preparation, safe handling and administration of parenterally administered chemotherapy drugs, there are few recommendations issued for safe handling and disposal of oral chemotherapeutics. The National Institute for Occupational Safety and Health (NIOSH) lists antineoplastics and other hazardous drugs. NIOSH recommends the use of single pair gloves for dispensing a single tablet. Repeatedly counting, cutting or crushing tablets may pose a higher risk of worker exposure and contamination to the workplace if proper precautions are not in place. If a containment device such as BSC (Class II biological safety cabinet) or CACI (compounding aseptic containment isolator) is not available, NIOSH recommends double gloves, a protective gown, respiratory protection, and a disposable pad. The aim of this study was to evaluate patients' attitudes in drug usage and handling of oral chemotherapeutics.

Materials and Methods:

This study was designed as a preliminary study and was conducted at an outpatient clinic in the Hacettepe University Oncology Hospital between 29th March and 19th April 2018. The patients who are older than 18 years of age and currently use oral chemotherapeutics were included in the study. The patients were asked 15 questions regarding demographics (age, gender and educational level), drug usage, storage/disposal conditions and hand-hygiene in the use of oral chemotherapeutics.

Results:

A total of 24 patients were included, of those 5 (20.8%) were male and the mean (± standard deviation) age was 54.29 (±8.73) years. Oral chemotherapy drugs used by all patients are on the NIOSH list. 37.5% of patients used tamoxifen, 25% used capecitabine, 16.6% used letrozol, 8.3% used thalidomide, erlotinib and 4.2% used regorafenib. Among the

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participants, 11 (45.83%) indicated to have an education on the use of oral chemoterapeutics, of those 9 (81.8%) indicated to have before the chemotherapy initiated. They stated that information was given by nurses (n=7, 63.6%) and doctors (n= 4, %36.3). While 87% of patients kept oral chemotherapy drugs in their original boxes, 12.5% of them kept oral chemotherapy drugs in dosette boxes. About 79% of patients stored oral chemotherapy drugs in the correct stroge conditions. Even though refrigerator conditions were not required, 21% of patients stored oral chemotherapy drugs in the refrigerator. All patients indicated not to crush / split their medication and 95.8%(n=23) take the dose correctly in terms of timing. About 45.8% and 33.3% of the patients indicated that they wash their hands 'before' and 'after' taking oral chemotherapy drugs, respectively. In terms of 12-steps correct hand-washing process; patients performed median (range) of 5 (1-10) steps. However, despite the NIOSH recommendation no patients use gloves while taking their oral chemotherapy drug.

Discussion*:*

There are studies that examine patients' attitudes and safe handing for the use of oral chemotherapy***.*** Chan et al., in study including 126 patients, showed that only 40% of patients had washed their hands after using oral chemotherapy drugs and no patients except 2 patients were using gloves when using oral chemotherapy drugs(1). Trovato et al., in a study involving 45 patients, reported that 26% of patients had washed their hands after using oral chemotherapy, and 86% of patients did not use gloves when using oral chemotherapy drugs (2).

Compared with other studies, this study found that hand washing rates after the use of oral chemotherapy drug were lower than those of Chan et al. but were relatively higher than those of Trovato et al. (1,2). When the use of gloves was assessed using oral chemotherapy, the results of this study were similar to those of Chan et al (1).

Trovato et al. showed that 76% of patients kept oral chemotherapy drugs in their original boxes and 90.5% of patients protected oral chemotherapy drugs from excessive heat and cold (2). In this study, 87.5% of patients keep medicines in their original boxes. Compared with the results of Trovato et al, it was observed that the rate of drug storage in original boxes was higher in this study.

Trovata et al. reported that 45% of patients did not receive any education for stroge and handling of the oral anticancer medication (2). In this study, the proportion of patients receiving education for oral chemotherapy was 45.88%. This ratio seems to be similar with the results of Travot et al.

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Clinical use deficiencies related to the use and storage of oral chemotherapy drugs are similar to the literatüre. Comprehensive studies are needed to measure patients' knowledge and attitudes towards the use and storage of oral chemotherapy drugs.

Conclusions:

Although, the concept of rational drug use includes aspects of safe handling and proper storages/disposal of medicine, unfortunately it can be ignored by patients and healthcare professionals in routine practice. Therefore, practice standards on each aspects of chemotherapy drug usage should be established and patients should be informed about the correct use of oral chemotherapeutics by healthcare professionals.

Acknowledgements:

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OP-096: RATIO DERIVATIVE AND DIFFERENCE SPECTROPHOTOMETRIC TECHNIQUES FOR SIMULTANEOUS DETERMINATION OF CARVEDILOL AND HYDROCHLOROTHIAZIDE IN MARKETED TABLETS

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Introduction:

Hypertension is the leading risk factor for cardiovascular disease and mortality worldwide. Carvedilol (CAR) and Hydrochlorothiazide (HYD) are used alone or as combination therapy in the treatment of patients whose blood pressure is not adequately controlled with any of the substances alone. CAR, (Fig.1) is used in the treatment of hypertension and stable angina pectoris. HYD, (Fig. 1) is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical formulations which decreases active sodium reabsorption and reduces peripheral vascular resistance. Aim of the study is to make the simultaneous determination without prior separation using some spectrophotometric techniques.

Figure 1: Chemical structure of **(A)** Carvedilol 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl]amino]-2-propanol and **(B)** Hydrochlorothiazide 6-choloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide

Materials and Methods:

CRV and HYD were obtained by *Pfizer* Pharm. Ind. as a gift and were used as received. Analytical grade methanol was purchased from Merck Chem. Ind. Stock solutions (0.2 $mgmL^{-1}$) of CRV and HYD were in methanol and were further diluted with the same solvent as appropriate. Stored at $+50^0C$ in the dark, these solutions were shown to be stable during the period of study. The spectrum was obtained using a Shimadzu 1800 double beam UV-Vis spectrophotometer with a fixed slit width (2 nm) connected to an PC computer loaded with Shimadzu UVProbe software, and equipped with a Lexmark 1020 model printer.

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Results:

Ratio derivative and difference spectrophotometric techniques were presented for analyzing a binary mixtures of CAR and HYD [1-3]. The zero order spectra of CRV, HYD and their binary mixtures exhibit overlapping as shown in Figure 2. In this technique the absorption spectrum of binary mixture was divided by the absorption spectrum of the standard solution. The ratio spectra were recorded. The ratio derivative amplitudes were measured at 236.70 nm for CAR (Fig. 3a,b) and 261.80 nm for HYD (Fig. 4a,b) respectively. On the other hands, in the ratio difference spectrophotometric technique which measures the difference in amplitudes between 241.3 -285.8 nm for CAR and 226.45 – 269.03 nm for HYD, respectively**.**

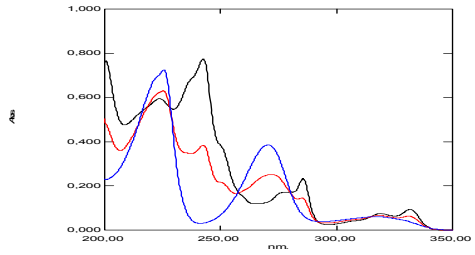

Figure 2. Zero-order absorption spectra of 6.0 µg/mL CRV (black line) 6.0 µg/mL HYD (blue line) and mixture of 3.0 µg/mL CRV with 3 .0 µg/mL HYD (red line) in methanol solution **(**$\Delta\lambda$ =2nm)

Figure 3: Curves which obtained by **a)** dividing the spectrums and **b)** first derivative of CRV calibrations (2-10 µg/mL) to spectrum of 6.0 µg/mL HYD.

Figure 4: Curves which obtained by **a)** dividing the spectrums and **b)** first derivative of HYD calibrations (2-12 µg/mL) to spectrum of 6.0 µg/mL CRV.

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The calibration curves were linear over the ranges of 2.0-10.0 µg mL(-1) and 3.0-20.0 µg mL(-1) for CAR and HYD, respectively (Table 1 and 2).

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Table 1. Analytical data of the calibration graphs for the simultaneous determination of CRV and HYD by the ratio derivative spectrophotometric technique

nm	m	n	r	Concentration range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
236.7	$1.98x10^{-3}$	$6.04x10^{-4}$	0.9988	2.0 - 10.0	0.101	0.333
261.8	$7.12x10^{-3}$	$-1.43x10^{-4}$	0.9991	2.0 - 10.0	0.216	0.712

Table 2. Analytical data of the calibration graphs for the simultaneous determination of CRV and HYD by the ratio difference spectrophotometric technique

In order to access the validity and applicability of the described method, recovery studies were performed by analyzing synthetic laboratory mixtures of each drug in different ratios.

nm	m	n	r	Concentration range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
241.4-285.8	$3.27x10^{-2}$	$1.94x10^{-2}$	0.9987	2.0 - 10.0	0.142	0.469
226.45-269.03	$1.08x10^{-2}$	$-2.86x10^{-2}$	0.9999	2.0 - 10.0	0.082	0.271

Table 3. Determination of CRV and HYD in laboratory prepared mixtures by the proposed by the ratio derivative spectrophotometric and ratio difference spectrophotometric techniques

	Ratio Derivative Spec. Tech.		Ratio Difference Spec. Tech.	
	CRV	HYD	CRV	HYD
Recovery (%)	99.8	99.2	100.1	99.7
Standart Deviation	0.85	0.53	1.07	1.10

Conclusion: Both the developed methods are specific, accurate, simple, precise and less time-consuming. Therefore these methods could be successfully applied for the routine analysis of binary mixtures in quality control laboratories without any preliminary separation steps.

Acknowledgements:

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OP-103: OPTIMIZATION THE PREPARATION PROCESS OF METHOTREXATE LOADED HUMAN SERUM ALBUMIN NANOPARTICLES

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Introduction:

Methotrexate (MTX) is one of the most widely utilized drug for the treatment of various forms of cancer. However, the clinical application of this drug is limited by its toxic dose related side effects and drug resistance by target cells. Since disadvantages of the MTX likewise the lack of selectivity, short half-life in the bloodstream and rapid diffusion throughout the body resulting in an essentially uniform tissue distribution, alternative drug delivery systems were developed (1). Nanoparticulate delivery systems are preferentially concentrated and retained in the leaky vasculature of tumor tissues due to the “enhanced permeability and retention” (EPR) effect. Due to their tumor-targeting abilities, a variety of nano-systems containing anticancer drugs have been developed in recent years (2). Human serum albumin (HSA) based nanoparticulate systems are also play an important role due to the ideal properties such as being non-toxic, non-antigenic, having active targeting ability (3,4). Many technologies for fabricating HSA based nanoparticles (HSA-NPs) have been reported in the literature, such as coacervation, emulsion and desolvation methods but difficulties remain in making albumin nanoparticles stable against dilution *in vivo*. Chemical cross-linking agents, such as glutaraldehyde, are often used to stabilize the albumin nanoparticles (5). The objective of the present study is the optimization of the preparation process of MTX loaded HSA-NPs.

Materials and Methods:

Materials:

MTX was obtained from Kocak Pharma (Turkey). HSA (fraction V, purity 96–99%) and glutaraldehyde 25% solution were obtained from Sigma (Steinheim, Germany). All other reagents were purchased from Merck (Darmstadt, Germany), they were of analytical grade and used as received.

Preparation of HSA Nanoparticles:

HSA nanoparticles were prepared by small modification on the desolvation technique as described previously (3). In principle, 150 mg HSA and 0.5 and 1 mg MTX dissolved in 2.0 mL 10 mM NaCl under constant stirring (550 rpm) for 2 h. By using 0.1 M NaOH the solution pH was titrated to 9. 8.0 mL of desolvating agent ethanol was added at a defined rate (1.0 $mL.min^{-1}$) drop wisely at room temperature. Particle crosslinking was induced by addition of 35 µL 8% glutaraldehyde in water. The crosslinking process was performed under stirring (550 rpm) of the suspension over a time period of 24 h.

Purification of HSA Nanoparticles:

24 h after incubation for crosslinking process, the resulting nanoparticles were purified by two cycles of centrifugation (10,000 × g, 20 min) and redispersion of the pellet with 10 mM NaCl to the original volume at pH 9. The supernatants of the washing steps were collected and the concentration of free MTX was analysed by HPLC.

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Measurement of particle size and zeta potential:

Particle size, PDI and zeta potential values of MTX-HSA-NPs were measured by a Zetasizer Nano ZS (Nano ZS, Malvern Instruments, UK). The samples were diluted 1:400 with purified water and measured at a temperature of 25 °C and a scattering angle of 173°.

HPLC analysis of MTX:

HPLC system for the quantification of MTX in the samples consisted of a Thermo Scientific Accela HPLC system. For the assay a reverse phase C18 column was used, and separation was obtained using an isocratic mixture of ammonium formate solution and acetonitrile (80:20). The flow rate was set to 1.0 $ml.min^{-1}$, and the peak was detected at 304 nm with a UV detector. Under these conditions**,** the retention time for MTX was about 2.3 min. MTX concentrations were calculated relative to a calibration curve.

Statistical methods:

All investigations were performed in triplicate. The results are displayed as average value with standard deviation. In order to compare independent groups, one-way ANOVA was performed using the software SPSS. Differences with *p*-values of $p < 0.05$ was considered as statistically significant.

Results:

Measurement of particle size and zeta potential:
The average particle size, PdI and zeta potential results of MTX-HSA-NPs were shown in Table 1-3.

Table 1. The particle size, PdI and zeta potential value of MTX-HSA-NPs

Drug amount	Particle size (nm±ss)	PDI (±ss)	Zeta potential (mV±ss)
0.5 mg MTX	250,67 ± 3,31	0,33 ± 0,05	-36,67 ± 0,75
1 mg MTX	338,33 ± 3,18	0,12 ± 0,03	-31,93 ± 0,50

Table 2. The particle size, PdI and zeta potential value of MTX-HSA-NPs at 25 ± 2 ºC

25±2ºC	Particle size (nm±ss)			PDI (±ss)			Zeta potential (mV±ss)		
Drug amount	$T_{initial}$	T_{1month}	T_{3month}	$T_{initial}$	T_{1month}	T_{3month}	$T_{initial}$	T_{1month}	T_{3month}
0.5 mg MTX	250,67 ± 3,31	211,87 ± 3,07	291,56 ± 4,64	0,33 ± 0,05	0,07 ± 0,02	0,15 ± 0,02	-36,67 ± 0,75	-13,37 ± 2,00	-24,10 ± 1,59
1 mg MTX	338,33 ± 3,18	230,33 ± 6,97	260,62 ± 5,35	0,12 ± 0,03	0,09 ± 0,03	0,09 ± 0,03	-31,93 ± 0,50	-38,9 ± 2,77	-30,15 ± 3,27

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Table 2. The particle size, PdI and zeta potential value of MTX-HSA-NPs at 40 ± 2 ºC

40±2ºC	Particle size (nm±ss)			PDI (±ss)			Zeta potential (mV±ss)		
Drug amount	$T_{initial}$	T_{1month}	T_{3month}	$T_{initial}$	T_{1month}	T_{3month}	$T_{initial}$	T_{1month}	T_{3month}
0.5 mg MTX	250,67 ± 3,31	234,25 ± 5,14	245,32 ± 3,21	0,33 ± 0,05	0,14 ± 0,01	0,13 ± 0,01	-36,67 ± 0,75	-23,54 ± 3,14	-25,23 ± 2,45
1 mg MTX	338,33 ± 3,18	245,16 ± 4,35	302,25 ± 6,15	0,12 ± 0,03	0,18 ± 0,08	0,17 ± 0,04	-31,93 ± 0,50	-30,25 ± 1,21	-32,26 ± 3,14

HPLC analysis of MTX: According to HPLC studies, with increasing MTX concentration, encapsulation efficiency (EE) increased too.

Table 4. The encapculation efficiency of MTX-HAS-NPs

Drug concentration	EE (%) ± ss
0.5 mg MTX	20,20% ± 1,25
1 mg MTX	25,64% ± 1,08

Conclusions: MTX-HSA-NPs could prove a promising tool for drug delivery system offering tumor-targeting strategy.

Acknowledgements: This study was supported by Ege University, Scientific Research Project Comission (Project number: 16-ECZ-016) and Aliye Uster Foundation. Also, the authors would like to thank to The Scientific and Technological Research Council of Turkey (TUBITAK-108S083) and T.R. Prime Ministry State Planning Organization Foundation (Project number: 09-DPT-001).

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OP-113: SYNTHESIS AND CHOLINESTERASE INHIBITORY POTENTIAL OF SOME PYRIDINIUM-3-CARBOHYDRAZIDE-HYDRAZONE DERIVATIVES

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Introduction:

Acetylcholinesterase (AChE) is an enzyme which catalyses the hydrolysis of acetylcholine (ACh) (1). Inhibition of AChE enyzme is the therapeutic target of drugs designed to manage various disorders such as myasthenia gravis, glaucoma, Lewy body dementia, and Alzheimer's disease (2). AChE inhibitors are used for the treatment of these disorders, which improve cholinergic functions by elevating ACh levels in cholinergic synapses. AChE is the primary enzyme responsible for the degradation of ACh, while butyrylcholinesterase (BuChE) plays a secondary role (3, 4). Therefore, targeting both AChE and BuChE is one of the most promising approach for the treatment of these disorders.

AChE inhibitors generally include aromatic-heterocyclic ring systems and a nitrogen atom as chemical structures (5). At present, among the clinically used drugs in the treatment of myastenia gravis, such as pyridostigmine and neostigmine, are quetarnary ammonium salts inhibitors of AChE. Pyridostigmine, a reversible AChE inhibitor used for the treatment of myasthenia gravis, includes a quaternary pyridinium-carbamate moiety in the molecule structure. Based on the AChE protein structure, this cationic nitrogen atom plays an important role in enzyme-ligand interactions (6). On the other hand, many compounds bearing hydrazide-hydrazone functional group have been reported to have ChE inhibitory activity. The hydrazones have hydrogen donor and acceptor nitrogen atoms and ability to make hydrogen bound with amino acids in the enzyme gorge.

Within this contex, in this study, pyridinium moiety was chosen as a core structure and a series of quaternary pyridinium derivatives bearing different alkyl groups on pyridine nitrogen and hydrazide-hydrazone structure at 3 position were synthesized as potential anti-ChE inhibitors (Scheme 1). At first, methyl derivative (**1a**) was synthesized and then, the side chain was extended from 1 to 4 carbon atoms. And then, to compare the effect on the interaction of an aromatic moiety, their benzyl (**1e**), phenylethyl (**1f**), phenylpropyl (**1g**) and phenylbutyl (**1h**) counterparts were also designed and synthesized.

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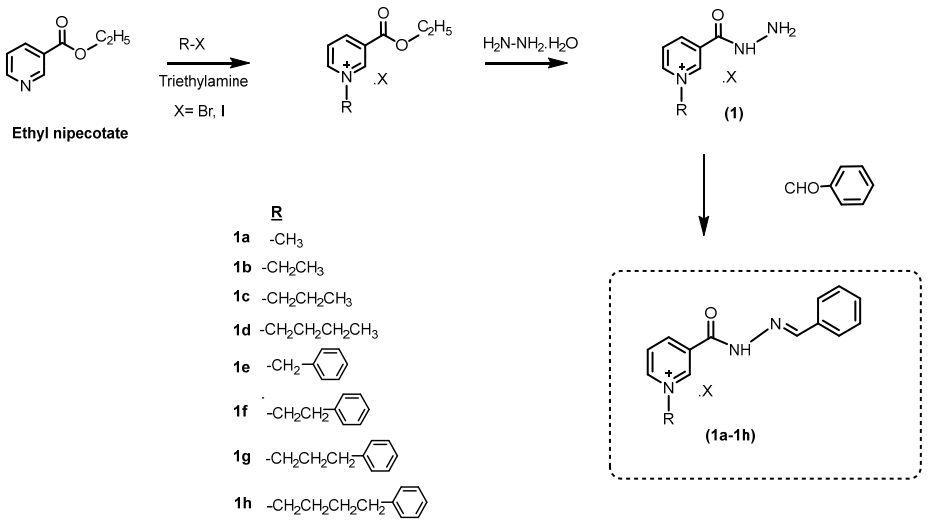

Scheme 1. Synthesis pathway of the final compounds

Materials and Methods:

The final compounds were synthesized according to the reported method (7). As shown in Scheme 1, their synthesis were realized in three steps. Initially, ethyl nipecotate and corresponding alkyl halide were stirred in acetonitrile in the presence of triethylamine to yield alkylpyridinium-3-carboxylate. After completion of the reaction, the obtained intermediate was reacted with hydrazinium hydroxide in ethanol under reflux condition. Lastly, to achieve the final compounds hydrazide intermediate was condensed with nonsubstituted benzaldehyde.The structures of the final compounds were confirmed by IR, ^{1}H NMR, and Mass spectra methods. The spectroscopic properties were in accordance with the proposed structures. And then, their AChE and BuChE inhibitory activities were assayed using the method of Ellman et al (8). Galantamine was used as the reference standard. The results were summarized as IC_{50} values in Table 1.

Table 1. *In vitro* AChE and BuChE inhibitory activities of the final compounds

Compound	IC_{50} (μM) ± SEM[a]		Selectivity
	***ee*AChE**	***eq*BuChE**	**AChE/BuChE**
1a	40.70 ± 0.59	6.09 ± 0.20	6.68
1b	>100	26.57 ± 0.27	
1c	>100	32.16 ± 0.75	
1d	11.94 ± 0.29	43.80 ± 2.56	0.27
1e	37.14 ± 2.27	1.61 ± 0.09	23.07
1f	12.83 ± 0.73	19.72 ± 0.49	0.65
1g	3.24 ± 0.14	22.29 ± 3.84	0.15
1h	11.93 ± 0.31	21.98 ± 0.91	0.54
Galantamine	0.43 ± 0.03	14.92 ± 0.57	0.03

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[a] Data are means ± standard error of the mean of triplicate independent experiments. The IC_{50} values were calculated by using GraphPad 5 software

Results: In the present study, a series of pyridiniumhydrazide-hydrazone derivatives were synthesized and evaluated for their anti-ChE activities. Chemical structures of the synthesized compounds were confirmed by spectral IR, ^{1}H NMR, and ESI-MS analysis. According to the biological activity results, most of the derivatives were found to inhibit ChE enzymes in micromolar range. The most active compound towards AChE was found to be the phenylpropyl derivative (**1g**) and it was also the most selective towards AChE enzyme. The most active and selective compound towards BuChE enyzme was the benzyl derivative (**1e**). Among the synthesized compounds, the derivatives bearing a phenyl moiety on the side chain displayed better activity than their analogues which dont have the phenyl ring. Thus, the introduction of a phenyl moiety to the alkyl chain positively influences the inhibitory capacity of the target compounds. The AChE inhibitory activity increased when the distance between the pyridinium nitrogen and the phenyl ring was extended from 1 to 3 carbon atoms. But extending to 4 carbon atoms led to a decrease in the inhibitory potency. In respect to BuChE activity results, extending the chain lenght from 1 to 4 carbons led to a decrease in the inhibitory activity.

Conclusions: A series of hydrazidehydrazone-containing pyridinium salts were synthesized and assayed for ChE inhibitory activity. Most of the compounds exhibited activity in micromolar range of concentration on both AChE and BuChE enzymes. Among the final compounds, the series including an aromatic phenyl ring on the side chain exhibited the best inhibitory activity when compared to their analogues. The distance between the pyridinium nitrogen and the phenyl ring seems to be important for both AChE and BuChE inhibitory activity. The obtained activity results suggested that these pyridiniumhydrazide-hydrazone derivatives might be promising leads for the development of anti-ChE drugs.

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OP-116: α-GLUCOSIDASE INHIBITORY EFFECTS OF SOME FUNCTIONALIZED AMINO ACID DERIVATIVES

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Introduction:

According to the first World Health Organization (WHO) Global report on diabetes, it caused 1.5 million deaths worldwide in 2012 and type 2 diabetes mellitus (T2DM) is the most common form in the society (1). Patients suffering from type 2 diabetes mellitus have been affected by numerous side effects such as impairment of functions of kidneys, heart, eye and nervous system (2). These damages are associated with insufficient insulin secretion and uncontrolled hyperglycemia. Controlling enhanced post-prandial glucose levels in these patients is shown to be essential to prevent progression of T2DM (3). One of the approaches for the preventation and treatment of T2DM is to reduce high glucose levels by inhibition of α-glucosidase, one of the major carbohydrate hydrolizing enzymes, located on the brush-border surface membrane of small intestine (4). α-glucosidase which catalyzes the cleavage of glucose from disachharides and polysaccharides, plays an important role in the carbohydrate digestion as monosaccharides are only ones absorbed from human intestine (5). Thus, the inhibition of this enzyme has been recognized an effective way to suppress the absorption of glucose (6). As it is known, only three agents namely acarbose, voglibose and miglitol are used in the treatment of T2DM by the way of inhibition of α-glucosidase but they have various side effects including diarrhea, abdominal pain and other gastrointestinal disorders (7). Therefore, there is an urgent need to discover and develop new chemical agents with enhanced activity profile and reduced side effects. For this purpose, in this study, as functionalized amino acid derivatives, fourteen 2/3-(acylamino)propionanilide derivatives (Figure 1) have been prepared and evaluated for their α-glucosidase inhibitory activities.

Materials and Methods:

The synthesis of the title compounds was realized in 2 steps. In the first step amino groups of α/β alanine was protected by BOC (8). Boc-alanines were reacted with anilines. Boc groups was removed from molcules. In final step, these intermediates were reacted with benzoyl chloride to obtain the target compounds. Melting points of the compounds were measured and are uncorrected. Stuructures of the compounds were confirmed by spectral (UV, IR, ^{1}H NMR, MS) and elemental analyses. All compounds were evaluated with regard to α-glucosidase inhibition compared to acarbose as reference compound. The enzyme inhibition assay was carried out spectrophotometrically by using slightly modified method of Zawawi et al (9) Briefly, α-glucosidase and substrate p-nitrophenyl-α-D-glucopyranoside (pNPG) were dissolved in 50mM phosphate buffer (pH:6.8) containing 100mM NaCl, 0.2 g/L NaN_3 and 2

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g/L bovine serum albumin. At first, 50µL of enzyme solution (50mU/ml) and 15µL of test compound dissolve in DMSO were mixed in a 96-well plate and incubated at room temperature for 15 min. Then, 50µL of solution of substrate (0.0625 mM) was added. After further incubation was conducted at 37 ^{o}C for 45 min. The sample solution was replaced by DMSO as a control. Acarbose was used as a reference inhibitor. All measurements were triplicated. The % inhibition has been calculated using the formula: Inhibition (%) = (1- $\Delta A_{sample}/ \Delta A_{control}$)* 100.

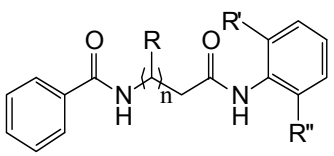
Results:

Following the assumption that functionalized amino acid derivatives have been reported to have wide range of biological activities, herein we investigated if the functionalized α/β-alanine derivatives might serve as potential α-glucosidase inhibitors.

With this aim a series of 2/3-(acylamino)propionanilide derivatives were synthesized and evaluated for their α-glucosidase inhibitory activity profile. For comparison of their inhibitory activities, inhibition% values of compounds and acarbose were determined. Based on biological activity results, most of the tested compounds displayed moderate to weak α-glucosidase inhibitory activity in comparison to acarbose.

Conclusions:

In conclusion, fourteen 2/3-(acylamino)propionanilide derivatives have been synthesized and evaluated for their α-glucosidase inhibitory activities. A preliminary study of synthesized compounds on α-glucosidase inhibition showed that further structural modification of this class of functionalized amino acid derivatives may lead to a promising anti-diabetic candidate molecules.



(n=1, R=CH_3)
(1) R'=H R"=H
(2) R'=H R"=Cl
(3) R'=H R"=CH_3
(4) R'=H R"=OCH_3
(5) R'=H R"=CH_2CH_3
(6) R'=H R"=$CH(CH_3)_2$
(7) R'=CH_3 R"=CH_3

(n=2, R=H)
(8) R'=H R"=H
(9) R'=H R"=Cl
(10) R'=H R"=CH_3
(11) R'=H R"=OCH_3
(12) R'=H R"=CH_2CH_3
(13) R'=H R"=$CH(CH_3)_2$
(14) R'=CH_3 R"=CH_3

Figure 1

Acknowledgements

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OP-122: NEW PURINE AND PYRIMIDINE NUCLEOSIDE ANALOGS: SYNTHESIS AND CYTOTOXIC ACTIVITY ON SELECTED HUMAN CANCER CELL LINES

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Introduction:

Cytotoxic nucleoside analogues and nucleobases are a pharmacologically diverse family, which has grown to include a variety of purine and pyrimidine nucleosides with activity in both solid tumors and malignant disorders of the blood (1, 2). These agents have many intracellular targets to induce cytotoxicity: they behave as antimetabolites, competing with physiological nucleosides during DNA or RNA synthesis, and as inhibitors of key cell enzymes (2, 3). Purine nucleoside analogs (PNAs) such as cladribine and fludarabine have been approved for the treatment of lymphoid malignancies and other hematological disorders (4). Recently, three novel second-generation PNAs, clofarabine, nelarabine and forodesine, have been synthesized and introduced into clinical trials (3).

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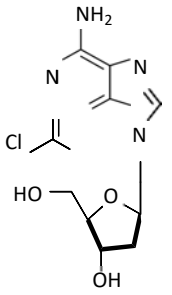
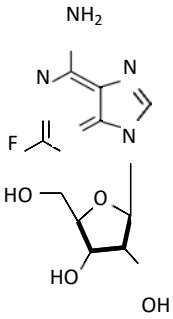
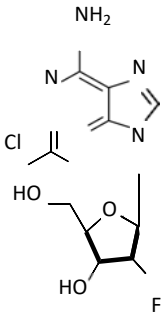


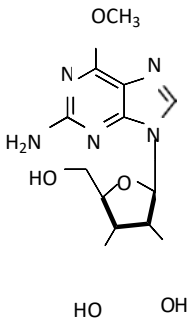
Cladribine



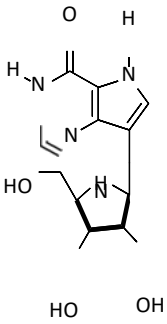
Fludarabine



Clofarabine

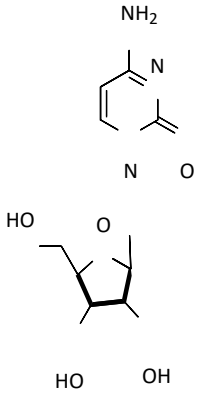


Nelarabine

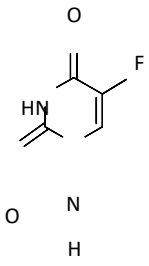


Forodesine

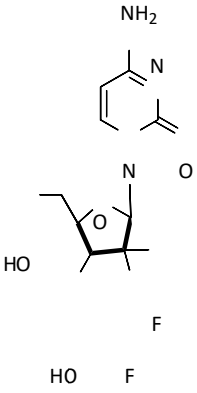
Among the currently available pyrimidine analogues, cytarabine is extensively used in the treatment of acute leukaemia; gemcitabine has activity in various solid tumors and some hematological malignant diseases; and the fluoropyrimidine fluorouracil has shown activity in colorectal and breast cancers (1).



Cytarabine, AraC

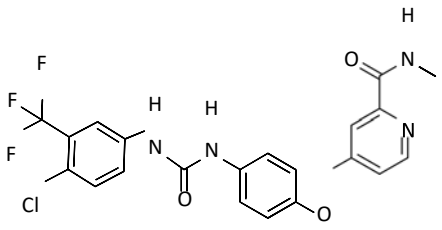


5-FU



Gemcitabine

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. The main risk factors for HCC are hepatitis B and C infection, heavy alcohol consumption and dietary aflatoxin exposure. HCC is often not diagnosed at an early stage due to tumor is slowly growing and patient is asymptomatic. Sorafenib is the first approved systemic drug for the treatment of HCC.



Sorafenib

Many drugs candidates have failed in the clinical trials in the past years (4, 5). Therefore, it is essential to identify new drug candidates for the treatment of HCC. In this study, we synthesized novel purine and pyrimidine ribonucleoside analogs and screened their anticancer activities on selected human cancer cells (liver Huh7, colon HCT116, breast MCF7); and the most potent nucleoside derivative was further tested on a panel of hepatocellular cancer cell.

Materials and Methods:

Herein, we prepared new series of substituted purine and pyrimidine ribonucleoside derivatives as putative cytotoxic agents. The 5', 6-disubstituted 9- (*β*-D-ribofuranosyl)purine derivatives were readily obtained from commercially available inosine in seven steps and the 4-substituted-1-(*β*-D-ribofuranosyl)-2(1*H*)-pyrimidinone derivatives were prepared from uracil/thymine in four steps in very cost effective synthesis approach. The newly obtained compounds were characterized for their cytotoxicity in human cancer cell lines.The cytotoxicities of the compounds were initially analyzed on liver (Huh7), colon (HCT116) and breast (MCF7) carcinoma cell lines by SRB assay for determining the IC_{50} values. The IC_{50} values after 72 hours of treatment with each molecule were also calculated in comparison with DNA topoisomerase inhibitor camptothecin (CPT), the nucleobase analog 5-fluorouracil (5-FU) and nucleoside analogs fludarabine, cladribine, pentostatine.

Results:

Among the purine nucleoside analogs N^6-Bromophenyl derivative demonstrated significant cytotoxic activity for the cell lines tested and was more cytotoxic (IC_{50} = 1.5-30.8 μM) than 5-FU, fludarabine on Huh7, HCT116 and MCF7 cell lines. In addition most of the compounds showed higher anticancer activitiy compared with 5-FU and known nucleoside drug Fludarabine, Pentostatine against Huh7 (liver) cancer cell line. We then tested the cytotoxic effect of the most potent nucleoside derivative on additional hepatocellular carcinoma (HCC) cell lines: Huh7, Hep3B, HepG2, PLC, Mahlavu, FOCUS, SNU475, SNU182, SNU387, SNU398, SNU423 and SNU449. N^6-(4-Bromophenyl) derivative displayed the best cytotoxic activity, with IC_{50} values of 15.5-40.7 μM against Huh7, Hep3B SNU475 and SNU423 cell lines and had a better cytotoxic activity than Fludarabine and Pentostatine. For the pyrimidine nucleoside analogs, we observed that our compounds had not showed significant activity. Notably, among the compounds synthesized, compound bearing a 3,4-dichlorophenyl substituent at C-4 position of the pyrimidine, had showed nearly similar IC_{50} value (17.5 μM) against MCF7 cell line than Fludarabine (15.2 μM).

Conclusions:

A series of new purine and pyrimidine nucleoside analogs were prepared and their cytotoxic activities identified. 6-(4-bromophenyl)purine derivative showed potent anticancer activity at low concentrations against Huh7, HCT116, MCF7cell lines when compared to 5-FU and Fludarabine as potent cytotoxic drugs. The second series of the pyrimidine nucleoside

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derivatives lacked antitumor activity. However, 3,4-dichloro analog, had showed nearly similar IC_{50} value against MCF7 cell line than Fludarabine (17.5 μM vs

15.2 μM, respectively). Among the 47 compounds investigated, the most potent purine and pyrimidine derivatives were further analyzed for their activity on HCC cells. The molecule bromo analog exhibited promising cytotoxic activity with IC_{50} value of 15.5 μM on Hep3B cell line.

Acknowledgements

This study was supported by a grant of TUBITAK (SBAG-112S182).

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OP-127: INVESTIGATION of CYTOTOXIC/APOPTOTIC EFFECTS of AZD3463, A NEW ALK/IGF-1R DUAL INHIBITOR, in BREAST CANCER CELL LINE

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Introduction:

Breast cancer is the most common cancer type and has the highest mortality in women worldwide (1). Therefore, more effective and less toxic strategies are needed for the management of breast cancer. Targeting multiple signalling pathways for development of new drugs seems the best strategy for this aim. AZD3463 is a potent ALK (anaplastic lymphoma kinase) inhibitor which inhibits other kinases including IGF-1R (Insulin–like growth factor-1 receptor) with malignant potential (2). ALK has been shown to have oncogenic activity via activation of signalling pathways such as PI3-kinase/AKT(Phosphoinositide-3-kinase) or by inhibition of apoptosis. The inhibition of ALK inhibits growth of breast cancer cell lines and also tumour xenografts in mouse models (3,4). IGF-1/IGF-1R system has been implicated with the development of several malignancies including breast cancer (5). Regarding these data, we aimed to investigate the cytotoxic/apoptotic activity of dual inhibitor AZD3463 in MCF-7 cell line.

Materials and Methods

Cell Culture: The study was conducted on breast cancer line (MCF-7 obtained from the ATCC. Cells were cultured in 25 ml cell culture flasks via RPMI-1640 medium (Biological Industries, USA) supplemented with 10 % fetal bovine serum, 1 % 2 mM L-glutamine 10000 U/mL penicillin and 10 mg/mL streptomycin. The cells were incubated in a cell culture incubator at 37 °C, 95 % humidity, and 5 % CO_2. Cell viability and proliferation controls were performed with trypan blue dye exclusion (Biological Industries, USA).

Cytotoxicity assay:

ALK/IGF-1R inhibitor AZD3463 was provided by MedKoo Biosciences. Time and dose based cytotoxic effect of AZD3463 on MCF-7 cell line were determined via WST-1 assay (Roche). The cells were incubated with different doses of AZD3463 (0,39μM to 50μM) for 24 h, 48 h and 72 h incubation periods. Untreated cells were used as control groups. Cell viability and proliferation controls were performed with trypan blue dye exclusion.

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Apoptosis Assay:

The apoptotic effects of AZD3463 on MCF-7 cell lines were discovered using Annexin V-FITC (BD Pharmingen) method. The cells were seeded into 6- well plates at 10^5 cells/ml concentration and incubated with IC50 doses of AZD3463 during 72 h. Results were evaluated by using BD Accuri C6 Flow Cytometer (BD Biosciences) according to untreated control groups.

Gene Expression Analysis by Real-time RT-PCR:

To determine AZD3463-dependent gene expression changes, the cells incubated with IC50 doses of AZD3463 during 72 h. Total RNA isolation for each period and cell type was carried out by using Rneasy Plus Mini Kit, and cDNA synthesis was performed by using RT2 First Strand Kit. Changes in gene levels associated with PI3K-AKT pathway will be determined by qRT-PCR instrument (LightCycler 480 Instrument II, Roche) using Profiler ™ PCR Array Human PI3K-AKT Signaling Pathway (PAHS-058Z RT²), RT2 SYBR Green qPCR Mastermix (Qiagen).

Statistical Analysis:

IC50 values of AZD3463 on the cell lines were calculated via CalcuSyn Version 2.0 software in a time- and dose-dependent manner. Fold-changes for RT-PCR and their significance were calculated by using the online software (https://www.qiagen.com/tr/shop/genes-and-pathways/data-analysis-center-overview-page). The fold change results of gene expressions were normalized by $\log_2$ transformation. Normalized results greater than 2-fold were considered as significant. Statistical significance was declared if the p value was<0.05

Results

Cytotoxicity Assay Results :

Cytotoxic effects AZD3463 on MCF-7 cells were determined using WST-1 assay compared to untreated control groups. IC_{50} doses of AZD3463 were determined as 0.765 mg/mL on 72th ($r = 0.87534$, $p < 0.05$) (Fig. 1).

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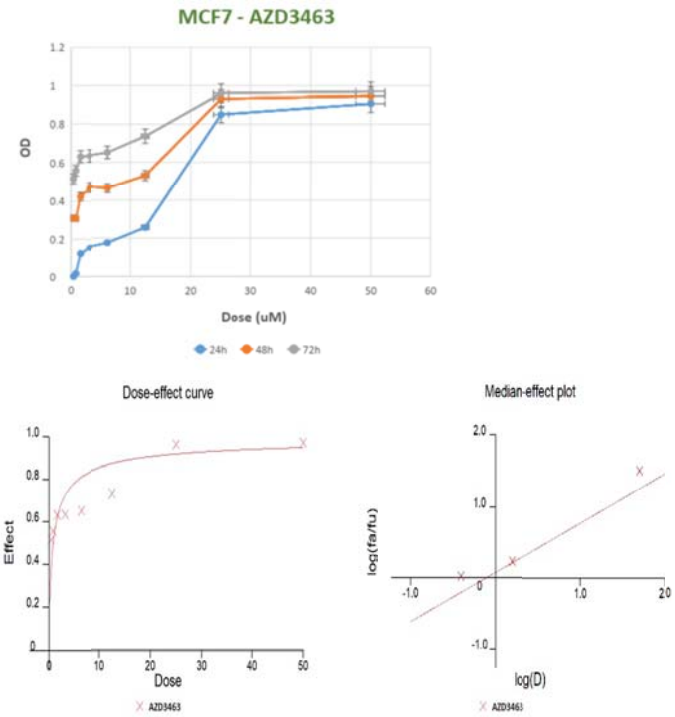
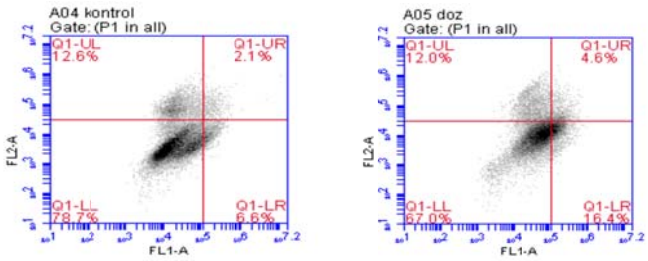


Figure 1: Cytotoxicity of AZD3463 (IC_{50}=0.765 mg/mL, r = 0.87534, p < 0.05)

Apoptosis Assay Results :

In order to evaluate the apoptotic or necrotic effects of AZD3462 on MCF-7ells treated by IC_{50} concentration of AZD3462 (0.765 mg/ mL) for 72 h, we performed the Annexin V-FITC (BD Pharmingen) staining assay. AZD3463 induced apoptosis 2.4 fold in MCF-7 cells treated with AZD3463, compared to control cells (Fig. 2).



	% of This Plot (FL1-1/FL2A)			
	Q1-UL	Q1-UR	Q1-LL	Q1-LR
Control	% 12.6	% 2.1	% 78.7	% 6.6
AZD3463	% 12.0	% 4.6	% 67.0	% 16.4

Figure 2: Apoptotic effect of AZD3463 (LL-live cells; LR-early apoptosis; UR-late apoptosis; UL-necrosis)

Gene Expression Assay Results:

qRT-PCR results showed that the expression of PTEN, an important tumor suppressor gene, increased 9,75 fold and ILK, IRS1, PIK3R2, MTCP1, HRAS, MAPK3 genes which were associated with PI3K/AKT pathway and cancer progression, decreased 2.03, 2.02, 3.7, 2.7, 2.02, 2.43 fold, respectively, in MCF-7 cells treated with AZD3463, compared to control cells (Fig. 3).

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	01	02	03	04	05	06	07	08	09	10	11	12
A	*ADAR*	*AKT1*	*AKT2*	*AKT3*	*APC*	*BAD*	*BTK*	*CASP9*	*CCND1*	*CD14*	*CDC42*	*CDKN1B*
	-2,858	1,3333	-1,6301	3,0001	-1,0755	-1,0461	4,2428	-2,9794	2,858	-1,0755	-1,3241	3,9313
B	*CHUK*	*CSNK2A1*	*CTNNB1*	*EIF2AK2*	*EIF4B*	*EIF4E*	*EIF4EBP1*	*EIF4G1*	*ELK1*	*FASLG*	*FKBP1A*	*FOS*
	-2,5937	1,3613	139,585	2,2423	179,147	1,4191	-2,2894	-2,9794	-1,0755	-1,0755	-2,3376	17,8147
C	*FOXO1*	*FOXO3*	*GJA1*	*GRB10*	*GRB2*	*GSK3B*	*HRAS*	*HSPB1*	*IGF1*	*IGF1R*	*ILK*	*IRAK1*
	1,1134	10,8153	1,0035	-1,21	1239,03	1,3996	-2,0209	1,4093	-1,0755	1,6876	-2,035	2,9383
D	*IRS1*	*ITGB1*	*JUN*	*MAP2K1*	*MAPK1*	*MAPK14*	*MAPK3*	*MAPK8*	*MTCP1*	*MTOR*	*MYD88*	*NFKB1*
	-2,0209	5025,32	1,3899	1,834	5537,43	3,9313	-2,4368	1,1368	-2,7606	4,7404	-1,0534	1,0981
E	*NFKBIA*	*PABPC1*	*PAK1*	*PDGFRA*	*PDK1*	*PDK2*	*PDPK1*	*PIK3CA*	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PRKCA*
	-2,42	12,5969	-1,1688	-1,0755	-1,0755	6,6576	-1,8987	-1,0246	43,5621	1,6415	-3,7192	-1,3149
F	*PRKCB*	*PRKCZ*	*PTEN*	*PTK2*	*PTPN11*	*RAC1*	*RAF1*	*RASA1*	*RBL2*	*RHEB*	*RHOA*	*RPS6KA1*
	-1,2879	56,2975	9,7473	1,0755	-2,0209	-1,3333	-2,858	-1,1527	11,6722	176,681	-1,8596	-1,7112
G	*RPS6KB1*	*SHC1*	*SOS1*	*SRF*	*TCL1A*	*TIRAP*	*TLR4*	*TOLLIP*	*TSC1*	*TSC2*	*WASL*	*YWHAH*
	-1,3241	1,0389	3,3753	-1,6994	-1,0755	-3,3987	2,0209	-2,3376	-1,0175	1,1688	-2,3867	1,8088
H	*ACTB*	*B2M*	*GAPDH*	*HPRT1*	*RPLP0*	*HGDC*	*RTC*	*RTC*	*RTC*	*PPC*	*PPC*	*PPC*
	-6,0002	2,5937	-3,2378	-2,6666	6,1688	-1,0755	-1,1851	-1,3333	-1,0035	9,4807	2,5228	-15233,9

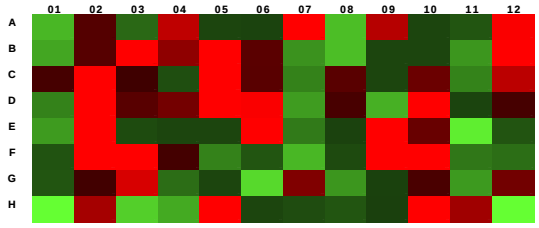

Figure 3: Heat map of PI3K-AKT Signaling Pathway-related genes expression changes with the AZD3463 in MCF7 cells compared with untreated control cells.

Conclusions:

These novel findings show that AZD3463 has an important role in preventing breast cancer progression and could be used as a pioneering target agent in breast cancer treatment.

Acknowledgements:

This study was partly supported by Ege University Coordinatorship of Scientific Research Projects (17-ECZ-018).

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OP-135: VOLTAMMETRIC DETERMINATION OF AN ANTIGUNGAL DRUG FROM PHARMACEUTICAL DOSAGE FORMS USING MODIFIED GLASSY CARBON ELECTRODES

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Introduction:

Fungal infections which affects skin, hair, and nails are treated using antifungal medicines. Antifungal medicines can be find as topical, oral, intravenous, and intravaginal (1). Oral antifungals used commonly are fluconazole, intraconazole, ketoconazole, and terbinafine. Terbinafine is a synthetic allylamine derivative used as an antifungal drug. It works by stopping the growth of fungi and used for the treatment of fungal infections of the groin area, toe and finger nails, the skin and the feet. It has a IUPAC name of (E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine (Figure 1).

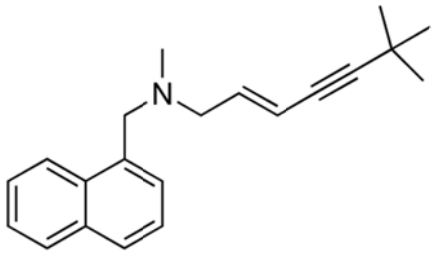

Figure 1. The structure of Terbinafine

The aim of this study to develop a more sensitive determination method than those in the literature (2-5) for terbinafine (TER) based on its oxidation using voltammetry at polymer modified electrodes prepared according to the literature and achieve its analysis from pharmaceutical dosage forms.

Materials and Methods:

All experiments were achieved using a three-electrode electrochemical cell containing a glassy carbon (GC) working electrode, a platinum wire as counter electrode, and an Ag/AgCl electrode as reference. Polymer film modification was achieved by potential cycling using the previous procedure. All measurements were performed using an Autolab Pgstat128n potentiostat/galvanostat with Nova 1.10 software (Metrohm). Stock solution of TER ($1x10^{-3}$

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M) was prepared in deionized water. Phosphate, Britton Robinson, and acetate buffer solutions at different pH values were used.

Results:

Electrochemical properties of TER were investigated on the anodic direction using cyclic (CV), differential pulse (DPV), and square wave (SWV) voltammetry methods, after the polymer film modification of GC electrode. It was seen that the peak current of TER increased by using the polymer modified electrode comparing to the bare GCE for both CV, DPV, and SWV techniques (Figure 2).

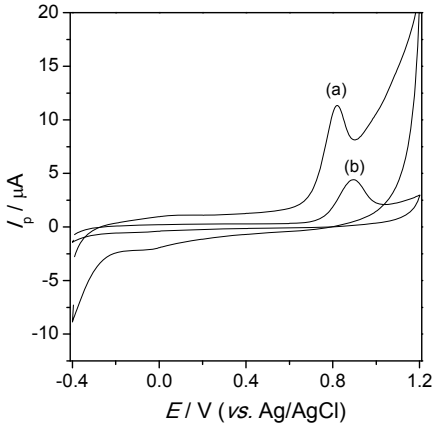

Figure 2. Cyclic voltammograms of 6×10^{-5} M TER solution in PB at pH 6.0 obtained with (a) poly(BCP) modified GCE and (b) bare GCE

The effect of pH on the redox process of TER was studied by CV. A well-defined anodic peak with the highest peak current was obtained in acidic acetate buffer solution. For this reason, this buffer was selected for the further studies. Scan rate study with CV showed a diffusion controlled process under some adsorption effect. The linearity range of the calibration graph (Figure 3) was determined as 40 - 20000 nM for DPV with the quantification limit of 24 nM. Repeatability (in the same day) and reproducibility (different days over a week) values were also calculated as relative standard deviation% from five repeated experiments for both the peak current and the peak potential of TER and found as below 2.0%.

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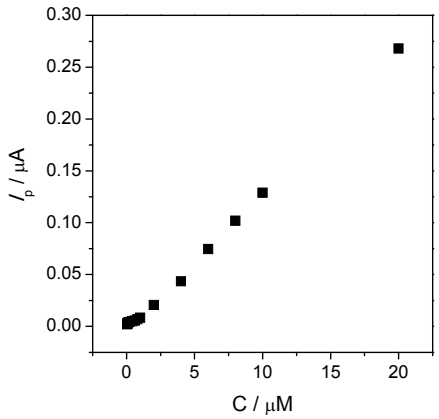

Figure 3. Plot of peak current (I_p) *vs.* concentration of TER (C), calculated from DP voltammograms

Quantitative analysis of TER from its pharmaceutical dosage form (250 mg TER per tablet) was performed using the standard addition method without any separation and filtration without the effect of the excipients. According to the results, the amount of TER found with the fully validated DPV method was ~250 mg and the recovery% was higher than 99%. RSD% of the recovery results obtained from five experiments was below 1.0%.

Conclusions:

Glassy carbon electrode was modified with polymer film using the procedure in the literature and used for the determination of TER. The modified electrode presented good sensitivity and wide linear range for TER. The proposed DPV method was successfully applied to determine TER from pharmaceutical dosage forms without any interference. Consequently, effective and economical modified electrode with high sensitivity and selectivity was obtained for the electrochemical analysis of TER.

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OP-145: ENHANCEMENT OF YAMANAKA FACTORS EFFECIENCY BY USING AXOLOTL OOCYTES

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INTRODUCTION:

To reprogram somatic cells, a complex mechanism is required in which chromatin environment alters by epigenetic remodelling and reactivating pluripotency genes (1, 2). Transfecting Yamanaka factors, Klf4, c-Myc, Oct4 and Sox2, into the differentiated cells can induce pluripotency to generate induced pluripotent stem cells (iPSCs) (2). Although iPSCs can be utilized in producing new adult cells and personalized treatment, it is very difficult to accomplish to receive efficiency rate more than 1% (3). Epigenetic inheretance of iPSCs is a limiting factor to establish pluripotency (4). Previous studies showed that reprogramming of differentiated cells by oocytes results rapidly and efficiently. The factors in oocytes expedite forming pluripotent environment in the somatic chromatin (5). Indeed, reprogramming of differentiated cells by using Axolotl oocyte extract (AOE) can reactivate the Nanog gene in mammalian cells, 'the gateway to the pluripotent ground state' (6, 7). In this study, we aimed to accelerate the efficiency of Yamanaka factors by using the capacity of Axolotl oocytes to remodel epigenetic pattern of somatic chromatin by providing AxNanog, an ortholog of mammalian Nanog.

MATERIALS AND METHODS:

An inducible NIH3T3 cell line was generated to express Oct4 and Sox2 factors upon doxycycline treatment. These cells were treated with AOE for 6 hours. Chromatin immunoprecipitation assay was carried out using Oct4 antibody. The Oct4-bound immunoprecipitated DNA was analysed by qPCR for the promoter and enhancer regions of the mouse Nanog gene.

RESULTS:

We first showed that AOE is capable of remodelling the epigenetic environment in the mammalian chromatin. For instance, the levels of the activating histone marks on the Nanog regulatory regions increased and that of the repressive histone marks were lessened. Moreover, somatic cell DNA is demethylated upon AOE incubation. These events start with binding of AxNanog on these regions. Then, we demonstrated that endogenous Oct4/Sox2 expression procure around 2 fold increase on the Oct4 binding on the mouse Nanog promoter and enhancer while AOE can enhance this binding to about 14 fold. This suggests that the factors AOE contains prepare the epigenetic profile of the somatic chromatin and make the regulatory regions of pluripotency genes more accessible to Oct4.

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CONCLUSIONS:

This study clarifies that the efficiency of iPSCs can be enhanced by reversal of epigenetic memory in somatic chromatin by using Axolotl oocytes. Axolotl oocytes provides AxNanog and other factors which are responsible for epigenetic alteration. Further experiments are still required to use this synergy between iPSCs and Axolotl oocyte in personalized therapeutics.

ACKNOWLEDGMENTS

This study was supported by a grant of Republic of Turkey Ministery of National Education.

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OP-148: DETERMINATION OF ACIDITY CONSTANTS AND THERMODYNAMIC PROPERTIES OF STATINS

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Hitit University, Faculty of Science and Art, Department of Chemistry, Corum, Turkey

Introduction:

Statins are primarily the most therapeutically effective drugs which reduce LDL cholesterol and triglyceride levels in the bloodstream of patients at risk of cardiovascular disease. Statins can lower cholesterol by 20 to 60%. Their water solubility depends to their chemical structures which affect their metabolism, distribution, absorption and excretion. Most drugs have ionization sites that can be protonated or deprotonated at different pH values. Hence, the pharmacodynamics and pharmacokinetic properties of drugs can be very important (1, 2). The acidity constant value may be helpful in predicting the behavior of a drug under in vivo conditions. Various analytical methods have been used for the determination of acidity constants such as spectrometry, potentiometry and chromatography. Up to date, there is no publish study about determination of acidity constants for rosuvastatine by electrochemical methods. Rosuvastatine calcium is a member of a drug group called statins. It can lower hypercholesterolemia. It is a synthetic lipid-lowering agent and inhibits 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG-CoA) so that prevent cardiovascular diseases.

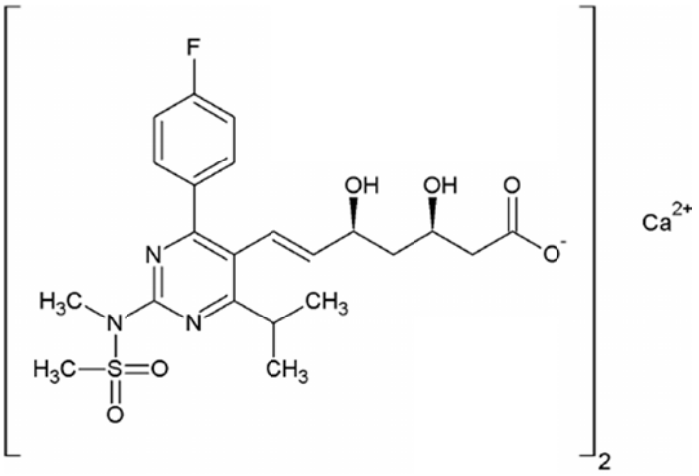

Figure 1. Chemical structure of rosuvastatine calcium

These methods have excellent features such as rapid, precise, cheap and do not require pretreatments. In this study, voltammetry and electrochemical impedance spectroscopy methods have been applied to investigation of acidity constants and thermodynamic parameters of statins in aqueous mediums.

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Materials and Methods:

Electrochemical experiments were performed by cyclic voltammetry and electrochemical impedance spectroscopy. Current-voltage curves were recorded by using Palmsens 5.2. Pyrolytic graphite electrode (BASi; ø: 3 mm, diameter) was used as working electrode, platinum as counter and Ag/AgCl (BASi; 3 M KCl) used as reference electrode. pH effect study was applied between pH 2.0-11.0 Britton-Robinson buffers (0.04 M). Rosuvastatine stock solution (1×10^{-3} M) was prepared with methanol. In measurement solution, it was fixed to 10% ratio. 0.01 M sodium dodecylsulphate solution (1 mL) was added to measurement solution.

Results:

Dissociation constant of the basic drug rosuvastatine was found by using cyclic voltammetry and electrochemical impedance spectroscopy. pH effect studied between pH 0.95-11.0 in pH 0.95 H_2SO_4 (0.1 M) and pH 2.0-9.0 Britton-Robinson buffers (0.04 M). Sodium dodecyl sulphate (SDS) effect was investigated on rosuvastatine response. The volume of SDS was fixed as 1 mL in measurement solutions (Figure 1).

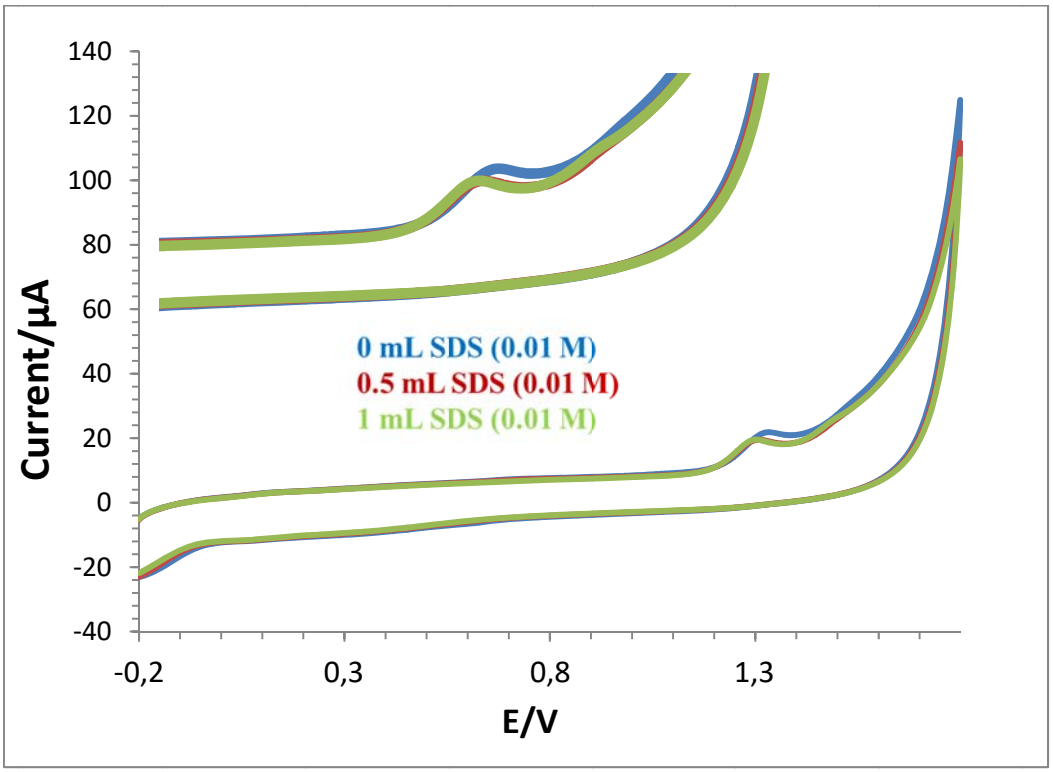

Figure 1. The investigation of SDS amount on rosuvastatine response by cyclic voltammetry (scan rate:0.1 V/s; potential range: -0.2 + 1.8 V)

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pKa value of rosuvastatine calcium was obtained as 3.8 and 3.9 by CV and EIS methods, respectively (Figure 2). The obtained pKa value is in close agreement with reported in the literature. ΔG, ΔH and ΔS parameters were calculated (Table 1).

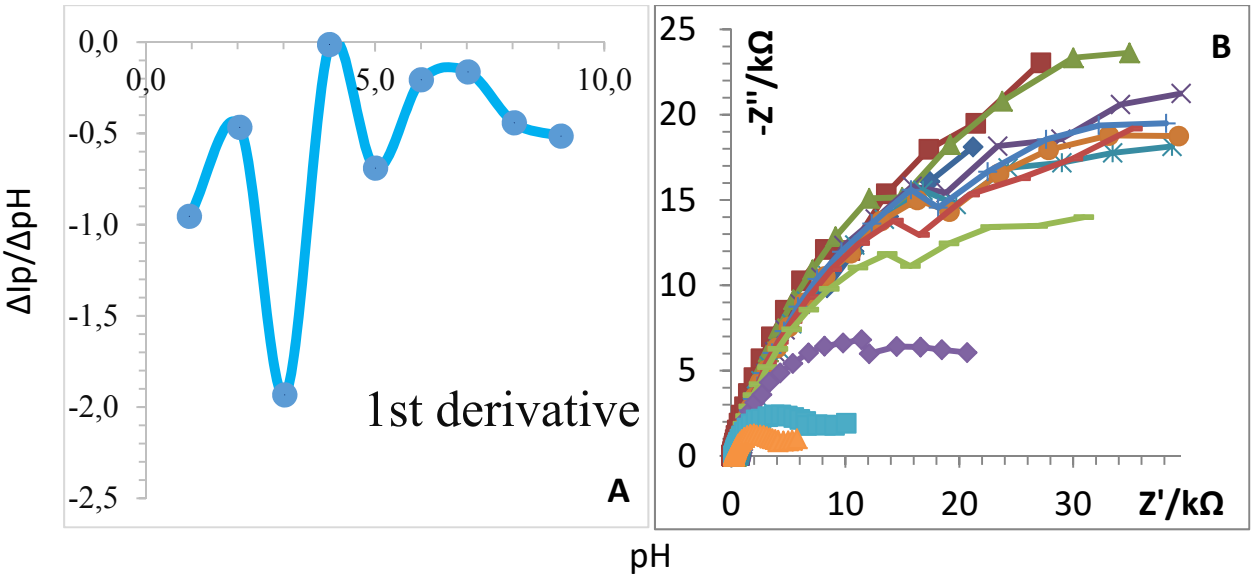

Figure 2. A) First derivative results of pH effect study on rosuvastatine **B)** Electrochemical impedance spectrums between pH 1.0 and 9.0 Britton Robinson buffer solutions.

Table 1. Thermodynamic parameters of rosuvastatine in pH 1.0 H_2SO_4 solution.

T (K)	Ea (kJ/mol)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol)
293		25.63	5.22	-69.64
308		25.76	5.10	-67.10
313		25.99	5.06	-66.87
318	7.66	26.33	5.02	-67.01
323		26.59	4.97	-66.91
328		26.88	4.93	-66.92

Conclusions:

This study shows that electrochemical methods are reliable and simple for pKa detection of drugs.

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