GAZİ ÜNİVERSİTESİ 1926

GAZİ FACULTY OF PHARMACY

GAZİ 2nd INTERNATIONAL SYMPOSIUM GPSS SERIES

GAZİ PHARMA SYMPOSIUM SERIES

PHARMA Turkey 13 11 2017 OCTOBER - ANKARA

2ND INTERNATIONAL GAZİ PHARMA SYMPOSIUM

ABSTRACT BOOK

GPSS-2017 IS SUPPORTED BY TÜBİTAK
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Special thanks to Turkish Association of Pharmacists for their kind support.

Dear Colleagues,

On behalf of the organizing committee, it is my great honor and privilege to invite you to the 2nd International Gazi Pharma Symposium Series (GPSS-2017), which will be organized by Faculty of Pharmacy, Gazi University in Ankara (Turkey) in October 11-13, 2017. Being a biennial symposium, the first series of Gazi Pharma Symposium was succesfully held in 2015 in Antalya (Turkey).

Discovery and development of drugs against human diseases involve a wide range of scientific approaches of pharmaceutical sciences. Therefore, the intention of GPSS-2017 is to emphasize the important roles of all fields related to the drug discovery and development process and also provide a wide platform for international collaboration and communication in the interdisciplinary fields of pharmaceutical sciences by bringing the experts together from all over the world. The scientific program includes plenary lectures as well as oral and poster presentations delivered by all partners contributing to all fields of pharmaceutical sciences.

We sincerely hope that this symposium will meet your expectations and stimulate new collaborations. Looking forward to welcoming you in Ankara, the capital of Turkey.

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Symposium Chairperson

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GPSS 2017 - Symposium Timetable

Wednesday - October 11, 2017

Opening Session | *Mimar Kemaleddin Hall (Gazi University Rectorate Building)*

10:30	**Welcome Speech**
11:00	**KL - New Analytical Technologies for Natural Product Research-Applications and Potentials in Phytopharmacy, Phytocosmetics and Phyto-Nutrition** Prof. Günther K. BONN *University of Innsbruck, Austria*
12:00	**SL - Effective Leadership** Assoc. Prof. Dr. Neşe Buket AKSU *Altınbaş University, Turkey*
12:20	**Lunch and Poster Session**
Session 1 *Conference Hall*	**Advanced Drug Delivery** **Session Chairs** Prof. Dr. Nevin ÇELEBİ, *Gazi University, Turkey* Prof. Gert STORM, *Utrecht Institute for Pharmaceutical Sciences, The Netherlands*
14:00	**IL1 - Targeted Nanomedicine: Past, Present and Future** Prof. Gert STORM *Utrecht Institute for Pharmaceutical Sciences, The Netherlands*
14:30	**IL2 - Quality by Design in Nanomedicine** Assoc. Prof. Camilla FOGED *University of Copenhagen, Denmark*
15:00	**IL3 - Overview of Development and Manufacture of Biologic Drug Substance** Thomas FELIX, MD *AMGEN, USA*
15:30	**Coffee Break and Poster Session**

Session 2 *Conference Hall*	**Obesity and Type-2 Diabetes** **Session Chairs** Prof. Dr. Fatma AKAR, *Gazi University, Turkey* Prof. Dr. İlhan SATMAN, *Istanbul University, Turkey*	**Session 3** *Hedef Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Sevgi TAKKA, *Gazi University, Turkey* Prof. Dr. Sema ÇALIŞ, *Hacettepe University, Turkey*
15:45	**IL4 - The Trends for Obesity and Type-II Diabetes in Turkey: Where Are We Now? Where Are We Going?** Prof. Dr. İlhan SATMAN *Istanbul University, Turkey*	**15:45**	**O1 - Hot Melt Extrusion and How to Use the Excipients for Successful Formulation Development** Cihan SANCAKTAROĞLU, MBA *BASF, Turkey*
16:15	**IL5 - Fructose-induced Diabetes and Obesity** Prof. Dr. Fatma AKAR *Gazi University, Turkey*	**16:15**	**O2 - Bioavailability Enhancement and In Vivo Evaluation of Olmesartan Medoxomil Self-Microemulsifying Drug Delivery System** Yelda KOMESLİ, PhD *Ege University, ARGEFAR, Turkey*
16:45	**IL6 - Diabesity as a Redox Disease** Prof. Dr. Çimen KARASU *Gazi University, Turkey*	**16:30**	**O3 - Optimization of Flurbiprofen Nanosuspensions with Polymeric Stabilizers for Dermal Application** Ayşe Nur OKTAY *Gazi University, Turkey*
17:15	**IL7 - Carbohydrate Intake and Nonalcoholic Fatty Liver Disease: Fructose as a Weapon of Mass Destruction** Prof. Dr. Metin BAŞARANOĞLU - not attended *Bezmialem University, Turkey*	**16:45**	**O4 - In Vitro Characterisation of Peptide-563 Conjugated Liposomes** Assist. Prof. Dr. Ongun Mehmet SAKA *Ankara University, Turkey*

Thursday - October 12, 2017

Session 4 *Conference Hall*	**Recent Advances in Toxicology** **Session Chairs** Prof. Dr. Sema BURGAZ, *Gazi University, Turkey* Prof. Javier ESTEBAN MOZO, *Miguel Hernández University of Elche, Spain*
9:00	**IL8 - Low Doses of Environmental Contaminants-Genotoxicity, Effect on Cell Processes and Possible Consequences** Assoc. Prof. Ksenija DURGO *University of Zagreb, Croatia*
9:30	**IL9 - Role of the Alteration of the Retinoid System Under Hazard Identification** Prof. Javier ESTEBAN MOZO *Miguel Hernández University of Elche, Spain*
10:00	**IL10 - Potential Role of Pharmacogenomics in Reducing Adverse Drug Reactions** Prof. Dr. Semra ŞARDAŞ *Marmara University, Turkey*
10:30	**Coffee Break and Poster Session**
Session 5 *Conference Hall*	**Recent Highlights in Medicinal Chemistry** **Session Chairs** Prof. Dr. Erden BANOĞLU, *Gazi University, Turkey* Prof. Gabriele COSTANTINO, *University of Parma, Italy*
11:00	**IL11 - Non Essential Targets, a Novel Route to the Next Generation Antibacterials** Prof. Gabriele COSTANTINO *University of Parma, Italy*
11:30	**IL12 - Enabling Synthetic Technologies for Chemical Libraries, Hit Optimization and Scale-up: In-flow Synthesis and Biological Activity of Quinoline-based Compounds** Assoc. Prof. Antimo GIOIELLO *University of Perugia, Italy*
12:00	**IL13 - New Advances in Isothermal Titration Calorimetry Applied to Drug Development: Going Far Beyond Traditional Analysis with the Affinimeter Software** Eva MUNOZ, PhD *AFFINImeter, Spain*
12:30	**Lunch and Poster Session**
Session 6 *Conference Hall*	**Pharmacognosy and Phytotherapy** **Session Chairs** Prof. Dr. Bilge ŞENER, *Gazi University, Turkey* Assoc. Prof. Krystyna SKALICKA WOZNIAK, *Medical University of Lublin, Poland*
13:30	**IL14 - Important Milestones in My 45 Years of Research into Natural Products** Prof. Dr. İhsan ÇALIŞ *Near East University, Turkish Republic of Northern Cyprus*
14:00	**IL15 - CLIPS (Chemical Linkage of Peptides onto Scaffolds) Technology Applied to Opioid Peptides Research** Assoc. Prof. Adriano MOLLICA *Università degli Studi G. d'Annunzio Chieti e Pescara, Italy*

14:30	**IL16 - Power of Liquids - The Case of Counter-Current Chromatography** Assoc. Prof. Krystyna SKALICKA WOZNIAK *Medical University of Lublin, Poland*						
15:00	**Coffee Break and Poster Session**						
Session 7 *Conference Hall*	**Cancer and Signaling** **Session Chairs** Prof. Dr. Sevgi AKAYDIN, *Gazi University, Turkey* Prof. Dr. Semra DEMOKAN, *Istanbul University, Turkey*						
15:15	**IL17 - Epithelial-Mesenchymal Transition in Cancer** Prof. Dr. Gülberk UÇAR *Hacettepe University, Turkey*						
15:45	**IL18 - Development of Novel Targeted Therapies for Highly Aggressive Cancers** Prof. Dr. Bülent ÖZPOLAT *MD Anderson Cancer Center, University of Texas-Houston, USA*						
16:15	**IL19 - Methylation Biomarker Studies in Saliva of Head and Neck Cancer Patients** Prof. Dr. Semra DEMOKAN *Istanbul University, Turkey*						
16:45	**Coffee Break and Poster Session**						
Session 8 *Conference Hall*	**Oral Presentations** **Session Chair** Prof. Dr. Sultan BAYTAŞ *Gazi University, Turkey*	**Session 9** *Hedef Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Figen TIRNAKSIZ *Gazi University, Turkey* Prof. Dr. Asuman BOZKIR *Ankara University, Turkey*	**Session 10** *Novartis Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Uğur TAMER *Gazi University, Turkey* Assoc. Prof. Dr. Aysel BERKKAN *Gazi University, Turkey*	**Session 11** *Ecz. Hikmet Türk Hall*	**Oral Presentations** **Session Chair** Assoc. Prof. Dr. Aysun HACIŞEVKİ *Gazi University, Turkey*
17:00	**O5 - Merging Different Drug Discovery Approaches to Offer Novel Therapeutic Options Against Mycobacterial Infections** Assist. Prof. Marco PIERONI *University of Parma, Italy*	**17:00**	**O9 - Passive Targeting of Gemcitabine HCl Using Poly(E-Caprolactone) Nanoparticles for Cancer Treatment** Kıvılcım ÖZTÜRK ATAR, PhD *Hacettepe University, Turkey*	**17:00**	**O13 - The Investigation of DNA Binding Profiles of Gold Nanoparticle Bound Lignan Species Called Secoisolariccresinol Using Spectrophotometry and Spectrofluorimetry** İsmail Murat PALABIYIK *Ankara University, Turkey*	**17:00**	**O17 - Effect of Pyrrolidine Derivatives Bearing Indole Ring as Substituted Moiety on COX2 on MCF-7 Cells** Assoc. Prof. Dr. Necmiye CANACANKATAN *Mersin University, Turkey*
17:15	**O6 - Coupling Spectroscopic and Molecular Dynamics Experiments to Elucidate Protein-Ligand Interaction** Agostino BRUNO, PhD *The FIRC Institute for Molecular Oncology Foundation, Italy*	**17:15**	**O10 - Nanocarrier-mediated Co-delivery of Pemetrexed and miR-21 Antisense Oligonucleotide for Glioblastoma Treatment** Berrin KÜÇÜKTÜRKMEN, PhD *Ankara University, Turkey*	**17:15**	**O14 - Dispersive Liquid-Liquid Microextraction of Some Capsaicinoids from Different Cultivars of *Capsicum annuum* Prior to Their Determination by HPLC** Jude CALEB *Near East University, TRNC*	**17:15**	**O18 - The Relationship Between Osteocalcin/Adiponectin Levels and Inflammatory Microenvironment in Colorectal Cancer Patients** Filiz BAKAR *Ankara University, Turkey*
17:30	**O7 - Synthesis of Some Pyridazine Derivatives, Investigation of Their In Vitro Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities** Burcu KILIÇ, PhD *Gazi University, Turkey*	**17:30**	**O11 - Design and In Vitro/In Vivo Evaluation of Smedds for Lymphatic Targeting Anticancer Peptide, LYP-1** Selin Seda TİMUR *Hacettepe University, Turkey*	**17:30**	**O15 - Simultaneous Detection of E. coli and Salmonella Based on Fluorescence Techinique in a Passive Type Microfluidic Chip** Üzeyir DOĞAN *Gazi University, Turkey*	**17:30**	**O19 - HSP70 Inhibitor PES, Down-regulate Several Cancer-related Genes on Breast Cancer Cells** Mustafa ERGÜL, PhD *Cumhuriyet University, Turkey*
17:45	**O8 - Size Exlusion Chromatograpy Applications for Protein Based Drugs** Shawn WELCH *Atomika, USA*	**17:45**	**O12 - Evaluation of IMT Labeling with Zr-89 (oxine) as New PET Radiopharmaceuticals** Assoc. Prof. Dr. Evren GÜNDOĞDU *Ege University, Turkey*	**17:45**	**O16 - Oligosacharrides Released from Milk Glycoproteins by a Novel N-Acetylglucosaminidase Act as Selective Prebiotic Source for Bifidobacteria Species** Sercan KARAV *Çanakkale Onsekiz Mart University, Turkey*	**17:45**	**O20 - Effects of Varying Degrees of Bladder Outlet Obstruction on Urinary Bladder Function of Rats: A Novel Link to Inflammation, Hypoxia and Oxidative Stress** Ecem KAYA SEZGİNER, PhD *Ankara University, Turkey*
20:00	**Gala Dinner**						

Friday October 13, 2017

Session 12 *Conference Hall*	**Recent Developments in Analytical Chemistry** **Session Chairs** Prof. Dr. Hasan BASAN, *Gazi University, Turkey* Prof. Boris MIZAIKOFF, *University of Ulm, Germany*
8:30	**IL20 - New Strategies for Synthesizing and Rationally Understanding Molecularly Imprinted Materials** Prof. Boris MIZAIKOFF *University of Ulm, Germany*
9:00	**IL21 - Biosensors for the Detection of Pesticides by Enhanced Raman Spectroscopy and Interactions with Gold Nanoparticles** Prof. Philippe DANIEL *University of Maine, France*
9:30	**IL22 - Next Generation Biosensors for Bioanalytical Measurements** Yıldız ULUDAĞ, PhD *BILGEM-TUBITAK, Turkey*
10:00	**IL23 - Particle Characterisation in the Pharma Industry** Stuart WAKEFIELD *Regional Manager Middle East & India at Malvern Instruments Ltd., UK*
10:30	**Coffee Break and Poster Session**
Session 13 *Conference Hall*	**Food Safety** **Session Chairs** Prof. Dr. Gülderen YENTÜR, *Gazi University, Turkey* Prof. Gustavo BARBOSA-CANOVAS, *Washington State University, USA*
11:00	**IL24 - Validation of Novel Technologies to Process Foods** Prof. Gustavo BARBOSA-CANOVAS *Washington State University, USA*
11:30	**IL25 - Fourier Transform Infrared Spectroscopy as a Potential Tool for Detection of Fradulent Raw Meat Mixtures** Prof. Dr. Kezban CANDOĞAN *Ankara University, Turkey*
12:00	**IL26 - Endocrine Disrupting Chemicals in Animal Origin Food** Assoc. Prof. Dr. Begüm YURDAKÖK DİKMEN *Ankara University, Turkey*
12:30	**Lunch and Poster Session**
Session 14 *Conference Hall*	**Current Issues of Antimicrobials** **Session Chairs** Prof. Dr. Berrin ÖZÇELİK, *Gazi University, Turkey* Prof. Dr. Selim BADUR, *GSK Vaccine, Belgium*

13:30	**IL27 - The Burden of Influenza: From Surveillance to Immunopathology** Prof. Dr. Selim BADUR *GSK Vaccine, Belgium*					
14:00	**IL28 - Recent Developments in Vaccinology - Taking into Account Vaccine Quality and Confidence** François MEURICE, PhD - not attended *GSK, Belgium*					
14:30	**Coffee Break and Poster Session**					
Session 15 *Hedef Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Ülkü ÜNDEĞER BUCURGAT *Hacettepe University, Turkey* Assoc. Prof. Dr. İlker ATEŞ *Ankara University, Turkey*	**Session 16** *Novartis Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Nurgün KÜÇÜKBOYACI *Gazi University, Turkey* Prof. Dr. Şükran KÜLTÜR *Istanbul University, Turkey*	**Session 17** *Ecz. Hikmet Türk Hall*	**Oral Presentations** **Session Chairs** Prof. Dr. Mustafa ARK *Gazi University, Turkey* Prof. Dr. Deniz Songül DOĞRUER *Gazi University, Turkey*	**Panel: Medical Devices** *Conference Hall* **(This session will be held in Turkish)** **Moderator** Prof. Dr. Füsun ACARTÜRK *Gazi University, Turkey*
14:50	**O21 - The Effects of Some Cytokine Gene Polymorphisms on Type 2 Diabetes and Its Complications** Assoc. Prof. Dr. İlker ATEŞ *Ankara University, Turkey*	**14:50**	**O29 - A Screening Study on the Wound Healing Activity of *Ribes* Species Growing inTurkey** Assist. Prof. Dr. Gülsen KENDİR *İstinye University, Turkey*	**14:50**	**O37 - Synthesis of New Diflunisal Derivatives as Potent Anti-HCV and Anticancer Agent** Assist. Prof. Dr. Sevil ŞENKARDEŞ *Marmara University, Turkey*	**Panel: Tıbbi Cihazlar** **(Oturum dili Türkçe olacaktır)** **P1 - Eczanelerde Satılan Tıbbi Cihazlar ve Yerli Üretimin Etkisi** *Dr. Recep USLU, TİTCK* **P2 - Tıbbi Cihaz Üretimi, Türkiye'de ve Dünyadaki Mevcut Durumun Değerlendirilmesi** *İ. Cem TÜRKER, SEİS* **P3 - Tıbbi Cihazların Eczaneler Açısından Değerlendirilmesi** *Ecz. Arman ÜNEY, TEB* **P4 - Tıbbi Cihaz Üretiminde ve Sertifikasyonda Karşılaşılan Sorunlar** *Osman Fikret KÜÇÜKDEVECİ, SEİS* **P5 - Türkiye'de Tıbbi Cihazlar için CE Sertifikasyonu** *Dr. Asım HOCAOĞLU, TİTCK*
15:05	**O22 - Effects of D-Limonene Against Complications of Diabetes in Rats** Merve BACANLI, PhD *Hacettepe University, Turkey*	**15:05**	**O30 - Investigation of Burn and Wound Healing Effects of Herbal Extracts** Emel Öykü ÇETİN UYANIKGİL *Ege University, Turkey*	**15:05**	**O38 - Studies on the Synthesis and Cytotoxic Activities of Platinum(II) Complexes Having 2-Substituted Benzimidazole Ligands** Mahmut GÖZELLE, PhD *Gazi University, Turkey*	
15:20	**O23 - Histopathological Examination of the Effects of Silymarin on Vancomycin-Induced Nephrotoxicity in Rats** Kezban KİBAR, PhD *Mersin University, Turkey*	**15:20**	**O31 - Ethanol Extract of Aerial Part of Salvia huberi Hedge Exhibited Antioxidant and Wound Healing Activities in Diabetic Rats** Ebru GÖKALP ÖZKORKMAZ *Ankara Yildirim Beyazit University, Turkey*	**15:20**	**O39 - Developing Multi-Target Inhibitors of Arachidonic Acid Pathway Based on the FLAP Inhibitor BRP-7** Zehra Tuğçe GÜR, PhD *Gazi University, Turkey*	
15:35	**O24 - The Serotonin 2A Receptor Gene -1438A/G Polymorphism and Sertraline Induced Nausea in Major Depressed Turkish Patients** Merve DEMİRBÜGEN *Ankara University, Turkey*	**15:35**	**O32 - Unani Treatment of *Tinea capitis*: A Case Study** Leena HAMEED - not attended *Hamdard University, Pakistan*	**15:35**	**O40 - Synthesis and In Vitro Anticancer Activities of Novel Pyrrolo[2,3-d]pyrimidine Derivatives Containing Urea Moiety** Zühal KILIÇ-KURT, PhD *Ankara University, Turkey*	
15:50	**Coffee Break and Poster Session**					
16:00	**O25 - Design and Discovery of Novel Melatonin Analogues as CYP1B1 Inhibitors** Elif İNCE *Ege University, Turkey*	**16:00**	**O33 - *Hypericum salsugineum* Inhibits Proliferation, Migration and Colony Formation Ability of Triple-Negative and Estrogen Receptor-Positive Breast Cancer Cells** Arzu ATALAY *Ankara University, Turkey*	**16:00**	**O41 - Computer Aided Drug Design Studies on Benzazoles Active Against Topo II Enzyme as an Anticancer Target** Andry Nur HİDAYAT *Ankara University, Turkey*	
16:15	**O26 - NOTCH1 and NOTCH3 Signaling Differentially Regulate Polarization of T Helper Subset Involved in the Development of Multiple Sclerosis** Assist. Prof. Dr. Furkan AYAZ *Mersin University, Turkey*	**16:15**	**O34 - Effects of *Urtica dioica* L. On Endometriosis Rat Model and Compounds Isolated from the Active Extract** Mert İLHAN *Gazi University, Turkey*	**16:15**	**O42 - Activation Of Liver X Receptors Prevents Cardiac Functional and Structural Changes in Doca-Salt Hypertensive Rats** Nur Banu BAL, PhD *Gazi University, Turkey*	
16:30	**O27 - Why Do Turkish People Use Electronic Cigarette (E-Cigarette)?** Awat Abdullah ALİ *Gazi University, Turkey*	**16:30**	**O35 - Chemotaxonomical Studies of Plantaginaceae Family** Vahap Murat KUTLUAY *Hacettepe University, Turkey*	**16:30**	**O43 - Applying Beers Criteria for Elderly Patients to Assess Rational Drug Use in Northern Cyprus** Sarah KHAMIS *Near East University, TRNC*	
16:45	**O28 - Determination of the Resistance Genotypes of Glycopeptide Resistant Enterococci Collected in Gazi University Hospital Between 2008-2017** İsmet KUTLUK *Gazi University, Turkey*	**16:45**	**O36 - High Performance Thin-Layer Chromatographic Method Applications for Qualitative and Quantitative Analysis of Flowering Aerial Parts of *Hypericum perforatum*** Esra SAÇICI *Yeditepe University, Istanbul*			
17:30	**Closing Ceremony** Prof. Dr. İlkay ERDOĞAN ORHAN *Symposium Chairperson*					

KL1

NEW ANALYTICAL TECHNOLOGIES FOR NATURAL PRODUCT RESEARCH - APPLICATIONS AND POTENTIALS IN PHYTOPHARMACY, PHYTOCOSMETICS AND PHYTO-NUTRITION

Gunther K. Bonn[1,2]

[1]*Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria*
[2]*ADSI - Austrian Drug Screening Institute, Innrain 66a, 6020 Innsbruck, Austria*

The achievements in natural product research are largely based on the constant development of highly selective and sensitive analytical technologies for monitoring active constituents. In particular sample preparation and separation have greatly facilitated the state-of-art research during the last years. Although numerous methods have been developed, still many challenges remain due to the complexity of sample matrix and diversity of natural products. Therefore, our main focus is placed on the development of novel analytical methods for phyto-pharmacy, phyto-cosmetics and phyto-nutrition. New isolation, enrichment and purification tools based on solid-phase extraction have been developed in order to reduce the complexity of plant extracts, while µ-HPLC is used for separation, pre-concentration and fractionation. The opportunity to hyphenate these techniques to robotic systems permits high-throughput screening. Significant progress has been made in the development of novel stationary phases which can be tailored to a specific application, allowing endless possibilities in terms of selectivity tuning. Further hyphenation to high-resolution mass spectrometry facilitates the identification and quantitation of active components in natural products. Near and mid infrared (NIR and MIR) spectroscopy enable a fast and simultaneous qualitative and quantitative analysis of raw plants and liquid extracts without destruction. On the other hand infrared imaging can be used to study the distribution of active ingredients in plant materials with a resolution of down to 5 µm. This presentation provides an overview about novel analytical technologies and methods for the investigation of active natural compounds in medicine, phyto-pharmacy, phyto-cosmetics and phyto-nutrition.

IL1

TARGETED NANOMEDICINE: PAST, PRESENT AND FUTURE

Gert Storm[1]

[1]*Dept. Pharmaceutics, Utrecht University Universiteitsweg 99, 3584 CG Utrecht, The Netherlands*

Perhaps the largest and most active sector of research within the field of nanomedicine has been the design of nanopharmaceutical platforms for the targeted delivery and controlled release of chemotherapeutic agents. And novel nanomedicines and drug delivery systems continue to flourish in the research laboratory, with a commensurate increase in expectations of their clinical impact. However, so far, only a handful of targeted nanomedicines have been approved, and the sceptism about their therapeutic usefulness is becoming stronger. The central question raised in this presentation is: How will targeted nanomedicine face tomorrow?

IL2

QUALITY-BY-DESIGN IN NANOMEDICINE: HIGHLY SYSTEMATIC OPTIMIZATION OF LIPID-POLYMER HYBRID NANOPARTICLES FOR EFFICACIOUS AND SAFE INTRACELLULAR DELIVERY OF CARGO SIRNA

Kaushik Thanki[1], Xianghui Zeng[1], Hanne Mørck Nielsen[1], Henrik Franzyk[2], Camilla Foged[1]

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[2]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Jagtvej 162, DK-2100 Copenhagen Ø, Denmark

Therapeutics based on RNA interference (RNAi) are highly target-specific and promising for the treatment of serious diseases lacking definite clinical management, e.g. certain inflammatory diseases. However, the efficacy of RNAi therapeutics like small interfering RNA(siRNA) are fully dependent on the development of safe delivery technologies that can mediate intracellular delivery of siRNA to the cytosol of target cells. A delivery system was engineered to consist of siRNA-loaded lipid-polymer hybrid nanoparticles (LPNs) composed of poly(DL-lactic-co-glycolic acid) and a lipidoid as the lipid component[1]. To account for the strong interplay between several contributing factors, the formulation was optimized using a highly systematic quality-by-design approach to define the optimal operating space(OOS), eventually resulting in the identification of a robust, highly efficacious and safe formulation[2]. A 17-run design of experiment with an I-optimal approach was performed to systematically assess the effect of selected variables on critical quality attributes(CQAs), i.e. physicochemical LPN properties and the biological performance in vitro. Model fitting of the obtained data was used to construct predictive models that revealed non-linear relationships for all CQAs, which readily may be overlooked in one-factor-at-a-time optimization approaches. The response surface methodology further enabled the identification of an OOS that met the desired quality target product profile. The optimized lipidoid-modified LPNs mediated highly efficient gene silencing in vitro at well-tolerated doses. Also, LPNs loaded with siRNA against the pro-inflammatory cytokine tumor necrosis factor α led to reduced experimental inflammation in mice. Thus, lipidoid-modified LPNs show highly promising prospects for efficient and safe intracellular delivery of siRNA.

References

[1] European PCT application number 57590PC01
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IL3

OVERVIEW OF DEVELOPMENT AND MANUFACTURE OF BIOLOGIC DRUG SUBSTANCE

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The manufacture of biologics is a highly demanding process. Protein-based therapies have structures that are far larger, more complex, and more variable than the structure of drugs based on chemical compounds. Plus, protein-based drugs are made using intricate living systems that require very precise conditions in order to make consistent products. The manufacturing process consists of the following four main steps: 1. Producing the master cell line containing the gene that makes the desired protein. 2. Growing large numbers of cells that produce the protein. 3. Isolating and purifying the protein. 4. Preparing the biologic for use by patients. The manufacturing process begins with cell culture, or cells grown in the laboratory. During the scale-up process, the cells are sequentially transferred to larger and larger vessels, called bioreactors. At every step of this process, it is crucial to maintain the specific environment that cells need in order to thrive. Even subtle changes can affect the cells and alter the proteins they produce. For that reason, strict controls are needed to ensure the quality and consistency of the final product. When the growth process is done, various filtering technologies are used to isolate and purify the proteins based on their size, molecular weight, and electrical charge. The final steps are to fill vials or syringes with individual doses of the finished drug and to label the vials or syringes, package them, and make them available to health care professionals and patients for injection or infusion.

IL4

THE TRENDS FOR OBESITY AND TYPE-II DIABETES IN TURKEY: WHERE ARE WE NOW? AND WHERE ARE WE GOING?

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Diabetes is one of the largest global health challenges, according to the NCD-RisC, 422 million adults estimated with diabetes in 2014, compared to 108 million in 1980. The prevalence has nearly doubled since 1980 (4.7% to 8.5%). Based on IDF, 415 million (8.8%) in 2015, and by 2040 642 million adults worldwide estimated to have diabetes. Diabetes prevalence goes hand-in-hand with population ageing, changing lifestyle, and increase in obesity. In Turkey, the prevalence of diabetes has nearly doubled within 12 years, rising from 7.2% (1998) to 13.7% (2010), and diabetes awareness decreased by 20%. Based on 2010 survey: age, BMI and hypertension in both sexes; waist, low-education and living environment in women appeared as risk factors; however, current smoking in women, and being single in men were preventing factors associated with diabetes. NCD-RisC reported obesity increased from 3·2% in 1975 to 10·8% in 2014 in men, and from 6·4% to 14·9% in women. In Turkey, obesity increased from 22.3% to 31.2% within 12 years, the prevalence was higher in women but the rate of increase was higher in men. As the population of Turkey getting older, more people will be affected, based on the adult population in 2016, it is estimated 8.2 million (15.2%) with diabetes, and 9.5 million (29.5%) with obesity in Turkey. The current situation shows that diabetes and obesity figures are alarming and underscore the urgent need for national programs to prevent these two conditions, to treat available cases, and thus prevent complications.

IL5

FRUCTOSE-INDUCED TYPE 2 DIABETES AND OBESITY

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Type 2 diabetes and obesity are becoming increasingly prevalent in the modern world. The excess sugar intake, especially as fructose, in daily human nutrition may contribute to the worldwide epidemic of type 2 diabetes and obesity [1]. High-fructose consumption has been shown to cause metabolic syndrome characterized with insulin resistance, hypertriglyceridemia, abdominal fat accumulation and fatty liver in human and animals. Data from our laboratory have shown that high-fructose diet leads to increase in plasma levels of insulin and triglyceride as well as produce endothelial dysfunction and vascular insulin resistance. Moreover, we showed that dietary high-fructose causes modulation in the expression of the genes and proteins with insulin signaling in target organs for insulin such as vascular, hepatic, adipose and pancreatic tissues of rats. This dietary intervention also produced an upregulation in inflammatory genes and proteins as well as an elevation in inflammatory markers of these tissues. Fructose-induced hepatic steatosis was linked to upregulation of lipogenic genes with induction of inflammatory mediators. Inflammatory mediators may possibly constitute a link between metabolic irregularity and insulin resistance. Fructose-induced metabolic disorders are more likely related to abdominal fat accumulation, but independent from the general obesity [2, 3, 4]. Here, we provide new insights into understanding the underlying mechanism responsible for fructose-induced metabolic disorders.

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IL6

DIABESITY AS A REDOX DISEASE

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The rapid increase in the prevalence of obesity, type-2 diabetes (T2DM) and associated complications (diabesity) is a major global health problem (1). T2DM is characterized by hyperglycaemia primarily associated with defective insulin secretion, insulin resistance, or both but often obesity, dyslipidaemia, hypertension, accelerated atherosclerosis and neurodegeneration are clinical comorbidities (2). High levels of circulating glucose and lipids can result in an excessive supply of energy substrates to metabolic pathways in cells, which in turn can increase the production of reactive oxygen species (ROS). While ROS are essential signaling molecules, if not well controlled they can damage nucleic acids, proteins, lipids and DNA. Oxidative stress, affecting the complex machinery of cell survival or apoptosis, are represented by oxygen- or nitrogen-derived free radicals and by byproducts of the mitochondria or endoplasmic reticulum stress response that are associated with an oversupply of reducing equivalents to metabolic pathways. The increased formation of advanced glycation and advanced lipoxidation end products (AGEs/ALEs) also contribute to the impaired redox signaling and abnormal energy metabolism in diabetic cells. Our laboratory not only investigate the ROS-induced damages in the cell signaling and their involvement in the progression of DM, but also focus on pharmacological interventions and targeting of ROS in DM. Our study also aimed to prevent ROS-derived injury in diabetic tissues, and to investigate the mechanisms of novel synthetic pyridoindoles and natural plant-derived polyphenols in regulating redox balance, modulating cellular stress response and influencing cell death pathways in models for glycolipotoxicity and DM (3-5).

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IL8

LOW DOSES OF ENVIRONMENTAL CONTAMINANTS - GENOTOXICITY, EFFECT ON CELL PROCESSES AND POSSIBLE CONSEQUENCES

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Cadmium and phthalates are found in the environment in nanomolar concentrations that are considered to be nontoxic. Cadmium is classified to be human carcinogen[1] and phthalates are not genotoxic compounds. If there are indices of their tumorigenicity it's suposed that it is the result of nongenotoxic mechanism of action[2]. Bis-2-ethylhexyl phthalate is widely used phtalate[3], and there are no experimental data concerning genotoxicity of environmentally significant concentrations. In this work, genotoxic potential of both compounds was investigated three different cell lines; human larynx carcinoma(HEp2) as a representative of the epithelial cells which are first in the contact with these contaminants after ingestion; human colon carcinoma(CaCo2) cells since all ingested food that contains traces of cadmium and bis-2-ethylhexyl phthalate will be in the contact with this type of cells. Human hepatocellular carcinoma(HepG2) cells were chosen as a suitable test system because of specificity of metabolic reactions and presence of certain endogenous compounds that can influence on overall (geno)toxicity of investigated compounds. Ames strains Salmonella typhimurium TA98 and TA100 were used to determine potential of these two chemicals to cause point mutations. Cadmium induced cell proliferation and the formation of free radicals after 24 hours at concentrations that corresponds to the measured concentrations of cadmium in the blood of children and adults. Proliferative and prooxidative effect of cadmium is reduced by longer exposure time. Low doses of cadmium caused point mutations, and chromosomal breaks were not detected. Bis-2-ethylhexyl phthalate showed prooxidant effect on CaCo2 cells after 24 and 48 hours of incubation.

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IL9

ROLE OF THE ALTERATION OF THE RETINOID SYSTEM UNDER RISK ASSESSMENT

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Brominated flame retardants (BFRs) are chemicals added to materials such as electronic equipment, textiles and plastics to reduce the risk of fire. Humans are exposed to BFRs such as polybromodiphenyl ethers (PBDE) and hexabromocyclododecane (HBCD), with are persistent, bioaccumulating and toxic substances. Thus, the aim of the current work was to compare doses of BFRs with effects on the retinoid system and other toxicological endpoints of experimental animals with human exposure levels. Wistar rats were exposed to pentaBDE, decaBDE or HBCD technical mixtures for 28 days according to the OECD repeated-dose test-guideline (TG407). Dose-response relationships were assessed by benchmark dose modelling and critical effect doses were calculated at the lower confident bound (CEDL). Margins of exposures (MOEs) for endpoints used to derive health-based guidance values and effect-exposure ratios (EERs) for retinoid concentrations were calculated by dividing CEDLs with human exposure to BFRs. Exposure to decaBDE and pentaBDE resulted in MOEs and EERs higher than total assessment factors (AFs) for general population, whereas MOEs and EERs were lower than AFs for occupational exposure in a recycling plant of electronic waste. Both MOEs and EERs were higher than AFs for median exposures to HBCD, whereas MOEs and EERs were lower than AF for exposures at the higher bound. From this communication, there is substantial support for the OECD-TG-project on analysing the possible need to incorporate the retinoid system in future toxicity testing methods for the risk assessment of chemical substances, including endocrine disrupting compounds.

IL10

POTENTIAL ROLE OF PHARMACOGENOMICS IN REDUCING ADVERSE DRUG REACTIONS

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Adverse drug reactions are a significant cause of morbidity and mortality. Although many adverse drug reactions are considered non preventable, recent developments suggest that these unexpected reactions may be avoided through individualization of drug therapies based on genetic information, which is an application known as pharmacogenomics. Health authorities are now more conscious of this issue and the reporting of adverse reactions has increased over time due to improved pharmacovigilance systems and better methods of signal detection. A considerable number of drugs were withdrawn from the world market in the last decades due to safety reasons. A total of 43 drugs were withdrawn between 1999 - 2016 in Turkey. The most frequently encountered safety issues leading to withdrawal (cardiac disorders 60%) and the therapeutic indication of the medicinal product (mostly analgesics), enzymes involved in their metabolic pathways (the most frequently found cytochrome P-450 enzymes were CYP3A4 and CYP2C9), causality assessments, interval between launch and withdrawal year in Turkey compared with the EMA and FDA regulatory actions will be presented to reveal the importance of developed risk management plans and drug safety monitoring systems starting from the clinical phases of drug development to protect public health safety. Greater co-ordination among drug regulatory authorities and increased transparency in the reporting of suspected adverse drug reactions would help improve decision-making processes.

IL11

DISCOVERY OF NEW, POTENTIAL ANTI-INFECTIVE COMPOUND BASED ON CARBONIC ANHYDRASE INHIBITORS BY RATIONAL TARGET-FOCUS REPURPOSING APPROACH

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Drug-repurposing (DR) denotes an ensemble of tasks aimed to the identification of new indications for existing drugs, and is an alternative strategy in drug discovery program, both in pharma and academia. Especially in academia, DR can be useful to re-invest compound library collections already available in-house [1]. We embarked in a project aimed at the repurposing of the compound libraries available in-house, looking for a new potential applications for our compounds. A rational target-based drug repurposing approach was applied [2], and the analysis of the data available allowed us to identify the Carbonic Anhydrase (CA, EC 4.2.1.1) metalloenzyme family as potential target of some of our series. We proceed to the analysis of the fragments by applying the Maximum Common Substructure (MCS) decomposition approach. A thoroughly validated docking screening protocol was combined with chemical synthesis [3] and in vitro assays to disclose new potential CA inhibitors. This method allowed us to identify eleven compounds as potential CA inhibitors (CAIs). They were tested in vitro for their ability to inhibit different isoforms of CA, leading to the discovery of inhibitors in the low µM range [4]. Additional rounds of optimization were carried out in order to improve both activity and selectivity. As such, we have disclosed a novel chemotype for the inhibition of CA that lacks the sulfone moiety and offers room for further investigation.

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IL12

ENABLING SYNTHETIC TECHNOLOGIES FOR CHEMICAL LIBRARIES, HIT OPTIMIZATION AND SCALE-UP: IN-FLOW SYNTHESIS AND BIOLOGICAL ACTIVITY OF QUINOLINE-BASED COMPOUNDS

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In the changing landscape of drug discovery, technological innovation in synthesis is playing a crucial role from early phase of lead identification to production [1]. Nowadays, synthetic strategies are designed to be versatile for a fast access to chemical libraries and, at the same time, easily scalable to support in vivo efficacy, safety testing, and clinical trials. The increasing number of reports and publications, the capital investments of pharmaceutical industries, and the availability of commercial equipments are qualifying continuous flow technology particularly suitable for these purposes. The potential advantages of the flow chemistry include high control of the reaction variables, which can translate into higher product quality; increased safety and ecosustainability; possibility of conduct reactions at supercritical conditions; feasibility of automation reducing manual handling and accelerating products throughput; reproducibility and easy scale-up; possibility of in-line purification, analysis and reaction telescoping; process intensification resulting in a smaller footprint in a manufacturing plant [2]. In this communications, our recent efforts directed towards the set-up and implementation of integrated flow platforms developed to accelerate the total compound-generation, to improve chemical processes, as well as to decrease the instrument face time by the operating scientist, will be presented. In particular, the prototype systems will be exemplifyed by case studies within our medicinal chemistry programmes showing the potential to reduce significantly the time and cost of preclinical drug discovery.

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IL13

NEW ADVANCES IN ISOTHERMAL TITRATION CALORIMETRY APPLIED TO DRUG DEVELOPMENT: GOING FAR BEYOND TRADITIONAL ANALYSIS WITH THE AFFINIMETER SOFTWARE

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Isothermal Titration Calorimetry (ITC) is an essential, but not full-exploited tool in drug discovery laboratories used right through the initial steps of hit validation and lead optimizatio [1]. Traditionally, it offers a direct, quantitative measure of the binding affinity (KA), enthalpy (DH) and stoichiometry; but the battery of information potentially available from ITC goes far beyond the thermodynamic profiling of binding events: a proper analysis of ITC data can yield rich thermodynamic and structural information that contributes to the elucidation of structure-activity relationships (SAR) and mechanistic aspects of interactions. Besides, the kinetic profiling can now be readily determined from standard ITC thermograms and the method KinITC [2]. Until recently, the main hurdles to exploit the full potential of ITC was not in the experimental facet but in the data analysis step. Herein we bring to the fore how the novel analytical tools developed in the software AFFINImeter are letting to exploit the full potential of the ITC and other biophysical techniques. This is illustrated with selected examples of ITC analyses of challenging ligand-receptor interactions of interest in pharmaceutical research.

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IL14

IMPORTANT MILESTONES IN MY FORTY-FIVE YEARS OF RESEARCH INTO NATURAL PRODUCTS

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My forty-five years of research in natural products and plant chemistry have generated isolation of more than 300 new compounds and novel structures. My research career on natural products followed my early interest in the diversity of nature. This interest guided by the discipline of “pharmacognosy” led my curiosity in the diversity of structural skeletons observed in nature, and, facilitated the establishment of my career in drug discovery methods from natural products.

My future research has been built upon my dissertation where I began to utilize chromatographical techniques and chemical methods - in addition to limited instrumental analysis. With this early study on saponins of Saponaria kotschyii, I began working on isolation of compounds and elucidation of a single oleanane-type bisdesmosidic triterpenoid saponoside.

In 1982 and 1983 I had studied Lonicera species. The aim of this project was to isolate monoterpene alkaloids [1]. This study commenced my research on iridoids and secoiridoids from plants of Caprifoliaceae, Gentianaceae, Globulariaceae, Oleaceae, Plantaginaceae, Rubiaceae, Scrophulariaceae and Verbenaceae families. Associated to chemotaxonomy, coexistence of caffeoyl ester glycosides, together with iridoids, made these studies much more exciting and motivating.

In the early 1990’s, my studies on the roots of a plant of an unknown origin that was effective against leukemia, resulted in the isolation of novel cycloartane-type triterpenic glycosides with immune-stimulant activities. Later, I was able to categorize this plant to the Astragalus species (Fabaceae) [2].

My recent studies resulted in the isolation of cardiac glycosides with remarkable anti-cancer activity [3].

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IL15

CLIPS (CHEMICAL LINKAGE OF PEPTIDES ONTO SCAFFOLDS) TECHNOLOGY APPLIED TO OPIOID PEPTIDES RESEARCH

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CLIPS (Chemical LInkage of Peptides onto Scaffolds) technology is a novel and still unexplored versatile method for constraining and functionalizing the 3D-conformation of peptides. This novel cyclization type involves the cyclization of linear peptides via reaction with a small scaffold like dibromoxylenes. The bromine anchor points react exclusively with the thiols of the Cys or Pen in the peptide and attach to the peptide via multiple thio-ether bonds. Six cyclic analogues of DPDPE, biphalin and three fluorescent probes were prepared and characterized as m/d-opioid receptors agonists. [1-3] The novel biphalin and DPDPE derivatives were tested by in vitro and by in vivo animal model of pain.

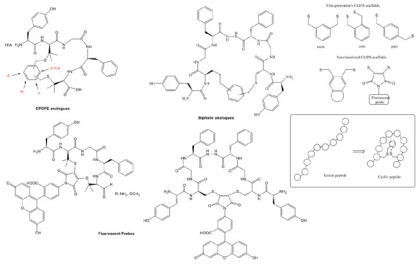

Figure 1. CLIPS Scaffolds

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IL16

POWER OF LIQUIDS – THE CASE OF COUNTER-CURRENT CHROMATOGRAPHY

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Medicinal plants have historically proven their value as a source of molecules with therapeutic potential, and nowadays still represent an important pool for the identification of novel drug leads [1]. Powerful analytical tools and technologies are still needed to provide new opportunities to identify and characterise such products, but most of all efficient tools able to ensure an efficient purification of singel molecules from complex plant matrix are important. In recent years, counter-current chromatography (CCC) has been broadly applied for the separation and purification, due to its unique advantages. CCC is a liquid–liquid partition chromatography process where both the mobile and stationary phases are liquids. The method is rapid and easy to scale up from analytical to a preparative, industrial scale. It also utilizes no solid support, and can be used for compounds with vastly different polarities, thus it is considered as one of the most efficient and economical separation techniques worldwide [2,3]. Due to numerous advantages CCC can be used for targeted purification, bioactivity-guided fractionation, followed by in vitro biological screening and many others, what will be presented during this lecture.
The work was financed from grant No 4/POLTUR-1/2016.

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IL17

EPITHELIAL-MESENCHYMAL TRANSITION (EMT) IN CANCER

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Epithelial-mesenchymal transition (EMT) is a core biologic process which is important in embryonic development. EMT has been implicated in several processes, such as organ morphogenesis, wound healing, fibrosis, and cancer pathogenesis. Polarized epithelial cells normally interact with the basement membrane via their basal surfaces, however they undergo multiple biochemical changes in EMT, lose their epithelial properties and gain mesenchymal cellular characteristics such as motility and invasiveness. Recent studies provide evidence that challenges the role of EMT as a critical mediator of cancer metastasis since tumor cells shed their differentiated epithelial characteristics, including cell–cell adhesion and polarity, and acquire mesenchymal traits attributed to those of stem cells [1,2]. It has been shown that cancer cells undergoing EMT resist conventional drug therapies possibly caused by a combination of several critical features such as relative dormancy, efficient DNA repair, high expression of multidrug-resistance-type membrane transporters, protection by a hypoxic environment and improvement of resistancy to apoptosis and oxidative stress. Since cancer stem cell/EMT related drug resistance involves complex molecular signaling pathways [2,3], the combined use of different gene products altered in EMT and cancer stem cells may represent potential strategies for improving the effectiveness of the diagnostic/prognostic methods and treatment efficacy for cancer patients. Understanding the molecular mechanisms responsible for EMT-mediated drug resistance will increase the sensitivity of cancer cells to chemotherapy and accelerate the discovery of therapeutic strategies including targeting directly to EMT pathways.

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IL18

DEVELOPMENT OF NOVEL TARGETED THERAPIES FOR HIGHLY AGGRESSIVE CANCERS

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Currently highly aggressive cancers such as triple negative breast cancer (TNBC), pancreatic cancer, ovarian and lung cancer have high mortality rates due to heterogeneous nature of the diseases and lack of therapeutic targets in advance and drug resistant cases. We recently discovered that eEF-2Kinase is highly overexpressed in these highly aggressive cancers and promotes cell proliferation, invasion and metastasis and tumor growth. We also developed therapeutics to target this EF2K and demonstrated for the first time that tin vivo targeting of this previously untargeted kinase by systemically administered-therapeutics inhibit tumor growth in preclinical tumor models. The talk will give background in targeted therapies used in cancer patients and our novel targeted therapies.

IL19

METHYLATION BIOMARKER STUDIES IN SALIVA OF HEAD AND NECK CANCER PATIENTS

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Head and neck cancer accounts for about 3% of all cancers. DNA promoter methylation of tumor suppressor genes and global DNA hypomethylation are common features of head and neck cancers. In one of our studies, we have analyzed genes by quantitative methylation-specific PCR method to identify methylation status in tissues and body fluids of patients and healthy individuals. Thus, the results demonstrated that EDNRB, KIF1A, p16 and DCC are frequently silenced by promoter methylation in the head and neck cancer (HNC) patients' samples. These genes may serve as a potential epigenetic biomarker for the early detection of HNC. Oral premalignant lesions (OPML) is the pre-cancerous lesion of OSCC, whose molecular mechanisms in OSCC tumorigenesis remain largely unclear. In our recent study, we aimed to identify DNA methylation changes in OPML for predicting early diagnosis, prognosis and recurrence. Therefore we identified candidate biomarkers predicting of the early OPML diagnosis using llumina Human Methylation 450 "(Illumina, CA, USA) arrays which comprise more than 450.000 CpG sites, in 12 OPML tumor and matched normal tissue samples. We found that the 370 genes were differentially methylated when we compared methylation status between tumor and adjacent normal tissues by using methylated gene profiles obtained from arrays. These potential candidate genes will be further validated in a larger group of patients.

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IL20

ADVANCEMENTS IN MOLECULARLY IMPRINTED POLYMERS: TOWARDS TAILORABLE BIOMIMETIC MATERIALS IN MEDICINE AND PHARMACEUTICS

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Biomimetic recognition utilizing molecularly imprinted polymers (MIPs) has proven its potential by providing synthetic receptors for numerous analytical applications including liquid chromatography, solid phase extraction, biomimetic assays and sensor systems [1-6]. The inherent advantages of synthetic receptors and functionalized membranes in contrast to biochemical/biological recognition and immobilization schemes include their robustness, synthetic versatility and potentially lower costs. In principle, molecularly imprinted/templated materials are an ideal molecular capturing matrix tailorable for selective recognition or immobilization of a wide range of molecules. Fundamental understanding of the involved processes via analysis of the governing interactions at a molecular level providing binding affinities, and facilitating pre-screening of optimized functional monomers and component ratios provides the basis for molecular dynamics simulations enabling modeling of the interactions of template molecules with functional monomers and cross-linkers in explicit solvent. While is anticipated that molecular templating based on rational synthetic design will significantly reduce the number of trial & error experiments currently required, it is evident that the complexity of simulating the generation of molecular imprints still requires extensive efforts toward rational design of next-generation synthetic receptors. Of particular interest is the development of biomimetic recognition schemes for selectively binding proteins and large biomolecules, e.g., at the surface of biomedical devices for promoting or preventing adhesion of selected biomolecules, as well as for controlled molecular release [7-10]. Recent developments on this new frontier in molecular imprinting will be highlighted with selected examples and novel routes toward tailoring polymeric receptors for biomolecular recognition facilitating innovative strategies in medical and pharmaceutical applications.

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IL21

BIOSENSORS FOR THE DETECTION OF PESTICIDES BY ENHANCED RAMAN SPECTROSCOPY AND INTERACTIONS WITH GOLD NANOPARTICLES

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This presentation focuses on the development of a new biosensors using surface-enhanced Raman spectroscopy (SERS) for the qualitative and quantitative detection of pesticides by measuring the acetylcholinesterase (ACHE) activity. The Raman SERS is not only used for measuring the ACHE activity, but also for the direct detection of pesticides individually and for their identification. Gold nanoparticles (AuNPs) were used as dynamic SERS substrates for sensitive monitoring of ACHE activity in the presence of very low levels of organophosphate and carbamate pesticides, chemical warfare agents that are known to be ACHE inhibitors. These results suggest that this biosensor could be used in the future for the non-selective detection of all ACHE inhibitors at very low concentrations with possible identification of the inhibitor.

In a second part, we established a new approach that consists in the development of another method for the detection of ACHE inhibitors by using unmodified gold nanoparticle-based dynamic light scattering (DLS). The hydrolysis of acetylcholine (ATC) mediated by ACHE yields the choline, which influences the AuNPs aggregation, and triggered the increase of their average diameter. The inhibition of the enzyme by pesticides (paraoxon) produces lower yields of choline, the gold nanoparticle remained unchanged. However, the use of this new method to detect ACHE inhibitors, such as organophosphates pesticides, has not been reported. These results suggest that this DLS-assay based unmodified AuNPs could be used in the future for measuring ACHE activity and the non-selective detection of trace amount of all ACHE inhibitors.

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IL22

NEXT GENERATION BIOSENSORS FOR BIOANALYTICAL MEASUREMENTS

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Currently, testing for analytes are typically performed in centralised laboratories using bulky automated devices. For laboratory based testing, samples need to be transferred to the testing site, requires trained staff and results in higher costs with longer waiting times.
The ever increasing demand of sensors for rapid on site results together with internet of things applications pushes the bioanalytical detection market to develop novel devices for everyday use. The complexity of biosensor devices range from simple tests such as enzymatic direct detection of analytes, to immunoassay or DNA based complex assays where multiple reagents has to be involved in sensing where in majority of the cases has to pass through a sensor surface. The biosensor market is dominated by glucose sensors for years, not only due to the high number of diabetes people who require blood glucose detection daily, but also detection can be achieved fairly simply by just adding a prick of blood on to an enzyme immobilized sensor chip. Whereas for tests such as pathogen detection or disease biomarker detection, immunoassay or DNA based complex assays are required, where the biosensor device need to have sensor integrated microfluidic system containing microchannels, microvalves, micropumps, miniaturized transducers with simple user interface for ease of use. In this presentation we will have a snapshot of the current technologies used in the biosensor devices on the market and will look into the future trends.

IL23

PARTICLE CHARACTERISATION IN THE PHARMACUETICAL INDUSTRY

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Anyone engaged in the development, formulation or manufacture of pharmaceuticals is likely to have some understanding of the role of particle size in determining the properties and efficacy of a wide range of pharmaceutical formulations. Understanding its effects, and being able to measure and control particle size is important at all stages of the drug development and production process, from early stage formulation through to the definition and assessment of parameters critical to quality. There are many techniques for the determination of particle size and size distribution, each one having advantages and disadvantages which are largely dependent upon the type of material to be measured and the type of information to be obtained from the material in question. In addition their are many different types of pharmacuetical delivery mechanisims. Inhalation, solid oral dose, emulsions are just a few that all require a different approach to measurement strategy and technique. Add to this the growing trend in biological products for theraputic use and the requirements for particle characterisation become even more varied and important. This presentation will focus on various techniques found in the pharmacuetical industry and how they are utilised. More importantly we will discuss how other characterisation techniques sit alongside particle size and how regulatory authorities are looking for more ‘orthoganal approaches” to characterisation. Labaoratory systems through to on line systems for controlling milling or granulation processes will be discussed along with examples that serve to demonstrate their use.

IL24

VALIDATION OF NOVEL FOOD PROCESSING TECHNOLOGIES

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There are a number of novel/emerging food processing technologies that are transitioning from pilot plants to industrial settings. Some of these technologies are microwave processing; high hydrostatic pressure, high pressure homogenization, pulsed electric fields. They are utilized in a number of applications such as sterilization, pasteurization, decontamination and extraction. In order to introduce in the market safe food products processed by these alternative technologies, proper validation studies followed by verification and monitoring are required and/or recommended. Data collection followed by scientific evaluation of all the critical processing points should render an answer whether a given product will be safe. The lack of enough scientific literature on the appropriateness of a given novel technology to process a specific product makes validation a very challenging proposition. Microorganisms of concern should be identified as well as proper surrogates; predictive microbial inactivation kinetic mathematical models have to be developed, suitable protocols should be implemented as well as some specific test to satisfy a performance criterion that will allow to reach a food safety objective (FSO). This presentation deals with the development of reliable validation protocols for novel food processing technologies highlighting Pressure Assisted Thermal Sterilization (PATS) and Microwave Assisted Thermal Sterilization. USFDA (United States Food and Drug Administration) approved these two sterilization techniques to process mashed potatoes and later on to other specific food products. Emphasis will be given as well to better quality food products rendered by these emerging technologies when compared to equivalent conventional processes.

IL25

FOURIER TRANSFORM INFRARED SPECTROSCOPY AS A TOOL FOR DETECTION OF FRAUDULENT RAW MEAT MIXTURES

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Fourier Transform Infrared (FTIR) spectroscopy has become a valuable analytical tool for structural studies in the identification of origin or species in food products. FTIR is a fast, sensitive, safe method and it is one of the first methods with environmentally-friendly sample preparation. Application of this unique technique has recently increased in food studies with the current research focusing on assessing possible adulteration in food products either by discrimination of the food fingerprints [1, 2]. Since ancient times, adulteration of food products to reduce manufacturing costs has been of great concern because, among other negative connotations, impacts consumer rights due to safety and ethical reasons and implies dishonest labeling and misdescription [3]. The most common worldwide adulteration found in meat products is intentional substitution of premium-quality meats by different types of low-cost meat species or by adding improper ingredients to improve the sensory and physical characteristics of the end product seeking financial gains [4]. One of the emerging areas in meat science and technology is to detect the existence of adulteration with proper authentication methods. A study supported by Scientific and Technological Research Council of Turkey (TÜBİTAK Project # 214O182) was conducted for the purpose of detecting adulteration in raw beef, chicken and turkey meat mixtures with FTIR. In this study, detection of changes in composition of these mixtures from the FTIR spectra yielded encouraging results. A large database was generated to facilitate the identification of fraudulent substitution of different meat species either for the meat industry or food control laboratories.

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IL26

ENDOCRINE DISRUPTING CHEMICALS IN ANIMAL ORIGIN FOOD

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Endocrine disrupting chemicals are attributed to various chronic disorders including metabolic syndrome, cancer, obesity, diabetes, infertility and other nervous/cardiovascular system diseases leading major health concern. The complexity of their mechanism of action compromises difficulties for their classification by toxicity tests and for authorities regarding legislation procedures; while the incidence of the mentioned health problems are globally increasing [1]. Among exposure routes, residues from animal origin food products (AOFP) are often overlooked. These compounds are introduced to AOFP during animal production indicated as ingredient-related contaminants and during slaughter, processing or packaging indicated as production related contaminants (facility related hazards) [2]. Residues of pesticides, metals, food additives, phthalates, industrial chemicals, polycyclic aromatic hydrocarbons, alkylphenols, growth promoters can occur in AOFP including meat, milk and eggs at very low concentrations and as mixed leading potential cocktail effect. An overview is presented of mechanisms contributing to the exposure routes through AOFP, monitoring methods, related legislation, critical control points in production units and risks in terms of veterinary public health with emphasis to our research groups' latest studies including persistent organic pollutant (PCBs, PBDEs, OCPs) and phthalate contamination in various matrices including processed meat, yoghurt, fish, milk and eggs.

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IL27

RATIONALE FOR VACCINATION: IMPORTANCE OF INFECTIOUS DISEASES SURVEILLANCE- EXAMPLE: SURVEILLANCE OF INFLUENZA

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Infectious diseases surveillance is an ongoing process that involves the systematic collection, analysis, interpretation, and dissemination of information regarding the occurences of diseases in defined population for public health action to reduce morbidity and mortality [1]. In this context, Influenza surveillance is designed to identify the onset of flu activity in a population at the earliest opportunity and to obtain virus isolates for characterization throughout the period of activity [2]. In fact surveillance provides valuable data on characteristics of Influenza activity, on types, sub-types, antigenic properties and antiviral resistance profile of circulating viruses, and also provides valuable data that can guide policy makers in developing programmes to prevent and reduce Influenza burden [3]. During this presentation I want to discuss the results of community-based sentinel surveillance of Influenza in western Turkey during 13 consecutive influenza seasons: 2003-2016. Analysis of the data revealed that, (a) influenza season has extended in Turkey and it lasts through May; (b) influenza peaks in different age groups depending on the season; (c) every year a different influenza type and subtype dominates the season; (d) influenza B has been circulating with increasing rate especially in the past six seasons. These results have important implications for vaccine composition and optimal vaccination timing [4].

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O1

HOT MELT EXTRUSION AND HOW TO USE THE EXCIPIENTS FOR SUCCESSFUL FORMULATION DEVELOPMENT

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Drug solubilization has drawn attention in recent years because large numbers of NCEs often fail in development due to their poor solubility and bioavailability. To circumvent these challenges and bring the compounds to the market, the pharmaceutical industry has a desire for novel solubilizers that can provide better opportunities for poorly soluble APIs by (1) lending better solubilization capacity than known solubilizers, (2) having unparalleled safety and toxicological standards, and (3) reducing time and cost in the drug development process. Classical solubilizers are usually polyethylene glycol-based surfactants that are well suited for liquid formulations (oral, parenteral). Hot melt extrusion (HME) technology has gained a significant interest in recent years. Even though this technique has been used in the plastics and food industries for decades, it is relatively new in the pharmaceutical industry, and only a few drug products (based on polyethylene glycol or copovidone) are currently available on the market. BASF has introduced a new polymeric solubilizer, Soluplus®, a graft copolymer composed of polyethylene glycol, polyvinylcaprolactam, and polyvinylacetate. Its unique chemistry coupled with granular and solubilization characteristics are important in the development of solid solutions by hot melt extusion. Soluplus outperforms many of the well-known surfactants and solubilizers for poorly soluble compounds and is potentially applicable to solid oral dosages. The low hygroscopicity and glass transition temperature of about 70°C makes it different from other polymers used as solubilizers.

O2

BIOAVAILABILITY ENHANCEMENT AND IN VIVO EVALUATION OF OLMESARTAN MEDOXOMIL SELF-MICROEMULSIFYING DRUG DELIVERY SYSTEM

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Olmesartan medoxomil is a hydrophobic antihypertensive drug with low bioavailability (26%) and is known to cause adverse effects such as celiac disease and enteropathy [1-3]. The purpose of this study was to develop self-microemulsifying drug delivery systems (SMEDDS) to increase bioavailability and decrease potential side effects of olmesartan. Hydrophilic lipophilic balance was calculatated by testing solubility of olmesartan in different oils, surfactants and cosurfactants to obtain the most suitable combination of microemulsion. Pseudoternary phase diagram was used to select the best O/W formulation of SMEDDS. After a test for 6-month stability, dissolution tests and PAMPA were conducted to investigate drug solubility and permeability. Biodistribution of fluorescent marked SMEDDS was observed by using InVivo Imaging System. The pharmacodynamics of drug were determined by measuring blood pressure in rats from tail. At the end of the experiment, intestines were examined for adverse effects of olmesartan. SMEDDS formulation showed 1.3 times improvement in solubility of olmesartan vs. tablet formulation according to the dissolution study. PAMPA studies suggested an at least 100 times faster permeability rate for olmesartan SMEDDS compared to the suspension form. Labelled microemulsion gave 24.1 times stronger fluorescent emission than control dye administered mice in IVIS studies indicating an increased bioavailability. Treating effect of microemulsion was 3.1 times more efficient compared to suspension in hypertensive rats. It did not cause neither enteropathy nor diarrhea, during 21- day non-invasive blood pressure (NIBP) assay. Our results suggest that SMEEDS formulation improves dissolution and oral bioavailability of olmesartan while reducing its adverse effects.

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O3

OPTIMIZATION OF FLURBIPROFEN NANOSUSPENSIONS WITH POLYMERIC STABILIZERS FOR DERMAL APPLICATION

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Flurbiprofen (FB) is a nonsteroidal antiinflammatory and BCS class II drug. Its poor solubility plays a critical role in limiting the dermal delivery and bioavailibility of FB. Nanosuspensions can be defined as nano-sized colloidal dispersions of crystal drug particles which are stabilized with polymeric stabilizers or surfactans. It can be increased the solubility of poor soluble drugs thanks to small size and large surface area of particles[1]. The aim of this study was to develop FB nanosuspensions with polymeric stabilizers using high pressure homogenisation method to improve dermal delivery of FB. A half, four factorial design $2^{(4-1)}$ with Design of Experiment (DoE)[2] was employed to determine the effect of formulation and process variables (drug concentration, polymeric stabilizer type, FB:stabilizer ratio and homogenization pass number) on the critical quality attributes (particle size(PS), polydispersity index(PDI) and zeta potential(ZP)) of nanosuspensions. The four independent variables and two levels studied in this investigation were concentration of FB (1-4%), type of polymeric stabilizer(HPMC-PVP), FB:stabilizer ratio (1:4-4:1) and homogenization pass number(10-30 cycle). 6 center points were also added to make the model more robust and predictable. PVP was found to be better than HPMC to improve stabilization of FB nanosuspensions. The optimum formulation parameters; PS, PDI and ZP were found 837,7±14,6 nm, 0,154±0,229 and -23,4±1,8 mV, respectively. This study demonstrated the effect of type of polymeric stabilizer, FB: stabilizer ratio and homogenization pass number on the final product using fractional factorial design with DoE.
This study was supported by Gazi University Scientific Research Foundation (Project no: 02/2017-05).

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O4

IN-VITRO CHARACTERISATION OF PEPTIDE-563 CONJUGATED LIPOSOMES

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Liposome-derived carriers have advantages to deliver DNA for therapeutic benefit. We have recently described a new peptide-based gene delivery system, prepared by cationic polymer which was covalently linked to dioleoylphosphatidylethanolamine (DOPE), and conjugated with peptide-563 which is specific for prostat cancer. Liposomes were prepared by using lipid hydration method and size extruder was used to obtain unilamellar nano-dimension liposomes. Their particle size and and zeta potential measurements were investigated. Also, their encapsulation efficiency and loading capacity of the formulations was calculated. Liposome formulations were also applied to agarose gel electrophoresis. Liposomes of DNA may cause positive zeta potential, low cytotoxicity (cells remaining 86-98%) , with a proper particle size (around 100nm), that might contribute to the high transfection efficiency and seemed to be more efficient carriers for in vitro gene transfer. Further studies were carried on to determine their stability and efficacy on animal model transfection process.

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Table 1: Characterisation of peptide-563(p563) conjugated liposomes, n=3.

Code	Polymer (used)	Ratio of pDNA	Encapsulation efficiency (%)	Particle size (nm)
Pk (control)	PEtOx5300-b-DOPE-coumarin, PEtOx5300-b-DOPE-p563 (1:1)	-	-	94.72±11.16
Pg	PEtOx5300-b-DOPE-p563	1:2	91.07±14.84	104.04±16.26

O5

MERGING DIFFERENT DRUG DISCOVERY APPROACHES TO OFFER NOVEL THERAPEUTIC OPTIONS AGAINST MYCOBACTERIAL INFECTIONS

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Tuberculosis remains one of the deadliest infectious diseases in the world, and the increased number of multidrug-resistant and extremely drug-resistant strains is a significant reason of concern [1]. We have previously addressed this need by reporting a series of substituted 2-aminothiazoles capable to inhibit the growth of actively replicating, non-replicating persistent and resistant Mycobacterium tuberculosis strains [2]. Clues from the structure–activity relationships lining up the antitubercular activity were exploited for the rational design of improved analogues. Two compounds belonging to this improved series were found to show high inhibitory activity toward susceptible M. tuberculosis strains, with an MIC_{90} of 0.125–0.25 µg/mL (0.33-0.66 µM) and 0.06–0.125 µg/mL (0.16-0.32 µM), respectively. Moreover, they maintained good activity also toward resistant and non-replicating strains, they were selective over other bacterial species and eukaryotic cells, metabolically stable, and apparently not susceptible to the action of efflux pumps. Along with this approach, we have also rationally synthesized a number of inhibitors of efflux pumps, in order to restore the susceptibility of mycobacteria to standard medicines [3].

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O6

COUPLING SPECTROSCOPIC AND MOLECULAR DYNAMICS EXPERIMENTS TO ELUCIDATE PROTEIN-LIGAND INTERACTION

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Early stage drug discovery includes an ensemble of challenging tasks aimed at the disclosure of new chemical entities (NCEs) as potential clinical candidate. Among these tasks hit expansion and hit-to-lead activities are usually hampered by several factors: (i) researchers have to deal with weak binders, with non-optimized physical-chemical properties; (ii) usually, for less explored targets it is challenging to obtain structural information (e.g. X-ray crystal structures) for the ligands and targets of interest; (iii) weak binders and their derivatives can exert different binding modes, which make difficult to rationalize SAR. Therefore, the availability of tools that can sustain/drive hit expansion and hit-to-lead activities are of great demand. In this scenario, we embarked in a project aimed at generation of an integrated approach, which can aid the identification and optimization of NCEs. This approach combines enhanced sampling techniques, Funnel-Metadynamics (Funnel-MetaD), with spectroscopic experimental data, such as Saturation Transfer Differences (STD) NMR experiments. The STD profile was reconstructed from the Funnel-MetaD simulations by applying an in-house developed script and compared with the experimental one. This integrated approach allowed us to identify a reliable binding mode instrumental to the rationalisation of the observed ligand activity.

O7

SYNTHESIS OF SOME PYRIDAZINE DERIVATIVES, INVESTIGATION OF THEIR IN VITRO ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES

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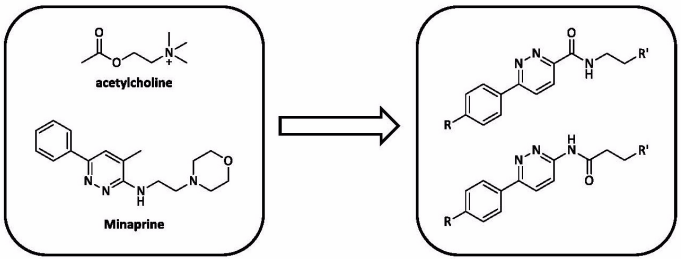
Alzheimer's disease (AD) is one of the most common neurodegenerative disorder in the elderly population. Cholinergic loss, amyloid-β (Aβ) deposition, τ-protein aggregation and oxidative stress are several hallmarks in multifactorial AD pathogenesis. That's why, drug design strategy for the complex diseases such as AD is based on multi-target-directed ligands (MTDLs). Especially, inhibition of cholinesterases activity and prevention of Aβ toxicity are primary targets for MTDLs researches [1]. Two enzymes that break down the acetylcholine to terminate cholinergic transmission are the acetyl- and butyrylcholinesterase (AChE, BChE). Inhibition of these enzymes clinically beneficial for symptomatic treatment of AD. Moreover, research shows that interaction between AChE and Aβ, through the peripheral anionic site (PAS) located at the entrance of the active-site gorge of the enzyme, promotes amyloid fibril formation and neurodegeneration. Compounds able to bind the catalytic and peripheral anionic sites of AChE, not only symptomatically improve the AD but also may have modifying effects on AD [2]. On the other hand, as the disease progresses, AChE levels decrease while the levels of BChE increase. Therefore, concurrent inhibition of both enzymes should provide additional benefits in the treatment of AD [3]. On the basis of these information novel 6-(substitutedphenyl)pyridazine-3-carboxamide and 6-(substitutedphenyl)pyridazine-3-il propionamide derivatives were designed and synthesized. Subsequently their cholinesterase and Aβ aggregation inhibitory activities were evaluated in the present study. Among the compounds we obtained cholinesterase inhibitors with sub-micromolar activities. Furthermore, several active compounds showed ability to inhibit AChE induced Aβ aggregation.



Design strategy and general structure of compounds

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O8

SIZE EXLUSION CHROMATOGRAPY APPLICATIONS FOR PROTEIN BASED DRUGS

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Biologicals are useful for the treatment of a wide range of illnesses. As biologicals come off patent, the opportunity increases for the production of biosimilars. Biosimilars have shortened licensing pathways, if they can be shown to be biologically similar to the already licensed innovator. For a product to be considered as biosimilar it must undergo analytical studies demonstrating its similarity, and include information to show that it is expected to produce the same clinical response. As part of analytical studies, the FDA requires the use of state-of-the-art technology to compare higher order structures, including aggregation, in addition to any formulation effects on purity, stability, product and process related impurities. A key tool in demonstrating biosimilarity is multi-detector SEC. Malvern's OMNISEC system includes refractive index and UV/VIS PDA detectors to measure concentration, a light scattering detector to measure absolute molecular weight and an online differential viscometer to measure intrinsic viscosity and size. The system allows the absolute Mw, oligomeric state, purity and size of a monoclonal antibody to be measured in a single injection. This talk presents a series of comparisons of innovator drugs with their respective biosimilars. Differences are revealed by the light scattering detector which were not visible using just a single concentration detector. Additionally, these measurements show increased susceptibility to stress testing in the biosimilar product. This technique therefore allows biosimilar manufacturers to develop and produce biosimilar drugs which more closely resemble the innovator, for presentation in any required filings.

O9

PASSIVE TARGETING OF GEMCITABINE HCL USING POLY(ε-CAPROLACTONE) NANOPARTICLES FOR CANCER TREATMENT

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A highly potent and hydrophilic anticancer drug gemcitabine HCl (GEM) has very short blood residence time and serious side effects. Novel, scalable, low cost, and biocompatible drug carrier system was designed to deliver GEM efficiently and safely as a candidate for various cancer treatments. Double emulsion solvent evaporation technique was developed and optimized for preparation of GEM loaded poly(ε-caprolactone) (PCL) nanoparticles. Obtained nanoparticle suspension was centrifuged 3 times at 10000 rpm, for 30 min at 15 °C to avoid PVA residue and free GEM. Before GEM loading studies, blank PCL nanoparticles were tested on L929 cells using (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) assay to evaluate cytotoxicity of nanoparticles and determine the subtoxic concentration. Zeta potential, particle size and polydispersity index (PDI) values of PCL nanoparticles were found -13,92±1,19 mV, 197,2±1,1 nm and 0,079±0,01 nm, respectively. Entrapment efficiency of PCL nanoparticles was found 38.74%. In a concentration range of 0,5-64 µg/ml, relative % of L929 cell viability for 24h and 48h ranged from 97,12±9,97-81,48±2,13% and 83,55±2,88-57,88±5,76%, respectively. According to the results, PCL nanoparticles were found to be non-toxic to L929 cells. Functionality studies of GEM loaded PCL nanoparticles on human cancer cell lines are evaluated. GEM loaded PCL nanoparticles would provide long circulation time in blood, passive targeting due to enhanced permeation and retention effect and lower side effect profile than commercial product.

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O10

NANOCARRIER-MEDIATED CO-DELIVERY OF PEMETREXED AND MIR-21 ANTISENSE OLIGONUCLEOTIDE FOR GLIOBLASTOMA TREATMENT

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Combination of miRNA-based therapy and chemotherapy is a promising strategy to overcome multidrug resistance in cancer [1]. In this study, a chemotherapeutic agent pemetrexed was combined with miR-21 antisense oligonucleotide (anti-miR-21) in different types of nanoparticular systems for treatment of glioblastoma, the most aggressive type of brain tumor. In this regard, lipid-polymer hybrid nanoparticles (LPNs), PEGylated nanoparticles and cationic solid lipid nanoparticles (cSLNs) were developed. Nanoparticles were well characterized by particle size distribution and zeta potential measurements, determination of encapsulation efficiency and in vitro release experiments. Spherical morphology of nanoparticles were determined by transmission electron microscopy. Nanoparticle sizes were measured as 93.9 ± 1.8 nm, 53.1 ± 3.7 nm and 124.9 ± 1.6 nm for LPNs, PEGylated nanoparticles and cSLNs, respectively. Nanoparticle formulations sustained the release of pemetrexed up to 8 h. Cell culture studies were performed using U87MG human glioblastoma cells. Compared with free pemetrexed solution, efficient encapsulation of pemetrexed into the nanoparticles increased its cellular uptake by 12, 9.2 and 9.9 times for LPNs, PEGylated nanoparticles and cSLNs, respectively. The lowest cell viability was obtained with LPNs. As a result of confocal microscopy analysis, nanoparticles were observed both in the nucleus and cytoplasm of U87MG human glioblastoma cells. These findings suggest that combination of pemetrexed and anti-miR-21 in a nanoparticular system represents a potential new approach for glioblastoma therapy.

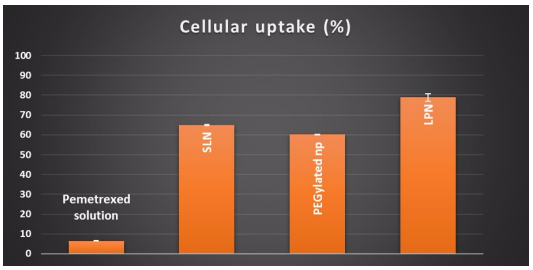

Figure 1.

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O11

DESIGN AND IN VITRO/IN VIVO EVALUATION OF SMEDDS FOR LYMPHATIC TARGETING ANTICANCER PEPTIDE, LYP-1

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The purpose of the present work was to evaluate the cytotoxicity and bioactivity of designed liquid SMEDDS formulations of the anticancer peptide, cyclic LyP-1 for the treatment of breast cancer. Optimized formulations were prepared with Labrasol®, Gelucire® 44/14, Labrafil®, D- tocopheryl polyethylene glycol succinate (TPGS) and Tween 80 as surfactants; PeceolTM and MaisineTM as oil phase and polyethylene glycol 300 and propylene glycol as cosolvents. The cytotoxicity and bioactivity of the formulations were evaluated in Caco-2, MDA-MB-231 and 4T1 cell lines by MTT assay. Permeability studies were carried out with Caco-2 cell monolayer as a model of intestinal barrier. Uptake studies were performed with MDA-MB-231 and 4T1 cell lines with fluorescein labeled peptide, alone and incorporated into novel SMEDDS formulations, to evaluate concentration dependent manner. The potential antitumor activity of peptide alone, in combination with doxorubicin hydrochloride (Dox HCl), within the formulations or in physiological saline solution were investigated with metastatic breast cancer model in 6-8 weeks old female BALB/c mice bearing 4T1 allografts and tumor sizes were measured during treatment. The droplet size of the optimized formulations was found between 17.84±2.24 nm with 0.21±0.05 PDI after two weeks of stability assessment. The apparent permeability coefficient (Papp, cm/s) of the peptide in U9-F21 formulation was found to be higher than the peptide solution ($p = 0.00005$). The %tumor volume change was observed to be statistically significant with the treatment group of Dox HCl in combination with LyP-1 in SMEDDS formulation ($p<0.05$).
Acknowledgement: This project is supported by TUBITAK (SBAG 1002-113S569).

O12

EVALUATION OF IMT LABELING WITH ZR-89 (OXINE) AS NEW PET RADIOPHARMACEUTICALS

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The clinical value of radiopharmaceuticals can be improved by introducing patient selection strategies based on noninvasive whole body imaging techniques such as positron emission tomography (PET) [1]. Thus a broad method to radiolabel and track chemotherapeutics such as imatinib (IMT) with PET radionuclides will have wide impact in cancer diagnosis. IMT is used to treat certain types of leukemia and gastrointestinal stromal tumors [2,3]. Here, we aim at radiolabeling of IMT with Zr-89 for cancer diagnosis and introduce simple and efficient PET radiolabeling method for IMT to achieve excellent radiolabeling purities and stabilities with Zr-89. Human serum stability, sterility and pyrogenicity of IMT-Zr-89 were estimated. The versatility, efficiency, simplicity of this labeling method was enable for IMT with high radiochemical purity (>%90) and the labeled compound was stable in human serum. IMT-Zr-89 was sterile and pyrogen free. Labeling method and IMT-Zr-89 may have clinical impact by facilitating the introduction of image-guided therapeutic strategies in current and future radiopharmaceuticals clinical studies.

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O13

THE INVESTIGATION OF DNA BINDING PROFILES OF GOLD NANOPARTICLE BOUND LIGNAN SPECIES CALLED SECOISOLARICIRESINOL USING SPECTROPHOTOMETRY AND SPECTROFLUORIMETRY

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Connection of lignan species namely secoisolariciresinol to gold nanoparticles modified by β-cyclodextrine were achieved and interactions of pure and bounded form of secoisolariciresinol with double strand DNA were investigated using with spectrophotometric and spectrofluorimetric methods in different pH, ionic strength and temperature. In spectrums obtained from spectrophotometry, there is an increase in absorbances, batochromic shift in bounded form and hypsochromic shift in pure form. Beside this, an isosbestic point was observed and binding constant was changed in different ionic strength. In spectrofluorimetric measurements, a quenching (was observed with increasing in DNA concentrations. Also, Ksv values are changed in different ionic strength. These findings are shown that there is a binding between double strand DNA and secoisolariciresinol and this binding is occurred both intercalation and groove binding way [1].

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O14

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION OF SOME CAPSAICINOIDS FROM DIFFERENT CULTIVARS OF CAPSICUM ANNUUM PRIOR TO THEIR DETERMINATION BY HPLC

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Dispersive liquid-liquid microextraction (DLLME) was used prior to HPLC for the extraction of three major capsaicinoids in pepper (i.e., capsaicin, dihydrocapsaicin and nordihydrocapsaicin). Optimum extraction conditions were: 100 µL chloroform (extraction solvent), 1.25 mL acetonitrile (disperser solvent) and 30 s extraction time. The analytes were back-extracted into 300 µL of 50 mM sodium hydroxide in methanol 45/55% (v/v) solution within 15 s for injection into HPLC. A reversed-phase column (Agilent Zorbax SB-Aq, 4.6 x 150 mm, 5 µm) was used for separating the analytes using a mobile phase consisting of 55/45% (v/v) methanol/0.5% (v/v) acetic acid at 25 °C and a flow rate of 1.2 mL/min, an injection volume of 5 µL. The analytes were monitored using a diode array detector (DAD) at 280 nm. Average enrichment factors were in the range of 4.4 to 10.2 and limits of detection ranged from 8.7 to 18.5 mg/kg. Calibration graphs showed good linearity with coefficient of determination (R^2) higher than 0.9930 and relative standard deviation (%RSD) lower than 6.9 and 7.8% for intraday and interday precision, respectively. Standards of the three capsaicinoids were isolated using reversed-phase medium pressure liquid chromatography (MPLC) and were characterized by LC-MS and 1D- (^{1}H- and ^{13}C-NMR) and 2D-NMR (COSY, HSQC and HMBC). DLLME-HPLC was applied to six capsicum samples with an average recovery of 48.7%. The proposed method was proven to be simple, rapid and efficient for the isolation and preconcentration of capsaicinoids from different cultivars of Capsicum annuum.

O15

SIMULTANEOUS DETECTION OF E. COLI AND SALMONELLA BASED ON FLUORESCENCE TECHNIQUE IN A PASSIVE TYPE MICROFLUIDIC CHIP

Uzeyir Dogan[1], Esin Nagihan Kasap[1], Ferah Cogun[2], Ender Yildirim[2], Demet Cetin[3], Zekiye Suludere[4], Ismail Hakki Boyaci[5], Nusret Ertas[1], Ugur Tamer[1]

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The detection and quantification of microorganisms is vital for human life in many fields such as diagnosis, medicine, pharmacy, food industry etc.[1]. Classical bacteria detection methods can give results in 12-48 hours which is very time consuming. Moreover, this procedure should be applied for the detection of each type of bacteria [2]. In this study, a rapid, sensitive and specific fluorescence based method has been developed by using magnetic nanoparticles and quantum dots (QDs) to enumerate Escherichia coli (E.coli) and Salmonella, simultaneously. Iron oxide core-gold shell (Fe_3O_4@Au) magnetic nanoparticles were synthesized and modified with biotinylated antibodies specific to target bacteria. Fluorescence labels have been constructed with biotinylated antibodies after chitosan coating of CdTe QDs and CdTeNi QDs. This novel method is applied to tap water and lake water samples for multiplex bacteria detection with a total analysis time less than 60 minutes, successfully.

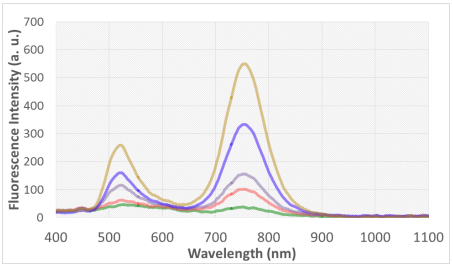

Figure 1. Fluorescence spectra obtained with different initial bacteria concentrations

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O16

OLIGOSACHARRIDES RELEASED FROM MILK GLYCOPROTEINS BY A NOVEL N-ACETLYGLUCOSAMINIDASE ACT AS SELECTIVE PREBIOTIC SOURCE FOR BIFIDOBACTERIA SPECIES

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Milk, in addition to nourishing the neonate, provides a range of complex glycans whose construction ensures a specific enrichment of key members of the gut microbiota in the nursing infant, a consortium known as the milk-oriented microbiome. Milk glycoproteins are thought to function similarly, as specific growth substrates for bifidobacteria common to the breast fed infant gut. However, the exact mechanism of this growth enrichment by glycoproteins is unclear. Recently,we isolated a cell wall-associated endo-β-N-acetylglucosaminidase (EndoBI-1) found in various infant-borne bifidobacteria was shown to remove a range of intact N-linked glycans. Here, EndoBI-1 was used to release N-glycans from concentrated bovine colostrum at the pilot scale. EndoBI-1-released N-glycans supported the rapid growth of Bifidobacterium longum subsp. infantis, a species which grows well on human milk oligosaccharides, but did not support growth of Bifidobacterium animalis subsp. lactis, a species which does not. Conversely Bifidobacterium longum subsp. infantis did not grow on the deglycosylated milk protein fraction clearly demonstrating that the glycan portion of milk glycoproteins provides the key substrate for growth. Mass spectrometry-based profiling revealed that B. longum subsp. infantis consumed 73% of neutral and 92% of sialylated N-glycans, while B. animalis subsp. lactis only degraded 11% of neutral and virtually no (<1%) sialylated N-glycans. These results provide mechanistic support that N-linked glycoproteins from milk serve as selective substrates for the enrichment of infant-borne bifidobacteria capable of carrying out the initial deglycosylation.

O17

EFFECT OF PYRROLIDINE DERIVATIVES BEARING INDOLE RING AS SUBSTITUTED MOIETY ON COX2 ON MCF-7 CELLS

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The pyrrolidine and indole ring system are important skeleton for preparation and modification of bioactive molecules which display as HIV integrase inhibitors, antitumoral, anti-tuberculosis and anticonvulsant properties. Thus the combination of the two ring system into compounds; 1a and 1b were attractive, we studied their effects on COX2 on the human breast cancer MCF-7 cells. The relationship between inflammation and cancer development was revealed in last decades and various studies point out the correlation between the expression of COX2 with existing clinical markers in breast cancer [1]. The corresponding pyrrolidine derivatives bearing indole ring were synthesized according to literature procedure [2] and characterized by NMR, FT- IR, MS and an X-ray crystal structure analysis. The synthesized compounds were tested for cytotoxicity MCF-7 cancer cell lines. After 24 and 48 h of treatment of 1a and 1b, COX2 enzyme activity was determined according to the manufacturer's instruction (Shanghai Sunred Biological Technology Co.,Ltd.). COX2 enzyme activity was decreased in 1b 24h group compared with the Control 24h groups ($p<0.05$). Although COX2 was decreased in 1a 24h group compared to Control 24h, unfortunately, this was not significant. COX2 over expression was found in many cancer types and COX2 selective inhibitors are one of the strategies in cancer treatment. Compound 1b may be considered as a promising anti-inflammatory agent in breast cancer.

This study was supported by Mersin University, Research Foundation with Project Number BAP 2015-AP2-1342

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O18

THE RELATIONSHIP BETWEEN OSTEOCALCIN/ADIPONECTIN LEVELS AND INFLAMMATORY MICROENVIRONMENT IN COLORECTAL CANCER PATIENTS

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Colorectal cancer is one of the most common malignancies worldwide and the epidemiological studies report that colorectal cancer renders 9% of cancer related mortalities [1]. The relation between inflammation and cancer has long been reported in several studies. In recent years several studies have focused on effects of osteocalcin and adiponectin on development of cancer [2]. At present study, we aimed to evaluate the relation of serum Gla-OC, Glu-OC and adiponectin levels with inflammatory microenvironment of patients with colorectal cancer. 114 patients who were diagnosed as colorectal cancer and 98 healthy volunteers as control were included for this study. Elisa method was performed for measurement of plasma Gla-OC, Glu-OC, adiponectin, interleukin-1α and interleukin-6. The mRNA expression of PLA_2 were analyzed by Real Time PCR technique. The correlation analyses between plasma levels of osteocalcin and IL-6, $cPLA_2$, $sPLA_2$ and interleukins were also performed. The results showed that adiponectin levels were significantly decreased in patients group ($p<0.05$). We also observed that patients had significantly higher Gla-OC levels when compared to control ($p<0.01$). The increase of Gla-OC levels were also correlated with in the increase of IL-6 and PLA-2 levels in patient group. The suggested relationship between inflammation and cancer based on the epidemiological and experimental data [3,4] has been verificated via determining the efficiency of anti-inflammatory treatment on prevention and management of cancer [5]. In conclusion, our results showed the relation between Gla-OC levels and inflammation in colorectal cancer patients. The further studies are required to explain the underlying mechanisms of the relation.

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O19

HSP70 INHIBITOR PES, DOWN-REGULATE SEVERAL CANCER-RELATED GENES ON BREAST CANCER CELLS

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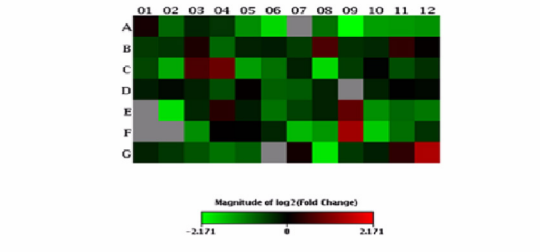
Heat shock protein 70 (Hsp70) is marginally expressed in healthy cells, but is aberrantly expressed in many types of tumor cells, helping tumor cells to survive in hard conditions [1, 2, 3]. This protein plays crucial roles in many processes related to poor prognosis in cancer treatment, including inhibition of apoptosis in tumor cells, resistance to chemotherapy, metastasis and, invasion. Thus, specific inhibition of Hsp70 in tumor cells is an important strategy in the treatment of cancer [4]. The present study aimed to evaluate quantitatively changes in cancer- and HSPs- associated gene expressions of 2-phenylethyenesulfonamide (PES) treated human breast cancer MCF-7 cells. Cell viability was evaluated by XTT assay. After PES administration, total RNA was isolated from MCF-7 cells and cDNA was synthesized using RNeasy Plus Mini Kits and RT^2 First Strand Kit respectively according to manufacturer's instructions. Pathway-focused gene expression profiling of MCF-7 cells was analyzed with the RT^2 Profile PCR Array System. According to our results, PES treatment dramatically inhibited cell proliferation of MCF-7 cells time- and dose-dependent manner. The IC_{50} value for the PES was calculated as 7,93 µM. PCR Array results revealed that seventeen cancer-associated genes were low expressed, while one gene was overexpressed. Besides, one Hsp- associated gene was low expressed, and one gene was overexpressed following PES administration. In conclusion, our results indicated that PES treatment significantly inhibit MCF-7 cell proliferation and suppress several cancer-related genes. However, further studies are needed to be able to utilize PES and PES derivatives in breast cancer treatment.



Comparison of the heat map between PES group and control group cancer-related PCR Array

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O20

EFFECTS OF VARYING DEGREES OF BLADDER OUTLET OBSTRUCTION ON URINARY BLADDER FUNCTION OF RATS: A NOVEL LINK TO INFLAMMATION, HYPOXIA AND OXIDATIVE STRESS

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An experimental animal model, partial bladder outlet obstruction (PBOO) mimics benign prostatic hyperplasia (BPH) development in men[1]. This study investigated the effects of the different degrees of obstruction and the roles of inflammation, oxidative stress as well as hypoxia parameters on bladder function. A total of 30 male Sprague-Dawley rats were divided into 3 groups equally: 1) Sham-operated control, 2) severe, and 3) moderate PBOO. PBOO was induced by urethral ligation using different-sized (3F and 4F) catheters for 6 weeks. The contractile responses of bladder strips were measured in vitro bath studies. The mRNA and protein expression of nuclear factor-κB (NF-κB), hypoxia inducible factor (HIF) and nuclear factor erythroid-2-related factor 2 (Nrf2) were determined by quantitative real-time (qRT)-PCR and Western blotting. Malondialdehyde (MDA) levels were measured with assay kits. The severe obstructed rats had the highest bladder weight. The detrusor strips from severely obstructed bladders exhibited 61-82% smaller contractile responses than those from sham-operated bladders. An inflammatory marker, NF-κB activity was increased in severe obstruction, while both HIF-1α and HIF-2β subunits were increased significantly in moderate obstruction. An oxidative stress indicator, Nrf2 expression was increased in all obstructed rats. MDA levels in severe obstruction were higher than the sham-operated group. Current results reveal the importance of oxidative stress-induced NF-κB signaling in bladder dysfunction with severe obstruction. Altered HIF signaling may contribute to the functional impairment after PBOO. Novel and evolving therapies targeting of oxidative and/or inflammatory pathways can be a reasonable strategy for the management of BPH.

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O21

THE EFFECTS OF SOME CYTOKINE GENE POLYMORPHISMS ON TYPE 2 DIABETES AND ITS COMPLICATIONS

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Diabetes Mellitus (DM) is a hyperglycaemic metabolic disease related with carbohydrate, protein and lipid metabolism dysfunction due to the decrease of insulin secretion. There are lots of genetic and environmental factors playing role in the development of the diabetic complications. In recent studies, it has been showed that, there is a relationship between the inflammation generation and diabetic complications. The inflammation induced-activation of the monocytes enhances the insulin resistance and decrease the insulin secretion due to the impairment of the pancreatic beta cells. Oxidative stress occurred after the disorder of the lipid metabolism also affects this fact. Following the formation of the oxidative stress, the levels of the reactive oxygen species (ROS) increase and insulin resistance develops consequently. Cytokines are neuromediators important in regulation of the homeostatic mechanisms including inflammation and tissue repair. Thus, variations in their levels and structures are the reasons for the occurrence of various diseases. The single nucleotide polymorphisms (SNPs) formed on the cytokine genes affect gene expression levels increasing the sensitivity to the diseases. Studies pointed out that there may be a possible relationship between TNF-α, IL-1β, IL-6 and IL-18 cytokine gene polymorphisms and the development of complications in patients with diabetes. Therefore this study is designed for searching and the evaluation of the possible relations between the TNF-α (-308), IL-1β (+3953), IL-6 (-174) and IL-18 (-607) gene polymorphisms and the development of the complications in a Turkish patient population with Type 2 diabetes by using PCR-RFLP method.

O22

EFFECTS OF D-LIMONENE AGAINST COMPLICATIONS OF DIABETES IN RATS

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It is known that diabetes caused some complications including alterations in lipid profile and hepatic enzyme levels and also oxidative stress. Limonene, a major component of Citrus oils, has important health beneficial effects due to its antioxidant activity. The aim of this study was to investigate the effects of D-limonene on streptozotocin (STZ)-induced diabetes in Wistar albino rats. For this purpose, DNA damage was evaluated by alkaline comet assay. Catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) activities and 8-hydroxy-2'-deoxyguanosine (8-OHdG), total glutathione (GSH) and malondialdehyde (MDA), insulin, total bilirubin and BCA protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol and triglyceride levels were also evaluated. D-limonene treatment was found to significantly decrease DNA damage, GR enzyme activities and MDA levels and significantly increase GSH levels and CAT, SOD and GSH-Px enzyme activities and altered lipid and liver enzyme parameters in diabetic rats. According to our results, it seems that D-limonene might have a role in the prevention of the complication of diabetes in rats.

O23

HISTOPATHOLOGICAL EXAMINATION OF THE EFFECTS OF SILYMARIN ON VANCOMYCIN-INDUCED NEPHROTOXICITY IN RATS

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Glycopeptide antibiotic Vancomycin (VCM) is widely used for the treatment of serious diseases caused by methicillin-resistant Staphylococcus aureus (MRSA) [1]. VCM has considerable nephrotoxic side effects which may limit its dose and duration of administration [2]. Silymarin (SLY) has strong free radical scavenging properties [3]. The aim of this study was to evaluate the effects of SLY on VCM-induced nephrotoxicity in rats histopathologically. Approval for the study was obtained from the Animal Experiments Local Ethics Committee in Mersin University (2016/HADYEK/E.98180). Adult male Wistar rats were randomly divided into seven groups, as follows: (i) Control group: saline was injected intraperitoneally (i.p.) at a dose of 2 ml for 8-day, (ii) DMSO group: DMSO was injected at a dose 0.5 ml i.p. for 8-day (iii) VCM group: VCM was injected at a dose of 400 mg/kg i.p. for 7-day, (iv) SLY group: SLY dissolved in DMSO was injected at a dose of 100 mg/kg i.p. for 8-day, and VCM plus SLY50 (v), SLY100 (vi), and SLY200 (vii) groups: VCM plus SLY injected at a dose of 50, 100, and 200 mg/kg i.p. for 8-day, respectively. On 9th day, all the rats were sacrificed under anesthesia. The kidneys were fixed in 10% formaldehyde, sampled and embedded in paraffin for light microscopic evaluation. Consequently, VCM administration caused histopathologically pronounced damage in the kidneys. The histopathological damages were reduced in the VCM plus SLY treatments.

This study was supported by the Research Fund of Mersin University in Turkey with Project Number 2016-2-AP3-1906.

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O24

THE SEROTONIN 2A RECEPTOR GENE -1438A/G POLYMORPHISM AND SERTRALINE INDUCED NAUSEA IN MAJOR DEPRESSED TURKISH PATIENTS

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Major depressive disorder (MDD) is the most common mental disorder and approximately 350 million people worldwide suffer from this disease [1] . Selective serotonin reuptake inhibitors (SSRIs), including sertraline, are a class of antidepressants, which are commonly used in the treatment of the MDD [2] . Despite, SSRIs are associated with less adverse drug reactions, nausea/vomiting is observed 20-46% of the MDD patients [3, 4] .There are different mechanisms for the occurrence of nausea/vomiting. Serotonin receptor polymorphisms might be one of these factors. Within this context, the purpose of this study was to evaluate relationship between the Serotonin 2A receptor (HTR2A) gene, -1438A/G polymorphism and sertraline induced nausea. A total of 65 patients participated to this study. The -1438A/G polymorphism was analysed by using PCR-RFLP techniques. In the full sample, HTR2A gene -1438A/G allele frequencies were A=0.47 and G=0.53. Genotype frequencies did not differ significantly from Hardy-Weinberg equilibrium (p=0.877).The genotype frequencies in the patients who experienced nausea/vomiting were: 1(7.69%), 7(53.85%), 5(38.46%) for AA, AG and GG genotypes respectively and those who did not experience nausea/vomiting were as follows: AA genotype 13(25.00%), AG genotype 26(50.00%) and GG genotype 13(25.00%). We did not find statistically significant difference between patients with and without nausea in genotypic distribution associated with the -1438A/G polymorphism of the HTR2A gene (p=0.340, X^2=2.157, d.f.=2). Our results demonstrate that HTR2A gene, -1438A/G polymorphism may not be associated with nausea/vomiting in MDD patients treated with sertraline.

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O25

DESIGN AND DISCOVERY OF NOVEL MELATONIN ANALOGUES AS CYP1B1 INHIBITORS

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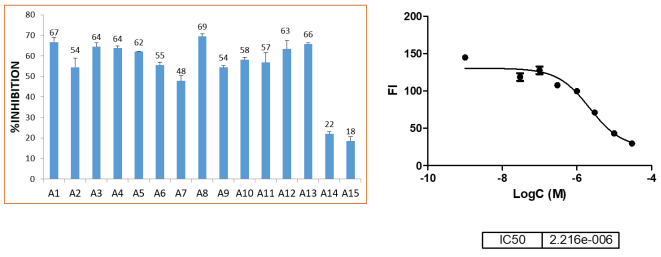
CYP1B1 is one of the main extra-hepatic enzymes which involves in the metabolic activation of procarcinogens. CYP1B1 also plays an important role in the metabolism of endogenous substrates such as estrogen which is converted into its reactive metabolite, 4-hydroxyestradiol. This reactive metabolite can bind covalently to adenine or guanine on DNA which may lead to mutations and breast cancer initiation. Natural indolic compound melatonin is reported to have inhibitor activity on bioactivating CYP1 enzymes, but its short half life as a result of its rapid metabolic inactivation is an important limitation in its therapeutic use. This drawback can be conquered by designing and synthesizing MLT analogues with longer half life than MLT. In the present study we have synthesized series of indole hydrazone derivatives, their docking positions were evaluated by molecular modelling and their potential inhibitory effect on CYP1B1 activity was further investigated via EROD assay. The results indicated that newly synthesized compounds strongly inhibited hepatic microsomal CYP1 activity. This result was confirmed by molecullar modelling studies. The selectivity of the compounds were further evaluated by using recombinant human CYP1A1, CYP1A2 and CYP1B1 enzymes. Mono halogenated compounds potently inhibited all CYP isosymes tested, the selectivity was CYP1A2 < CYP1A1< CYP1B1. Position of the halogenation did not effect the inhibitor activity. Among mono halogenated derivatives, o-chloro substituted one was found to be more potent than alizarin, a known CYP1B1 inhibitor. In conclusion, some of the tested compounds seem to be promising candidates for preventing estrogen induced carcinogenesis mediated by CYP1B1.



Effect of compounds on the microsomal CYP1 activity and CYP1 inhibition curve of the compund A1

O26

NOTCH1 AND NOTCH3 SIGNALING DIFFERENTIALLY REGULATE POLARIZATION OF T HELPER SUBSET INVOLVED IN THE DEVELOPMENT OF MULTIPLE SCLEROSIS

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Multiple Sclerosis (MS) is an autoimmune disorder that occurs upon inflammatory damage to myelin sheath around nerve cells. Th1 and Th17 subsets of $CD4^+$ T cells (T Helper) are major adaptive immune cells involved in inflammation during the development of MS. Development and differentiation of Th1 and Th17 cells are regulated by the Notch family of trans-membrane proteins (Notch1, 2, 3 and 4). Interaction of Notch protein with cell bound ligands, Delta like (1, 3, 4) and Jagged (1, 2), causes cleavage and activation of Notch intracellular signaling pathway. We and others have shown that pharmacological inhibition of Notch activity impairs Th1 and Th17 differentiation. When a mouse model of MS, Experimental Autoimmune Encephalomyelitis (EAE), was examined in the presence of Notch inhibitors, a decrease in Th1 and Th17 polarization was coupled with a decrease in the development of EAE as compared to controls. Pharmacological inhibition of Notch prevents the activation of all four family members of Notch (1, 2, 3, and 4) as well as other type 1 trans-membrane proteins. Therefore, we have been investigating whether the observed effect of the inhibitor is due solely to inhibition of Notch activity and furthermore, which specific Notch family members are involved in MS development by regulating Th1 and Th17 polarization. Our recent data support the hypothesis that Notch1 and Notch3 differentially regulate Th1 and Th17 development as well as diseases dependent on these cell types.

O27

WHY DO TURKISH PEOPLE USE ELECTRONIC CIGARETTE (E-CIGARETTE)?

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Electronic cigarettes are the most common type of a category of products called electronic nicotine delivery systems (ENDS). E-cigarettes are designed to simulate smoking by heating a solution that typically includes nicotine, flavorings, and a delivery system like propylene glycol or glycerin, or both. The first commercialized product officially entered the marketplace in 2007, then evolved rapidly in the past years and this rapid evolution has been accompanied by dramatic increases in use prevalence in many countries among adults, especially adolescents. The market and usage prevelance also is in increase in Turkey. Once delivered to the markets, manufactures introduced e-cigarette as a replacement tool for quitting conventional cigarette smoke. In respects of being a tobacco quitting method, some studies proved that e-cigarette can help quite smoking but usually users of this product follows a new addiction of nicotine intake. In this study, aim of use of e-cigarette smokers and statistical relation of conventional cigarette quitting evaluated withtrying to understand and demonstrate the main reason of beginning this product and other related social and personal trends and choicesvia questioning 29 e-cigarette smokers between May 2016 - August 2017. A widespread debate continues about the possible individual and public health effects of e-cigarette, many questions that need to be clarified in order to evaluate the suggestion that ENDS users have less harmful effects rather than conventional smokers.

O28

DETERMINATION OF THE RESISTANCE GENOTYPES OF GLYCOPEPTIDE-RESISTANT ENTEROCOCCI COLLECTED IN GAZI UNIVERSITY HOSPITAL BETWEEN 2008-2017

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Enterococcus faecalis and Enterococcus faecium, the most clinically important species of Enterococci, are known to cause serious nosocomial infections such as endocarditis, sepsis, meningitis and urinary tract infections. Glycopeptide antibiotics such as vancomycin and teicoplanin are commonly used for the treatment of severe infections caused by Gram (+) bacteria, such as Enterococci. Vancomycin-resistant enterococci (VRE) have shown an increase in prevalence in recent years. A total of nine mobile gene clusters were discovered that mediate resistance to glycopeptides in enterococci, named vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM and vanN. Determination of the resistance genotypes is particularly important for the rapid and effective treatment of some severe infections. It will be beneficial for prevention of hospital-acquired infections. Aim of this study is to determine the glycopeptide resistance genotypes of VRE that were isolated in Gazi University Hospital during 2013-2017 using Polymerase Chain Reaction (PCR). 3.882 of 45.160 bacterial isolates (%8,596) were identified as Enterococci. 78 clinical isolates of the Enterococci (%2,009) were determined as VRE using the E-test method. DNA of the VRE isolates were extracted using the “boiling” method. 55 of the samples were investigated for vanA and vanB resistance genotypes using PCR. 46 isolates (83.6 %) were found to carry vanA, while only one (2 %) carried vanB genes.

O29

A SCREENING STUDY ON THE WOUND HEALING ACTIVITY OF RIBES SPECIES GROWING IN TURKEY

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Ribes species are shrubs, usually evergreen and represented by eight species in Turkey namely Ribes alpinum L., R. anatolica Behçet, R. biebersteinii Berl. ex. DC., R. multiflorum Kit. ex Romer & Schultes, R. nigrum L., R. orientale Desf., R. rubrum L., R. uva-crispa L. Among these species, R. anatolica is an endemic and R. rubrum is cultivated in Turkey [1-3]. This species are distributed mainly in the Northeastern Anatolia and known as “frenk üzümü, it üzümü or bektaşi üzümü” [4]. Although Ribes species are known especially due to their fruits with commercial importance, their leaves have also been used in the folk medicine because of various medicinal properties including skin diseases [5]. In the present study we aimed to assess wound healing activity of the methanol extracts obtained from the leaves of eight Ribes species growing in Turkey. To induce in vivo wound model, linear incision (6 cm length) and circular excision (6 mm diameter) wounds were created on the dorsal parts of the rats and mice, respectively [6]. The effects of the extracts on the rate of wound healing were evaluated by tensile strength and reduction in wounded area by comparing the results with the reference ointment containing Triticum vulgare extract. Histopathological and antioxidant assays were also conducted on the skin tissues. The results have shown that R. nigrum, R. multiflorum and R. anatolica displayed enhancement in the tensile stregth and reepithelialization indicating potential wound healing effect.

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O30

INVESTIGATION OF BURN AND WOUND HEALING EFFECTS OF HERBAL EXTRACTS

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Plants are used ethnobotanically by the people for wound and burn treatment. The aim of these studies is to develop formulations for the treatment of wound and burn clinically. In our country, raw drugs are usually supplied from natural plants. They are mostly delivered to the public by the seller of herbs and folk remedies. It is necessary to develop formulations containing herbal extracts for wound and burn treatment scientifically. Turkey is a country which has a rich diversity of medicinal and aromatic plants. In most of the studies, experiments were not designed well. Herbal extracts containing new pharmaceutical formulations are not developed. In our previous works several herbal extracts and natural source compound were studied. These studies were supported by Research Fund of Ege University. The extracts were obtained from herbs and analysis was done. Semi solid dosage forms were prepared by Hypericum perforatum, Alpinia officinarum, Momordica charantia extracts and silk sericin for the treatment of wound and burn. The formulations were applied to the burn and incision wound models in rats. Contact or thermal type burn was formed. The incision wound was formed on the dorsal side of rats, a caudally- based dorsal skin flap was drawn and pulled up by sharp dissection. Histological and biochemical analysis were done for evaluating the experimental results for burn or wound healing.

O31

ETHANOL EXTRACT OF AERIAL PART OF SALVIA HUBERI HEDGE EXHIBITED ANTIOXIDANT AND WOUND HEALING ACTIVITIES IN DIABETIC RATS

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The effects of ointment prepared with Salvia huberi extract that was topically applied on excisional and incisional skin wounds with experimental diabetes were studied in this work. Male Wistar albino rats weighting 200-250 g were used in this study (n:60). Rats were divided into 5 groups consist of 12 animals. A single dose of streptozotocin (45 mg/dl) was given to rats intraperitoneally to introduce diabetes. After 7 days of STZ injection, blood glucose levels above 250 mg/dl were accepted as diabetic. At regular time intervals blood glucose levels were measured for diabetes follow-up. Excisional wound was created with 1.5 cm and also incisional wound was created with 4 cm diameters in diabetic rats under anesthesia. Wounds were cleaned with saline solution and disinfected with Betadine. Glycol stearate, propylene glycol and paraffin were added in the ratio of 3:6:1 to prepare a simple ointment base. Extracts of 0.5 % and 1% of S. huberi were added respectively in mixtures to prepare the ointments and were topically applied to wounds. After the treatment period was ended, tissues were evaluated with macroscopic, histopathological and biomechanical analysis. NO, MDA and glutation levels were investigated as well. Macroscopic and biochemical studies revealed that both in excisional and incisional skin wounds, wound healing of S. huberi groups were statistically significant compared to control groups ($p<0.05$) according to dosage and time. According to histopathological results, treatment with ointment of S. huberi extract induced significant histological changes in diabetic and treated groups compared to control groups in the meaning of reepithelialization, granulation tissue thickness and angiogenesis . In conclusion, ointment prepared with extract of S. huberi has a healing effect on excisional and incisional diabetic wounds when histopathologically compared to control groups. It was also seen that S. huberi changed antioxidant levels. When all results were combined it can be said that healing ratio changes according to application of dosage and time.

This study was supported from Adiyaman University Scientific Research Center TIPFBAP/2015-0004.

O33

HYPERICUM SALSUGINEUM INHIBITS PROLIFERATION, MIGRATION AND COLONY FORMATION ABILITY OF TRIPLE-NEGATIVE AND ESTROGEN RECEPTOR-POSITIVE BREAST CANCER CELLS

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Hypericum species are pharmacologically bioactive natural products that have great impact on various types of cancers and their therapeutic effects have been extensively studied in the anti-cancer drug discovery field. Breast cancer is a very heterogeneous and multifactorial disease, therefore, the benefit from common treatment protocols vary among different types of disease. In this study, the effects of Hypericum salsugineum, a local endemic species that grow in Salt Lake, Turkey, were investigated on breast cancer cells for the first time. The anti-proliferative effects of Hypericum salsugineum methanolic extract on triple-negative MDA-MB-231 and estrogen receptor-positive MCF-7 cell lines were determined by using iCELLigence cell analysis system, enabling real-time and label-free monitoring of cell proliferation. As a result of this analysis, cell viability decreased in both cell lines and close IC_{50} values (~350 µg/ml) were obtained. Next, wound healing assay was performed to test the effects of Hypericum salsugineum on the migration capabilities of the cells, and the colony formation assay was performed to test the effects on the ability to colonize. 350 µg/ml Hypericum salsugineum significantly reduced the ability of cells to migrate and colonize on both cell lines. Our results demonstrated the inhibitory effects of Hypericum salsugineum on both triple-negative and estrogen receptor-positive breast cancer cells and may be evaluated as a potential therapeutic agent for breast cancer. Our ongoing efforts are focused on characterization and quantification of the compounds responsible for the inhibitory effect.

O34

EFFECTS OF URTICA DIOICA L. ON ENDOMETRIOSIS RAT MODEL AND COMPOUNDS ISOLATED FROM THE ACTIVE EXTRACT

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Leaves of Urtica dioica L. have been used to regulate menstrual cycle and for the treatment of rheumatism, cancer and stomachache in folk medicine [1,2]. The objective of this study was emphasized on the beneficial effect of U. dioica in the treatment of endometriosis. The effects of n-hexane, ethyl acetate and methanol extracts prepared from the aerial parts of U. dioica were investigated on experimental endometriosis model in rats in order to find scientific evidence for the folkloric use of this plant. Results showed that the methanol extract of this plant significantly decreased the volumes of endometriotic implants and cytokine levels when compared to the control group on surgically rat endometriosis model. Therefore, phytochemical studies were conducted on the methanol extract. Subsequently, methanol extract yielded two compounds, rutin and isoquercitrin by using chromatographic separation techniques. The results of the present study revealed that the leaves of Urtica dioica could be beneficial in the treatment of endometriosis. The effect could be partly attributed to flavonoids.

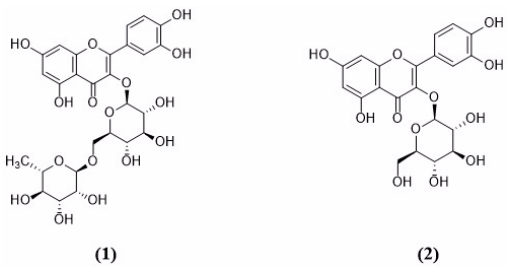

Figure 1. Isolated compounds from MeOH Extract of Urtica dioica

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O35

CHEMOTAXONOMICAL STUDIES OF PLANTAGINACEAE FAMILY

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In early 21th century Digitalis and Veronica which are formerly members of Scrophulariaceae have been moved to Plantaginaceae according to chemotaxonomic and phylogenetic studies [1]. The genus Digitalis has been found to be closely related to the genus Veronica and Plantaginaceae. Digitalis has been known for its cardioactive glycosides. But this group of compounds are not common in Veronica, Plantago or other Plantaginaceae family members. So they could not be used as a chemotaxonomic marker. Phytochemical studies showed that phenylethanoid glycosides (PGs) are common in all three genus. Although PGs could be found widely in other families, 3'-O-glucosyl caffeoyl glycosides have been found only in Plantaginaceae [2]. In our study, Digitalis, Plantago and Veronica genus were compared through their PGs. The aim of this research is to compare the phytochemical study results of Digitalis and Veronica with the Plantago genus which is a member of Plantaginaceae family. Results showed that all three genus were rich in PGs. Digitalis, Plantago and Veronica species have 28, 25 and 23 reported phenylethanoid glycosides respectively. Acteoside, isoacteoside and plantamajoside are common in all. Calceolarioside A and B, plantainoside D were found in Digitalis and Plantago. Cornoside, poliumoside, salidroside were found in Digitalis and Veronica. Our isolation studies on three different Digitalis species resulted in isolation of a total 11 phenylethanoid glycosides, 6 of them are 3'-O-glucosyl caffeoyl glycosides, and 8 different phenylethanoid glycosides from Plantago species, which are specific for Plantaginaceae. Our findings support chemotaxonomic and phylogenetic studies on Digitalis genus and Plantaginaceae family.

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O36

HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD APPLICATIONS FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF FLOWERING AERIAL PARTS OF HYPERICUM PERFORATUM

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The use of herbs increasingly continues to be a popular healthcare preference among patients and society. The genus Hypericum L. (Hypericaceae, Guttiferae) has been used as traditional remedy for eras. Among this genus, Hypericum perforatum L. (St. John's wort) is one of the significant medicinal plant and has got a widespread distribution in Turkey [1]. The flowering aerial parts of the plant (Hyperici herba), St. John's wort, is a well-known natural remedy to reduce the symptoms of the mild to moderate depressions [2]. However, antimicrobial [3], antibacterial [4], topical anti-inflammatory [5] and wound-healing [6] activities have been evidenced through scientific investigations. In European Pharmacopoeia, the chemical constituents in Hyperici herba are analyzed by thin layer chromatography (for qualitative analysis) and high performance liquid chromatography methods (for quantitative analysis). This study aims to develop a validated high performance thin-layer chromatography (HPTLC) method for quantitative and qualitative analysis of Hyperici herba materials as an alternative to those techniques described in European Pharmacopoeia. Quality assessment of Hyperici herba samples were assigned based on the concentration of three active markers, including hyperforin (phloroglucinol), hypericin (naphthodianthrone) and hyperoside (flavonoid) by HPTLC system. Three different mobile systems were used in HPTLC analysis; for hyperforin analysis hexane-ethyl acetate (8:2, v/v), for hypericin analysis toluene-chloroform-ethyl acetate-formic acid (8:5:3.5:0.6, v/v/v/v) and for hyperoside analysis ethyl acetate-formic acid-acetic acid-water (15:2:2:1, v/v/v/v). Developed methods were validated for each solvent system by sufficient repetition and also their reliabilities were practiced on purchased samples.

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O37

SYNTHESIS OF NEW DIFLUNISAL DERIVATIVES AS POTENT ANTI-HCV AND ANTICANCER AGENT

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It is evident that in hydrazide-hydrazone derivatives the -CO-NH-NH=CH- linkage is an essential structural requirement for biological activity. Hydrazones possess remarkable antibacterial, antifungal, anticancer, anti-HCV and antiviral activities [1]. Also, 4-thiazolidinone ring system is a core structure in various synthetic compounds displaying broad spectrum of biological activities [2], including NS5B RdRp inhibition [3] and anticancer effect [4]. Diflunisal derivatives had been synthesized in our laboratory exhibited antimicrobial [5], antiviral [6], anti-HCV [3] activities. It has been decided to synthesize new diflunisal derivatives in light of this information. We synthesized 2',4'-difluoro-4-hidroxy-N'-(arylmethylidene)biphenyl-3-carbohydrazides [3a-o] and 2',4'-difluoro-4-hydroxy-N-(2-aryl-4-oxo-1,3-thiazolidine-3-yl)biphenyl-3-carboxamide [4a-o] via diflunisal. The characterization of the synthesized compounds were identified by the, UV, IR, ^{1}H-NMR, ^{13}C-NMR, DART-MS and HR-MS spectral data while the purities of them were proved by elemental analysis, TLC and HPLC. As a result of biological activities, the compound 4a containing thiophen-2-yl substituent at C2 position of the 4-thiazolidinone ring, possessed anticancer activity on leukemia cell lines K562 and induced apoptosis via caspase activation pathway with IC_{50}=5.2 µM. Furthermore, the compounds were screened for their anti-HCV activity at 50 µM, the hydrazone compound 3b bearing 2-pyridyl moiety appeared the most promising with an EC_{50} of 3.9 and SI>25.6. Also, this compound exhibited promising cytotoxic activity against liver cancer cell lines with IC_{50} values of 10.00, 10.34, 16.21, 4.74, 9.29 and 8.33 µM for Huh7, HepG2, Hep3B, Mahlavu, FOCUS and SNU-475 cells respectively and produced dramatic cell cycle arrest at SubG1/G0 phase as an indicator of apoptotic cell death induction [7,8].

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O38

SYNTHESIS, CYTOTOXICITY AND DNA INTERACTIONS OF PLATINUM(II) COMPLEXES HAVING SUBSTITUTED BENZIMIDAZOLE LIGANDS

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Cisplatin is one of the well-known effective antitumor drugs with a wide spectrum of activity, such as testicular, ovarian, and head and neck cancers. Nevertheless, this drug induces nephrotoxicity, ototoxicity, and neurotoxicity [1]. Although common usage of cisplatin in the clinical treatment, new platinum complexes have been synthesized and tested for their antitumor activity to achieve its therapeutic benefits while lowering its toxicity [2]. In the previous studies, with the consideration that variations in the chemical structure of the "carrier-ligands" of the cisplatin can have a significant effect on the activity and toxicity of platinum complexes we synthesized some platinum complexes with the structure $[PtCl_2L_2]$ (L=2-substituted benzimidazole). It was determined that some of these compounds have in vitro cytotoxic activities on RD, HeLa and MCF-7 cancer cell lines. In this proposal, as an extension of the planned investigation into the antitumor activity of platinum(II) complexes with 2-substituted benzimidazole ligands, it is aimed to synthesize some new platinum complexes having 2-substituted benzimidazole ring as "carrier-ligands" and chloro and oxalato groups as "leaving-ligands". Synthesized platinum(II) complexes have been characterized and evaluated for their in vitro cytotoxic activities against the human HeLa cervix cancer cell line. The plasmid DNA interactions and inhibition of the BamHI and HindIII restriction enzyme activities have been also studied by using gel electrophoresis method. Some of new platinum complexes found to be noteworthy for further studies, due to moderate in vitro cytotoxic activity close to carboplatin.

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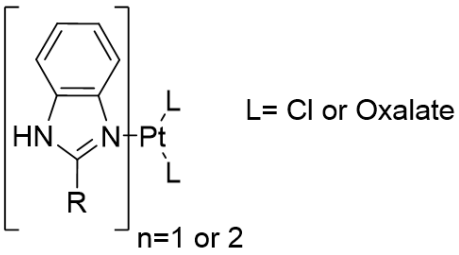

General structure of platinum complexes

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O39

DEVELOPING MULTI-TARGET INHIBITORS OF ARACHIDONIC ACID PATHWAY BASED ON THE FLAP INHIBITOR BRP-7

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Leukotrienes (LT) and prostaglandins (PG) are important lipid mediators produced from arachidonic acid (AA), which play important roles in the pathophysiology of chronic inflammatory diseases. LTs are formed by 5-lipoxygenase (5-LO) with the aid of 5-LO activating protein (FLAP) while microsomal PGE_2 synthase-1 (mPGES-1) acts as a terminal enzyme for the formation of inflammatory PGE_2. Therefore, these are promising biological targets for intervening with the AA pathway, and their simultaneous inhibition may result an increased therapeutic effect. Hence, there is a growing interest on developing multi-target inhibitors of AA pathway which may lead to balanced inhibition of both LTs and PGE_2 to obtain more effective anti-inflammatory drugs while minimizing the observed side-effects resulting from single inhibition of one of these targets [1]. In this presentation, we will present the evolution of selective FLAP inhibitor BRP-7, previously discovered by us [2], towards a new chemical class which is able to interfere with the biosynthesis of both LTs and PGE_2. To identify the pharmacophore groups which are responsible for inhibition of FLAP/5-LO and mPGES-1 activities, structure-activity relationship studies were performed at C(5) position of the BI core introducing polar substituents. Eventually, introduction of the 1,3,4-oxadiazole-5-thione group at C(5)-BI of BRP-7 resulted for potent inhibition of all three therapeutic targets in AA pathway. The obtained results showed for the first time that compounds with multiple ligand properties towards simultaneous inhibition of these three proteins may have potential for developing novel and safer anti-inflammatory drugs.

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O40

SYNTHESIS AND IN VITRO ANTICANCER ACTIVITIES OF NOVEL PYRROLO[2,3-D]PYRIMIDINE DERIVATIVES CONTAINING UREA MOIETY

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Pyrrolo[2,3-d]pyrimidine compounds have been reported to possess significant antitumor activities by inhibiting different targets such as epidermal growth factor receptor (EGFR) tyrosine kinase [1], Janus kinase (JAK) [2], mitotic check point protein kinase (Mps1) [3], carbonic anhydrase [4] and MDM-2 [5]. To date numerous heterocyclic scaffolds bearing urea moiety have been identified as potential anticancer agents [6-7]. In this study, a series of new pyrrolo[2,3-d]pyrimidines containing urea moiety have been synthesized and evaluated for their anticancer activities against cancer cell lines including A549, PC3, SW480 and MCF-7. Among the synthesized compounds, 9e, 10a and 10b exhibited the most potent cytotoxic activity with IC_{50} values ranging 0.19-4.86 µM against treated cancer cell lines. Our data also suggest that the cytotoxic activity of the 9e and 10b in A549 and MCF-7 cells might be mediated by apoptosis revealing a significant increase in the percentage of late apoptotic cells and causing cell cycle arrest in $G/G_1$and G_2/M phases, respectively. Additionally, western blot analysis regarding the expression levels of apoptotic and proapoptotic markers have also revealed the apoptotic efficiency of the compounds.
In conclusion, synthesized compounds displayed potent cytotoxic and apoptotic efficiency, thus they might be potential candidates for anticancer treatments.

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O41

COMPUTER AIDED DRUG DESIGN STUDIES ON BENZAZOLES ACTIVE AGAINST TOPO II ENZYME AS AN ANTICANCER TARGET

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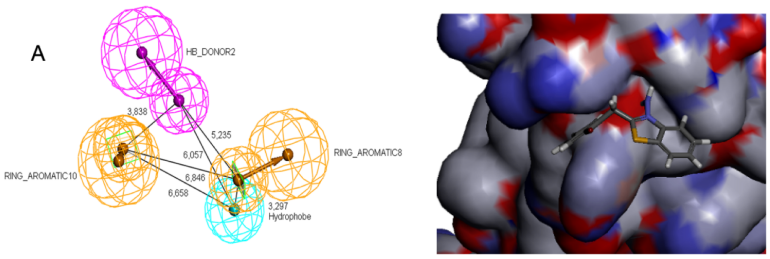
Rational drug design area covering the computer aided drug studies by which mostly therapeutically active drugs put into market [1]. Anticancer drugs gaining resistance to the drugs by the cancer cells is a rising problem for the last decade. DNA topoisomerases, which catalyze the interconversion of various topological states of DNA, were originally discovered to change the superhelical structure of closed circular DNAs. Since the activity of topoisomerases is essential for several cellular processes such as replication, transcription and chromosome condensation, they are widely used in anticancer drug development as one of the important targets[2]. Formation of protein-concealed DNA strand breaks, resulting in the stabilization by the drug of an intermediary complex of the Topo II reaction is mainly related to the antitumor activity. Our group has been working on the heterocyclic compounds, such as benzoxazoles, benzimidazoles and benzothiazoles and their DNA Topoisomerase II inhibitory activities in the recent years which some of them were found to be more active than the reference drug etoposide [3-5]. Herein, some applications of the molecular modeling studies such as docking and pharmacophore analysis as computational techniques were applied for the lead optimisation and generation of some benzazoles active against DNA-Topoisomerase II [6].



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O42

ACTIVATION OF LIVER X RECEPTORS PREVENTS CARDIAC FUNCTIONAL AND STRUCTURAL CHANGES IN DOCA-SALT HYPERTENSIVE RATS

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Liver X receptor (LXR) plays a critical regulatory role in metabolism and inflammation and involved in cardiovascular physiology/pathology. In the present study, the effect of LXR agonist GW3965 on cardiac functional and structural changes in DOCA-salt hypertensive rats was investigated. Hypertension was induced by unilateral nephrectomy and DOCA+salt treatment in male rats for 6 weeks. Blood pressure was measured by tail-cuff method. GW3965 was given by intraperitoneal injection last one week. At the end of treatment, right atrium (RA) and left papillary muscle (LPM) were isolated and contractions were recorded. Biochemical parameters were assessed in the plasma and left ventricle. Expression of various proteins such as markers of endoplasmic reticulum stress (ERS), inflammation, fibrosis and apoptosis were examined by Western Blotting. Histopathologic examination was performed in the left ventricle and the liver. GW3965 treatment reduced systolic blood pressure in hypertensive group. In calcium-free medium, noradrenaline-induced contractions and rhythmic activity of RA were similar in all groups but contractions of LPM were greater in hypertensive groups. While additional calcium-induced contraction of RA was lower, sinus rate of RA was higher in hypertensive group and GW3965 treatment reversed these responses. Plasma lipid levels increased whereas nitric oxide level unchanged with hypertension. GW3965 treatment elevated the plasma NO levels but did not affect plasma lipids. GW3965 treatment remarkably restored hypertension-induced structural remodeling but not hypertrophy and apoptosis. These data demonstrated that LXR stimulation by GW3965 attenuates systolic blood pressure and improves functional and structural changes induced by hypertension without affecting plasma lipid levels.

O43

APPLYING BEERS CRITERIA FOR ELDERLY PATIENTS TO ASSESS RATIONAL DRUG USE IN NORTHERN CYPRUS

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Several regulations for potentially inappropriate medications (PIMs) have been published that are designed specifically for elderly patients to rationalize drug use in such vulnerable communities. The aim of this study was to determine the rational use of prescribed drugs and to assess the prescribing patterns of various drugs in elderly patients and also to evaluate rational prescribing according to the Beers Criteria 2015 recommendations. A cross-sectional prospective analysis of 451 in-patients admitted to the hospital between September and October 2016 was conducted. Data were extracted from the patient medical records using special forms. The prevalence of potentially inappropriate medications (PIMs) prescribed to both hospitalized and discharged patients. 119 geriatric patients were identified (26.4%) and evaluated, of which 107 were eligible and 12 were excluded. Out of the 1,039 prescribed medicines, 16.9% were potentially inappropriate medications during hospitalization, while 10.6% were on discharge. The most prevalent PIM group during hospitalization was identified as the “medications to be avoided in older adults” (50%), while it also formed 38.1% of medications prescribed on discharge. PIMs of the class “drugs used with caution” formed 18.2% of prescribed medicine during hospitalization and 11.9% on discharge. The prevalence of polypharmacy was 79.4%, mainly identified as unpreventable polypharmacy of elders. A significantly higher prevalence of PIMs was observed in inpatients than at discharge, with a high prevalence of polypharmacy. These results necessitate a nationwide assessment and responsible bodies should act to reduce and adopt strategies that could reduce or overcome the aforementioned high prevalence in Northern Cyprus.

P1

STABILITY OF KOLLIDON® SR AND EUDRAGIT® RS 100 POLYMERIC NANOPARTICLES

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Polymeric nanoparticles based on biocompatible and biodegradable polymers are preferred for drug delivery owing to their property of being adsorbed intact in the gastrointestinal tract after oral administration [1]. Stability of polymeric nanoparticles are generally described as physical, chemical, long-term "pharmaceutical" and colloidal stabilities [2]. Objective of this study was to evaluate the stability of dexketoprofen trometamol-loaded Kollidon® SR and Eudragit® RS 100 polymeric nanoparticles. Polymeric nanoparticles were prepared by the spray-drying (Büchi, Nano Spray Dryer B-90) and stored at different temperatures (4±1°C, 25±1°C, 40±1°C) for six months [3]. Stability parameters studied within the scope of the study were: physical appearance, particle size, polydispersity index, zeta potential, SEM imaging, thermal analyses (DSC), entrapment efficiency (EE %), FT-IR and NMR spectroscopic analyses. Time dependent variation in EE % was calculated using a validated high performance liquid chromatography (HPLC) method. No significant difference ($p>0.05$) in dexketoprofen trometamol-loaded polymeric nanoparticles stored at 4±1°C, 25±1°C, 40±1°C was found after 6 months and also DSC, FT-IR and NMR analyses were found to be similar to the freshly prepared sample. The results demonstrated some variation in particle size of nanoparticles stored at 40±1°C. Results indicated that optimum temperature for long-term storage of the nanoparticles prepared is room temperature (25±1°C).

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P2

ELETRIPTAN HYDROBROMIDE-PLGA NANOPARTICLES: EFFECT OF FORMULATION VARIABLES ON SIZE DISTRIBUTION

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Eletriptan hydrobromide is a second generation triptan class drug designated as R-3-[(1-methyl-2-pyrrolidinyl) methyl]-5-[2-(phenyl sulfonyl) ethyl]-1hindole mono hydrobromide. It is used to treat migraine but not prevention. Eletriptan hydrobromide is freely soluble in water and methanol and well absorbed after oral administration [1]. Currently, eletriptan hydrobromide is available in oral formulations. Yet, due to problems like twice in a day administration and side effects tablet dosage form may not be the ideal route of administration. In order to enhance the bioavailability and reduce the side effects, it is beneficial to use prolonged release formulations or other routes. Eletriptan hydrobromide micro and nano-sized drugs have been developed as described in the literature aiming to apply the drug to various administration types [2]. The aim of this study is to optimize the formulation of PLGA nanoparticles, containing eletriptan hydrobromide, by trying to determine the factors affecting the physicochemical properties of the nanoparticles. The prepared nanoparticles were evaluated by particle size and polydispersity index (PDI). D-optimal response surface methodology was used perform the experiments. The effects of variations in the drug, polymer, surfactant concentrations, sonication time and energy were evaluated through changes in the size of the nanoparticles. 75 mg PLGA, 1 mg eletriptan hydrobromide, 10 mg polyvinyl alcohol with sonication time 30 sec and 30 W sonication energy was predicted as the optimal formulation with minimum polydispersity index. However encapsulation efficiency was found 6.70 ± 0.84% and this result can be enhanced by changing polymers, surfactants or preparation methods.

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P3

DIPYRON-LOADED POLYVINYLPYRROLIDONE (PVP) BASED POLYMERIC NANOPARTICLES: FORMULATION AND IN VITRO CHARACTERIZATION

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Acute and chronic pain occur as a result of tissue damage either accidentally because of an injury or in consequence of surgery. The treat of acute/chronic pain and inflammation is a crucial component of patient care [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most extensively used drugs worldwide owing to their efficacy in reducing inflammation and pain. Basic mode of pain relieving is inhibition of pro-inflammatory enzyme cyclooxygenase (COX) [2]. NSAIDs were used in clinical disorders such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, dysmenorrhea, dental pain and headache. Dipyrone (metamizole) is a NSAID with a rather short half-life [1]. PVP with varying molecular weights is a non-toxic and non-ionic polymer used in formulating nanoparticles. PVP molecule contains a highly hydrophilic component and a considerably hydrophobic group [3]. Preparing and characterizing dipyrone-loaded polymeric nanoparticles for oral controlled release analgesic delivery was aimed in this study. Spray-drying (Büchi B-190) was used as the preparation method and characterization was achieved by particle size/PDI and zeta potential measurements, DSC thermograms, SEM imaging, XRD, FT-IR and NMR spectroscopic analyses for the comparison of different types of PVP (MW: 10.000 and MW: 58.000).

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P4

PREPARATION AND IN VITRO CHARACTERIZATION OF MOXIFLOXACIN HYDROCHLORIDE-LOADED EUDRAGIT RL® NANOPARTICLES FOR OCULAR DELIVERY

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Moxifloxacin hydrochloride (MOX) is a fourth generation fluoroquinolone with enhanced activity in vitro against Gram-positive bacteria and maintenance of activity against Gram-negative bacteria. It is an effective agent useful in the treatment of ocular infection such as bacterial conjunctivitis, keratitis and keratoconjunctivitis [1]. Topical instillation is the most accepted and the most convenient route for ocular delivery. Poor ocular bioavailability compels frequent instillation to achieve the desired therapeutic effect leading sometimes to undesirable side effects. Efficacy of drugs is closely related to their ocular bioavailability where corneal penetration and precorneal residence time may be enhanced [2]. Several formulations were designed to improve ocular bioavailability through enhancing permeation, prolonging residence time, inserts and colloidal systems such as liposomes and nanoparticles [1-3]. The objective of the current study was to formulate and evaluate positively charged ocular nanoparticles of MOX using Eudragit RL® to overcome the problems mentioned above. Eudragit RL 100® is a suitable inert vehicle for ophthalmic drug delivery due to its capability of forming nanodispersion with rather small particle size, positive surface charge, good stability and biocompatibility [4]. Spray-drying was used for preparing nanoparticles and characterization was achieved by particle size/PDI and zeta potential measurements, DSC thermograms, SEM imaging, FTIR, XRD and NMR spectroscopic analyses. Conclusively, MOX could be incorporated successfully into cationic polymeric nanoparticles which seems to be a promising approach to prolonging contact time, enhancing ocular bioavailability and reducing dosing frequency and side effects.

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P5

ENTRAPMENT EFFICIENCY AND IN VITRO DISSOLUTION OF DIFFERENT POLYMER-BASED MOXIFLOXACIN HYDROCHLORIDE NANOPARTICLES FOR OCULAR DELIVERY

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Fluoroquinolones are one of the promising antibacterial chemotherapeutic agents for the treatment of infections of different etiology and localization in humans and animals [1]. Fourth generation fluoroquinolones with 8-methoxy substitution such as moxifloxacin hydrochloride (MOX) are used in ocular infections such as conjunctivitis, bacterial keratitis and keratoconjunctivitis [2]. Following drug encapsulation into nanoparticles which are promising vehicles, ocular absorption is enhanced significantly when compared to eye drop owing to the much slower ocular elimination rate of particles [3]. Smaller particles applied are better tolerated by patients and thus nanoparticles may represent comfortable ophthalmic prolonged release delivery systems [4]. In this study, Eudragit RS® 100 and Eudragit RL® 100 were used as polymeric ingredients to prolong ocular residence time resulting in sustained drug release pattern [5]. MOX-loaded polymeric nanoparticles were prepared by spray-drying method aiming prolonged drug release by increasing corneal residence time which may lead to extended antibacterial effect. MOX entrapment efficiency was calculated using a validated high performance liquid chromatography (HPLC) method. Release characteristics of the particles were evaluated with dialysis bag at 37°C ± 1°C in phosphate buffer solution (pH 7.4) during 24 hours. As a result, MOX release from nanoparticles in pH 7.4 phosphate buffer was much lower than its intact form dependent on encapsulation efficiency since drug release was found to decrease as the encapsulation efficiency increased.

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P6

DEVELOPMENT AND CHARACTERIZATION OF INDOMETHACIN AND CURCUMIN CONTAINING NANOFIBERS

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Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) [1]. Curcumin is antibacterial substrate isolated from Curcuma Longa [2]. The aim of this study was to develop and characterize nanofiber formulations containing indomethacin and curcumin for treatment of wounded skin. Gelatin nanofibers (25% w/v) were prepared in the absence and presence of indomethacin (1% w/w) and curcumin (1-2% w/w), employing flow rate of 5 µL/min with 25 kV voltage and 12-cm distance from needle tip and the collector. Nanofibers were then crosslinked with glutaraldehyde. DSC results showed that melting peaks of indomethacin and curcumin were disappeared [3, 4]. FTIR spectra showed that characteristic peaks of curcumin, indomethacin and gelatin were at same wavelength interval as reported in literature [5, 6]. SEM analysis demonstrated that nanofibers (approximately 500 nm) were smooth with no drug crystals. Tensile strengths indicated that 2% curcumin containing fibers were stronger than 1% curcumin, and gelatin containing fibers. MTT analysis showed that viability of fibers were between 92%-107%. Content uniformity of 10 mg fiber was 0.090±0.010 mg and 0.069±0.011 mg indomethacin; 0.052±0.009 mg and 0.080±0.005 mg curcumin for 1% and 2% curcumin containing fibers, respectively. Complete drug release was achieved within 24 hours for indomethacin, 48 hours for curcumin at PBS (pH 7.4). Nanofibers are successfully fabricated for treatment of wounded skin.

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P7

DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMES CONTAINING OVALBUMIN FOR NASAL DELIVERY

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In order to avoid hepatic first pass metabolism, enzymatic or acidic degradation of the active copmpounds, nasal route is an alternative to conventional routes. In addition to that, nasal vaccination is an emerging strategy to prevent both respiratory and other mucosal sites diseases[1,2]. The aim of this study was to develop an optimum nasal delivery system for ovalbumin. For this purpose we have developed ovalbumin containing liposome formulations using L-alpha-Phosphatidyl choline (from egg yolk) (egg-PC) and cholestrol (chol) which are composed of phospholipids. Liposomes of ovalbumin were prepared according to the film formation method. The resulting multilamellar liposomes were extruded 12 times back and four times through polycarbonate membranes to yield 100-200 nm liposomes, which is the size required for increased uptake from the nasal mucosa. The average mean particle size and zeta potential of the liposomal vesicles was measured using Zetasizer. Encapsulation efficiency was determined with an indirect method by determination of non-entrapped ovalbumin in liposome suspensions. Prepared liposomes were characterized for their morphology using transmission electron microscopy(TEM) and scanning electron microscopy(SEM). The developed liposomes have negative zeta potential(-5.33 mV ± 2.02), 200-250nm particle size and 73% entrapment efficiency. All of the prepared liposomes had a spherical shape and a rough surface in TEM image (Fig.1). The film formation method has been proven to be simple, reproducible and appropriate for encapsulation of ovalbumin for nasal delivery.

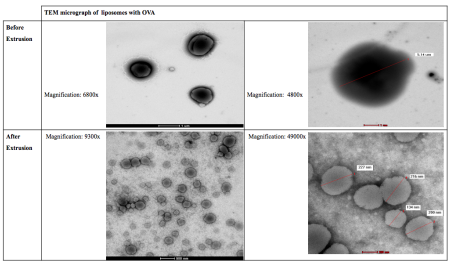

Figure 1.TEM Micrograph of Ovalbumin loaded liposomes before extrusion and after extrusion

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P8

DEVELOPMENT OF EMBRYONIC STEM CELL AND INSULIN-LOADED LIPOSOMES AND EVALUATION OF EFFICIENY ON PANCREATIC BETA TC CELLS

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Diabetes Mellitus (DM) is one of the most common diseases that occur globally and also a useful treatment for DM is cell replacement therapy [1,2]. Embryonic stem cells (ESCs) isolated from the inner cell mass, have the potential of self-renewal and to differentiate into various types of cells [3,4]. Liposomes (LPs) are biocompatible, non-toxic, can be formulated in nano size, and enhanced permeability [5]. In this study, we determinated ESC and insulin-loaded LPs effiencies on diabetic pancreatic beta TC cell line. LPs were prepared using dry film hydration method. For amounts of captured insulin and ESC, Lowry protein determination method was used. In additon, for ESC encapsulation efficiency, cell counting and Western Blot were performed. The type and characterization of LPs were determined. In cell culture studies, cytotoxicity test was carried out. Insulin solution with different concentrations and blank and insulin-loaded LPs were not found to be toxic to cells at any concentrations. In vitro insulin release and transport experiment from LPs were performed. We investigated the relationship between glucose and insulin concentration in diabetic cells incubated with glucose and streptozocin (STZ). After applying ESC and insulin-loaded LPs to diabetic cells for 48 hour, insulin levels were increased for both glucose and STZ-induced diabetic cell groups and difference with control group is insignificant ($p>0.05$).
It was concluded that ESC and insulin-loaded LPs may be used in the repair of pancreatic cells and this ESC treatment is a potential source for cell replacement therapy in the treatment of diabetes.

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P9

MULTIWALLED CARBON NANOTUBE-CHITOSAN SCAFFOLD: CYTOTOXIC, APOPTOTIC, AND NECROTIC EFFECTS ON CHONDROCYTE CELL LINES

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In the present study, we report on a composite preparation, involving the use of CNT(carbon nanotube)-chitosan as scaffold for bone repair and regeneration. Through the use of water-soluble tetrazolium salt (WST-1) and double staining methods, the cytotoxic, necrotic, and apoptotic effects of chitosan–multiwalled carbon nanotube nanocomposites on the chondrocyte ATTC cell line have been exhibited. WST-1 assay for cytotoxicity studies were performed by using chondrocytes cells in 12.5-200 µL concentration range. To predict the number of apoptotic and necrotic cells in culture, the technique of double staining with Hoechst dye was performed with PI on the basis of scoring cell nuclei. The mechanical properties such as tensile strength and elongation at break values of the chitosan only and chitosan/CNT scaffolds were evaluated on Texture Analyzer. Based on the results of the WST-1 assay procedure, the amount of cell viability was not significantly affected by nanocomposite concentrations and the lowest mortality rate of cells was obtained at a concentration of 12.5 µg/mL, whereas the highest mortality rate was obtained at a rate of 200 µg/mL. In addition, the effects of chitosan-CNT nanocomposites were not found to cytotoxic on chondrocyte cells. The apoptotic and necrotic effects of the combined compounds had varied within the concentrations. In a similar manner to the outcome of the control groups, apoptosis was obtained at a percentage of 2.67%. We also compared the strain-stress curve measurements results. The results indicated that the mechanical properties of scaffold were not different. Elongation at break values increased by addition of CNT.

P10

DEVELOPMENT OF SOLID DOSAGE FORMS WITH 3D PRINTING TECHNOLOGY

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3D printing is a layer-by-layer deposition process which is used to manufacture a 3D object utilizing computer aided design data[1, 2]. Pramipexole is a class 1 drug which used for the treatment of Parkinson's disease[3]. The aims of the present study are to prepare pramipexole loaded filaments by hot melt extrusion (HME) and the tablets by fused deposition modeling (FDM) 3D printing. In this study, pramipexole containing Soluplus®:Polyethylene oxide N80 polymer mixture was prepared in a 60:40 (%) ratio. The mixture was hot melt extruded using single screw extruder (Filabot Filament Maker, USA) at 120°C to generate filaments. The extruder and platform temperature of printer (MakerBot® Replicator_ 2X, USA) were set at 140°C and 30°C, respectively to fabricate 3D printed tablets with infill density of 100% and infill layer height of 0,1 mm using filaments. Images of the drug loaded filaments and the tablets were taken with a scanning electron microscope (SEM). USP-2 Paddle method was used for in vitro dissolution studies of the pramipexole loaded tablets. The test was conducted using 500 ml pH 1,2 buffer solution maintained at 37.0±0.5°C at a rotation speed of 75 rpm. Drug release at different time intervals was measured by UV-visible spectrophotometer at 265 nm. The layered structure of tablets and the smooth surfaced filaments were observed in SEM images (Figure 1A). In vitro dissolution studies showed that within two hours pramipexole was released from the tablets completely (Figure 1B). In conclusion, drug loaded filaments and tablets were successfully fabricated by HME and 3D printing process.

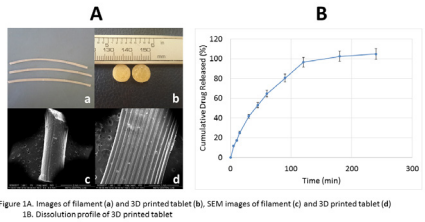

Figure 1A. Images of filament (a) and 3D printed tablet (b), SEM images of filament (c) and 3D printed tablet (d)
1B. Dissolution profile of 3D printed tablet

Figure

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P11

STABILITY STUDIES OF GEMCITABINE HYDROCHLORIDE LOADED LIPOSOMES AND NANOPARTICLES IN VARIOUS MEDIA

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Analysis of properties of nano-sized drug carrier systems in different physiological conditions is crucial for ex vivo and in vivo applications. Serum components, particularly proteins would alter the colloidal characteristics of nanocarriers such as particle size and zeta potential. The incubation time is also a critical factor to determine colloidal stability of particles [1]. The aim of this study was to evaluate colloidal stability of gemcitabine hydrochloride loaded liposomes and nanoparticles in various media. In our study, PEGylated liposomes and PEGylated nanoparticles were incubated at 37±1°C for 5 days with pH 7,4 phosphate buffer solution, 100% fetal bovine serum (FBS) and phosphate buffer solution containing 10% FBS. Periodically, the mean particle size, polydispersity index and surface charge were determined using the dynamic light scattering (DLS) technique in a Zetasizer Nano ZS (Malvern Instruments, UK). In pH 7,4 phosphate buffer solution media, the particle size of the liposomes and nanoparticles did not statistically change (p values >0,05) for a period of 5 days. The nanoparticle formulations showed the particle size stability for a period of 3 days, while the liposome formulations showed the particle size stability for a period of 2 days in 100% FBS media. In 10% FBS media the nanoparticle formulations had a size increase from 207±10 nm to 258±18 nm within 5 days of incubation, in contrast the liposome formulations had a size increase from 188±10 to 705±35 nm at the same incubation time. All formulations had negative zeta potential in all media.

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P12

ACCELERATED AND LONG-TERM STABILITY STUDIES FOR GEMCITABINE HYDROCHLORIDE LOADED LIPOSOMES AND NANOPARTICLES

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Liposomes and polymeric nanoparticles are two important classes of the nano-sized drug carrier systems [1]. The aim of this study was to investigate accelerated and long-term stability of PEGylated liposomes and PEGylated nanoparticles containing highly hydrophilic drug gemcitabine hydrochloride. The stability of gemcitabine hydrochloride loaded liposomes and nanoparticles was monitored at 4°C, 25±2°C for 12 months and 40±2°C for 6 months. Periodically, the samples were withdrawn and the particle size, polydispersity index as well as drug content (%) were determined. Also the surface morphology of the liposomes and nanoparticles were examined using Transmission Electron Microscopy (TEM) at different time intervals. Statistically, the particle size of the nanoparticles showed physical stability for a period of 9 months; however, the liposomes showed stability for just a period of 3 months at 4°C (p values>0,05). By the end of 12 months, the particle size increased from 215±4 nm to 250±17 nm for PEGylated nanoparticles at 4°C, whereas the particle size of PEGylated liposomes formulation increased from 206±18 nm to 651±47 nm at the same time. Both of the formulations were unstable when stored at 40±2°C for 6 months. The mean particle size of the nanoparticles and liposomes increased to 360±18 nm and 1294±253 nm, respectively for 6 months at 40±2°C possibly due to aggregation. The results showed that the nanoparticles were more stable than the liposomes in all conditions.

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P13

POLYMERIC GELS AS A PROMISING CARRIER FOR VAGINAL DELIVERY: DEVELOPMENT, CHARACTERIZATION AND EX-VIVO MUCOADHESIVE EVALUATION

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The vagina, because of its anatomical location and physiological structures, is ever more being preferred for drug delivery. Vaginal application of bioadhesive drug forms has reveal positive results in delivering drugs both locally and systemically[1].The aim of this study is to determine in vitro characterization and ex-vivo mucoadhesive properties of gels for vaginal tissue. Mucoadhesive gels were prepared using different polymers such as chitosan, carbopol, HPMC and guar gum. The rheological properties such as viscosity, flow type, loos modulus and storage modulus of gels were determined. In addition, texture profile analysis and spreadability test were performed using different features of the texture analyser. Mucoadhesion testing of gels was carried out using a texture analyzer. Freshly excised bovine vaginal mucosa was the model mucosa. When rheological properties of gels were evaluated, it was determined that the most suitable gels for vaginal administration were HPMC and carbopol gels. According to the results of textural profile analysis, HPMC and carbopol gels had an appropriate adhesiveness(174±4,176±2g.sec), cohesiveness(1.01±0.02, 1.00±0.05) and elasticity(0.124±0.002, 0.138±0.002) for application to vaginal mucosa. The spreadability properties of the gels were evaluated and carbopol gel found to have the most suitable spreadability value(574±18g.sec).Carbopol and guar gum gels showed the best mucoadhesive properties to the vagina. It was concluded that rheological and textural and spreadability properties of HPMC and carbopol gels are suitable for vaginal mucosa.Carbopol and guar gum gels shows good mucoadhesive properties in vagina.

This study was supported by a research grant(SBAG 3001-215S155) from the Scientific and Technical Research Council of TURKEY(TÜBITAK).

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P14

SKIN PENETRATION ENHANCEMENT MECHANISM OF MIXED MICELLES

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The aim of present study was to investigate skin penetration enhancement mechanism of mixed micelles consisted of benzyl alcohol. The thin film hydration method was used to prepare of mixed micelles (Bz-MMs) in absence/presence of benzyl alcohol (1%). Pluronics and nile red were chosen as copolymers and model fluorescence dye, respectively. The hydrodynamic size of nile red loaded Bz-MMs were determined by ZetaSizer Nano ZS. The in vitro penetration of nile-red loaded Bz-MMs across pig skin was examined using Franz-diffusion cells for 24 hours. At the end of experiment, the localization of Nile-Red loaded Bz-MMs on skin layers was observed by confocal laser scanning microscopy (CLSM). ATR-FTIR spectroscopy was also used to elucidate penetration enhancing mechanism of Bz-MMs on conformational structures of lipid layers in skin. The hydrodynamic size of Bz-MMs was approximately 20 nm. CLSM images have depicted that nile red loaded Bz-MMs localized intensively into hair follicles, (especially in infundibulum) compared to mixed micelles in absence of benzyl alcohol (Fig. 1). ATR-FTIR spectroscopy data have revealed that both Bz-MMs and mixed micelles not including benzyl alcohol caused to change liquid phase from hexagonal phase on conformational structures of lipid layers in skin. As a result, mixed micelles in presence and absence of benzyl alcohol could improve drug penetration into deeper layers of skin with changing conformational structures of lipid layers in skin. Bz-MMs have also localized in hair follicles, indicating increase in drug penetration into deeper skin layers via shunt route.

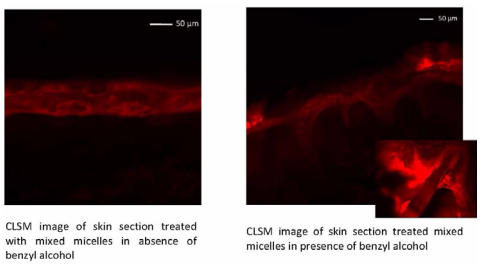

Fig.1.CLSM images of skin sections treated with mixed micelles in absence/presence of benzyl alcohol

P15

SURFACE MODIFICATION OF POLYMER-BASED NANOPARTICLES CAPPED BY CHITOSAN CHLORIDE: IN-VITRO CHARACTERIZATION AND STABILITY STUDIES

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Polymer-based nano-scaled drug carriers have been a rapidly developing area in the last few decades for the controlled and/or sustained release of conventional hydrophobic and hydrophilic active substances. Surface modification of these carriers provides several advantages to improve their physicochemical and pharmaceutical activities. The most significant superiorities of surface coating provide to the nanocarriers include targeting potencial and/or ability, changed biodistribution, increased stability in the storage conditions and also blood stream, and longetivity in the circulation half-time, and improved the accumulation in the tumor tissues to a higher level [1]. In this research, nimesulide-loaded chitosan chloride modified polymer-based nanoparticles were prepared using emulsion solvent evaporation-diffusion-salting out technique. In situ coating technique was utilized for the surface modification of nanoparticles with chitosan chloride [2]. The influence of chitosan chloride as a surface modifier on nanocarriers were evaluated with respect to production yield, particle size, surface charge, morphological properties, storage stability and in vitro dissolution studies of the formulations. The results showed that surface modification with chitosan chloride statistically affected the final physicochemical characteristics of the polymer-based nanoparticles. A concentration of 0.3 mg/ml chitosan chloride coated polymer-based nanoparticles were obtained at a particle size of 137.30 nm with a zeta potential of + 23.54 mV, while unmodified nanoparticles were achieved with 221.58 nm particle size and – 21.04 mV zeta potential value. This study indicated that surface modification of nanocarreirs capped by chitosan chloride was a key point in the design and attainment of nimesulide-loaded polymer-based nanoparticles with different surface properties.

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P16

EFFECT OF VARIOUS PARAMETERS ON PRODUCTION OF ATENOLOL-ION EXCHANGE RESIN COMPLEX

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Ion exchange resins are cross-linked, water insoluble polymers carrying ionizable functional groups on their repeating positions which make them alternative drug carriers in pharmaceutical technology to obtain rapid, sustained or site-specific release, to enhance gastrointestinal stability of the drugs, and also to mask bitter taste of drugs. In this study, atenolol, a cationic drug with pKa 9.6 was chosen as model drug because of its suitability to produce drug-ion exchange complex (resinate) with anionic resins. Anionic resin Dowex 50W X2 was used in production of atenolol resinate in order to ensure a pH-dependent release. Resinates were produced by using batch method [1]. Effect of pH and counter ion existence in medium (PBS 6.8, PBS 7.4 and HEPES 7.4, respectively), drug:resin ratio (1:1; 1:2; 2:1 for single loading and 1:1 for dual loading), method of drying (oven drying at 45°C or lyophilization) were evaluated in means of loading capacity, manufacturing efficiency and mechanical stability of resinates.Best loading capacity results were achieved by single loading of drug for mixing short time (< 30 min) using low ion capacity buffer (HEPES) (Fig. 1). Due to the low crosslinking characteristics of resinates, generally low mechanical strength was observed by optical microscopy. Mechanical stability of resinates prepared in HEPES were also found better as it allows less mechanical force over the resins. Thus, atenolol-ion exchange resins were prepared with a loading capacity over 90% by batch method using HEPES medium with single loading.

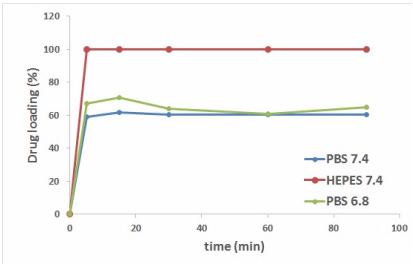

Figure 1. Effect of ion capacity and pH of buffer solutions on drug loading

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P17

PREFORMULATION STUDIES FOR DEVELOPMENT OF ETOFENAMATE TOPICAL EMULGELS BY QBD

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Etofenomate is a non-steroidal anti-inflammatory drug. Several preparations of etofenomate are available in the market as different topical preparations. In spite of many advantages of gels, a major limitation is the delivery of hydrophobic drugs. So to overcome this limitation, emulgels are prepared and used for hydrophobic drugs [1]. They have many favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, long shelf life, biofriendly, transparent and pleasing appearance [2]. In this study, different etofenamate emulgel formulations were prepared and characterization studies were performed as preformulation studies for QbD. Two types of oil with two ratios were used for preparation of emulsion phase. The gel formulations prepared with 0.25, 0.5 and 1 % of Carbopol 940 as polymer. The formulations were characterized via several parameters such as pH, conductivity, viscosity and flow properties. As results, 12 formulations were prepared with paraffin liquid (PL) and oleic acid (OA). The ratios of components are shown in Table 1. All formulations have non-Newtonian behavior and pseudoplastic flow. The viscosity and rheological behavior showed that both formulations could apply on skin. The pH of formulations was between 5.5 and 7.5 that are acceptable for topical application on skin. As conclusion, etofenamate emulgel formulations were prepared successfully for the first time by QbD approach.

Codes	*Oil ratio*	*Polymer ratio*
F1	PL %20	0.5
F2	PL %20	1
F3	PL %20	0.25
F4	PL %30	0.5
F5	PL %30	1
F6	PL %30	0.25
F7	OA %20	0.5
F8	OA %20	1
F9	OA %20	0.25
F10	OA %30	0.5
F11	OA %30	1
F12	OA %30	0.25

Table 1. Ratio of oils and polymer

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P18

ALGINATE BEADS CONTAINING CARBAMAZEPINE AND LEVETIRACETAM: FORMULATION AND IN VITRO CHARACTERIZATION

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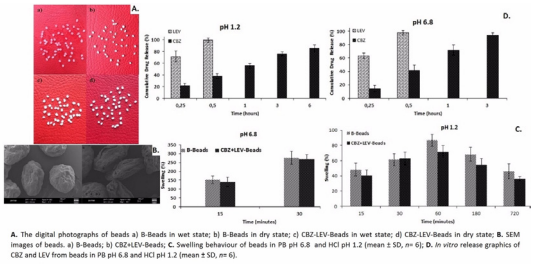
Levetiracetam (LEV) is used in the treatment of partial seizures [1]. When LEV is used in combination with Carbamazepine (CBZ), a synergistic effect is observed [2]. The aim of this study is to formulate and in vitro characterize the CBZ-LEV-loaded-alginate beads for oral drug delivery. The beads containing CBZ+LEV (CBZ-LEV Beads) was prepared using ionotropic gelation method [3]. Their digital photographs and SEM images were obtained (Figure 1-A and 1-B). The drug contents were measured using the validated HPLC method (230 nm). Swelling study for dried beads and also in vitro release studies were carried out in PB pH 6.8 and HCl pH 1.2 at 37±0.5 °C. The mean sizes of the Blank Beads (B-Beads) and CBZ+LEV-Beads (150; wet state) were 1.61±0.07 mm and 1.74±0.08 mm, respectively ($p<0.05$). The encapsulation efficiency (EE%) values of CBZ+LEV-beads were found 93.45±3.76% (for CBZ) and 24.65±1.69% (for LEV). The obtained low value of EE% for LEV may be due to the solubility of LEV in water and leakage to the aqueous medium. The weight change % for the beads were shown in Figure 1-C. The beads exhibit maximum water uptake at 30 minutes in PB pH 6.8. The water uptake of beads in HCl pH= 1.2 was very low compared to that in PB pH 6.8 at 30 minutes ($p<0.05$). The results of in vitro release studies were given Figure 1-D. The beads might be useful for combination therapy in epilepsy.



A. The digital photographs of beads a) B-Beads in wet state; b) B-Beads in dry state; c) CBZ-LEV-Beads in wet state; d) CBZ-LEV-Beads in dry state; **B.** SEM images of beads. a) B-Beads; b) CBZ+LEV-Beads; **C.** Swelling behaviour of beads in PB pH 6.8 and HCl pH 1.2 (mean ± SD, n= 6); **D.** *In vitro* release graphics of CBZ and LEV from beads in PB pH 6.8 and HCl pH 1.2 (mean ± SD, n= 6).

Figure 1. The figures of the characterization studies

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EVALUATION OF ALENDRONATE SODIUM PERMEABILITY IN NOVEL SOLID AND LIQUID MICROEMULSION DRUG DELIVERY SYSTEMS BY USING TECHNETIUM-99M

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Alendronate sodium (ALD) is a second generation amino bisphosphonates which selectively inhibits osteoclast mediated bone resorption, increases bone mineral density and reduces the incidence of vertebral, hip and other fractures. Like all bisphosphonates, ALD is poorly permeated from the gastrointestinal tract, with oral bioavailability of around 0.9–1.8% [1]. The aim of this study was to develop liquid and solid microemulsion systems (L-ME and S-ME) of ALD and evaluate its permeability properties in these systems. Two liquid formulations (F1 L-ME and F2 L-ME) and solid microemulsion systems (F1 S-ME and F2 S-ME) containing oleic acid, Span 80, Labrafil 2125 M, Transcutol HP were prepared by using pseudo-ternary phase diagram and spray-drying method, respectively. Their physicochemical and permeability properties of ALD from newly developed formulations were evaluated. Firstly, ALD was directly labeled by using ^{99m}Tc as previously described [1-3]. ^{99m}Tc-ALD was added to F1 L-ME, F2 L-ME, F1 S-ME and F2 S-ME. The radiochemical purity of ^{99m}Tc-ALD solution and whole formulations were analyzed by chromatographic studies. According to results, newly developed formulations are stable. The particle size of all formulations was found to be between 200 and 500 nm. ALD solution and all formulations provided high radiochemical purity (˃90%). F1 S-ME and F2 S-ME showed higher permeability than F1 L-ME, F2 L-ME and ALD solution in permeability studies. The results have displayed that these drug carrier systems can be used to improve the permeability properties of ALD.

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P20

NEWLY DEVELOPED MICROEMULSIONS SYSTEM OF RISEDRONATE SODIUM: CHARACTERIZATION AND IN VITRO RADIOACTIVE PERMEABILITY STUDIES

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Risedronate (RSD) is a novel bisphosphonate, potent inhibitor of osteoclast-mediated bone resorption and has permeability problem [1]. The objective of this study was to formulate novel liquid and solid microemulsion (L-ME and S-ME) to evaluate the permeability of RSD. L-ME was developed by using pseudo-ternary phase diagram, composed of oleic acid, Span 80, Labrafil 2125 M, Transcutol HP. Spray drying method was used to prepare S-ME from L-ME. New microemulsion system was characterized by droplet size, viscosity, refractive index, conductivity and pH. RSD was radiolabeled by ^{99m}Tc, previously described [2]. After that, ^{99m}Tc radiolabeled RSD was put to L-ME and S-ME. The labeling efficiency, radiochemical purity and stability of L-ME and S-ME containing ^{99m}Tc-RSD were examined by using thin layer chromatography. The permeability of ^{99m}Tc-RSD from L-ME and S-ME formulations was investigated with Caco-2 cells. This study achieved that complete labeling procedure, high labeling efficiency (>90%) and good stability for all formulations. Additionally, L-ME, S-ME and solution obtained a similar permeability for RSD. In conclusion, this lipid carrier system can be used as a new formulation for RSD.

This study was supported by Ege University Scientific Research Projects Coordination Unit, project number: 16-ECZ-017.

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DEVELOPMENT OF A SELF EMULSIFIYING DRUG DELIVERY SYSTEM BY DESIGN OF EXPERIMENT (DOE) APPROACH

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DoE is an approach that is used to determine the relationship between the independent variables and the response variables in order to obtain highest amount of information[1, 2]. In this study we used DoE approach to optimize a self emulsifying drug delivery system (SEDDS) for oral peptide delivery. Surfactant/Co-surfactant (S/Co-S) ratio was selected as 3:1 according to preliminary studies. The amounts of oil (A) and surfactant/Co-surfactant mixture(B) were selected as independent variables. Droplet size (DS), polydispersity index (PDI) and zeta potential (ZP) were evaluated as response variables. For the statistical design, Design Expert® Version 9.0.6 was used; 3^2(3 levels, 2 factors) with randomized designs, two replicates, total number of 18 experiments were performed. As the interaction between A and B was found significant ($p<0.05$) for DS and ZP, full factorial design was chosen. DS and ZP were effected by both A and B. As the insignificant interaction between A and B for PDI, main effects were considered. PDI was effected only by the amount of oil. According to our experimental design, higher amount of the S/Co-S and lower amount of oil is recommended for SEDDS which yields a small droplet size with a PDI below 0.2 and zeta potential of ±30-50 mV. When the ratio of A: B was 0.5:2.5 (w:w); DS, PDI and ZP were found as 20.423±0.093 nm, 0.079±0.010 and -12.503±7.812 mV respectively for optimum formulation. Consequently, it can be said that DoE approach is useful for selecting optimum formulation of SEDDS.

This study was supported by Erciyes University Scientific Research Foundation (Project No: 7087).

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PREPARATION AND CHARACTERIZATION OF CYCLOSPORINE A NANOSUSPENSION FOR ORAL ADMINISTRATION I: EFFECTS OF FORMULATION PARAMETERS

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Cyclosporine A (CsA) is used for the suppression of the immune system after organ transplantation. CsA has low solubility and bioavailability and therefore is classified as BCS Class II. Nanosuspensions are great of interest because they can increase the solubility, dissolution and bioavailability by reducing the particle size [1, 2]. The aim of this study was to prepare CsA nanosuspensions by microfluidization method using Design of Experiment (DoE) for oral administration. HPMC and Poloxamer 188 were chosen as stabilizing agents. CsA:HPMC ratio, CsA:Poloxamer 188 ratio and homogenization cycles were selected as independent variables and particle size (PS), particle size distribution (PDI) and zeta potential (ZP) were selected as response variables in a scope of DoE. 3^2 (3 levels, 2 factors) full factorial design, with two replicates, was applied the formulations. CsA was used 1% (w/w) and CsA:stabilizing agent ratios were 1:1, 1:2 and 1:4. HPMC or Poloxamer 188 were dissolved in distilled water and CsA were added. The UltraTurrax (at 15.000 rpm-10 minutes) was used to minimize particle size. Different homogenization cycles (5, 15 and 30 cycles) were applied at 30.000 psi pressure to obtain nanosuspensions. PS, PDI and ZP values of nanosuspensions were measured after the microfluidization process. The particle sizes of the initial suspensions were about ~40 µm. CsA:HPMC 1:1 formulation (after 30 cycles) showed better results according to PS, PDI and ZP which were 707.73±52.28 nm, 0.76±0.08, -23.57±0.61 mV, respectively. This study showed that independent variables were found effective on PS, PDI and ZP of CsA nanosuspension.

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PREPARATION AND INVESTIGATION ON ERLOTINIB HCL AND DEXKETOPROFEN TREMETAMOL LOADED CHITOSAN NANOPARTICLES FOR CANCER TREATMENT

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The goal of this project is to develop erlotinib HCl loaded chitosan nanoparticles for cancer treatment. Erlotinib HCl (ERLO) is classified as a BCS II drug substance (low aqueous solubility and high permeability)[1]. ERLO and dexketoprofen trometamol (DEX) co-loaded nanoparticles (ERLODEX-NPs) were prepared using spray drying method. Briefly, the certain amount of ERLO and DEX were dispersed in the chitosan aqueous solution. Two more nanoparticle batches were also prepared using either polyethylene glycol (ERLODEX-PEGNPs) or polyethylene glycol folic acid (ERLODEX-PEGFANPs). All the solution were sprayed via Nano Spray Dryer B-90 (Buchi) in following conditions, 4µm spray cap, 100% spray capacity, 120ºC. Dried samples were investigated. Surface morphology was studied using a TEM. Particle size and zeta potential were measured using laser diffraction. Dissolution studies were performed using a dialysis tube in acetate buffer (pH 3). The amount of drug in the samples was analyzed using UPLC. All three different nanoparticles showed the excellent efficiency of encapsulation (higher than 90%). The average particle size ranged from 100 and 206 nm with the positive surface charge at about 26 mV. TEM images of nanoparticles were smooth and spherical. According to the dissolution data, the nanoparticles showed the burst effect and the surface modification improved the dissolution rate. The nanoparticles fitted to Higuchi kinetic. TEM images supported it. The permeabilities of nanoparticles were so similar to the dissolution data. ERLODEX-PEGFANPs showed the highest dissolution rate(58,9%) and permeability(83,8 %).
This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, No. 213M675).

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FORMULATION AND IN VITRO CHARACTERIZATION STUDIES OF LEVOFLOXACIN HEMIHYDRATE INCORPORATED PLGA BASED NANOPARTICLES

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Polymeric nanocarriers find versatile applications in minimization of drug toxicity, improvement in drug stability, modulation of pharmacokinetics, sustained drug release, targeting, intracellular trafficking, and theranostics [1]. The most widely used polymer in FDA-approved drug products and medical devices is poly(lactide-co-glycolide) (PLGA) as nanoparticle- forming material [2]. PLGA a biodegradable and biocompatible polymer, has been extensively used for developing an array of nanoparticulate drug delivery systems and has several advantages, such as good mechanical properties, low immunogenicity, low toxicity, excellent biocompatibility, and predictable biodegradation kinetics [3]. Levofloxacin hemihydrate (Levo-h), a third generation fluoroquinolone antibacterial agent, has a broad spectrum of activity against gram positive and gram negative bacteria. Fluoroquinolone derivatives are used mainly for the treatment of urinary tract, respiratory tract, skin, soft tissue and eye infections [4]. The aim of this study is to formulate Levo-h loaded nanoparticles with high bioavailability and prolonged effect for optimizing antibacterial drug concentrations that protects its inactivation. Also, it may be good idea to formulate Levo-h loaded nanoparticles as a controlled release drug delivery system to reduce dose frequency, toxicity and improve patient compliance. Nanoprecipitation technique was used for preparing nanoparticles and characterization was achieved by particle size/PDI and zeta potential measurements, DSC thermograms, SEM imaging, FTIR spectroscopic analyses.

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DEVELOPMENT AND CHARACTERIZATION OF NOVEL CO-DELIVERY SYSTEM BASED ON DEXAMETHASONE SODIUM PHOSPHATE (DEX-NP) LOADED CHITOSAN WITH PLASMID DNA

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Recently co-delivery of therapeutics by nanocarriers have been popular to enhance gene expression or to achieve the synergistic/combined effect. Dexamethasone sodium phosphate (DEX-NP) is water soluble steroid and have transient dilatory effect on nuclear pores that enhanced the gene transfection efficiency [1]. Based on this, in our study we aimed to develop a novel optimize nanoparticle system by using two different molecular weight (low and medium)of chitosan polymer to prepare a co-delivery system by DEX-NP and psv-β-gal pDNA. Blank nanoparticles were prepared by ionotropic gelation method [2]. DEX-NP loaded chitosan formulations were prepared with three different amounts (0.5, 1 and 1.5 mg/mL) of DEX-NP with same method. Optimum chitosan formulations were selected for complexation with pDNA based on nanoparticle size and encapsulation efficiency. Complexation of DEX-NP loaded chitosan nanoparticles with pDNA carried out at various weight ratios and analyzed with agarose gel electrophoresis. The results indicated that 1.5 mg/mL DEX-NP loaded LMW and MMW chitosan nanoparticles had high encapsulation efficiency and optimum particle size with 45.7% and 40.8% encapsulation and 163.6 nm, 0.3PDI, 34.6 mV and 133.8 nm, 0.3 PDI and 29.8 mV, respectively. These formulations were selected for complexation. The complexation results showed that increasing weight ratio caused decreasing particle size and increasing zeta potential. Both nanoparticles effectively condansed the pDNA at increased weight ratio. Agarose gel images revealed that nanoparticles inhibited pDNA migration on gel even at 1:1 weight ratios. Our novel optimize co-delivery nanoparticle system can be a promising candidate for gene therapy with effective characteristic features.

Weight ratio (Chitosan: pDNA)	Size(nm)		Poly dispersity index (PDI)		Zeta Potential (mV)	
	(LMW) DEX-NP loaded chitosan	(MMW) DEX-NP loaded chitosan	(LMW) DEX-NP loaded chitosan	MMW) DEX-NP loaded chitosan	(LMW) DEX-NP loaded chitosan	(MMW) DEX-NP loaded chitosan
1:1	2505.3±1417,8	3103.3±312,4	0.2±0,1	0.3±0,1	8.4±4,5	3.1±0,4
2:1	2942.7±1836,2	1153.7±161,6	0.7±0,3	0.4±0,4	0.1±3,3	0.9±3,0
4:1	148.3±3,2	1029.3±231,2	0.3±0,0	0.4±0,1	15.5±0,7	10.5±2,8
8:1	165.0±9,8	468.2±211,7	0.4±0,0	0.6±0,0	25.3±0,8	20.3±1,3
16:1	148.7±4,7	201.7±4,7	0.2±0,0	0.4±0,0	35.4±2,9	24.1±0,5
19:1	166.0±8,8	166.4±1,6	0.4±0,0	0.3±0,0	30.8±0,5	23.9±0,4

Table 1. Particle size, polydispersity index and zeta potential of various weight ratios of DEX-NP l

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PULLULAN BASED ORALLY DISINTEGRATING FILMS OF TELMISARTAN

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Orally disintegrating films (ODF) are preferred to conventional oral solid dosage forms because of ease of administration and their ultra-thin strip form. ODF is more useful to the patients who have the fear of taking solid tablets [1,2]. We aimed to develop an ODF containing telmisartan, which is an antihypertensive agent. ODF was prepared using solvent casting method and different amounts of polymer (Pullulan), and plasticizer (PEG 400) were used for optimization. Tween 80, $NaHCO_3$ and aspartame were also used as surfactant, alkalizing agent and sweetener, respectively. The appearance of films was evaluated by visual inspection. Formulation which was non-sticky, non-brittle and had better homogeneity was selected for further characterization. Film thickness was measured by a micrometer. Folding endurance was measured by folding film from the same place until it broke. Drug content was analyzed with UV spectrophotometry at 295 nm. Dissolution studies were performed using USP pedal method and pH 7.5 phosphate buffer. Selected film showed suitable thickness (0.1± 0.0) and folding endurance (210±22). Disintegration time was determined as 1.05 min when using USP conventional method. Drug content was found to be between 94.5% and 105.6% for six different films. Films showed acceptable mass and content uniformity. Dissolution test result showed that there was 27.6 % drug release at the end of the 60 min (Figure 1). In conclusion, alkalizing agent amount or type change is needed to improve dissolution of developed formulations.

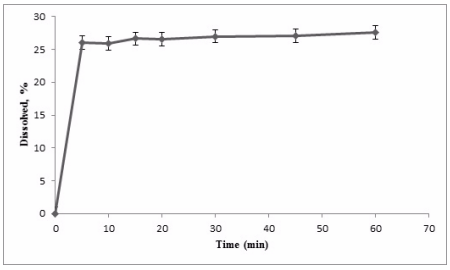

Figure 1. Dissolution profile of telmisartan ODF

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EVALUATION OF CYTOTOXICITY OF OF BUDESONIDE-LOADED SNEDDS ON HUMAN COLON CANCER CELL LINES

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The intra-subject, inter-subject variability and dose profile problems of newly developed drug active substances in the finished product are very troublesome in the development step. Self emulsifying systems are quite advantageous in terms of getting over such problems [1]. In addition to its advantages, it has potential toxicity due to surfactants and co-surfactants used [2]. The aim of this study was to evaluate cytotoxicity of budesonide-loaded self-nanoemulsifying drug delivery systems (SNEDD) formulation in two human colon cancer cell lines (Caco-2 and HTC-116). Cytotoxicity of budesonide loaded SNEDD in HCT 116 and Caco-2 cells was investigated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) cell proliferation assay. Caco-2 and HTC-116 were cultured in DMEM containing 10% (v/v) fetal bovine serum and antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin) at 37 ˚C under 5% CO_2. Cells (2×105) were incubated in 96-well plates. After 24 h, cells were exposed to different concentrations (0.05, 0.10, 0.25, and 0.50% (v/v)) of drug-loaded SNEDD and empty SNEDD for 2h and 24 h. Response of Caco-2 and HTC-116 cells to the cytotoxic effect of formulations were concentration-dependent and time-dependent manner. Drug-loaded SNEDD formulation at concentration of 0.25% (v/v)) and 0.50% (v/v)) exhibited more cytotoxic effect in Caco-2 compared to HCT-116 cells. Budesonide-loaded SNEDD formulation at a concentration of 0.05 (v/v) can be regarded as non-toxic for HCT-116 and Caco-2 cells during 2 h and 24 h and convenient for oral administration.

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PREPARATION AND EVALUATION OF OXICONAZOLE NITRATE LOADED PROLIPOSOME

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Oxiconazole nitrate (ON) which is frequently used for treatment of chronic fungal infections such as candida infections, athlete's foot, Jock itch, tinea and vaginal fungal infection is a topical imidazole derivate antifungal drug[1,2]. It is widely used as cream and lotion forms in the concentration of 1 %[3]. However, these dosage forms do not present a prolonged duration of action and decrease the efficacy of ON[4]. Moreover, topical treatment cause side effects such as pruritus, burning sensation, irritation, allergic contact dermatitis, folliculitis, erythema, fissure, rash, stinging etc. in some patients[3]. Alternative drug delivery systems are needed to overcome poor aqueous solubility, short half-life and low bioavailability of ON which limit antifungal activity of the drug and cause several systemic toxicity problems[2,4]. The aim of present study was to formulate and characterize proliposomal formulations to overcome these problems and enhance the bioavailability of ON for topical administration. ON loaded proliposomes were prepared using various drug:maltodextrin ratios(w/w),egg lecithin and cholesterol by slurry method. The liposomes derived from proliposomes were characterized for particle size, zeta potential, entrapment efficiency, drug loading, production yield and microscopic imaging. Differential scanning calorimetry(DSC) analysis were conducted to investigate drug-proliposome excipients interactions. The particle size and zeta potential of the derived liposomes were found to be between 98.85±1.32 nm–194.70±9.39 nm and -40.0±0.6 mV– -20.3± 0.3 mV, respectively. The encapsulation efficiencies were 95.27±0.134-58.69±0.10 %. In conclusion, an optimum formulation was chosen for further in vitro and/or in vivo evaluations. Proliposomes may be beneficial and alternative drug delivery systems for poorly water soluble drugs.

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FORMULATION AND IN VITRO EVALUATION OF CHITOSAN BASED BUCCAL TIZANIDINE HYDROCHLORIDE MINI-TABLETS

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Tizanidine hydrochloride (TZN) is an active ingredient that has monolithic effect on skeletal muscle as α-2 adrenergic receptor agonist. Because of rapid and excessive first-pass effect, the oral bioavailability of TZN remains around 30–40% [1, 2]. The purpose of this study was to prepare and assess the potential use of mini-tablets for buccal delivery of TZN. Tablets were prepared using direct compression method. The mini-tablets were formulated using chitosan salt and magnesium stearate. Moreover ethyl cellulose (EC) was used to prepare bilayered mini-tablets for unidirectional release (Table 1). To prepare mini-tablets; TZN, chitosan salts and magnesium stearate mixed and pre-compressed, then final compression was performed adding EC. Weight, thickness, pH, friability, and hardness of mini-tablets were evaluated. Also in vitro release studies were carried out. The uniformity of weight in all mini-tablets was found to be within the acceptable range. The thickness of tablets was detected 2.48±0.02mm while the diameter of the tablets was found 8.00±0.01 mm. All tablets exhibited friability values ranging between 0.13 and 0.81%, less than the limit of 1%. The surface pH of tablets was within a range of 6.07–6.52, close to neutral pH. Hardness test indicated good mechanical strength, the hardness of tablets was found to be in the range of 72–92N. The release of TZN from the tablets was in the range of 65.34 to 81.29% at the end of 8h. TZN tablets may be a good way to bypass the hepatic first-pass metabolism and to improve the bioavailability of TZN through buccal mucosa.

Formulations/ Materials (mg)	TC-7	TC-10	TG-7	TG-10
Tizanidine hydrchloride	2.288	2.288	2.288	2.288
Magnesium stearate	2	2	2	2
Chitosan glutamate (7 cP)	-	-	95.712	-
Chitosan glutamate (10 cP)	-	-	-	95.712
Chitosan chloride (7 cP)	95.712	-	-	-
Chitosan chloride (10 cP)	-	95.712	-	-
Ethyl cellulose	50	50	50	50

Table 1. The formulations of buccal bioadhesive minitablets

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INFLUENCE OF EXPERIMENTAL PARAMETERS ON THE CHARACTERISTICS OF CATIONIC NANOPARTICLES PREPARED BY NANOPRECIPITATION AND DOUBLE EMULSION METHODS

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Cationic nanoparticles with net positive surface charge emerge as a promising alternative to nanomedicine due to their very strong cellular interaction properties and good cellular uptake. Especially, a more effective treatment can be achieved by preparing nanoparticles carrying various anticancer agents, especially for cancer therapy, as cationic nanoparticles [1]. One of the most important parameters affecting the choice of nanoparticle preparation method is the hydrophobic or hydrophilic nature of the anticancer drug to be used. In our study, cationic nanoparticles with positive surface charge, which can be used in cancer chemotherapy, are prepared by changing various formulation parameters by using nanoprecipitation method [2] which is mostly used for hydrophobic drugs and double emulsion [3] methods which are frequently used for hydrophilic drugs. Effects of the changing formulation parameters on the characteristics such as particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of the obtained nanoparticles were evaluated. For this purpose, drug-free poly-L-lysine (PLL) coated Poly-ε-caprolactone (PCL) and chitosan (CS) coated Poly-ε-caprolactone (PCL) nanoparticles were prepared by changing various formulation parameters with both nanoprecipitation and double emulsion methods. At the end of the study, cationic nanoparticles with positive net surface charge were obtained and the effects of changing formulation parameters on PS, PDI and ZP were collectively evaluated.

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IN VITRO RELEASE AND EX VIVO PERMEATION/PENETRATION STUDIES OF TETRAHYDROZOLINE HYDROCHLORIDE LOADED MICROEMULSIONS FOR OCULAR DELIVERY

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Tetrahydrozoline (THZ) is a sympathomimetic agent with α adrenergic activity and is used as a conjunctival decongestant [1]. The unique physiology, anatomy and biochemistry of the eye makes this organism impermeable to foreign substances, making it difficult for the formulation to permeation barriers [2]. Microemulsions are thermodynamically stable colloidal systems and they consist of water, oil, surfactant and cosurfactant [3]. The aim of this study was to formulate and evaluate THZ microemulsions for ocular delivery to be used in the treatment of allergic conjunctivitis. Isopropyl myristate(IPM) as oil, Span 80, Tween 80(ME-1,ME-4), Cremophor EL(ME-2,ME-5), Tween 20(ME-3,ME-6) as surfactant, propylene glycol, PEG 400, ethanol as cosurfactant and distilled water as the water phase were used for preparation of THZ microemulsions. To determine the sterility of the microemulsions a microbiological control study was conducted. All microemulsions were determined to be sterile. In vitro drug release study was carried out in artificial tear fluid. Diffusion cells were used for ex vivo permeation/penetration studies. The release profiles obtained from microemulsions revealed sustained release(Figure 1). THZ permeation through cornea was determined between 40-57%. The penetrated amount in the cornea was determined as 12.08% for ME-1, 8.55% for ME-2, 4.41% for ME-4, 4.161% for ME-5 and 5.631% for commercial formulation (VISINE®). Microemulsions are innovative systems for ocular drug delivery and they are suitable to eye application. On the basis of in vitro and ex vivo studies, it could be concluded that THZ could be successfully administered via microemulsions for treatment of allergic conjunctivitis.

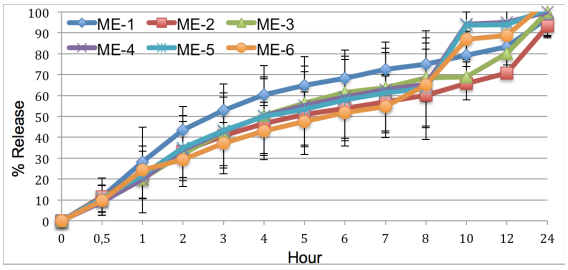

Figure 1. In vitro drug release results of microemulsions

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P32

DEVELOPMENT AND EVALUATION OF SEDDS PELLET FOR ATORVASTATIN CALCIUM

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Atorvastatin calcium is poorly soluble in water that anti-hyperlipidemic drug, having a biological half-life of 14 hours [1] . Lipid-based formulations have attracted attention in recent years because it improves the solubility of active substances showing low solubility and low bioavailability in gastrointestinal tract [2]. These formulations has many advantages such as easy to prepare, can reduce the impact of food and reduce interpersonal variation [3]. Self-emulsifying pharmaceutical pellets combine the advantages of emulsions and solid dosage forms, improved absorption of low solubility drugs and better stability in the gastric fluids. Among the approaches to improve the oral bioavailability, the use of self-emulsified drug delivery pellet systems (SEDDS-pellet) has been shown to be reasonably successful in improving the oral bioavailability of poorly water-soluble and lipophilic drugs[4,5]. The aim of this study is to develop a new dosage form, alternative to the classical tablet forms of atorvastatin. In this study, oleic acid was used as the oil phase, Tween 20 and Span 80 were used as the surfactants, N-methylpyrrolidone was used as the cosurfactant and both Avicel and Aerosol were used as the solid phase. The prepared SEDDS-pellet formulations are characterised for size, shape, density, stability and dissolution studies. Permeation studies were examined with Caco-2 cell culture. According to results, SEDDS-pellet formulation had a higher permeability value than the conventional tablet formulation (Figure 1).

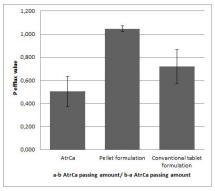

Figure 1. The graph of Pefflux values of different formulations (± SD)

References

[1] Bhumika S et al. J Pharm Drug Deliv Res 1(3), 1-7, 2012.
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DEVELOPMENT AND EVALUATION OF SELF MICROEMULSION DRUG DELIVERY SYSTEM FOR ATORVASTATIN CALCIUM

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Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood. People with hypercholesterolemia have a high risk of developing a form of heart disease called coronary artery disease [1]. Atorvastatin is a BCS class 2 classification group and displays low resolution with high permeability [2]. It has almost 14 % bioavability absolutely [3] . As a consequence of modern drug discovery techniques, there has been a steady increase in the number of new pharmacologically active lipophilic compounds that are poorly water-soluble [4,5]. The aim of this study is to develop a new dosage form, alternative to the classical tablet forms of atorvastatin. In this study, atorvastatin calcium was used as the active ingredient, oleic acid was used as the oil phase, Tween 20 and Span 80 were used as the surfactants, ethanol was used as the cosurfactant. The prepared self microemulsion drug delivery system (SMEDDS) formulations are characterised for size, shape, density, stability and dissolution studies. Permeation studies were also examined with Caco-2 cell culture. According to the obtained results, SMEDDS formulation had a higher dissolution profile and permeability value than the conventional tablet formulation (Figure 1).

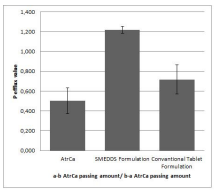

Figure 1. The graph of Peflfux values of different formulation (± SD)

References

[1] Bhumika S et al. J Pharm Drug Deliv Res 1(3), 1-7, 2012.
[2] Talegaonkar S. et al. J Disper Sci Technol 31(5), 690-701, 2010.
[3] Colin W et al. Adv Drug Deliver Rev 60, 625–637, 2008.
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P34

EVALUATION OF THE PROLIFERATIVE EFFECTS OF CHITOSAN NANOPARTICLES CONTAINING ANTHOCYANIN ON NEURO BLASTOMA CANCER CELLS

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Anthocyanins are responsible for the color of black carrots and has intensified due to health promoting ability by reducing the risk of atherosclerosis, cancer, diabetes and neurodegenerative disorders. Black carrots are reported to have high anthocyanin content up to 1750 mg/kg fresh weight exhibiting antioxidant and anti-inflammatory activities [1-5]. The aim of this study was to develop anthocyanin loaded chitosan nanoparticles and evaluate the inhibition of proliferative effects of neuroblastoma (Neuro 2A) cancer cells. Chitosan nanoparticles were prepared by ionic gelation method. For the characterization of the formulation particle size, zeta potential, entrapment efficiency and in vitro release kinetics were determined. DSC analysis and SEM imaging were done. Cytotoxicity studies were carried out using the Neuro 2-A cell line to determine the cytotoxic effects of the black carrot extracts containing high levels of anthocyanins. The particle size and zeta potential of nanoparticles were found 123.6 nm and 30.38 mV, respectively. Entrapment efficiency was 85%. The cumulative percentage release of anthocyanin from chitosan nanoparticles was 90%. The release results were evaluated kinetically and it was observed that all of the results fitted to the Higuchi square root of time order kinetic model. Cell viability was observed below 50% in the A549, Neuro 2A and NA2A cell lines at the concentration of 6 µg / mL.
This project is supported by the Scientific and Technical Research Council of Turkey (TUBITAK) (113M196).

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P35

ORALLY DISINTEGRATING TABLETS OF TELMISARTAN; FORMULATION DEVELOPMENT AND EVALUATION

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Orally disintegrating tablets (ODT) are advantageous for patients who have difficulty in swallowing or patients who have limited access to water. Especially for geriatric and pediatric patients easy administration is important to improve patient compliance [1]. Telmisartan is an angiotensin II receptor antagonist and is used in the treatment of primary hypertension which is a disease common in geriatric patients [2]. In this study we attempted to develop ODT formulations of telmisartan. Formulations (F1-F4) were prepared by using four different superdisintegrants (croscarmellose sodium-F1, crospovidone-F2, low-substituted hydroxypropyl cellulose-F3, PEARLITOL® Flash-F4) by direct compression. Bulk powder properties of formulations were investigated and quality control tests were performed on tablets. Dissolution studies were performed using USP apparatus II and pH 7.5 phosphate buffer as media at 37 °C. Angle of repose was in the range of 23.9-35.4° and flowability of bulk powder was between fair and excellent for all formulations. Compressibility index values were 21.3, 17.0, 23.5, 4.7 for F1, F2, F3 and F4 respectively. All formulations exhibited suitable hardness between 43.5-55.6 N. Friability was under 1 % for all formulations except for F4 (6.8%). Disintegration time was between 12-37 s for all formulations. Dissolution profiles of all tablets were similar but drug release was below the 25 % for all formulations after 60 min (Figure 1). This result is attributed to the pH dependent solubility of telmisartan. Further studies such as addition of an alkalizing agent to formulations should be conducted to improve dissolution of the ODTs.

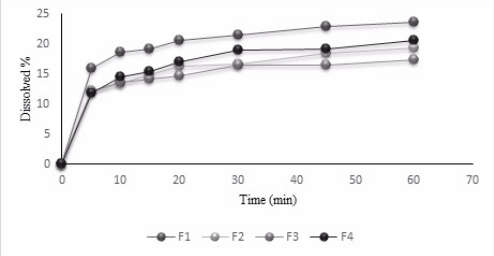

Figure 1. Dissolution profiles of ODT formulations

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P36

QBD-BASED DEVELOPMENT AND CHARACTERISATION OF MICROEMULSION FORMULATIONS FOR IMPROVED SKIN DELIVERY OF FLURBIPROFEN

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Non-steroidal anti-inflammatory drugs are widely used via topical route in treatment of rheumatoid arthritis, osteoarthritis, low back pain and some joint diseases. However, conventional topical formulations could be inefficient to pass the skin and to reach effective drug levels in the target tissues due to the barrier properties of skin and unfavorable physicochemical characteristics of drugs. The purpose of the present study was to formulate and characterize topical microemulsion formulations of flurbiprofen, a highly lipophilic non-steroidal anti-inflammatory drug (Log P =4.16), in order to increase its penetration to the skin. The microemulsions were prepared using oleic acid (oil phase), Labrasol (surfactant), Transcutol (co-surfactant) and water. Optimized microemulsions containing flurbiprofen (5% w/w) were determined by using quality by design (QbD) approach. After physicochemical characterization studies, in vitro skin permeation of flurbiprofen from the microemulsion formulations was studied. The drug deposition in the skin was also quantified. Our results showed that the microemulsions exhibited uniform size distribution. Electrical conductivity and refractive index of all microemulsion samples confirmed the formation of oil-in-water type microemulsions. The optimized microemulsions showed significantly higher skin deposition of flurbiprofen with GEP (Genetic expression programming) and ANN (Artificial neural network) analysis (263,76±32,66 µg/cm^2 and 322,82±53,14 µg/cm^2, respectively) over its commercial gel preparation (54,66±5,05 µg/cm^2). Our results showed that using QbD framework could be considered as an efficient implementation of flurbiprofen microemulsion formulations.

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CHONDROITIN SULPHATE/ POLYVINYL ALCOHOL HYDROGELS FOR VAGINAL FORMULATIONS. I. TEXTURE-PROFILE ANALYSIS

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In the development of ideal vaginal dosage forms optimal mechanical properties contribute to the patient acceptability and clinical efficacy of the product [1]. Crosslinking of polymers in an hydrogel can be carried out by physical or chemical methods. Freeze-thawing is an easy, chemical-free method capable of producing physically crosslinked hydrogels with a wide range of mechanical properties. The resulting mechanical properties are correlated to the usage and therapeutic outcome of the hydrogel formulations [2]. The aim of this study is to evaluate the mechanical properties of hydrogel formulations for vaginal application of econazole nitrate (ECO). For this purpose TPA studies were performed on freeze-thawed polyvinyl alcohol (PVA) - chondroitin sulfate (CSF) hydrogels. Also chemically crosslinked hydrogels were prepared for comparison. To prepare hydrogels, varying ratios of PVA and CSF were used. Different freeze–thawing process parameters resulted in changes in final mechanical properties. Chemically crosslinked hydrogels of PVA and CSF were prepared by adding glutaraldehyde (GA). TPA studies were performed using software-controlled penetrometer (TA-XT Plus Stable MicroSystems UK) at 25°C. Each experiment was repeated six times. The results are given in Table 1. As vaginal gels require a lower hardness and compressibility but a higher elasticity, cohesivity and adhesivity, it was found that PVA/CSF-30/70(v/v/%) and PVA/CSF-70/30(v/v/%) gels prepared by freeze-thawing with 2 hours freezing and 2 hours thawing were the most suitable formulations.

	Hardness (N) ± SD	Compressability (N.sec) ± SD	Adhesivity (N.sec) ± SD	Cohesiveness ± SD	Elasticity ± SD
PVA-CSF (30-70) 2DH4H	0,406±0,0049	0,016±0,0097	0,004±0,0001	0,941±0,0059	0,905±0,0047
PVA-CSF (30-70) 2DH4H +ECO	0,391±0,0017	0,103±0,0019	0,007±0,0001	0,963±0,0019	0,963±0,0055
PVA-CSF (50-50) 2DH4H	0,360±0,0053	0,263±0,0123	0,005±0,0001	0,999±0,0062	0,958±0,0031
PVA-CSF (50-50) 2DH4H +ECO	0,372±0,073	0,166±0,0012	0,005±0,0001	0,954±0,0069	1,014±0,0042
PVA-CSF (70-30) 2DH4H	0,362±0,0177	0,205±0,0002	0,013±0,0005	0,948±0,0068	0,987±0,0028
PVA-CSF (70-30) 2DH4H +ECO	0,336±0,0012	0,031±0,0066	0,003±0,0001	0,940±0,0021	0,968±0,0058
PVA-CSF (30-70) 2H2H	0,010±0,0001	0,044±0,0003	0,006±0,0003	0,994±0,0028	0,976±0,0022
PVA-CSF (30-70) 2H2H +ECO	0,010±0,0014	0,009±0,0005	0,006±0,0001	0,997±0,0004	0,991±0,0024
PVA-CSF (50-50) 2H2H	0,011±0,0004	0,052±0,0007	0,006±0,0001	0,995±0,0062	0,941±0,0029
PVA-CSF (50-50) 2H2H +ECO	0,010±0,0004	0,036±0,0001	0,005±0,0001	0,992±0,0002	0,962±0,0064
PVA-CSF (70-30) 2H2H	0,011±0,0011	0,045±0,0012	0,005±0,0002	0,988±0,0018	0,964±0,0049
PVA-CSF (70-30) 2H2H +ECO	0,013±0,0001	0,052±0,0001	0,005±0,0001	0,989±0,0012	0,968±0,0068
PVA-CSF+GA	0,012±0,0011	0,052±0,0006	0,004±0,0001	0,992±0,0097	0,965±0,0069
PVA-CSF+GA+ECO	0,012±0,0013	0,051±0,0018	0,004±0,0001	0,995±0,0077	1,041±0,0055

Table 1 Mechanical Properties of Formulations

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P38

THE OPTIMIZATION OF THE FORMATION OF ANTIBACTERIAL NASAL FILM STRUCTURE IN BECLOMETHASONE DIPROPIONATE METERED-DOSE SPRAY FORMULATION AND THE PREVENTION OF BACTERIAL GROWTH DURING ALLERGIC RHINITIS IN PATIENT USE

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The objective of this research was to design and optimize the formation of nasal film structure embedded in beclomethasone dipropionate metered-dose spray formulation. Owing to its antibacterial characteristics nasal film structure be able to prevent possible bacterial growth in alkali pH values in the case of allergic rhinitis during patient use. It also simulates the behaviour of Lysozyme enzyme which lose its activity in the symptoms of rhinitis due to the change of pH from acidic to alkali values, greater than pH 6.5. The present study also investigate the local tolerance and the in-vitro nasal permeation of beclomethasone dipropionate in nasal spray, nasal film formulations. For this purpose monolayers of the human bronchial cell line Calu-3 were used. Calu-3 cells represent a suitable model to study respiratory drug delivery in vitro in order to quantify the drug's absorption the cumulative transport from the apical to the basolateral side of the cell monolayers. Antimicrobial Effectiveness Test (AET) were performed for nasal film suspension and TEER (Trans epithelial electrical resistance) values for Calu-3 monolayers were determined. TEER values were constantly higher than 300 $\Omega \bullet cm^2$ over the whole period of the transport studies. The adhesion during the 30 minute incubation phase was low but after 270 minutes no active ingredient be detected in the donor. Test formulations, nasal film, had suitable results in tolerance test with good stability of the active ingredient and yielded similar absorption behaviour with spray formulations.

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FLEXIBLE NANO-SIZED VESICLES AS NOVEL OCULAR DELIVERY SYSTEMS FOR VORICONAZOLE: OPTIMIZATION AND CHARACTERIZATION STUDIES

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Topical treatment of infectious eye diseases is required an effective drug delivery to overcome the limitations of eye's defense mechanism. Nano-sized novel drug delivery systems seem to be promising to enhance ocular permeability of drugs and to improve treatment of patients with fungal keratitis. In this context, the aim of this study was to investigate the potential of flexible nano-sized vesicles as an ophthalmic delivery system for voriconazole to improve its corneal permeability. Voriconazole loaded vesicles were prepared by the thin-film method. Optimized vesicles were characterized in terms of their particle size, polydispersity index and drug entrapment efficiency. In vitro release behavior and ex vivo membrane permeation of voriconazole across sheep cornea using Franz-diffusion cells were also assessed. Voriconazole loaded vesicles have mean size of about 100 nm with narrow polydispersity index and negative zeta potential and over 85% of encapsulation efficiency. Sustained drug release was observed via nano-sized vesicles in comparison with control formulation. Voriconozole permeation from vesicles across cornea increased compared to its control formulation. Overall, the data obtained showed that flexible nano-sized vesicles can be considered as promising delivery systems for potential topical treatment of fungal keratitis.

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INCLUSION COMPLEXES OF ROSUVASTATIN CALCIUM WITH SULFOBUTYLETHER-BETA-CYCLODEXTRIN (CAPTISOL®)

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Rosuvastatin calcium (RCa) is a dyslipidemic agent, which exhibits unsatisfactory water solubility, problems in absorption which result in poor bioavailability. Thus, the aim of this study was to enhance the solubility of RCa by preparing the inclusion complexes of API with sulfobutyl ether-beta-cyclodextrin (Captisol®) [1] Phase solubility studies were conducted and (1:1) molar ratio was selected as the proper concentration (Figure 1) [2]. Kneading and lyophilization methods were used to formulate RCa/Captisol® complexes. Scanning electron microscope was used for morphological analyses. The physicochemical properties of the complexes were evaluated by particle size, polydisperisty index (PDI), zeta potential analyses and the analyses results revealed that the complexes were within the nanometer range (210.30 ± 5.70 nm and 321.77 ± 5.23 nm, mean±SE) with homogenous size distribution (PDI data of 0.382 ± 0.01- 0.448 ± 0.02, mean±SE). DSC, XRD, FT-IR and ^{1}H-NMR analyses were accomplished to evaluate the structural properties. Drug content % and the sample analyis of in vitro release studies were performed by a validated HPLC method [3]. Similarity factors (f_2) were also determined. The molar solubility studies results demonstrated that in the form of inclusion complexes the solubility of API has been enhanced in a great extend [4]. After physicochemical characterization of RCa/Captisol® inclusion complexes, CDs-F6 was nominated for further cytotoxicity and permeation studies. According to MTT studies IC_{50} value was determined as 117.071 µM. The statistical analyses were performed using two way ANOVA method.

This study was financed by Anadolu Univeristy Scientific Research Project Foundation (Pr. No: 1404S289).

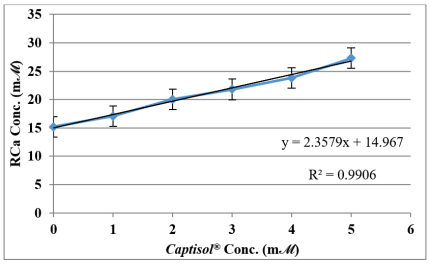

Figure 1. Phase Solubility Diagram of RCa/Captisol® (mean ± SE), (n = 3)

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PREDNISOLONE-LOADED DELAYED-RELEASE NANOFIBER DRUG DELIVERY SYSTEMS

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Electrospinning is as a simple and common method for fabrication of nanofibers from natural and synthetic polymers [1]. Prednisolone is a synthetic corticosteroid and anti-inflammatory drug. Long-term corticosteroid treatment can cause some gastric side effects. Therefore, the delayed-release formulation of prednisolone can avoid high peaks of plasma prednisolone level [2]. The aim of this study is to prepare delayed-release prednisolone nanofibers using Eudragit®L100-55, Eudragit®S100, Kollicoat®MAE 100P and MAE 100-55 polymers and to evaluate their in vitro release profiles. Viscosity, conductivity and surface tension values of drug-free and drug-loaded electrospinning solutions were measured. Polymer concentrations were kept constant at 10% in ethanol (w/v). Electrospinning process parameters of polymer solutions were shown on Table 1. The amount of prednisolone loaded into nanofibers was assayed by UV-Vis spectrophotometer at 247nm. Mean nanofiber diameters were measured from SEM images using ImageJ software. Dissolution profiles were evaluated using USP-I basket method at pH 1.2 and 6.8 buffer solutions at 37°C and rotational speed of 100 rpm. The viscosity and conductivity values of prednisolone-loaded polymer solutions were lower than those of prednisolone-free polymer solutions (Table 1). Uniform and smooth nanofibers were produced from all polymer solutions, except Kollicoat®MAE 100P. In-vitro dissolution studies showed that in acidic media, Eudragit®L100-55 nanofibers released more than 60% prednisolone, although the Eudragit®S100 and Kollicoat®MAE 100-55 nanofibers released less than 40% prednisolone. Prednisolone-loaded nanofibers were successfully produced and characterized for the first time in this preliminary study. Further studies are carrying out with different polymer concentrations to achieve desired release profile.

Table 1. Electrospinning process parameters, viscosity, conductivity and surface tension values of polymer solutions and mean nanofiber diameter and drug loading values of produced nanofibers

Formulation code	Voltage (kV)	Feed rate (mL/h)	Distance of Needle tip to collector (cm)	Viscosity (cPs)	Conductivity (μs/cm)	Surface tension (mN/m)	Mean nanofiber diameter (nm)	Drug loading (%, w/w)
Eudragit® L100-55	13,5	1	10	315	51,9	23,15	252	-
Eudragit® S100	14,5	2,2	10	353	47,5	22,93	1155	-
Kollicoat® MAE 100P	-	-	-	737	41,0	23,35	-	-
Kollicoat® MAE 100-55	15,0	7	15	341	39,0	23,2	650	-
Prednisolone-Eudragit® L100-55	13,5	1,5	10	31,7	43,1	21,96	344	34
Prednisolone-Eudragit® S100	14,0	4	15	31,7	43,5	22,54	2526	14,78
Prednisolone-Kollicoat® MAE 100P	-	-	-	421	37,4	24,9	-	-
Prednisolone-Kollicoat® MAE 100-55	13,5	7,5	15	6,6	27,2	23,22	964	27,16

Table 1.

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DEVELOPMENT AND ANTIMICROBIAL EVALUATION OF VETERINARY INTRAMAMMARY OINTMENT CONTAINING FUSIDIC ACID

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Mastitis is an inflamatory udder disease and commonly seems in milker livestock. It effects their milk yield and productions and so causes serious economical losses. Our purpose in this study is to develop a veterinary ointment formulation containing fusidic acid and test its in vitro efficacy against mastitis. Fusidic acid has a steroidal structure and is derived from Fusidium coccineum and it has been used in clinics since 1962. It is effective against especially methicillin-resistant Staphylococcus strain and other aerobic and anaerobic bacterias. In our study, we prepared different ointment formulations containing % 1 fusidic acid using different surfactants and their different ratios and all of the formulations were sterilized by gamma radiation. The antimicrobial activity of the formulations were tested with Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212 strains. Fusidic acid reference standard and previously prepared 5 g intramammary suspension formulation containing 100.000 IU of penicillin G procain, 100 mg streptomycin sulphate, 100 mg neomycine sulphate and 10 mg prednisolone were used as positive controls in this antimicrobial test studies. Disc diffusion method was used for the in vitro studies. In conclusion, veterinary intramammary ointment formulations that contain fusidic acid were prepared, and their stability and the other parameters of their specifications were tested. The analysis results of our each formulation were conformed to the specifications. All of the formulations developed in this study showed similar antimicrobial activity to the positive control groups according to the antimicrobial test results.

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DESIGN AND OPTIMIZATION OF FLUTICASONE PROPIONATE LOADED SOLID LIPID NANOPARTICLES BY FACTORIAL DESIGN

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Fluticasone propionate (FP) is a potent topical corticosteroid and widely used for the treatment of skin diseases such as atopic dermatitis, psoriasis and vitiligo because of its anti-inflammatory, immunosupressive and antiproliferative effects [1]. Nanosized drug delivery systems are extensively investigated for dermal drug delivery into the different skin layers. Solid lipid nanoparticles (SLNs) are colloidal drug delivery systems consisted of lipids or lipid mixtures which are in solid state at body and room temperature. SLNs get a great attention because of their potential for dermal delivery of drugs. In this study, FP loaded SLN formulations were prepared using high pressure homogenization method. Nine formulations were prepared using a 3^2 factorial design to select the optimum SLNs. Tristearin percentages (1%, 2% and 4%) and homogenisation cycle numbers (2, 4, 8 cycle) were investigated based on two responses: encapsulation efficiency (Q1) and particle size (Q2). The Q1 response values of the SLNs varied widely from 27.07% to 94.65%. When the Q2 responses were examined, the mean particle size of SLNs was found to range from 130.9 to 352.9 nm. All of the SLNs exhibited negative surface charge between -19.5 and -29.7 mV. According to factorial design study obtained in this research, the optimum formulation could be achieved with the content of 4% tristearin and the homogenisation cycle 4.

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P44

DEVELOPMENT AND IN VITRO EVALUATION OF PH TRIGGERED HOLLOW SILICA NANOPARTICLES AS A DRUG DELIVERY SYSTEM

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Hollow mesoporous silica particles (HMSPs) are quite impressive due to its superior properties such as easy modification, high biocompatibility, low toxicity and mechanical and thermal stability among the hollow structured materials. HMSPs are advantageous with regarding to high internal volume, controlled release of water-soluble drugs and convenience to design as a stimuli-responsive system [1, 2]. In this study, it is aimed to achieve an effective breast cancer treatment with decreased side effects by decreasing the amount of drug in systemic circulation and increasing doxorubicin concentration in tumor tissue via preparing an efficient pH-triggered system by exploiting acidic tumor environment [3]. To achieve this purpose, HMSPs were synthesized and optimized by design of experiment. The following steps were taken to synthesize the particles: First of all amorphous silica nanoparticles were prepared as templates, which will create the interior cavity by Stöber method [4], [5]. Then, these particles were coated with mesoporous silica shell by modified Stöber method which includes a surfactant as a structure directing agent. After that, template was removed by selective etching. Finally, surfactant was removed by acidic ethanol extraction. Characterization of the obtained particles was performed by DLS, FTIR, SEM and drug loading capacities were examined. Average nanoparticle size was 200nm according to DLS measurements and SEM images indicate particle shape is spherical. FTIR analysis was performed to determine the surfactant removal from the particles, and it was understood the extraction procedure was successful. Doxorubicin was loaded into HMSPs and drug release studies were performed at different pHs.

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CHARACTERIZATION OF BEVACIZUMAB BY DYNAMIC LIGHT SCATTERING TECHNIQUE

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Antibody loaded nanoparticles can provide many advantages such as controlled release, targeted delivery and avoiding from protein inactivation. However thermal and mechanical stress during the encapsulation process can cause antibody stability problems [1]. Bevacizumab is a recombinant monoclonal antibody that approved by Food and Drug Administration, for treatment of patients with, metastatic renal cell cancer, non-small-cell lung cancer, metastatic colorectal cancer and cervical, epithelial ovarian and fallopian tube cancer in the USA and Europe [2]. Bevacizumab loaded nanoparticles were prepared in many studies [3, 4] for improved treatment. Dynamic Light Scattering (DLS) technique, which is based on the measurement of intensity and variation of light emitted from small particles in a dilute solution, is a rapid and non-invasive method for protein size determination and a reliable way to see protein aggregation [5]. In this study, DLS technique was used to determine bevacizumab stability in different conditions that may be encountered during preparing nanoparticles. For this aim, the hydrodynamic diameter of bevacizumab was measured by DLS technique at different temperatures and pHs and under different sonication conditions. The hydrodynamic diameter of bevacizumab varies depending on the concentration of the protein solution and is in the range of 12-15nm. Average particle size of bevacizumab after stress conditions was 30nm according to DLS measurements which indicates antibody aggregation. It was determined that the pH change had a great effect on bevacizumab stability. The average particle size exceeded 100nm, with the PDI approaching 1, the presence of aggregation was observed under varying pH conditions.

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SYNTHESIS AND CHARACTERIZATION OF SILICA NANOPARTICLES: OBTAINING NANOPARTICLES SMALLER THAN 100 NM

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Nanoparticular drug delivery systems have significant advantages such as controlled drug release and targeted drug delivery [1]. For understanding the mechanisms of targeted drug delivery, interaction between cells and nanoparticles is critical. Various studies showed that caveolin-mediated endocytosis could be a better strategy than others, since it may allow nanoparticles payload to escape from lysosomal degradation. Although it is indicated that nanoparticles with a particle size below 100 nm are internalized by caveole-mediated endocytosis, preparation of such small nanoparticles is more complicated [2]. Mesoporous silica nanoparticles (MSN) have been highlighted as an interesting drug delivery platform, due to their tunable particle size and pore diameter, flexible surface functionalization, biocompatibility and biodegrability. MSNs are also known as carriers with high loading capacity for both hydrophobic and hydrophilic drugs [3,4]. In this study, it is aimed to prepare doxorubicin loaded MSNs which are smaller than 100 nm to escape from lysosomal degradation. For this aim, MSNs were synthesized by modified Stöber method. The average particle size of nanoparticles and zeta potentials were measured by zetasizer. Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analyses also performed. Results show that, spherical nanoparticles were obtained with a particle size of 60-90 nm and size distributions were consistent between SEM and DLS results. FITR analyses confirmed that surfactant was successfully removed from nanoparticles. Encapsulation efficiency of MSNs was more than %90. Studies, to overcome rapid elimination of the obtained small sized MSNs via hybrid nanoparticle approach, are ongoing.

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THE BIODISTRIBUTION OF FARNESYLTHIOSALICYLIC ACID INCORPORATED HYBRID NANOPARTICLES FOLLOWING INTRANASAL ADMINISTRATION IN RATS

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The blood-brain barrier (BBB) limits the entrance of many drugs into the brain. To bypass the BBB, intranasal drug delivery can be used to directly enter the brain via systems such as the olfactory bulb. In this study the biodistribution of our previously developed farnesylthiosalicylic acid (FTA) incorporated hybrid nanoparticles (HNPs) was investigated in female Wistar rats. Following intravenous (IV) or intranasal (IN) administration of either free FTA or FTA loaded HNPs with a FTA dose of 500 µM, blood samples were taken at 1, 4, 24, 72 and 120 hr after application. Also, after 4, 24 and 120 hr, a number of animals were sacrificed and the brain, olfactory bulb, liver and spleen were collected. Blood plasma and organ homogenates were analyzed for FTA content with LC/MS/MS method [1]. FTA loaded HNPs remained present in plasma for at least 24 h, and even for 120 h. Moreover, the half-life for IV and IN FTA loaded HNPs was increased with 5- and 6-folds, respectively compared to IV administered free FTA. The highest accumulation of FTA in the brain was observed for IN and IV applied FTA loaded HNPs. Moreover, IN FTA loaded HNPs were able to accumulate in a higher degree in the olfactory bulb. FTA is delivered to the brain via the olfactory bulb following IN drug delivery. Both IN and IV routes cause equal accumulation of the drug in the brain. However, with regard to systemic toxicity, the IN route is superior to the IV route.

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DEVELOPMENT AND IN VITRO CHARACTERIZATION OF THERMOSENSITIVE HYDROGEL SYSTEM FOR OCULAR DELIVERY OF RIBOFLAVIN-5 PHOSPHATE SODIUM

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Kerataconus is a noninflammatory disease which is characterized with bilateral ectasia of cornea [1]. Riboflavin, also known as vitamin B2, is a vitamin which has been used as photosensitizer in UVA mediated collagen crosslinking. The aim of this study was to develop thermosensitive hydrogel formulations to extend residence time of riboflavin-5-phospahate sodium on eye surface. Riboflavin-5-phospahate sodium was used 0.1% (w/v) concentration in the formulations while Pluronic F-127 was used in different ratios (15%, 18% and 20%, w/v for formulation A1, A2 and A3, respectively) as polymer. Sodium chloride (0.7%) and benzalkonium chloride (0.01%) were added to formulations as osmolarity adjusting agent and antibacterial preservative, respectively. Viscosity measurements of the developed formulations were performed with the Brookfield RV2T instrument. The gelation temperatures of the formulations were determined by the tube inversion method. Viscosity values of the formulations were determined as 196.26 cP, 17908 cP and 37500 cP for A1, A2 and A3 formulations, respectively at 32°C. Also, A2 formulation had the optimal gelation temperature which was found as 31.4°C. A2 formulation was found as promising candidate among the developed formulations for the ocular delivery of riboflavin-5-phospahate sodium since it provided a wide range of confidence for the formulation to remain liquid at room temperature and was in gel form at the surface temperature of the eye.

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STUDIES ON ABSORPTION ENHANCEMENT OF TAMOXIFEN BY LIPOSOME FORMULATIONS

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Breast cancer is known to be the most common type of cancer among the women worldwide. The utilization of drug delivery systems for anticancer therapy provides enhanced efficacy and/or reduced toxicity and side effects. Liposomes are the most common vesicular lipid-based carrier systems and have been used to enhance permeability and bioavailability of the drugs with low solublity. Their main advantage is to show structural similarities with biological cell membranes. Hence, liposomes are safe, biodegredable, biocompatible and can be used as both systemic, oral, topical dosage forms [1]. Tamoxifen is the first representive of the SERM (Selective Estrogen Receptor Modulators) group drugs and approved by FDA for the treatment of estrogen positive breast tumors [2]. In this study, new liposome formulations of tamoxifen was developed using dimethyl-β-cyclodextrin (DM-β-CD) and sodium taurocholate as absorption enhancers. Caco-2 (Human colorectal carcinoma cell line) model was used to investigate oral absorption properties of developed liposome formulations. The liposome formulations' cytotoxic properties, Caco-2 transportation properties were investigated comparatively with solutions and permeability coefficients were calculated. On the other hand, formulations were given to mice orally and tamoxifen analyzed from plasma samples (at the same time intervals with Caco-2 transportation studies) to evaluate in vitro/in vivo correlation. According to the results, DM-β-CD successfully enhanced tamoxifen transportation. While the initial TEER value was 285 Ω, 226 Ω was measured for tamoxifen+ DM-β-CD liposomes at the end of 24 hours. This result indicated that DM-β-CD in liposome formulation, increased tamoxifen transport by opening tight junctions.

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ANTITUMORAL AND MMP-2 ENZYME INHIBITION ACTIVITIES OF RALOXIFENE NANOCOCHLEATES ON MCF-7 AND MDA-MB-231 CELL LINES

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Raloxifene-HCl is a SERM (Selective Estrogen Receptor Modulators) group drug which approved by FDA for the treatment of osteoporosis. It binds to estrogen receptors on breast tissue and endometrial cells but unlike tamoxifen, it has no proliferation effect on the endometrial cells [1]. Because of this feature, raloxifene has an extensive area of clinical studies on breast cancer therapy. Raloxifene is known to be a BCS Class II drug. Its absolute bioavailability is about 2% due to poor solubility and extensive first pass metabolism [2]. Cochleates are cylindrical lipid-based delivery systems micro or nanostructures that consist of a series of lipid bilayers. In this study, raloxifene nanocohleates were formulated using absorption enhancers (dimethyl-β-cyclodextrin and sodium taurocholate). The antitumoral activity studies were evaluated on estrogen receptor positive (MCF-7) and negative (MDA-MB-231) cell lines. Matrix metalloproteinase-2 (MMP-2) enzyme inhibition properties of formulations were also determined because it is known that raloxifene inhibits MMP-2. This enzyme is responsible for tumor invasion and the initiation of angiogenesis during the tumor growth. Among the formulations, the highest antitumoral activity (41.6%) and MMP-2 inhibiton (51.9%) results were obtained with raloxifene+dimethyl-β-cyclodextrin cochleates on MCF-7 cell line.

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EFFECTS OF PVA CONCENTRATION ON THE SIZE DISTRIBUTION OF NANOPARTICLES PREPARED BY EMULSION–SOLVENT EVAPORATION METHOD

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Levodopa (L-3,4-dihydroxy phenylalanine) is utilized for drug therapies for Parkinson disease. Although levodopa is still the most effective drug in Parkinson therapy, when it is administered orally, it has low oral bioavailability (30%) [1-3]. Main objective of our study is enabling accession of levodopa into the target tissues via novel nanocarrier systems, in order to provide more effective, safe and qualified treatment of Parkinson disease. For this purpose, obtaining the effective passage of nanocarriers into the brain parenchyma and thereby optimal thereputic effect has been aimed, using mucoadhesive compound modified nanoparticles. In order to increase the bioavalibility of levodopa, poly(d,l-lactide-co-glycolide) acid (PLGA) nanoparticulate drug delivery may be a solution. In this part of our study, nanoparticles were prepared by double emulsion-solvent evaporation method (w/o/w), using methylene chloride as an organic solvent and polyvinyl alcohol (PVA) as the surfactant. Experimental parameters such as the surfactant concentration were investigated for its effect on particle size and the polydispersity index (PDI) on levodopa loaded PLGA nanoparticles. Among the tested PVA concentrations, it was found that when the PVA concentration was incerased (5%), both PDI and the particle size decreased.

Formulation	PVA concentration %(a/h)	PDI	Size (nm)
F1	1	0,627±0,047	432,1±20,96
F2	2,5	0,619±0,158	417,2±70,2
F3	5	0,480±0,045	357,7±40,90

Table 1. Effect of PVA concentration on the size distribution of nanoparticles

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USING LIPOSOMES CONTAINING ERLOTINIB-DEXKETOPROFEN FOR THE TREATMENT OF CANCER

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Liposomes are developed as drug delivery system that enable the active substance to remain stable, facilitate uptake by cells or tissues, improve access to the target site and they have a great influence from many biomedical fields [1]. It is important to evaluate the release rates of active substance from drug delivery systems in order to determine the characterization of formulations as well as to predict their in vivo behaviors [2,3]. Although many methods have been used in the literature, the dialysis bag method is the most popular and versatile one [4]. Apart from this, Caco-2 cells are frequently used to study intestinal absorption of orally administered drugs [5]. Erlotinib-dexketoprofen loaded liposomes were prepared by Bangham method. In vitro release study was carried out using dialysis bag. Caco-2 cell permeation studies were carried out. The release media were pH 3 acetate buffer and DMEM with 40% propylene glycol for dialysis bag and Caco-2 cell studies, respectively. The mixing rate was 100 rpm. The temperature was kept at 37°C. The studies were done in 3 triplicate. The results have been interpreted on graphics. Although higher release values were obtained for solutions in dialysis bag studies, the percentage of drug permeations were found to be higher for formulations with PEG in Caco-2 cell studies. This is thought to be caused by PEG which can reduce the resistance of cells.

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COMPARISON OF INTESTINAL PERMEABILITY OF NEBIVOLOL HYDROCHLORIDE IN SOLID LIPID NANOPARTICLES AND COMMERCIAL TABLET

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Hypertension, or high blood pressure, is one of the main risk factors for cardiovascular diseases. Nebivolol (NBV) is a third generation cardioselective beta 1-blocker that combines beta-adrenergic blocking activity with a vasodilating effect. The oral application of drugs is the most popular route for achieving systemic effects; nevertheless it is limited by difficulties related to physicochemical properties of the drug. Solid lipid nanoparticles (SLNs) are of great interest due to their ability to increase the solubility, and also to improve the oral bioavailability via different mechanisms [1]. The aim of the present study was to compare and evaluate the in-situ intestinal permeability of NBV loaded SLN and its commercial tablet formulation using Single-Pass Intestinal Perfusion (SPIP) method. NBV loaded SLNs were prepared by homogenization technique using compritol, poloxamer, lecithin, and modified with polyethylene glycol (PEG). Particle sizes of blank and loaded SLN were 213.4±17.5 and 264.1±18.8 nm, respectively with polydispersity index values of approximately 0.3 for each. NBV loading resulted in positive electrical charge on SLNs. The encapsulation efficiency was 98.04±0.2 %. Permeability coefficient values were tripled when NBV was incorporated in SLNs, and doubled when pure NBV was given separately with a blank SLN (Figure 1). PEG modified SLN can be used to enhance oral absorption of NBV, and SLNs alone can be used as permeation enhancer in oral drug delivery.

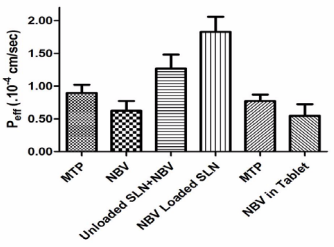

Figure 1. The permeability coefficients (Peff; cm/sec) of NBV obtained from perfusion of rat ileum.

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DEVELOPMENT AND CHARACTERIZATION OF SELF MICROEMULSIFIED DRUG DELIVERY SYSTEMS FOR CISPLATIN

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Cancer, today, is one of the most threatening diseases to society. Ovarian cancer is the ninth most common cancer in women and the most lethal gynecologic cancer [1]. Cisplatin is indicated in primary, advanced stage and refractory ovarian cancer. Classical drug application routes in chemotherapy are oral and intravenous. There are some disadvatages of these methods. Absorption of the drugs used for the oral route is not complete [2]. The aim of this study is to develop a new self microemulsion dug delivery system (SMEDDS) for cisplatin to enhance the oral bioavailability of drug. In this study, isopropyl myristate was used as the oil phase, Kolliphor were used as the surfactant, propylene glycol was used as the co-surfactant. SMEDD formulations were characterised (Table 1) and dissolution studies were evaluated in pH 6.8 phosphate buffer. Permeation studies were examined with Caco-2 cell culture.

CHARACTERIZATION STUDIES		
	DEVICE MODEL	VALUE
pH	METTLER TOLEDO	7,24
CONDUCTIVITY	METTLER TOLEDO	21,3
REFRACTIVE INDEX	ATAGO	1,4489
VISCOSITY	AND-VIBRO VISCOMETER	273 cP
EMULSIFICATION TIME	USP PADDLE APP	10 seconds
DISPERSITY	USP PADDLE APP	LEVEL A
% TRANSMISSION	LAVIBOND	2,88
TURBIDITY*	SHIMADZU SPECTROFOTOMETER	0,319
DENSITY	PIKNOMETER	1,282
PARTICLE SIZE	MALVERN ZETASIZER	24 nm
ZETA POTENTIAL**	MALVERN ZETASIZER	-2,07

* Wavelength set to 254 nm to measure absorbance value.
** Formulation was diluted with water by the ratio of 1:500.

Table 1: Characterization Studies of Cisplatin SMEDD

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DETERMINATION OF CRITICAL MICELLE CONCENTRATION OF LIPID BASED SYSTEMS WITH CISPLATIN

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Surfactant solutions which are prepared by different concentrations, could show rapid changes of its osmotic pressure, conductivity, turbidity and surface tension at higher concentrations [1]. McBain associated these rapid changes with the formation of micelles or aggregates. The lyphophyllic hydrocarbon chains which have hydrophyllic groups that interacts with water phase, move to the inner part of the micelles. The critical micelle concentration (CMC) is defined as the concentration of surfactants above which micelles form and all additional surfactants added to the system go to micelles [2]. The aim of this study is determination of critical micelle concentration of lipid based system with cisplatin by surface tension method. Isopropyl myristate was used as the oil phase, Kolliphor were used as the surfactant, propylene glycol was used as the co-surfactan to produce lipid based system. Surface tension is typically measured in dynes/cm, the force in dynes required to break a film of length 1 cm. There are several methods to measure the surface tension such as Wilhelmy plate, capillary rise method, drop weight method. In this study Traube Stalogmometre is used for drop weight method. Water, HCl buffer with pH 1,2 and phosphate buffer with pH 6,8 were used as solvents. $0.24x10^{-3}$, $1.2x10^{-3}$, $2x10^{-3}$, $4x10^{-3}$ ve $6x10^{-3}$ g/ml concentrations of smedds (self microemulsifying drug delivery system) with cisplatin are prepared to measure surface tension in these solvents.

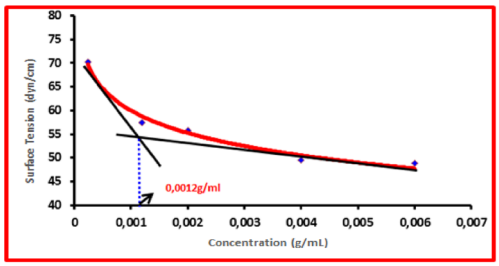

Figure 1: Surface tension-concentration graph for lipid based system with cisplatin

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P56

IN VITRO PERMEATION STUDY FOR ORALLY DISINTEGRATING TABLETS CONTAINING MIRTAZAPINE

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In present study, in vitro permeation study of mirtazapine in developed orally disintegrating tablets (ODTs) was carried out to investigate the apparent permeability coefficients, to have a preliminary opinion about the absorbance of drug for oral administration. Five tablets of developed formulations (Table 1), reference product (Remeron SolTab) and pure mirtazapine were dissolved in DMSO using ultrasonic bath for 15 min. Samples were diluted with pH 6.0 HBSS to obtain 50 µM final concentration. Both donor and receiver sides of the wells were aspirated with HBSS before starting permeability studies and Caco-2 cells (ATCC) were pre-incubated for 15 min. Samples (n=3) were added to apical side as 500 µL, PBS (pH 7.4) was added to basolateral side as 1000 µL. After 2 h, samples were taken from both apical and basolateral sides and analyzed using a previously validated HPLC method. Apparent permeability coefficients (Papp) were calculated. According to acquired results, A3 and B2 formulations (including different coating ratio or different fillers) showed highest apparent permeability coefficients. This could be attributing the positive effect of the excipients (type and ratio) in both formulations on Papp values of mirtazapine. A3 and B2 formulations had the closest Papp values to reference product. Developed mirtazapine ODTs were found promising in terms of showing the better or similar Papp values to the original formulation.

Materials used in formulations	A1	A2	A3	A4	B1	B2
mg/tablet						
Mirtazapine	45	45	45	45	45	45
Kollidon® CL (core)	40	40	40	40	40	40
Eudragit E® 100	2,70 (%6)	2,70 (%6)	2,70 (%6)	2,70 (%6)	3,60 (%8)	3,60 (%8)
Citric acid anhydrous	20	40	20	20	20	20
Pharmaburst™ C1	270	250	264	245	264	245
Aspartame	2,5	2,5	2,5	2,5	2,5	2,5
Orange flavor	2	2	2	2	2	2
Kollidon® CL	25	25	25	25	25	25
Microcrystalline cellulose	-	-	-	130	-	130
Kollidon® CL-F	130	130	130	-	130	-
Sodium bicarbonate	-	-	6	6	6	18
Sodium stearyl fumarate	10	10	10	15	10	15
Aerosil® 200	2,5	2,5	2,5	2,5	2,5	5

Table 1

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EVALUATION OF THE EFFECT OF POLYMER VISCOCITY ON DRUG RELEASE FROM PULSATILE RELEASE TABLETS

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Conventional oral controlled release systems may not always be suitable for biological systems or disorders which require specific drug concentrations at specific times according to circadian rhythm [1]. Some functions of cardiovascular system like blood pressure and heart rate are associated with circadian rhythm [2]. Blood pressure is at its lowest level during sleeping period and increases gradually during early morning hours. Pulsatile drug delivery systems are characterized by a lag time followed rapid and complete drug release which would be effective in treating morning surge hypertension with minimum side effects [1-3]. Press-coated tablets can obtain therapeutic drug concentrations at the time which the symptoms of the diseases arise by bed-time administration of drugs. The purpose of this study was to improve solubility of the poorly water soluble drug telmisartan by using solid dispersions technique and then formulate into press-coated tablets for pulsatile delivery. Core tablets were prepared with crosscarmellose sodium as a superdisintegrant for immediate release by the direct compression method. Core tablet was press coated by using a hydrophilic polymer, hydroxypropyl methylcellulose (HPMC) and a hydrophobic polymer, cellulose acetate propionate (CAP) in various proportions, which prolong the lag time. The press coated tablets showed different release profiles with clear lag times followed by different release phases depended on coating layer compositions. Incorporation of a pore forming agent, HPMC, in CAP coating layer modulated the lag times and drug release profiles and resulted in release profiles with different lag times and release phases.

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P58

DETERMINATION OF OXICONAZOLE NITRATE'S ANTIFUNGAL ACTIVITY CHARACTERIZATION AGAINST CANDIDA ALBICANS TYPES AND COMPARISON WITH DEVELOPED THERMOSENSITIVE GEL FORMULATION

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Superficial fungal infections caused by C. albicans species are common skin diseases [1]. Therefore, in this study, it was aimed to develop a new formulation containing oxyconazole nitrate, which is an azole group derivative for antifungal treatment, as a thermosensitive gel instead of transdermal conventional dosage forms and the MIC value of the developed thermosensitive formulation against the C. albicans agent was calculated and time-kill analysis was performed. Viscosity, transition temperature $T_{SOL-GEL}$ (C) and gelation time of the thermosensitive gel formulation were also determined in the viscometer (Haake Viscotester 7 Plus). The measurements performed on the tensilometer device were analyzed for adhesion hardness and elongation percentages of the formulation. In the FT-IR spectrometer (Perkin Elmer), the spectrum of solution and gel state were compared between 650-4000 cm^{-1} and it was found that there is no difference between them. Therefore it has been determined that the temperature is recycled on the formulation and does not cause any disruption of its components. Characterization parameters of the thermosensitive gel formulation containing oxiconazole nitrate and time activity against C. albicans yeast is proved to be the same as those of the solution containing only oxyconazole nitrate, it will be appropriate to support with future studies for use in superficial fungal infections.

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P59

IN VITRO & IN VIVO INVESTIGATIONS ON ROSUVASTATIN SOLID DISPERSIONS PREPARED WITH SKIMMED MILK

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Rosuvastatin(RVS) is a crystalline, poorly water soluble drug and therapeutically HMG-CoA reductase inhibitor. To improve the solubility of the drug and dissolution rate, the complexation of RVS with skimmed milk (SM) from different sources (cow and goat milk) was studied. A physical mixture (PM) and solid dispersion of RVS (SD) with SM were prepared. The lyophilization method was used to prepare the SD. Detection of SD formation was performed in the solid state using differential scanning calorimetry (DSC), powder X-ray diffractometry, and SEM. The diffractogram of the SD differed from that of the PM, where the characteristic peaks of RVS, particularly at 80° and 164°, nearly disappeared, indicating the formation of an inclusion complex. These observations were in accordance with the results of the DSC analysis. Disappearance of the specific DSC peaks of the drug in the DSC curve of the SD showed that the drug interacts with the carrier. Solubility studies were also conducted on PM and SD of RVS in comparison to pure drug. The results indicated enhaced solubility with SD formulations. In order to determine therapeutic effect of SD formulation, in vivo anti-hyperlipidemic activity was assessed on the hyperlipidemic rat model. Oral administration of the RVS-SD for a period of 15 days resulted in significant lowering of the levels of total cholesterol (7.31 mmol/L) and LDL (4.16 mmol/L) with an increase in HDL (45.3 mmol/L) in HFD-induced hyperlipidemic rats. In conclusion, the SD of RVS could effectively reduce or control the amount of serum cholesterol and LDL.

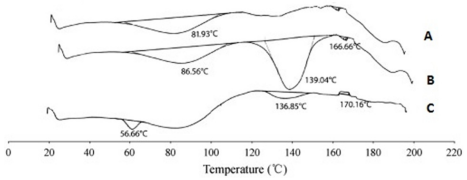

Figure 1. DSC chromatograms of SM (A), PM(B) and SD (C).

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P60

EFFECT OF PROPOLIS & CO-ENZYME Q10 CO-ADMINISTRATION ON THE THERAPEUTIC EFFICACY & GENOTOXICITY OF METHOTREXATE IN RATS WITH RHEUMATOID ARTRITIS

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Rheumatoid arthritis (RA) is a common severe joint disease affecting patients in all age groups. The aim of the present study is to examine the combined effect of propolis (PPS), coenzyme Q10 (CoQ10) and methotrexate (MTX) on the progression of collagen induced arthritis (CA) in rats and also genotoxicity of MTX. The experiments included 7 groups of rats: I: healthy animals (control group), II: arthritic rats (AR) not treated (sham group), III: AR treated with CoQ10, IV: AR treated with PPS, V: AR treated with MTX alone, VI: AR treated with a combination of CoQ10 and MTX, VII: AR treated with a combination of PPS and MTX. The latter groups received a daily oral dose of 20 mg/kg b.w. of CoQ10 or PPS, either alone or with MTX (0.3 mg/kg b.w. twice a week). It was found that CoQ10 and PPS potentiated both the antiarthritic (decrease of hind paw volume) and the antioxidant effects of MTX on the level of oxidation of proteins as well as lipoperoxidation. Moreover, the combination therapy improved the functionality of peripheral blood neutrophils in CA. Genotoxicity was investigated using micronucleus test (MN). MN frequency values were determined for each group. In conclusion, combined administration of CoQ10 or PPS and methotrexate suppressed arthritic progression in rats more effectively than did MTX alone. On the other hand, it was determined that genotoxicity of MTX was reduced by co-administration of CoQ10 and PPS during MTX treatment. This findings may help improve treatment of rheumatoid arthritis.

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BREAKING STRENGTH OF THREE DIFFERENT ABSORBABLE SUTURES

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Sutures are the medical devices to approximate the tissues and hold wound edges together until the healing process has completed. Sutures are used for reapproximate the tissues, incision and ligation of blood vessels. Sutures can be classified into different groups as to their physical and the other properties. One of this properties is absorbability. After implantation, an absorbable suture loses most of their breaking strength within in 60 days. These sutures may be monofilament or braided construction derived from synthetic or natural origin. Synthetic bioabsorbable polymers have been investigated for several biomedical, pharmaceutical and industrial applications. Polylactides, polyglycolide co-lactide(PGLA), poly(ε-caprolactone) (PGCL), polydioxanone(PDO) and their copolymers at varying rates are used to produce surgical sutures. Sutures can be described their properties such as tensile strength, breaking strength, elasticity, capillarity and memory. In this study we compare breaking strength of three different absorbable sutures at the same gauge number. Measurement of breaking strength of the tree different absorbable sutures has carried out with tensilometer device. In the measurements of the USP 1 and metric 4 gauge sutures, mean of breaking strength and standard deviation has been found respectively in PDO 67,357 and 1,511; in PGCL 69,149 and 1,189; in PGLA 63,416 and 1,240. The results of the breaking strength has evaluated according the European Pharmacopoeia.

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THE RELATIONSHIP BETWEEN INSULIN LIKE GROWTH FACTOR-1 (IGF-1) THERAPY AND ANTI-OXIDANT PARAMETERS DURING WOUND HEALING

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Insulin like growth factor-1(IGF-1) belongs to the family of growth factor that orchestrate metobolizm, growth and cell differentiation as well as cell survival. IGF-1 is distributed in various tissues, including skin and it is strongly expressed in the injury area [1]. There are limited numbers of studies related to IGF-1 application effect of antioxidant levels in dermal wound healing. The objective of this study was to evaluate the relationship between IGF-1 therapy and antioxidant parameters during wound healing. Wistar-albino male rats were used for this study. A linear full thickness excisional wounds were made under anesthesia in rats. Twenty-four male rats divided into 4 groups: control, untreated wounds, control BSA and IGF-1 treatment (1.5 ng/ml). All groups were sacrificed under anesthesia on the 3rd day. Tissue glutathione (GSH) and ascorbic acid (AA) levels were determined by spectrophotometric methods. The GSH levels were decreased in the wound tissue by IGF-1 treatment when compared to BSA control group ($P<0,05$). The wound tissue AA levels were increased by IGF-1 treatment when compared to BSA control ($P<0,05$). IGF-1 application may arrange wound tissue antioxidant capacity in the early phase of wound healing.

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P63

POTENTILLA ERECTA EXTRACT INCREASES THE GLUTATHIONE LEVELS OF WOUND TISSUE IN STZ-INDUCED DIABETIC RATS

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Oxidative events are one of the important factors affecting the wound healing process. We investigate to effect of extract of Potentilla erecta (EPE) on glutathione (GSH) levels in diabetic wound healing process. Plant samples were extracted with methanol by using soxhlet apparatus. After extraction, the methanol solvent was evaporated by using rotary evaporator. Experiments were performed on 36 adult male Wistar-albino rats (200–250 g).The animals were divided into 3 main group: non-diabetic (NDM), diabetic (DM) and P. erecta (EPE) groups. Diabetes was induced by a single dose intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Using an 8-mm punch, six uniform full-thickness dorsal excisional skin wounds were created in all rats. The wounds were topically treated with 50 mg/kg P. erecta methanolic extract in the EPE group. No treatment was applied to the NDM and DM groups. After the procedures, on the 3th and 7th days of healing, the rats were sacrificed. At the same time skin punches indicating initial day were also collected from non-wounded animals of NDM and DM groups. The GSH levels were measured spectrophotometrically at 412 nm [1]. In the DM group, the GSH levels were significantly decreased on all days compared with the NDM group (P<0,001). EPE administration significantly increased the GSH levels on days 3 and 7 compared to DM group (P<0,001). The findings of this study suggest that extract of P. erecta increases the antioxidant capacity of diabetic wound tissue through glutathione. It may be useful for the development of new drugs in diabetic wound healing.

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P64

LC METHOD DEVELOPMENT FOR DETERMINATION OF OBIDOXIME FROM PARENTERAL PRODUCTS AND STABILITY OF OBIDOXIME AT DIFFERENT PHS

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The organophosphate nerve gas agents are a serious threat in the battlefield. Obidoxime [N,N′-oxydimethylene bis(pyridinium-4-aldoxime) dichloride], a fast reactivating oxime used in organophosphate intoxication at several countries [1]. The storage of these pharmaceuticals is often complicated. The given shelf-lives and stability prediction may become impossible. It is important to control their identity and quality [2]. Reversed-phase HPLC techniques were developed in order to determine the compound in one run at a single wavelength using UV detection. The aim of this study to develop a LC method for the selective and reliable determination of obidoxime from parenteral preparations and to evaluate the effect of solution pH on stability of obidoxime. For this purpose, Zorbax SB-C8 (150 mm, 4.6 mm, 3.5 µm particle size) column with a flow rate of 1.0 mL/min, at 25°C with mobile phase composition of buffer containing PBS (pH 6.0):ACN (80:20 v/v) at 230 nm was developed. Caffeine was used as internal standard. The analysis of obidoxime was obtained within 4.5 min. After the optimization of parameters, the proposed method has been extensively validated. Linear range, limit of detection and quantification values, are also calculated. The linearity of the detector response was determined by plotting peak areas vs concentration. Sensitive determination method for the obidoxime with LC was achieved. The method is further used for the determination obidoxime in developed parenteral formulations. Obidoxime was obtained from parenteral with an acceptable recovery results.

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P65

DEVELOPMENT OF VALIDATED UV SPECTROPHOTOMETRIC ASSAY OF TENOFOVIR

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Tenofovir is nucleotide reverse transcriptase inhibitor with activity against chronic Hepatitis B virus. Tenofovir disoproxil fumarate is a water soluble ester prodrug which is rapidly converted in vivo to tenofovir[1] . It is a BCS Class 3 drug with high solubility low permeability. The aim of this study was to developed a validated spectrofotometric assay of tenofovir. Two solutions (distilled water and ethanol) and compendial buffers (pH 1.2, pH 4.5, and pH 6.8) were used. Analytical method validation parameters (accuracy, precision, repeatability, reproducibility, specificity, sensitivity, limit of detection(LOD), limit of quantitation(LQD), calibration curve, analysis range and recovery) were assesed. Acceptance criteria and coefficient of variation(CV) were determined. The maximum absorbance (λ_{max}) was determined at 260 nm in distilled water, pH 4.5, pH 6.8, and ethanol. λ_{max} was determined at 258 nm in pH 1.2. The all calibrations were an eight point plot. The concentration range was 10-40 µg/mL in distilled water, pH 1.2, pH 4.5, pH 6.8. The concentration range was 10-35 µg/mL in ethanol. Linear correlations between absorbance and concentration of tenofovir were found in drug assay. The standard curve equations were found and a regression coefficient (r^2) was 0.999 for each media. The average recovery was determined as 98-103%. The samples were stable for at least three days when kept at 4°C. As an application drug concentrations were found using this validated spectrofotometric method in a solubility and dissolution studies of tenofovir.

This study was supported by a research grant(115S405) from the Scientific and Technical Research Council of TURKEY(TÜBİTAK).

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P66

DEVELOPMENT AND VALIDATION OF A SIMPLE AND SENSITIVE HPLC ASSAY METHOD FOR DETERMINATION OF CISPLATIN

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Cisplatin is an important chemotherapeutic drug for cancer treatment. A novel HPLC method has been developed and validated for the determination of assay of cisplatin self microemulsion drug delivery system. HPLC system with UV detector consisted of an isocratic pump and thermostable column part (Shimadzu- 20A). The column was a C18 column HICHROM LICHROSPHER RP18-5 25x4,6 cm [1]. All UV-Vis spectrums were moniterized between 200-400 nm and quantification was performed at 254 nm. The injection volume was 20 µL and the retention time of cisplatin was about 9.27 min. The mobil phase was mixture of water:methanol:acetonitrile (30:40:30) (v/v) pumped at 1 mL/min. The method was validated partially with respect to specificity, linearity, accuracy, precision, repeatability, limit of quantitation (LOQ) and detection (LOD) [2]. In addition, the solubility of cisplatin in water, ethanol, various oils, and surfactants, gastric and intestinal mediums were determined. There is no interference observed on the cisplatin peak, so specificity is verified. 2, 10, 30, 45 and 60 µg/mL were choosen for the linearity (r^2>0.999) (Figure 1). 24, 30 and 36 µg/mL were injected three times at different levels as a test sample for accuracy. Six samples prepared at the same concentration (30 µg/mL) to evaluate method precision and one sample prepared at 30 µg/mL and injected six times for repeatability. LOQ is 0,29 µg/mL and LOD is 0,09 µg/mL.

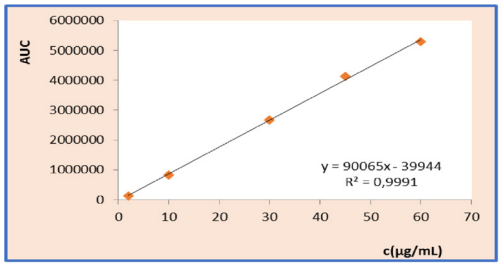

Figure 1: Calibration curve for quantitative analysis of cisplatin

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HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTIFICATION OF TAZAROTENE

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Tazarotene is a topical retinoid that appears to exert its effects via retinoic acid receptors specifically. In psoriasis, tazarotene normalizes abnormal keratinocyte differentiation, reduces their hyperproliferation and downgrades inflammation [1]. The aim of the study was to develop and validate a High-Performance Liquid Chromatography (HPLC) method in order to investigate novel pharmaceutical formulations of tazarotene. The HPLC system consisted of a gradient pump, thermostable column and UV detector supplied by Agilent 1100. The chromatographic separation was achieved on a C18 column (5µm,150×4,6mm) at 25°C in isocratic mode, and column effluent was monitored by UV detector at 346 nm. The mobile phase used was methanol at a flow rate of 1.5 ml/min. The volume of injection was adjusted to 10µl. Method was partially validated according to ICH guidelines with respect to system suitability, linearity, accuracy, precision, specificity, stability and robustness [2]. The linearity between peak area and concentration was analyzed using calibration curve, obtained from the analysis of different concentrations of tazarotene ranging from 1 to 6 ppm (Fig.1). The retention time of tazarotene was about 2.03 min. This method was linear over the range of 1 to 6 ppm with regression coefficient greater than 0.9993. A simple, precise, accurate, reproducible, effective, stability indicating HPLC method was developed and validated for quantitative determination of tazarotene. In this study high recovery, low relative standard deviation confirms suitability of the method for determination of tazarotene. In conclusion, this method could be useful for the routine determination of tazarotene in pure and pharmaceutical formulations.

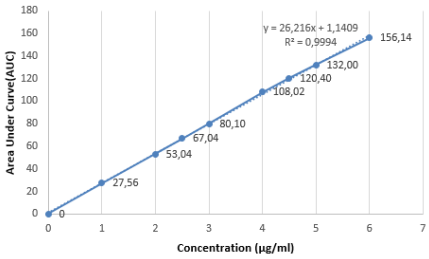

Figure 1. The regression line for tazarotene

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PHARMACOKINETIC STUDY OF VALPROIC ACID

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The valproic acid is an anticonvulsant drug widely used in psychiatric treatments. Monitoring of plasma concentrations is essential for its proper use because there is a wide inter- and intraindividual variability. Its pharmacokinetics profile shows a high protein binding. This characteristic causes that proportional increases in the plasma concentrations are not produced when the dose increases. The purpose of this research is to demonstrate the high pharmacokinetics variability of the valproic acid on steady doses and the lack of proportionality between an increasing dose and the plasma drug levels. We conducted a retrospective observational analysis of patients with serious mental disorders taking valproic acid in a social welfare centre. The recorded data was: demographic data, the doses of the valproic acid, the plasma concentrations, the concomitant medication as well as data from their medical history. The patients were divided in two groups depending on the doses. On one hand, the results showed that the valproic acid has a non-linear pharmacokinetics in the majority of cases. On the other hand, the great intraindividual variability of the plasma concentrations of the anticonvulsant was confirmed. This research shows the need to introduce modifications in the monitoring pharmacotherapeutic follow-up of the valproic acid, because the overall plasma concentration is not a good indicator of the amount of drug in the body.

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EVALUATION OF SPICE-DRUG INTERACTIONS ON THE BIOAVAILABILITY OF ORALLY ADMINISTRATED DRUGS

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Bioavailability of drugs can be affected by many factors including physicochemical, formulation and physiological factors. Also, concomitant use with foods (and spice) may influence the bioavailability of drugs. Phytochemicals in commonly used spices (e.g. black and chili peppers, clove, garlic, ginger, turmeric, cumin) can interact with certain influx (e.g. OATPs, PEPT, MCT) and efflux (e.g. P-gp, BCRP, MRPs) intestinal membrane proteins and also drug metabolizing enzymes. Therefore, bioavailability of substrate drugs may be influenced by spice-drug interactions. The aim of this study was to examine potential spice-drug interactions by surveying the literature using key words such as 'phytochemicals', 'drug transporters' 'drugs interactions' and spice of interest. The search results were then gleaned to identify reports of spice-drug interactions. Results revealed that P-gp is inhibited by black pepper, chili pepper and turmeric, however, garlic increases P-gp activity. On the other hand, both BCRP ve MRPs are inhibited by black pepper, cumin, garlic and turmeric. Several influx transporters (OATP, MCT, PEPT1) are inhibited by garlic only. Except cumin and chili pepper, other spices either inhibit or suppress expression of CYP enzymes. In regard to UGT enzymes, while clove induces liver UGT enzymes, certain UGTs are inhibited by black pepper. All these results demonstrate that spice-drug interactions at the drug transporter/metabolizing enzyme levels may affect the bioavailability of interacting compounds. These interactions could be particularly important in the populations where all these spices are highly consumed on a daily basis.

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EVALUATION OF WATER AND OIL SOLUBILITIES OF BOSENTAN, A BCS CLASS IIA DRUG

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Bosentan monohydrate is a endothelin receptor antagonist specifically for oral pulmonary arterial hypertension, which reduces blood pressure without affecting the sympathetic system or renin-angiotensin system in patients with essential hypertension. Bosentan is a BCS Class IIa drug with low solubility, high permeability and its bioavailability of 50%[1]. For this purpose, the design of lipid-based self-emulsifying nanosystems has been considered. The aim of this study was to compare solubility behaviors of bosentan in water, dissolution media, and different types of synthetic and vegetable oils. 1% SLS, as suggested by FDA[2], was added to water for preparing the dissolution media. Shake-flask method was used for solubility experiments. Bosentan is dispersed in distilled water or oils and shaken for 24 and 72 hours, respectively, at 25 ºC for solubility studies. Solubility in dissolution medium by FDA recommended was performed in 24 hours at 25 ºC and 37 ºC. Samples were analyzed by validated UV spectrophotometric method. The water solubility of bosentan was not found. The dissolution medium solubility of bosentan was found 1,21±0,02 mg/mL at 25 ºC and 1,48±0,08 mg/mL at 37 ºC. The dissolution media and long chain mono and diglyceride synthetic oils were shown higher solubility compare to vegetable oils. Maisine 35-1, which is synthetic oil, was shown the highest solubility (19,5 mg/mL). The interpretation of the solubility values in the light of these results will lead to the selection of the most suitable oils for the preparation of the nanoemulsion formulations which will provide increased solubility of bosentan.

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EVALUATION OF CYTOTOXIC EFFECT OF BIORELEVANT MEDIA ON CACO-2 CELL LINE

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Biorelevant media, which are similar to physiological fluids in terms of various components and physiological factors such as pH, osmolality and buffer capacity are widely used in dissolution studies. The most commonly used biorelevant media are Fasted State Simulated Intestinal Fluid (FaSSIF) and Fed State Simulated Intestinal Fluid (FeSSIF) [1]. In many studies it was observed that solubility and dissolution of low soluble drugs were increased dramatically in biorelevant media, comparing with compendial media. These studies have emphasized the importance of using biorelevant media for particularly BCS class II drugs. The aim of this study was to evaluate cytotoxic effect of biorelevant media on Caco-2 cells in comparison with levofloxacin (BCS Class I drug) and flurbiprofen (BCS Class IIa drug). Standart Caco-2 cell culture procedures were applied. MTT assay was performed to determine effect of the formulations on cell viability. Measurements were performed at 1st and 24th hours. The color density was measured in 570 nm with a multiwell Elisa reader. The results were calculated as a percentage using the control group (DMEM) values. Results were evaluated using cell viability (%) parameter. After 24 hours cell viability values for blank FaSSIF, FaSSIF, blank FeSSIF, and FeSSIF were 73.2 %, 77.5 %, 70.8 %, 69.1 %, respectively. Flurbiprofen (72 - 87 %) and levofloxacin (71 - 89 %) did not show any additional cytotoxic effect on cells. In conclusion, FaSSIF and FeSSIF have shown “slightly cytotoxic” [2] effects on cells. They could be safely used in Caco-2 permeability studies.

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ENHANCEMENT OF SOLUBILITY OF ROSUVASTATIN CALCIUM BY COMPLEXATION WITH METHYL-BETA-CYCLODEXTRIN

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Rosuvastatin calcium(RCa) was defined as a "super-statin" group's member which used in the treatment of elevated low-density lipoprotein(LDL) levels. RCa belongs to the BCS class II having low solubility and high permeability. RCa low solubility causes its elimination from body as such, and desired therapeutic levels are not achieved[1]. The solubility of API can be enhanced by complexation with methyl-beta-cyclodextrin (M-β-CD). The inclusion complex was prepared by two techniques; kneading and lyophilization methods in selected molar ratios of the API and M-β-CD (1:1) according to the results of phase solubility studies (Figure 1)[2].The PS, PDI, ZP and drug content % of the RCa/M-β-CD inclusion complexes were analyzed and the results demonstrated that the prepared complexes were nanosized within the range of 192.60 ± 4.78 nm - 532.80 ± 15.35 nm (mean±SE) with homogenous size distribution considering PDI data (0.310 ± 0.03-0.364 ± 0.02, mean±SE). The RCa/M-β-CD inclusion complexes were also evaluated by SEM, DSC, XRD, FT-IR and 1H-NMR analyses for physicochemical characterizations. A validated HPLC method was used for the quantification of RCa in the samples in in vitro release studies, and similarity factors (f2) were also determined. The molar solubility results demonstrated that the RCa in form of inclusion complex was more soluble than pure RCa nearly four folds. According to the results of physicochemical characterization of complexes, CDs-F2 complex was selected for further cytotoxicity and permeation studies. The results of the MTT cytotoxicity studies demonstrated that the IC50 cytotoxic value was 161.898 µM. Two-way ANOVA was used for statistical evaluations.

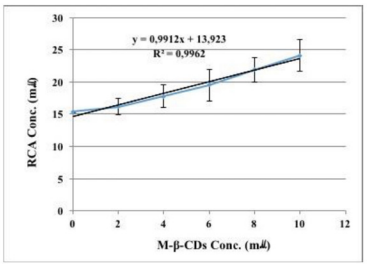

Figure 1. Phase Solubility Diagram of RCa / M-β-CDs (mean ± SE), (n = 3)

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IN VITRO DISSOLUTION AND CYTOTOXICITY STUDIES ON QUERCETIN AND RUTIN LOADED HP-BETA-CYCLODEXTRIN COMPLEXES

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Quercetin is one of the most important flavonoid has been shown to exert anticancer and antiinflammatory effects [1]. Rutin is also a flavonoid has demonstrated a number of pharmacological activities like antioxidant, cytoprotective, vasoprotective, anticarcinogenic effects [2]. Different drug delivery materials are being constantly developed to overcome the undesirable properties of drug molecules. Amongst them, cyclodextrins (CDs) have been found as potential candidates due to their ability to alter physical and chemical properties of guest molecules through the formation of inclusion complexes [3]. 2-hydroxylpropyl-beta-cyclodextrin (HP-beta-CD), a hydroxyalkyl derivative, is an alternative to alpha-, beta- and gamma-cyclodextrin, with improved water solubility properties [4]. In this study, different inclusion complexes of quercetin, rutin and quercetin/rutin with HP-beta-CD were prepared by both evaporation and lyophilization methods in ethanol:water (1:1,v/v) solvent system. After the evaluation of the characteristic properties of complexes; the optimum formulations were selected for further studies. Release characteristics of the complexes were evaluated with dialysis method at 37°C±1°C in pH 1.2 buffer solution during 2 hours, and analysis results demonstrated that quercetin's release characterictics remained unchanged while the release rate of pure rutin (36.03%) enhanced up to 64.50% and 75.81% for the selected two formulations. In vitro release kinetics were investigated using DDSolver software. Cytotoxicities of complexes on NIH-3T3 (mouse embryonic fibroplasts) cell line were assessed using tetrazolium salt reduction (MTT) assay for 48 hours and analyses results indicated that all formulations showed dose and time-dependent toxicity.

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OXIDATIVE STRESS, FOXO3A AND BIM EXPRESSIONS IN AGING CEREBRAL CORTEX: MODULATION BY CURCUMIN

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Curcumin is a polyphenol derived from turmeric (Curcuma longa), which is known to have anti-oxidant, anti-apoptotic and anti-aging activity. FOXO transcription factors play crucial role in stress resistance, cell cycle arrest and apoptosis. In the current study, we investigated the effects of curcumin treatment on oxidative stress parameters, FOXO3a and pro-apoptotic BIM expressions in the cerebral cortex of young and aged rats. Twenty four wistar albino male rats (young: 3 months old, aged: 22 months old) were divided into following groups: young-control, young-curcumin, aged-control, aged-curcumin. Curcumin (30 mg/kg/day) was dissolved in 4% DMSO-PBS. Intraperitoneal injections of control and curcumin groups were maintained for 21 days. Total oxidant status (TOS) and total antioxidant status (TAS) were measured using commercial kits. The ratio of TOS to TAS, i.e. the oxidative stress index (OSI), was calculated. FOXO3a and BIM expressions were tested by Western blotting, and FOXO3a protein levels was also measured by a sandwich ELISA method. ANOVA, LSD, Pearson's r were used for statistical analysis ($p<0.05$). Our findings showed that aging increased TOS, OSI, FOXO3a and BIM, and decreased TAS in cerebral cortex ($p<0.05$). Curcumin administration in aged rats decreased age-related increase in TOS, OSI and FOXO3a ($p<0.05$). In aging, curcumin caused slight, but not-significant decrease in BIM, which was positively correlated with FOXO3a. Curcumin seems to be effective in reducing oxidative stress in the aging brain, which may result in suppression of the FOXO3a transcription factor that upregulates target genes involved in stress resistance and cell death.

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ANTICANCER EFFECTS OF CANNABINOID AGONISTS ACEA AND ACPA ON TT THYROID CANCER CELLS LINE AND L929 CELLS.

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Cannabinoids and their derivatives have been used for years in folk medicine and later in the field of palliative care. Recently, they were found to show pharmacologic activity in cancer (1). In this study we aimed to evaluate the anticancer effects of ACEA and ACPA CB1 agonists on TT thyroid cancer cell line and L929 fibroblast. We also aimed to observe the changes that these agonists will create on proliferation stages. To determine the effects of cannabinoid agonists on cell proliferation, cytotoxicity of ACEA and ACPA were tested on TT thyroid cancer cell line and L929 fibroblasts by using XTT assays. 200 µM, 100 µM, 50 µM, 10 µM, 1 µM and 0,1 µM concentrations of ACEA and ACPA were administered. Results were evaluated as absorbance at 450 nm and IC_{50} values were calculated according to the absorbance data. Both ACEA and ACPA produced concentration dependent cytotoxic effect on TT and L929. IC_{50} values for ACEA and ACPA were 22 µM and 7 µM, respectively on TT cells. There was no significant cytotoxic effect observed at the indicated IC_{50} values on the healthy fibroblast cell line L929. These results clearly show that cannabinoid agonists ACEA and ACPA have potential cytotoxic effects and these agonists may be alternative treatment option in thyroid cancer with further studies underlying the mechanisms behind the positive effect.

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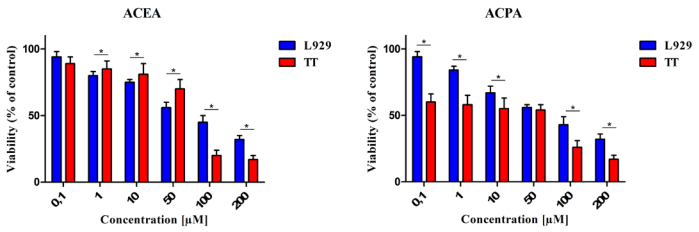

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EFFECT OF TOFISOPAM ON PROLIFERATION OF GRANULAR NEURONS IN RAT HIPPOCAMPUS

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Tofisopam, an anxiolytic drug, has been reported to inhibit the phosphodiesterases isoenzymes such as PDE-2, PDE-4, and PDE-10 [1]. Based on the therapeutic potential of PDE-4 inhibitors on cognition [2,3], we have recently reported beneficial effects of this drug on scopolamine-induced amnesia model in rats. Due to the close relationship of the hippocampus with cognitive processes [4], in this study, we aimed to investigate the effects of tofisopam on the proliferation of granular neurons in the dentate gyrus. Amnesia was induced by scopolamine administration (0,5 mg/kg, i.p). Then, rats were treated with tofisopam (po, 50 mg/kg/day) for one week. After anesthesia, rats were perfused transcardiacally with PBS and 4% paraformaldehite solution. Brain hemispheres comprising the dorsal hippocampus were embedded in paraffin, sectioned at 3 µm thickness and stained immunohistochemically for quantitative analysis. The boundaries of the dentate gyrus were defined according to the stereotaxic rat brain atlas. Photomicrographs were taken under light microscope by integrated camera. Ki-67 positive neurons per unit area was estimated by using Image J analysis program. Our data indicated that the density of Ki-67 immunoreactive cells was lower in the scopolamine-treated group than those of control. However, tofisopam administrations increased the proliferation of neurons in the sub-granular zone of dentate gyrus with respect to the scopolamine group. These results suggest that induction of the hippocampal neurogenesis by tofisopam might contribute to its nootropic effect. However, further detailed studies are required to validate these preliminary findings.
Keywords: Hippocampus, Ki-67, tofisopam

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EFFECT OF EVEROLIMUS ON CIRCADIAN RHYTHMS OF REST-ACTIVITY AND LIVER PER2 GENE EXPRESSION IN FREELY MOVING MICE

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The circadian timing system (CTS) controls many biological functions in mammals including xenobiotic metabolism and detoxification, DNA repair, cell proliferation and apoptosis (1). Everolimus is an immunosuppressant/anticancer agent that is active against many cancers (2). The aim of this study was to investigate dosing time-dependent effects of everolimus on the circadian rhythms in clock gene Per2 expression and locomotor activity, a well-established biomarkers of CTS. KI/KI Per2::Luc male mice were singly housed in an innovative, non-invasive monitoring device, Real Time-Biolumicorder (RT-BIO, Lesa Technology, Switzerland) units which let us to monitor real-time and long-term gene expression in freely moving mice. Mice were synchronized with Light-Dark (LD) 12h:12h, with L onset at Zeitgeber Time (ZT) 0. D-luciferin (1.5 mg/mL) was dissolved into drinking water. After 3-days baseline recordings, vehicle or everolimus (5 mg/kg/d) was orally administered at ZT1-resting span- or ZT13-activity span- for 6 days. Liver bioluminescence reflecting Per2 gene expression and mouse rest-activity were monitored every minute with RT-BIO photomultiplier tube and infrared sensor respectively. Body weight was measured every day as an index of toxicity. Data were analyzed with ANOVA and Spectral Analysis. Circadian rhythms were validated Cosinor Analysis. We found that mean body weight loss was 2-fold as large in mice received everolimus at ZT1 compared to ZT13 (ANOVA; $p<0.05$). Everolimus altered or disrupted both rest-activity and Per2 expression rhythms at ZT1. It was not the case following drug dosing at ZT13. Chronotherapy of everolimus is an effective way to increase tolerability and decrease toxicity on circadian physiology outputs.

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THE EFFECTS OF RESVERATROL ON UTERINE ESTROGEN AND PROGESTERONE RECEPTOR GENE EXPRESSION IN A RAT MODEL OF ENDOMETRITIS

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Endometritis is an endometrial inflammatory disease that causes infertility[1]. We aimed that to investigate the activity of resveratrol in experimental endometritis model in rats. Twelve-week-old female, nonpregnant, Sprague Dawley rats were used. Thirty-six rats were randomly divided into six groups: the negative (NaCl 0.9%) and positive control (NaCl 0.9%), reference treatment (marbofloxacin and PGF_2alfa), antibiotic (marbofloxacin), antibiotic (marbofloxacin)+resveratrol and resveratrol groups. Endometritis model was induced through progesterone (5 mg/kg/s.c., 5 days) and then Escherichia coli (50 µl $1.2x10^5$ CFU/rat) injection in right cornu uteri following laparotomy. The treatment protocols was applied for 14 days after 16 hours from bacterial inoculation. At the end of experiment, rats were sacrificed under general anesthesia. Severity of inflammation in uterine tissues and follicular activity in ovaries were histopathologically evaluated. In uterus tissues, mRNA levels of estrogen (ESRα-ESRβ) and progesterone receptors (PR) were measured by real time-PCR method. Inflammation of endometrium and the number of corpus luteum in positive control group was higher than negative control ($p<0.05$). Resveratrol improved inflammation in uterine and follicular activity. ESRα expression was significantly reduced in resveratrol groups. While ESRβ expression was decreased in endometritis group, resveratrol administration enhanced the expression level ($p<0,05$). PR gene expression was similiar in all groups. It is known that ESRα plays an important role in proliferative and estrogenic effects while ESRβ involve in endometrial regeneration[2]. Our results suggest that resveratrol may have beneficial effects in the treatment of endometritis through reducing ESRα gene expression and increasing ESRβ gene expression.
This research was supported by TUBITAK (215S660).

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THE INVOLVEMENT OF TRPV-1 CHANNELS IN ANTIHYPERALGESIC EFFECTS OF GABAPENTINOIDS

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Involvement of vanilloid receptor-1 (TRPV1) channels in thermal hyperalgesia, which revealed to develop in STZ induced diabetic neuropathy, was shown[1]. To investigate the participation of TRPV1 channels in the antihyperalgesic effects of pregabalin and gabapentin, indicated in diabetic neuropathic pain[2], the antihyperalgesic effects of gabapentin (100 mg/kg, p.o.) and pregabalin (30 mg/kg, p.o.) were investigated in capsaicin-induced thermal hyperalgesia in rats[3]. Before drug administration, hind paw withdrawal latencies evoked by thermal stimuli were assessed using a plantar test apparatus and recorded as the baseline values. Drugs were administered 45min prior to intraplantar capsaicin injection (20µg in 30µL). Thermal withdrawal thresholds were reassessed 15,30,45 and 60min after capsaicin[4]. Data showed that thermal hyperalgesia occurs 15min after capsaicin and persists for 30min. Gabapentin treatment improved thermal thresholds at 15,30 and 45min after capsaicin, whereas pregabalin was effective only at 45th min compared to capsaicin-treated control group. Since capsaicin is a TRPV1 agonist and gabapentinoids significantly prevented the thermal hyperalgesia induced by capsaicin, it is suggested that antihyperalgesic effect of gabapentinoids involves the mechanisms related to TRPV1 channels. The fact that pregabalin was effective only 45min after capsaicin injection, which was the end point of the induced thermal hyperalgesia, suggests that the effects of pregabalin developed later than the anticipated onset of action. Other studies show different onset of antihyperalgesic action for pregabalin[3,5].Studies by changing administration timing and including mechanistic studies are currently under investigation by our team.The evaluation of action mechanisms of drugs which are indicated in neuropathic pain will provide guide findings for novel drug development studies.

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CYTOTOXIC EFFECT OF GALLIC AND 4-HYDROXYBENZOIC ACIDS ON PROSTATE CANCER CELLS (DU145)

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Prostate cancer is an important disease causing death in men. Natural and synthetic agents are being investigated for the treatment of prostate cancer as well as the majority of cancer types. It is known that phenolic acids have antioxidant activity. But, the study which about their cytotoxic effect on prostat cancer cells (DU145) are limited. Hence, in this study we aimed to determine the cytotoxic effects of gallic, valinic, hydroxybenzoic and trans-cinnamic acids on DU145 cells. Dose-dependent (0.2, 1.0, 5.0 ve 25 µg/ml) cytotoxic activities of phenolic acids were determined using MTT stain. Valinic, gallic , and hyrdroxybenzoic acid exhibited the cytotoxic effect at doses of 0.2, 5.0 and 25 µg/ml, respectively. Trans-cinnamic acid did not exhibited the cytotoxic effect on DU145 cells. Consequently, gallic, vanillic and hydroxybenzoic acids were found to have cytotoxic effects on DU145 cancer cells. But, the effects on the apoptotic pathway of these phenolics should be determined in future studies

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ACTIVATION OF MTOR/MEK1/ERK1/2/IKKβ/IκB-α//NF-κB P65 PATHWAY CONTRIBUTES TO ZYMOSAN-INDUCED SYSTEMIC INFLAMMATORY RESPONSE IN RATS.

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The mammalian target of rapamycin (mTOR), a serine-threonine kinase regulates both cell growth, proliferation, survival, and metabolism [1]. Dysregulation of mTOR has been implicated in cancer, neurodegeneration, metabolic disorders and inflammation [2,3]. Zymosan, derived from the cell wall of the yeast Saccharomyces cerevisiae plays an important role in the systemic inflammation [4]. In this study, we aimed to investigate the contribution of mTOR/MEK1/ERK1/2/IKKβ/IκB-α/NF-κB p65 pathway activation to zymosan-induced systemic inflammatory response in rats. Male Wistar rats received saline (4 ml/kg, i.p.) or zymosan (500 mg/kg, i.p.) at time 0. Rapamycin (1 mg/kg, i.p.) was given 1 h after saline or zymosan injections. At time 0 and 1, 2, 3 and 4 h after injection of saline or zymosan, mean arterial pressure (MAP) and heart rate (HR) were measured. Rats were sacrificed 4 h after zymosan challenge and sera, kidney, heart, thoracic aorta, and superior mesenteric artery were collected. Ribosomal protein S6, MEK1, ERK1/2, IKKβ, IκB-α, NF-κB p65, iNOS, and COX-2 expression and/or activities with TNF-α, IL-1β, and nitrite levels were measured. Zymosan administration decreased MAP and increased HR. Expression and/or activities of ribosomal protein S6, MEK1, ERK1/2, IKKβ, IκB-α and NF-κB p65 were increased associated with proinflammatory and vasodilator mediator formation. These effects of zymosan were reversed by the specific mTOR inhibitor, rapamycin. Rapamycin alone had no effect on the parameters measured. These results demonstrate that zymosan-induced systemic inflammation is most likely mediated by mTOR/MEK1/ERK1/2/IKKB/IκB-α/NF-κB pathway activation.

Financial support was provided by the Research Foundation of Mersin University 2016-2-AP3-1900.

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CARDIOVASCULAR SAFETY EVALUATION OF SIBUTRAMINE IN A METABOLIC SYNDROME MODEL VIA PATCH CLAMP TECHNIQUE

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Metabolic syndrome (MetS) is a complex of disorders (including diabetes mellitus and obesity) and eventually leads to cardiovascular diseases, a major cause of mortality. Action potential duration (APD) prolongation, which may evoke arrythmias, occurs in MetS related disordes (1). Sibutramine is an antiobesity drug that has been banned since 2010 due to cardiovascular safety issues. However, slimming products or counterfeit drugs which include sibutramine are still available. It has been reported that illegal sibutramine-contained pharmaceutical products induce cardiovascular crisis (2). In this study, it is aimed to evaluate possible electrophsiological mechanisms that participates in cardiovascular adverse effects of sibutramine, regarding effects on APD, in a MetS model of rat. MetS model was generated via a high-carbohydrate diet (1). Isolation of cardiac ventricular myocytes from adult rat hearts was performed enzymatically. AP recordings were performed by current clamp method. Membrane potential changes were observed. 25,50,75,90 % ($APD_{25,50,75,90}$) durations of repolarization phases of obtained APs were recorded (3). The statistical significance was determined by two-way ANOVA. $P<0.05$ was considered statistically significant. As shown in Figure.1., sibutramine lengthened the APDs significantly in both control and MetS group. Excessive AP prolongation leads to arrhythmic activity (4). In diabetic hearts, prolongation of APD was shown (5). The enhancing effect of sibutramine on APD prolongation may lead to an increased risk for arrythmias when used in patiens with diabetic conditions. The action pathway of sibutramine on APD in MetS-related disordes still needs further investigations.

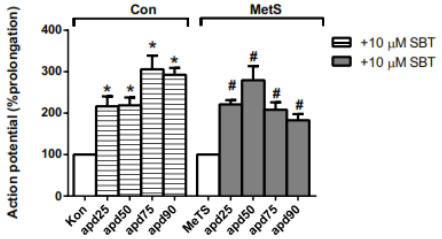

Figure.1 Effect of sibutramine on action potential durations in rat cardiomyocytes

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INHIBITION OF ENDOPLASMIC RETICULUM STRESS REVERSED DOCA-SALT HYPERTENSION-INDUCED STRUCTURAL CHANGES IN CARDIAC AND HEPATIC TISSUES

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Tauroursodeoxycholic acid (TUDCA) is a bile acid and has protective effect on endoplasmic reticulum stress (ERS). In the present study, the effects of TUDCA on the hypertension-induced inflammation, hypertrophy, fibrosis and degeneration were determined in DOCA-salt hypertension model of rats by using histopathological examination. Hypertension was induced by unilateral nephrectomy and DOCA+salt treatment in male rats for 12 weeks. TUDCA was given by intraperitoneal injection for the last 4 weeks. At the end of treatment, left ventricle and liver samples were fixed with 10% neutral formalin and embedded in paraffin blocks. After sections were obtained, they were performed with Hematoxylin and Eosin and Masson's Trichrome staining. Inflammatory cell infiltrations in the left ventricle of hypertensive rats were higher than control group. TUDCA treatment significantly decreased inflammatory cell infiltration in hypertensive heart. Collagen content in both interstitial and perivascular areas of the left ventricle was increased in hypertensive group. TUDCA attenuated perivascular fibrosis but unchanged intensity of interstitial fibrosis. Also, ventricular hypertrophy score were similar in all groups. In hypertensive animals, both lobular and portal inflammation were found, TUDCA alleviated these alterations in liver tissue. Moreover, hepatic fibrosis was induced by DOCA-salt hypertension. TUDCA treatment markedly ameliorated the hepatic fibrosis. In hypertensive group, hepatic balloon degeneration that seen in the previous phase of liver steatosis was observed while TUDCA did not affect balloon degeneration score. These data suggest that TUDCA may protect detrimental effect of DOCA-salt hypertension on the structure of the heart and the liver.

P84

EFFICACY OF TIANEPTINE ON DIABETES INDUCED MECHANICAL HYPERALGESIA AND ALLODYNIA PARAMETERS OF RATS

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Peripheral neuropathy is a common form of diabetic neuropathy. Main symptoms of peripheral neuropathy can be listed as sharp pains or cramps; augmented sensitivity to touch; tingling or burning sensation; reduced ability to feel pain or temperature variations; muscle weakness; loss of reflexes, balance, and coordination, and serious foot problems. Progression of diabetic neuropathy can be slow down with a tight glycemic control and a healthy lifestyle. Moreover, some drugs are prescribed for the symptomatic treatment of pain. We previously reported the favorable efficacy of tianeptine, an atypical antidepressant, on diabetes-induced thermal hyperalgesia and allodynia in rats. In this present study, we examined the therapeutic potential of tianeptine on nociceptive pathways carrying mechanical stimuli. Wistar rats (3 months age) were administered a single dose of streptozotocin (60 mg/kg) in the tail vein to induce diabetes. The treatment started after a 4-week waiting period for the occurrence of neuropathy. Following the 2 weeks treatment period, efficacy of tianeptine (20 mg/kg/day) on mechanical hyperalgesia and allodynia was examined using the Randall-Selitto and Dynamic plantar tests, respectively. The experimental protocol of this study was approved by the Anadolu University Animal Experiments Local Ethics Committee. In both of the tests, paw-withdrawal thresholds of diabetic rats were significantly lower than that of the control animals and tianeptine treatment induced significant increase in these reduced values. These data suggested that subacute tianeptine treatment improved mechanical hyperalgesic and allodynic responses in diabetic rats.

Keywords: Diabetic neuropathy, mechanical nociceptive pathways, tianeptine.

P85

IN VITRO POTENTIAL ANTICANCER EFFECTS OF CANNABINOID AGONISTS ACEA AND ACPA IN NEUROBLASTOMA.

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In recent years, interest in the role of cannabinoids, mainly tetrahydrocannabinol, in cancer therapy has been renewed because of the ability of these molecules to limit tumour cell proliferation and to induce selective cell death (1). Cannabinoids bind to their receptors and control cell growth and migration in cancer (2). Our study aimed to evaluate the anticancer effects of ACEA and ACPA CB1 agonists on the human SH-SY5Y neuroblastoma cancer cell line and L929 fibroblast cells. We also aimed to observe the changes that these agonists will create on proliferation stages. To determine the effects of cannabinoid agonists on cell proliferation, cytotoxicity of ACEA and ACPA were tested on SH-SY5Y cells and L929 fibroblasts by using XTT assays. 200 µM, 100 µM, 50 µM, 10 µM, 1 µM and 0,1 µM concentrations of ACEA and ACPA were administered. Results were evaluated as absorbance at 450 nm and IC_{50} values were calculated according to the absorbance data. Both ACEA and ACPA produced concentration dependent cytotoxic effect on SH-SY5Y and L929. IC_{50} values for ACEA and ACPA were 24 µM and 14 µM, respectively on SH-SY5Y cells. There was no significant cytotoxic effect observed at the indicated IC50 values on the healthy fibroblast cell line L929. The present study shows that these agonists may be alternative treatment option in neuroblastoma with further studies underlying the mechanisms behind the positive effect.

Acknowledgement
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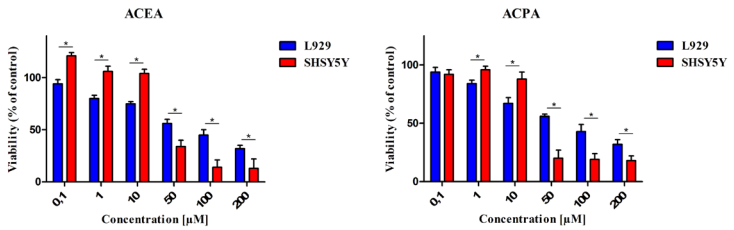

Comparison of the cytotoxic effects of ACEA and ACPA between SH-SY5Y and L929 cell lines at the same

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AN INVESTIGATION ON THE EFFECT OF REBOXETINE TREATMENT ON THERMAL HYPERALGESIA AND ALLODYNIA IN DIABETIC RATS

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Diabetic neuropathy is a chronical syndrome resulting from the exposure of peripheral neurons to long-term hyperglycemic conditions. Hyperalgesia and allodynia are of high incidence among the undesirable consequences of diabetic neuropathy. We previously reported the beneficial effects of reboxetine, a potent and selective noradrenergic reuptake inhibitor, on diabetes induced mechanical hyperalgesia and allodynia in rats. In this following research, we examined whether there is a therapeutic potential of reboxetine also on nociceptive pathways carrying thermal stimuli. Experiments were conducted with male Sprague Dawley rats. Diabetes was induced by a single dose of streptozotocin (50 mg/kg, iv). By the occurrence of peripheral neuropathy in the end of 4 weeks, reboxetine (8 and 16 mg/kg/day) treatment was initiated and continued for 2 weeks. Thermal stimuli-induced hyperalgesia and allodynia were evaluated by Hargreave's and warm-plate tests, respectively. Rota-Rod apparatus was used to assess the motor coordination of the animals. Both Hargreave's and warm-plate test results show that paw-withdrawal thresholds of diabetic rats were lower than that of normoglysemic rats. Moreover, treatment with reboxetine significantly enhanced the reduced paw-withdrawal thresholds of diabetic rats. Obtained data indicates that, reboxetine treatment improved the thermal hyperalgesia and allodynia responses induced by diabetes with no effect on the motor coordination of the rats. This data points out a possible beneficial therapeutic effect in the diabetic neuropathic pain; however, further clinical studies are necessary to verify these preclinical findings.
Key Words: allodynia, Diabetes mellitus, hyperalgesia, neuropathy, reboxetine

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THE EFFECT OF DIETARY FRUCTOSE ON INFLAMMATORY FACTORS IN LIVER AND ADIPOSE TISSUE

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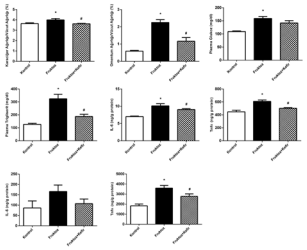
The increased consumption of fructose may contribute to the worldwide high prevalence of metabolic syndrome. The aim of the present study was to investigate the influence of dietary high-fructose on inflammatory markers in liver and adipose tissue as well some metabolic parameters. Four-week-old Wistar male rats were divided into two groups: control and fructose. Fructose was given to rats in drinking water (20 %) for 15 weeks. TNFα and IL-6 levels were measured in liver and fat homogenates using ELISA kits. Plasma triglyceride, glucose and insulin levels were determined by standard enzymatic techniques or ELISA kit. Dietary fructose increased plasma triglyceride, insulin and glucose levels. This dietary intervention also led to elevations in TNFα and IL-6 levels in liver and adipose tissue. Moreover, liver and omental fat weights were augmented in rats subjected to fructose feeding. High fructose-induced metabolic changes are positively correlated with the increases of inflammatory parameters. These findings could be relevant for the understanding of the pathogenesis of metabolic syndrome.



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P88

GLUCOSE INCUBATION CHANGES INSULIN SIGNALING GENE EXPRESSIONS IN ISOLATED AORTIC SEGMENTS OF RATS.

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A temporary rise in plasma glucose level occurs at the post-prandial period in diabetes which may lead to change in vascular function and insulin signaling pathway. The aim of the present study was to investigate the influence of high-glucose challenge on mRNA levels of possible effectors of insulin signaling in aortic segments of rat. Aortic segments of rats were incubated with high-glucose (30 mM). The expression of genes involved in insulin signaling including IRS-1, Akt, PI3K, eNOS, FOXO3a, mTOR and PPARg were assessed in the aorta by using the real-time PCR. Glucose incubation significantly decreased IRS-1, Akt, PI3K, eNOS and FOXO3a mRNA expressions in aorta of rats ($P < 0.05$ versus their corresponding control groups), whereas no changes were observed in expression levels of mTOR and PPARg mRNAs. Our results showed that acute exposure to high-glucose suppressed several components of insulin signaling pathway, namely IRS-1, Akt, PI3K, eNOS and FOXO3a, in rat aorta.

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THE EFFECT OF DIETARY FRUCTOSE ON TESTICULAR INFLAMMATORY FACTORS IN RATS

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The excess fructose intake in daily human diet may contribute to the worldwide epidemic of metabolic syndrome. In experimental study, high-fructose consumption has been shown to cause insulin resistance, hypertriglyceridemia, abdominal fat accumulation and fatty liver. In this study, we aimed to investigate the effect of dietary fructose on inflammatory factors in testicular tissue of rats. Herein, a model of metabolic syndrome was produced by fructose given in drinking water (20%) for 15 weeks period in Wistar rats. According to our purpose, TNFa, IL-1ß, IL-6, IL-8, iNOS, IL-31 and vitamin D levels in the samples of testis were measured by using commercial ELISA kits. In our model, high-fructose administration increased the level of pro-inflammatory factors namely IL-1ß and iNOS, while a marked decrease was observed in anti-inflammatory cytokines IL-6 and IL-31 levels. However, there was no change in level of testicular TNFa and IL-8. Concentration of vitamin D, an important infertility indicator, was diminished in fructose group, when compared to controls. Our results can provide useful data on the relationship between high-fructose consumption and infertility-related pathology, which shows worldwide increasing prevalence.

P90

IMMUNOHISTOCHEMICAL DEMONSTRATION OF EXPRESSIONS OF CYPS AND GSTS IN HUMAN TUMOR AND NON TUMOR TISSUES OF NON-SMALL CELL LUNG CANCER

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Lung cancer is the leading cause of cancer mortality. Non-small cell lung cancer (NSCLC) represents the majority of lung cancer cases and NSCLC is classified mainly as adenocarcinoma (AC) and squamous cell carcinoma (SCC) subtypes histologically. Metabolic activation of polycyclic aromatic hydrocarbons (PAHs) and nitrosamines in cigarette smoke to carcinogenic metabolites are mediated by Cytochrome P450 1A1 (CYP1A1), CYP1B1 and CYP2E1. On the other hand, glutathione S-transferases (GSTs) are involved in the inactivation of mutagenic and carcinogenic molecules which can bind to DNA. In addition, these CYPs and GSTs play a role in the metabolism of a number of chemotherapeutic agents and thus involve in drug resistance. In this study, we investigated the protein expressions of CYPs and GSTs in tumor and surrounding tumor free (control) lung tissues in 50 pairs of samples obtained from NSCLC patients. Protein expressions of CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTM1 and GSTT1 were assessed using immunohistochemistry. CYP1A1 expression was statistically higher only in lung AC tissues than normal tissues whereas CYP2E1 expression was found to be significantly higher in tumors than in normal lung tissues in overall NSCLC, AC and SCC tissues ($p<0.05$). GSTM1 expression was statistically higher in tumor tissues than normal tissues in overall NSCLC patients and SCC tissues ($p<0.05$). These results show that the elevation of expressions of CYP1A1, CYP2E1 and GSTM1 in tumors versus control tissues depends on histopathological subtype of NSCLC.

P91

EFFECT OF CELL CONFLUENCY ON PER2 GENE EXPRESSION OSCILLATORY PROFILES IN SYNCHRONIZED MOUSE PER2::LUC HEPA1-6 HEPATOCARCINOMA CELL CULTURES

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Cell confluency may affect the gene expression patterns of cells (1, 2). In this work, we aimed to investigate the effect of cell confluency on Per2 clock-gene expression oscillatory profiles in synchronized mouse Per2::luc Hepa1-6 hepatocarcinoma cell cultures. Per2:luc Hepa1-6 cells were obtained following stable introduction of a luciferase reporter gene under the control of Per2 promoter in Hepa1-6. Cells were cultured at 10%, 40%, 70%, and 100% confluence and synchronized with 100 nM dexamethasone for 2h. The offset of the synchronization defined as Circadian Time 0 (CT0). Dexamethasone-containing medium was replaced with luciferin-containing one. The bioluminescence oscillatory profiles reflecting Per2 expression were recorded as counts/sec for 63h with Lumicycle32 (Actimetrics, USA). Data were analyzed with Cosinor for rhythm detection and ANOVA. Increase of cell confluence from 10% to 100% has changed the rhythm characteristics and resulted in enhancement of the oscillation amplitudes and periods, and slight advances in the acrophases. The real-time bioluminescence pattern of Per2 revealed a circadian rhythm with a 23h-dominant period (T) (Cosinor, $p<0.001$) at 10% percent confluence. We found a significant period lengthening in 40% ($T=24h$, $p<0.001$ Cosinor; $p<0.01$ ANOVA), 70% ($T=25h$, $p<0.001$ Cosinor; $p<0.001$ ANOVA), and 100% ($T=25h$, $p<0.001$ Cosinor; $p<0.001$ ANOVA) confluence as compared to 10%. Advances in the acrophase values in 40% ($j=02:45$, $p=0.05$), 70% ($j=01:23$, $p<0.001$), and 100% ($j=00:37$, $p<0.001$) were found to be significant with ANOVA as compared to 10% ($j=03:54$). Consequently, circadian oscillation profile of clock gene Per2 and rhythm characteristics can be changed depending on the cell confluence.

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DRUG INTERACTIONS DURING THE TREATMENT OF LUNG CANCER, BREAST CANCER AND SOME GASTROINTESTINAL SYSTEM CANCER PATIENTS

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In this work, investigation of major and moderate drug interactions for drugs and cures taken by 30 patients in total, of whom 10 having breast cancer, 10 having lung cancer, 10 having cancer in their gastrointestinal tract (4 gastric cancer, 4 pancreatic cancer, 2 colon cancer) who possess appropriate criteria for the research and had chemotherapy and radiotherapy at Erciyes University Kemal Dedeman Hematology-Oncology Hospital Medical Oncology Service between August-December 2014, and of whom therapy is proceeding is targeted. In the work, suggestions about how to develop a treatment plan, as a pharmacist, to prevent major drug interactions for patients suffering from solid tumors are presented. In the view of the fact that negative effects may be dominant than healing effects in the presence of major drug interactions, suggestions aiming enhancement of treatment plan considering pharmaceutical treatment and good pharmacy applications are introduced. Besides, mechanisms of the interactions are mentioned. A total of 30 cancer patients were monitored for 7 days and 1cure. A total of 35 major and 153 moderate interactions were observed in these patients. As a result, it was determined that the complications due to polypharmacy may be severe. The results of this work led for changing drugs of patients having major drug interactions. The treatments for patients treated with drugs showing major interactions were revised. Thus, providing decreasing the interaction to the minimum level important contributions were achieved for increasing both the treatment activity and patient life quality.

	Number of patients	Number of major interactions	Number of moderate interactions
Lung cancer	10	12	44
Breast cancer	10	12	75
GIS cancer	10	11	34

Major and moderate interactions observed in patients

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HEPATOPROTECTIVE ACTIVITY OF S. CRYPTANTHA AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS

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This study was designed to determine the effects of extract of Salvia cryptantha on antioxidant and apoptotic enzymes in carbon tetrachloride (CCL4)-induced liver injury. METHOD: 30 animals were divided into five groups as following: control (group I), CCl4 group (group II), olive oil group (group III), CCl4 + S. cryphantha 200 mg/kg group (group IV) and CCl4 + S. cryptantha 400mg/kg group (group V). Apoptotic proteins (Bax and Caspas3) were determined by using immunohistochemical staining method while apoptotic index was determined by using TUNEL assay. mRNA expression levels of antioxidant enzymes (SOD, CAT and GPx), antiapoptotic (BCL2) and Caspas3 were determined by using real-time PCR while MDA level was determined by using colorometic assay. Bax and Caspas3 protein levels were found to be increased in the CCL4 control group, whereas the expression of these proteins and consequently the apoptosis were decreased in the extract of Salvia cryptantha groups. mRNA expression levels of antioxidant and antiapoptotic enzymes were decreased in all groups. Caspase3 levels were not statistically altered. S. cryptantha extract suplementation causes a decrement of MDA levels when compared with controls group. S. cryptantha extract significantly inhibits apoptosis, especially at immunohistochemical staining, and those results leading us thus to judge that S. cryptantha extract might be efficiently used as a hepatoprotective agent after successful clinical trials.
This work is supported by Adıyaman University Scientific Research Center in TIPAAAP/2015-0002 project.

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THE EFFECTS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPAR) AGONISTS ON PROTEINS ASSOCIATED WITH OXIDATIVE STRESS IN PARKINSON'S DISEASE MODEL

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Oxidative stress plays an essential role in the pathogenesis of Parkinson's Disease (PD). Cells have developed protective systems like antioxidant molecules and detoxifying enzymes against oxidative stress including antioxidant response element (ARE). In case of oxidative stress, the nuclear factor E2-related factor 2 (Nrf2) binds to the ARE and induces antioxidant enzymes. Peroxisome proliferator-activated receptor gamma (PPARg) contributes to antiinflammatory response mechanisms. Pioglitazone and tideglusib, thiazolidinedione compounds, are PPARg agonists. Recently tideglusib and pioglitazone are thought to have a neuroprotective role in neurodegenerative diseases. Our aim is to evaluate the effects of PPARg agonists on proteins associated with oxidative stress in MPP^+-induced cellular PD model. A human neuroblastoma cell line (SH-SY5Y) was used in this study. Glutathione S-transferase (GST), glutathione reductase (GR) and lactate dehydrogenase (LDH) enzyme activities and amount of total glutathione (GSH) were measured. Western blot technique was used for PPARg protein analysis. The changes in Nrf2 mRNA levels were analyzed by RT-PCR technique. MPP+ treatment triggered cell death by increasing LDH enzyme activity ($p<0.001$). Tideglusib and pioglitazone treatments significantly increased GR and GST enzyme levels and amount of total GSH ($p<0.01$) against MPP^+. MPP^+ treatment significantly increased Nrf2 mRNA levels. PPARg protein levels are significantly decreased following MPP^+ treatment at 3, 6, 12 and 24 hours ($p<0.001$). Our study provides the evidence that PPARg activation may exert neuroprotection through increasing cellular antioxidant defense systems in cell culture model of PD.

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P95

EFFECT OF INTRACAVERNOUS CHOLINE INJECTIONS ON ERECTILE DYSFUNCTION IN HEALTHY AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Diabetes mellitus (DM) is one of the common risk factors for erectile dysfunction (ED). In this study, we aimed to investigate the possible beneficial effect of choline treatment on ED in streptozotocin-induced diabetic rats. Adult male Wistar rats were divided into control and streptozotocin-induced diabetic groups. Intracavernous pressures (ICP) and intracavernous pressure/mean arterial pressure (ICP/MAP) values were recorded in vivo after intracavernosal injections of choline in doses of 100, 150 and 200 µmol in anesthetized rats. In non-diabetic rats, choline administration in 100 and 150 µmol doses reduced the ICP values significantly ($p<0.05$ and $p<0.05$; respectively), whereas 200 µmol choline injection did not show any significant effect in stimulation with 7.5 V. Choline in three doses did not show any significant effect with 5 and 2.5 V stimulations. There were no significant changes in ICP/MAP values following the choline administration with three doses. In diabetic rats, ICP and ICP/MAP values were significantly lower than the non-diabetic rats following the cavernous nerve stimulation with only 7.5 V ($p<0.001$ for ICP and $p<0.01$ for ICP/MAP). Choline administration did not result in any statistically significant change in ICP and ICP/MAP values in 7.5, 5 and 2.5 V stimulations. The results of our study firstly revealed the effects of intracavernosal administration of choline in erection function in healthy and diabetic rats. The effects of different doses of choline and choline donors in diabetes-related erectile dysfunction require to be clarified in further studies.

P96

INVESTIGATION OF THE MUTAGENICITY OF THE TIO2 NANOPARTICLES BY MODIFIED AMES II ASSAY

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Titanium dioxide nanoparticles (TiO2 NPs) have been used increasingly for various cosmetics (especially sunscreens), food and drug products. Recently, researches on nanoparticle toxicity focus on importance of their size and various toxic effects of nanoparticles have been investigated by using different toxicity methods. The Ames II assay is a second generation bacterial reverse mutation assay developed as a predictive screening assay for genotoxicity. In this study we aimed to modify the Ames II assay by treating the bacterial strains (Salmonella typhimurium TA98, TAMix) with a low-ionic strength solution at the pH 5.5 value which is below the nanoparticles isoelectric points to increase the bacterial cell wall permeability. The nanoparticles characterized for their size by using transmission electron microscopy (TEM) and zeta potential by using Zeta Sizer. We investigated the mutagenic effects of three different nanosize (20 nm, <100 nm and <150 nm) of TiO2 NPs in 6 different concentrations. We conducted both modified and original Ames II assay and compared the results of the both assay. Our results demonstrated TiO2 NPs did not create a frameshift or base-pair substition type mutation in the modified and original Ames II assay.
This study has been supported by TUBITAK (Project no: 113S229).

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GENOTOXICITY OF SPHERICAL TIO2 NANOPARTICLES IN HUMAN PERIFERAL LYMPHOCYTES ASSESSED BY CHROMOSOMAL ABERRATION ASSAY IN VITRO

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Nanoparticles are at the leading edge of the rapidly developing field of nanotechnology. TiO_2 nanoparticles, one of them, are widely used in dyes, cosmetic, textile, food, plastic, medical, space, and military industries. However, nanoparticles may have toxic effects on human cells, bacteria's, and rodents. Therefore this study was planned to investigate genotoxic potential of spherical TiO_2 NPs (STNPs) by using chromosome aberration (CA) assay in human lymphocytes (HLs) in vitro. HLs (from two men+a woman, between 21-25 years) were treated with 125, 250, 500, and 1000 µg/ml concentrations STNPs. A negative and a positive control (mitomycin-C) were also maintained. At the 24 h treatment, the frequency of aberrations and CA/cell increased dose-dependently compared to control but it was only significant for CA/cell at 500 µg/ml concentration. At 48 h, the frequency of aberrations slightly increased while it decreased at 1000 µg/ml concentration. CA/cell also slightly increased but it was nonsignificant. On the other hand, STNPs decreased mitotic index (MI) does dependently at both treatment durations. This decrease was significant for only 1000 µg/ml at 24 h and for all the concentrations (except 125 µg/ml) at 48. The data observed show that STNPs may increase the frequency of aberrations and CA/cell in dose dependent but nonsignificant manner. STNPs were also found to be cytotoxic at longer treatment nearly at all the concentrations. This study stated that other genotoxicity assays as sister chromatid exchange and micronucleus should also be performed to evaluate genotoxic potential of STNPs in HLs.

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EVALUATION OF GENOTOXIC EFFECTS OF NEEDLE-LIKE TIO2/POLYRHODANINE CORE/SHELL HYBRIDE NANOSTRUCTURES IN HUMAN LYMPHOCYTES IN VITRO BY SISTER CHROMATID EXCHANGE ASSAY

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Human exposure to chemicals is increasing day by day together with developing technology. Therefore, the safety of nanostructure use is questioned among some researchers. TiO_2 nanoparticles are widely used in pharmaceutical and medical diagnosis because of its chemical stability, photo catalysis, antibacterial, and white pigment properties. Rhodanine is used because of its antibacterial and antiviral activity, corrosion inhibition, and chemical sensor properties. Needle-Like TiO_2/Polyrhodanine core/shell (TiO_2/PRh) nanocomposite synthesized by Özkan et al. [1] showed a strong antibacterial effect. The aim of this study is to investigate genotoxic effect of needle-like TiO_2/PRh nanocomposite by using sister-chromatid exchange (SCE) assay in cultured human lymphocytes. Lymphocytes obtained from one healthy donor were treated with different concentrations of TiO_2/PRh nanocomposite (50, 100, 200, 300, 400, and 500 µg/ml) for 24 and 48h. A negative (ultra-distilled water) and a positive control (mitomycin C-MMC) were also maintained. Data obtained from the treatment groups were compared with the negative and positive controls. Sister chromatid exchange test results were analyzed by t test. TiO_2/PRh nanocomposite did not significantly increase the frequency of SCE/Cell in human lymphocytes at 50, 100, 200, 300 and 400 µg/ml concentrations either at 24 h or 48 h treatments compared to negative control. At 500 µg/ml concentration, cells could not be evaluated because NPs covered them. NPs used in this study did not show toxicity in these conditions.

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IN VITRO GENOTOXIC EFFECT OF ROD TIO2 NANOPARTICLES BY SISTER CHROMATID EXCHANGE ON HUMAN LYMPHOCYTES

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Nanotechnology is defined as the design, production,characterization,and application,taking into accounts the shape and size of nanoscale structures. TiO_2 nanoparticles (NPs) have a wide range of applications in the nanotechnology. They have very high strength, weight ratio, and superior resistance to corrosion, susceptibility to bio marketing applications, non-silicate and flammable properties. However, NPs can cause adverse effects in human health and environment. Therefore, there is a great concern about the risks related to their use. Recently, TiO_2 NPs have been used in various genotoxic studies, but there are still controversies on the effects of these NPs. The aim of this investigation was to evaluate the genotoxic effect of Rod TiO_2 ($RTiO_2$) on human peripheral lymphocytes by sister chromatid exchange (SCE) test. Peripheral blood obtained from 1 healthy volunteer cultured for 72 h. Cell cultures were exposed to four different concentrations of $RTiO_2$ NPs (50, 100, 250, and 500 µg/ml) for 48 h. This study indicated that all the concentrations of $RTiO_2$ NPs increased the mean number of SCEs compared to negative control. However, this increase was significant at the two highest concentrations. This result shows that $RTiO_2$ NPs may reach to nucleus and may interact with DNA causing genetic damage. $RTiO_2$ NPs may also damage DNA by generating reactive oxygen species in cell. However, some more studies should be conducted to evaluate in vitro and in vivo genotoxic mechanism of $RTiO_2$ NPs.

P100

CYTOTOXICITY INDUCED BY MIXTURES OF ZINC OXIDE AND COPPER OXIDE NANOPARTICLES IN HUMAN NEUROBLASTOMA CELL CULTURES AND PROTECTIVE EFFECTS OF DTPA, NAC AND TAURINE

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In the present study, possible cytotoxic effects of ZnO and CuO nanoparticles (NPs) in SH-SY5Y cell cultures were evaluated. For this purpose, cell cultures were exposed to various concentrations of these nanoparticles between the ranges of 0.01 to 800 µg/ml either alone or as a mixture for 24 hours, and cytotoxicity was determined by MTT assay, following characterization of nanoparticles using TEM. Individual effects of these NPs were as follows: A slight cytotoxic effect was noted between the concentrations of 5 to 50 µg/ml for ZnO NPs, and 12.5 to 50 µg/ml for CuO NPs. At the highest concentration, cell survival was determined as >50% for ZnO and 29% for CuO NPs. Cytotoxicity results of binary mixtures of these NPs showed generally a similar profile to individual effects of ZnO and CuO NPs. Pretreatment of cells with two different concentrations of DTPA or N-acetyl cysteine for 4 hours provided a slight protection against cytotoxicity induced by CuO NPs alone or in combination of ZnO and CuO NPs. Minor preventive effects of two different concentrations of taurine were also noted against cytotoxicity induced by CuO NPs alone. Considering limited data about the toxic effects of either individual or as mixtures of these nanoparticles in nervous system, the results of this study suggest that detailed investigations are required in order to explain toxicity mechanism of these nanoparticles.

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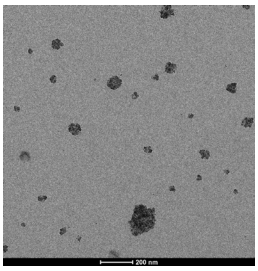
TEM images were obtained in Central Laboratory of METU in Ankara, Turkey.



TEM image of ZnO NPs.

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CYTOTOXIC EFFECTS OF COPPER OXIDE NANOPARTICLES IN HUMAN NEUROBLASTOMA CELL CULTURES: A PRELIMINARY STUDY

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In the present study, possible time- and dose-dependent cytotoxic effects of CuO NPs were examined in human neuroblastoma (SH-SY5Y) cell cultures using neutral red (NR) assay. Particle sizes, and dispersion of CuO NPs were also analyzed. Incubation of cell cultures with various concentrations of CuO NPs (0.01- 800 µg/ml) for 24, 48, 72 and 96 hours results in non-linear dose-response curves: At all incubation periods, particularly with 12.5 and 50 µg/ml of CuO NPs, survival percent of cells increased (110- 125%). However, lower concentrations of CuO NPs were found more cytotoxic, and time-dependent decreases in cell survival were noted only at 800 µg/ml following all incubation periods. Possible protective effects of DTPA, NAC and taurine against 800 µg/ml of CuO NPs-induced cytotoxicity were also assayed following incubation of cell cultures for 24 hours. For these experiments, cell cultures were pre-incubated with two different concentrations of DTPA, NAC or taurine for 4 hours, and lysosomal cytotoxicity was determined by NR assay. Results showed that both concentrations of DTPA, NAC or taurine provided significant protection against CuO NPs-induced cytotoxicity. Present results suggest that cytotoxicity of CuO NPs might be related to oxidative stress, and prevention provided by all substances should be considered when studying mechanism of lysosomal cytotoxicity of CuO NPs.

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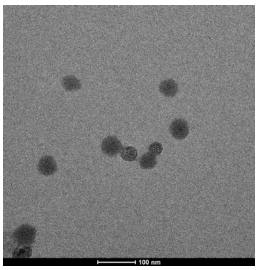
TEM images were obtained in Central Laboratory of Middle East Technical University (METU) in Ankara, Turkey.



TEM image of CuO NPs.

P102

COMPARATIVELY ASSESSMENT OF EPIGENETIC ALTERATIONS ON DNA AND RNA IN MULTI-WALLED / SINGLE-WALLED CARBON NANOTUBES AND ASBESTOS EXPOSED HUMAN BRONCHIAL EPITHELIAL CELLS

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Carbon nanotubes (CNTs) are widely used nanomaterial. Considering their similarity with structure of asbestos, there is growing concern about their cancer risk CNTs. Changes in DNA/RNA methylation are promising biomarkers of exposure related carcinogenesis. This study aimed to comparatively investigate epigenetic effects of CNTs and asbestos. Human bronchial epithelial cells (16HBE14o-) were treated with 25 and 100 mg/ml of CNTs (SWCNTs and MWCNTs) and 2.5 mg/ml of asbestos (chrysotile, amosite, crocidolite) for 24 hours. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to assess global DNA and RNA methylation/hydroxymethylation in cytosines. Colorimetric ELISA-like assay was used for global RNA methylation in adenines. Gene specific DNA methylation status in certain CpG sites of CDKN1A, ATM, and TRAF2 were assessed using the bisulfite pyrosequencing technology. MWCNTs exposed cells showed significant global DNA hypomethylation and global RNA hydroxymethylation on cytosines and global RNA hypomethylation on adenosine ($p<0.05$). Only chrysotile induced 5-hydroxymethlation on cytosine in RNA. Significant hypomethylation in CDKN1A gene was observed in amosite, SWCNTs, and MWCNTs. Significant hypomethylation in ATM gene was determined for chrysotile and SWCNTs. On the other hand, SWCNTs caused significant hypermethylation in TRAF2. Overall CNTs and asbestos exposure cause methylation changes in genes involved in several cellular pathways which have been related with disease progression including cancer. However, asbestos and CNTs do not show a similar pattern in terms of epigenetic alterations in DNA and RNA.

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ASSESSMENT OF INDIVIDUAL AND COMBINED CYTOTOXIC EFFECTS OF SODIUM ARSENITE AND CADMIUM CHLORIDE IN VERO CELLS

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The aim of the present study was to investigate possible cytotoxic effects of $NaAsO_2$ and $CdCl_2$ either alone or as a mixture in African green monkey kidney epithelial (Vero) cell cultures. The cultured cells were incubated with various concentrations of $NaAsO_2$ and $CdCl_2$ for 24, 48 and 72 hours, and the mixtures of them for 48 hours, then cytotoxicity was determined by MTT assay. When the cells were exposed to ten different concentrations of $NaAsO_2$, dose-dependent decreases were noted in cell viability with >1 µM and >5 µM concentrations of $NaAsO_2$ following 24 and 48 hours, and >5 µM following 72 hours. Time-dependent decreases were observed only following 24 and 48 hours. Dose-dependent diminutions in cell viability were also seen following >5, 0.05 and 0.5 µM concentrations of $CdCl_2$ for 24, 48 and 72 hours, respectively. Between the ranges of 80-300 µM of $CdCl_2$ for all incubation periods, survival percent were found <6%. A dose-dependent cytotoxic effect were more evident when the cells exposed to the mixtures of these compounds between the ranges of 0.05:0.05 to 120:150 µM ($NaAsO_2$:$CdCl_2$) for 48 hours. A fluorescent probe 2′,7′-dichlorofluorescein-diacetate was used to determine the level of ROS following 48 h of incubation of cell cultures with three different concentrations of $NaAsO_2$, $CdCl_2$ or mixtures of them. Results showed that production of ROS was induced by lowest concentration of $CdCl_2$, and two different concentrations of mixtures. In accordance with literature, these results suggest that oxidative stress might be involved in cytotoxicity mechanism of these substances.

P104

THE EFFECTS OF COLCHICINE ON CELL GROWTH AND APOPTOSIS OF SW480 HUMAN COLORECTAL CANCER CELLS

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Colchicine, an alkaloid isolated from Colchicum autumnale, is a drug used for the treatment of familial mediterranean fever and gout through the anti-inflammatory and anti-fibrotic effects of the drug (1,2). In literature, there is limited study regarding the mechanisms of effects of colchicine in cancer types. Since colchicine is toxic to normal tissues at higher concentrations, the researchers have focused on investigating the anticancer effects of the drugs in lower concentrations (3). In our study, anti-cancer activity of colchicine on SW480 human colon cancer cell line was investigated at 10, 25 and 50 ng/ml concentrations and the results were compared to untreated control. In order to evaluate the effect of colchicine on cell growth, MTT method was used. The apoptosis was detected by Annexin V binding assay. The nuclear and cytosolic Nf-Kb levels were measured by western blot method. And the effects of colchicine on cell cycle was evaluated using flow cytometry-based Muse cell analyzer. The results have shown that colchicine decreased cell growth of SW480 cells in a dose dependent manner. Additionally, a correlation has been observed between the apoptotic activity and cytotoxic activity in association with time and concentration. In cell cycle analysis, it's been detected that colchicine induced a cell cycle arrest on G2/M phase in SW480 cells. In conclusion, our findings suggest that colchicine has anticancer activities on SW480 cells at lower concentrations and further studies are needed to clarify the underlying mechanisms.

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P105

THE EVALUATION OF GENOTOXIC EFFECT OF ANTIHYPERTENSIVE DRUG ACTIVE INGREDIENT USING MICRONUCLEUS AND COMET ASSAYS

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Indapamide is an orally administered diuretic antihypertensive drug. Physicians' Desk Reference report (2005) demonstrated that indapamide was not carcinogenic in mouse and rats for long-time administration. However, these data very limited and not detailed (in terms of doses, treatment period etc.). In addition, no data are available on the genotoxic effects of indapamide in human peripheral lymphocytes (HPLs). The purpose of this study was to evaluate the potential genotoxic effects of indapamide using micronucleus (MN) and comet assays in HPLs. For this purpose, peripheral blood obtained from three healthy young donors, a man and two women, was treated with four different concentrations (18.75, 37.50, 75.00, and 100.00 µg/mL) of indapamide for MN assay. Effect of indapamide on nuclear division index (NDI) was also investigated. In the comet assay, isolated lymphocytes were treated with the same concentrations. The results showed that indapamide did not affect the frequency of micronucleus and NDI in all concentrations. In the comet assay, indapamide increased the comet tail intensity, tail length, and tail moment at the highest concentration only. In our previous study indapamide did not affect the frequency of chromosomal aberrations and SCE/cell (except two highest concentrations for 48 h period). Therefore, this and the previous study together indicate that this compound does not represent a significant risk at the genetic level in in vitro human lymphocytes.
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INVESTIGATING GENOTOXIC EFFECT OF SWEETENER XYLITOL USING SISTER CHROMATID EXCHANGE TEST

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Sweeteners, sugar substitute, are a food additive that provides a sweet taste like that of sugar while containing significantly less food energy. The need to avoid the adverse effects of sugar can not stop feeling the taste on humans has resulted in intense use of sweeteners nowadays. There are positive and negative opinions about their effects on human health. Xylitol is a sugar alcohol that is similar in taste to sugar but with about 40% fewer calories and is used as sweetener. The purpose of this study was to evaluate the potential genotoxic effect of xylitol by using in vitro sister chromatid exchange (SCE) test in human peripheral lymphocytes. Replication index was also determined. Peripheral blood samples were collected from three healthy (1 male and 2 female) non-smoking (of ages, 25-27 years) donors. Peripheral lymphocytes were incubated with different concentrations of xylitol (125, 250, 500, and 1000 µg/mL) for 24 and 48 hours. A negative and a positive control (MMC) were also maintained. Results showed that xylitol significantly increased the SCE/cell ratio at two highest concentrations at both 24 h and 48 h periods compared to negative control. However, xylitol did not affect replication index. These data demonstrated that xylitol may have genotoxic risk to human lymphocytes in vitro at high concentrations. However, other toxicity tests should also be applied for detail analysis.

P107

GENOTOXICITY OF MONOPOTASSIUM GLUTAMATE ASSESSED BY CHROMOSOMAL ABERRATION ASSAY IN VITRO

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Flavour enhancers are used to bring out the flavour in foods without adding a flavour of their own. Monopotassium glutamate (MPG) is a flavour enhancer, a kind of food additive. However, the effect of its use on human health is controversial. The purpose of this study was to evaluate the potential genotoxic effect of MPG by using in vitro chromosomal aberration test in human peripheral lymphocytes. Mitotic index was also determined. Lymphocytes obtained from three healthy young donors (a man and two woman) were treated with four different concentrations (125, 250, 500, and 1000 µg/mL) of MPG in culture conditions for 24 and 48h. A negative and a positive control (mitomycin-C) were maintained for each treatment. At the 24h treatment, the percentage of abnormal cells significantly increased in two highest concentrations (500 and 1000 µg/mL) compared to negative control. At 48h treatment, only the highest concentration showed significant increase for the percentage of abnormal cells. On the other hand, MPG decreased the mitotic index. However, this decrease was significant at the two highest concentrations at both 24h and 48h. In our previous studies, MPG significantly increased the SCE/cell ratio at all the concentrations at both 24 h and 48 h periods and also significantly increased the frequency of micronucleus in the two highest concentrations. All these data we obtained show that MPG is genotoxic to human lymphocytes in vitro especially at high concentrations. However, to clarify these results, other genotoxicity tests such as in vivo tests need to be done.

P108

CYTOTOXIC EFFECTS OF HELICOBACTER PYLORI EXPOSURE AFTER DIFFERENT MULTIPLICITIES OF INFECTION IN HUMAN GASTRIC ADENOCARCINOMA CELLS

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Helicobacter pylori is helix-shaped gram-negative bacteria that is both microaerophilic and neutralophilic. It usually colonizes in upper gastrointestinal tract and chronically infects the human gastric mucosa. This bacterium can be found in 50% of the world's population. Chronic infection with Helicobacter pylori leads to gastritis and peptic ulcer, and later to gastric cancer and gastric mucosa associated lymphoid tissue (MALT) lymphoma. The aim of this study was to investigate the cytotoxic effects of Helicobacter pylori (NCTC 11637) exposure after different multiplicities of infection (MOIs) in human gastric adenocarcinoma cells. Cell viability was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After incubation of the adenocarcinoma cells with different MOIs (25, 50, 75, 100, 200 and 400) of Helicobacter pylori, we observed that this bacterium causes dose-dependent cytotoxicity. The median inhibitory concentration 50 (IC50) was found to be 236 bacterium/cell and IC70 was 153 bacterium/cell. These results show that Helicobacter pylori can lead to cell death at high MOIs. Further in vitro and in vivo studies are needed to show the mechanism of cytotoxicity of Helicobacter pylori in human gastric cells.

P109

SELENOMETHIONINE CAN INHIBIT NUCLEAR AND CYTOPLASMIC OXIDATIVE STRESS IN HUMAN BLADDER UROTHELIAL CELLS TREATED WITH ALKYLANILINE METABOLITES

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Alkylanilines are widespread environmental chemicals and humans are exposed to these chemicals through different routes, including cigarette smoke. Alkylanilines are suggested to be mainly toxic to human bladder. 3,5-dimethylaniline (3,5-DMA) is mainly used in azo dyes, pharmaceuticals, antioxidants, detergents, wood preservatives, textiles, metal complexes and antiozonants. Its main metabolite is 3,5-dimethylaminophenol (3,5-DMAP) and it was shown to cause oxidative stress in CHO cells. Selenium is an essential trace element which was shown to be protective against several cancers, including cancer of the bladder. Selenomethionine is an organic selenocompound and As the main target of 3,5-DMAP is bladder, we have investigated its toxicity in different fractions of human bladder urothelial cell line. Protective effect of selenomethionine against its toxicity was also assessed. We observed that at half maximal inhibitory concentration 50 (IC50) dose of 3,5-DMAP was ~100 µM and this substance caused a dose dependent increase of intracellular ROS generation. In both cytoplasm and nucleus, 3,5-DMAP altered in the antioxidant enzyme activities, caused decreases in cellular redox ratio. Lipid peroxidation and as a consequence protein oxidation were elevated in particularly cytoplasm. Selenomethionine was found to be partially protective against the toxicity of 3,5DMAP in both cytoplasmic and nuclear fractions. More studies are needed to show the underlying toxicity mechanisms for alkylanilines and different protection strategies should be developed against their toxicity.

P110

HIGH THROUGH-PUT COMET ASSAY TECHNIQUE IN INVESTIGATING THE TOXIC EFFECTS OF BIOLOGICAL AND CHEMICAL AGENTS

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The single cell gel electrophoresis assay, also known as the comet assay, is a versatile, sensitive and easy method for detecting many types of DNA damage, including single and double strand breaks. Classic Comet assay is time-consuming, has limited throughput and reproducibility. In order to overcome these limitations, a high through-put comet assay is created. In this assay, use of a micrometer scale array of cells increases the number of analyzable comets/cm2 and provides automated imaging and analysis by using a special program. In addition, high through-put comet assay gives compatible with standard the classic Comet assay. It can be used to detect different types of DNA damage caused by different biological and chemical agents. We have used high throughput Comet assay to detect the DNA strand breaks caused by alkylaniline metabolites in different mammalian primary cells/cell lines as well as for detecting the DNA-damaging effects of biological agents like Helicobacter pylori in human gastric adenocarcinoma cells. We observed that alkylaniline metabolites caused significant single strand breaks by using alkali high through-put Comet assay while Helicobacter pylori mainly caused DNA double strand breaks as evidenced by neutral high throughput Comet assay. In addition, we have observed that different antioxidants may be beneficial in reducing the DNA damage caused by these agents. We can suggest that this relatively new technique can bring easiness, convenience and speediness to the researchers and it can be used trustfully in the near future in labs that use classic Comet assay technique.

P111

CELL VIABILITY AND INTRACELLULAR REACTIVE OXYGEN SPECIES PRODUCTION AFTER EXPOSURE TO BISPHENOL DERIVATIVES IN HEPG2 CELL LINE

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Bisphenols are widely used as plasticizers in different polycarbonate-based products in the production of epoxy resins. Bisphenol A (BPA) is the most abundant bishenol derivative in the environment, with well-documented toxicity on liver, testis, pancreas and breast. Due to the high toxicity of BPA, society mainly choses “BPA-free products”, such as BPA-free carboys, feeding bottles and water bottles. These products usually contain other bisphenol derivatives [bisphenol F (BPF) and bisphenol S (BPS)]. However, due to limited evidences in literature, these analogues also may cause toxicity and their toxicity mechanisms are not well-documented yet. The aim of this study was to investigate the alterations in cell viability and intracellular reactive oxygen species (ROS) production after exposure to bisphenol derivatives in HepG2 cell line. Cell viability was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and intracellular ROS production was determined by a fluorometric intracellular ROS kit that detects intracellular ROS (especially superoxide and hydroxyl radicals) in live cells after a 1 hour incubation. After incubation with different concentrations of BPA, BPF and BPS, MTT assay was performed and inhibitory concentration 50 (IC50) doses for BPA, BPF and BPS were found to be 623 µM, 611 µM and 428 µM, respectively. Intracellular ROS levels were significantly higher after exposure to BPS (52%), BPF (78%) and BPA (75%) (vs. control, $p<0.05$, all). These results show that BPS is the most cytotoxic compound to HepG2 cells while BPF leads to the highest intracellular ROS levels. Therefore, using other bisphenol derivatives instead of BPA should be reconsidered.

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GENOTOXICITY ASSESSMENT OF CHILDREN WITH β-THALASSEMIA MINOR BY USE OF MICRONUCLEUS ASSAY IN PERIPHERAL BLOOD LYMPHOCYTES

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Chronic anemia and iron overload lead to the development of oxidative stres and genotoxicity in β-Thalassemia. In the carrier status of the disorder as β-Thalassemia minor (β-Tm), unbalanced globin chain ratio and lower antioxidant capacity have been also documented. However, the status of genotoxicity in β-Tm has not yet been elucidated. The aim of this study was to assess genotoxicity in the children with β-Tm. For this purpose, 79 children with β-Tm and 74 children as healthy controls were involved in the study. A considerable part of the control group was formed from healthy siblings of the subjects. Cytokinesis blocked micronucleus assay has been used to assess the genotoxicity. Questionnaires investigating environmental and lifestyle factors were completed by the parents. The groups were similar according to demographics and general characteristics ($p>0.05$). The micronucleus and micronucleated cell frequencies were slightly higher in β-Tm children than that of control group children (Micronucleus frequencies per 1000 binucleated cells; mean ± standart deviation: 6,38±3,85 vs. 5,82±4,05, respectively) which was not statistically significant difference ($p=0,274$). In the present study, genotoxicity by micronucleus assay was for the first time investigated in pediatric subjects with β-Tm. Reassessment of the subjects at appropriate intervals extending to adulthood could be crucial in order to elucidate the interaction of additional life style risk factors.
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P113

EFFECTS OF USING SEVOFLURANE WITH NITROUS OXIDE AND REMIFENTANIL ON DNA DAMAGE

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In this study, the possible effects of sevoflurane anesthesia along with remifentanil and nitrous oxide, on DNA damage was investigated by alkaline comet assay. The study was performed on 52 patients aged 20-50 years, who were undergoing operation for elective myringoplasty and tympanoplasty. The patients were randomly divided into three groups to provide different anesthesia maintenance. Anesthesia induction was carried out with propofol and vecuronium bromide in all groups. After intubation, Group SN (Sevoflurane-Nitrousoxide) recieved 50%02+50%N2O and 2-4% sevoflurane. Group SR (Sevoflurane-Remifentanil) received remifentanil by infusion at rate of 0,2 mcg/kg/min, sevoflurane 2-4%+100% O2. Group C (Control) received sevoflurane 2-4% and 100% O2 for maintenance. Blood samples were obtained from all patients before induction, at 120 minute of operation, and on first and fifth postoperative days. The basic alkaline technique was followed with minor modifications. We demonstrated that the patients receiving remifentanil have shown increased DNA damage in 120th minutes of the operation, which is maximized on postoperative first day, decreased to preoperative values on postoperative fifth day. In nitrous oxide and control groups, the DNA damage was detected at 120th minute of the operation, was maximized on postoperative first day, and decreased on postoperative fifth day however was still higher than the preoperative values.

P114

THE ROLE OF NICOTINE ON LIVER GLUTATHIONE AND LIPID PEROXIDATION LEVELS IN MOUSE MODELS OF PARKINSON'S DISEASE

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This study was undertaken to investigate whether neurodegeneration may regulate hepatic functions, and how nicotine influences chemically-induced liver oxidative stress in the condition of neurodegeneration. C57Bl/6 and Swiss albino mice were given daily injections of MPTP, which is a neurotoxin, (30 mg/kg/day, i.p.) for four days and mice were observed for twenty days after the injections. For nicotine treatment, the mice were injected 2.0 mg/kg nicotine (i.p.), twice a day at 2-h intervals for seven days and following this period for the next fourteen days, 1.0 mg/kg nicotine (i.p.), twice a day at 2-h intervals. In the procedure in which MPTP and nicotine were administered together, the mice were intraperitoneally given daily injections of 2.0 mg/kg nicotine, twice a day at 2-h intervals for seven days and following this period for the next fourteen days 1.0 mg/kg nicotine (i.p.), twice a day at 2-h intervals and after this twenty-one days period for the next four days, 30 mg/kg MPTP (i.p.) once a day. The effects of nicotine on the levels of liver glutathione and lipid peroxidation, which are important indicators of oxidative stress, were measured in control, MPTP-induced, nicotine-treated and MPTP+nicotine-treated groups of Swiss albino and C57Bl/6 mice. In both species, nicotine-treated mice showed the significant decrease in liver glutathione levels. However, we monitored various profiles for both species in the context of liver lipid peroxidation levels. The results obtained thus suggest that nicotine may modulate MPTP-induced alterations in the levels of liver glutathione and lipid peroxidation.

P115

DETERMINATION OF CYP2E1 METABOLIC ACTIVITY IN C57BL/6 BRAIN MICROSOMES

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Research conducted in recent years has demonstrated that CYP450 enzymes are associated with many diseases. Among the CYP450s, CYP2E1 is an important enzyme involved in the metabolism of various xenobiotics. It is also known to generate reactive oxygen species and promote oxidative stress. Optimization of CYP2E1 activity may offer new insight into the unknown mechanisms of brain diseases. This study was aimed at investigating optimal conditions (the amount of protein, substrate (p-Nitrophenol) concentration and incubation time) for CYP2E1 activity in C57Bl/6 mouse brain microsomes. For this purpose, microsomal fractions were obtained from C57Bl/6 brain tissues by differential centrifugation and then brain CYP2E1 (p-Nitrophenol-O-hydroxylase) activity was analyzed in different reaction conditions spectrophotometrically at 546 nm. In the present study, optimum conditions for C57Bl/6 brain CYP2E1 assay were found to be as follows: 4.0 mg protein, the temperature of 37°C, 125 µM of the substrate, and a reaction time of 30 minutes.

P116

THE PROTEIN EXPRESSIONS OF DRUG/XENOBIOTIC METABOLIZING ENZYMES IN HUMAN COLON TUMOR AND NON-TUMOR TISSUES

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Colon cancer is a frequent type among all cancers, and is one of the leading causes of cancer-related deaths in many countries. Xenobiotics, including carcinogens, are metabolised by phase I and phase II biotransformation enzymes including cytochrome P450s (CYPs) and glutathione S-transferases (GSTs). The aim of the study was to assess the level of expressions of CYP1A1, CYPB1, CYP2E1, GSTP1, GSTT1, GSTO1, and GSTK1 in colon adenocarcinoma tissues obtained from 50 patients with using immunohistochemistry. Tumor and surrounding tumor free colon tissues of patients were compared according to their staining intensities. Mann Whitney-U test was used to examine the association between CYP and GST expressions in adenocarcinoma tissue, and Spearman correlation rank test was utilized to examine the clinicopathological data. Colon cancer cells were found to express significantly higher CYP1A1, CYP1B1, CYP2E1, GSTP1, GSTT1, GSTO1 and GSTK1 than those of colon normal epithelial cells ($p<0.05$). On the other hand, no statistically significant association was found between the protein expressions studied and age, gender, smoking status, tumor grade and tumor stage ($p>0.05$).

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EXPRESSIONS OF GSTS1, GSTZ1 IN HUMAN COLON TUMOR TISSUES

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The colonic epithelium is in continuous contact with numerous xenobiotics, which people take in as part of their diet. The glutathione S-transferase (GST) is a superfamily of enzymes which are important in detoxification. GSTs have been shown to be overexpressed in many tumours. Among them, GSTS1 and GSTZ1 are not well-studied enzymes which can be overexpressed in response to oxidative stress. GSTZ is important in the metabolism of dichloroacetic acid which is usually found as a contaminant in chlorinated water supplies, certain drugs and chlorine containing industrial solvents. In this study we investigated the immunohistochemical staining characteristics of glutathione GSTS1, GSTZ1 in colon tumor and surrounding tumor free (normal) colon tissues obtained from 60 patients with colon adenocarcinoma who underwent colon cancer surgery. Tumor and control tissues of patients were compared according to their staining intensity. Relationships between GSTS1 and GSTZ1 expressions in adenocarcinoma tissue were examined by the Mann Whitney-U test, and the clinicopathological data were examined by the Spearman correlation rank test. GSTZ1, GSTS1 expressions in colon cancer cells were significantly higher than those in colon normal epithelial cells ($p<0.05$). However, no significant relationship was found between the GSTZ1, GSTS1 expressions and clinical parameters ($p>0.05$). In colon cancer patients the higher expressions of GSTS1, GSTZ1, proteins in tumor than normal colon tissues might be important in colon cancer development.

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INVESTIGATION OF GST ISOENZYMES AND APOPTOTOTIC EFFECT IN DLD-1 HUMAN COLON CANCER CELL LINE BEFORE AND AFTER 5-FLUOROURACIL TREATMENT

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Cancer cells that develop resistance to chemotherapeutic agents are a major clinical obstacle in the successful treatment of cancer. Glutathione S-transferases (GSTs) play an important role in the detoxification of various drugs used in cancer chemotherapy. p38, bcl-2 and caspase-3 have a role in apoptosis. In this study, immunocytochemical expressions of GST Alpha-1 (GSTA-1), GST Mu-1 (GSTM1), GST Theta-1 (GSTT1), GST Pi-1 (GSTP1), GST Omega-1 (GSTO1), GST Zeta-1 (GSTZ1), GST Sigma-1 (GSTS1), GST Kappa-1 (GSTK1), p38, bcl-2 and caspase-3 were examined in DLD-1 human colon cancer cell line before and after 5-fluorouracil (5-FU) treatment. 5-FU was added at a concentration of 0, 1, 2,5 or 5 µM and cells were continuously treated at 37°C and 5% CO2 for 10–12 days for colony formation. After incubation, colonies were washed in PBS, fixed with methanol for 15 min and washed in PBS. The harvested colon cells were immunostained. Treated and untreated cancer cells were scored according to their immunostaining intensity. The GSTP1, GSTT1, GSTM1, GSTA1, GSTO1, GSTZ1 and GSTK1 expressions were higher in treated colon cancer cells than those in untreated DLD-1 human colon cancer cells. Similarly, the p38, bcl-2 and caspase-3 expressions were higher in treated colon cancer cells than that in untreated DLD-1 human colon cancer cells. However, there was no statistical difference in GSTS1 expression. In conclusion, elevated expressions of GSTP1, GSTT1, GSTM1, GSTA1, GSTO1, GSTZ1, GSTK1, p38, bcl-2, p53 and caspase-3 might be important in 5-FU resistance in colon cancer.

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THE ROLE OF FOLATE DEPENDENT GENETIC SUSCEPTIBILITY IN THE RISK OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). Aberrations in one-carbon metabolism may have an impact on MS pathophysiology due to genetic susceptibility and thus increase MS risk. Furthermore, genetic factors such as polymorphisms of folate – dependent enzymes may lead to inadequate vitamin B and folate levels and elevated plasma homocysteine levels. Methylene tetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR) are key enzymes in one carbon metabolism pathway. Therefore, MTHFR C677T, MTRR G66A and MTR A2756 genetic polymorphisms were analyzed in 149 MS patients and 126 matching healthy controls by PCR-RFLP. We did not find any significant associations between genetic polymorphisms and MS risk. The CT and TT genotypes of C677T polymorphism showed no statistically significant difference (p=0.201 and p=0.112) and were not associated with MS risk (COR=1.717, 95% CI (0.749-3.936) and AOR=2.029, 95% CI (0.847-4.859), respectively). The AG and GG genotypes of A2756G polymorphism showed no statistically significant difference (p=0.932 and p=0.564) and were not associated with MS risk (COR=1.043 95% CI (0.394-2.765), and AOR=0.737 95% CI (0.261-2.078), respectively). The GA and AA genotypes of MTRR G66A polymorphism showed no statistically significant difference (p=0.759 and p=0.554) and were not associated with MS risk (COR=0.826 95% CI (0.244-2.799), and AOR=0.707 95% CI (0.223-2.236), respectively). In conclusion, our findings suggest that the mutant genotypes of MTHFR, MTR and MTRR genes were not associated with MS susceptibility in Turkish population. Our findings provide additional support to genetic basis for MS development.

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TOXICOLOGICAL EVALUATION OF EFFECTS OF BORIC ACID AND ZINC BORATE ON HUMAN SERTOLI CELLS

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Zinc borate is typically composed of 45% ZnO and 34% boric anhydride (B2O3), with 20% water of hydration. Zinc borate readily breaks down in the stomach to zinc oxide and boric acid. Boron compounds have been considered as being toxic to reproduction system in animal experiments. In addition, reproductive data of boron exposure is very limited. Results of epidemiological studies in Turkey and China showed that normal daily boron intake have no adverse effect on human reproductive system. Because of the limited information in the literature on the toxicity of zinc borate, this study is substantial as we use the reproductive system cell which is the target of boron exposure. In this study, it is aimed to investigate the cytotoxic and apoptotic effects of boric acid and zinc borate on Sertoli cell culture in vitro. The cytotoxicity of boric acid and zinc borate was determined by using Neutral Red Uptake (NRU) assay. Apoptosis was performed by Muse Annexin V& Dead Cell Kit using Muse Cell Analyser. Acording to our results, boric acid has no cytotoxic effect and does not induce apoptosis on human Sertoli cells up to 1000 µM. Besides, zinc borate is cytotoxic on human Sertoli cells (IC50=90µM) and significantly induces apoptosis at 100 µM. From the results of our study, it was concluded that zinc borate induces cell death and apoptosis at relatively high concentrations.

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EFFECTS OF PRENATAL EXPOSURE TO BISPHENOL A AND / OR DI-2-ETHYLHEXYL PHTALATE ON SPERM PARAMETERS AND REPRODUCTIVE HORMONES

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Early-life exposure to endocrine disrupting chemicals (EDCs) can lead to significant and persistent reprotoxicity, hormonal and metabolic problems with serious consequences in adulthood. Bisphenol A (BPA) and di-(2-ethylhexyl) phthalate (DEHP) are widely used EDCs. The aim of this study was to evaluate the reprotoxic effects of prenatal and postnatal exposures to DEHP and/or BPA in male Sprague-Dawley rats. Pregnant rats were divided randomly in four groups (n=3/group): Controls received corn oil; DEHP-group received 30 mg/kg /day DEHP; BPA-group received 50 mg/kg/day BPA and DEHP+BPA-group received 30 mg/kg /day DEHP and 50 mg/kg/day BPA (by intragastric lavage) through 6-21 gestational days and lactation period. Male offsprings (n=6/group) were fed until the end of the twelfth postnatal week. Reproductive hormones [estradiol, testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS)] and sperm parameters (count, motility and morphology) were determined. No significant differences were found between the groups, concerning all of the hormones. Only DHEAS was significantly lower in the DEHP (37%) and BPA+DEHP (40%) groups compared to the control. Both sperm counts and motilities were significantly lower in all of the study groups vs. control. Normal sperm morphology was also significantly lower in all of the study groups vs. control (91% in control, 72% in DEHP group, 61% in BPA group and 42% in DEHP+BPA group). These results indicate early-life exposure to EDCs can cause significant effects on sperm parameters, with more pronounced changes after combined exposures.

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EXPOSURE OF THE TURKISH POPULATION TO BISPHENOL A, 4-T-OCTYLPHENOL AND 4-NONYLPHENOL AND: A PILOT STUDY

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Bisphenol A (BPA), 4-t-octylphenol (4-t-OP) and 4-nonylphenol (4-NP) are manmade alkylphenols (APs) used in high volumes in industry. The results of in vitro and in vivo toxicological studies have shown that they act as hormone disrupters. Because of this reason they have attracted public attention due to their negative effects on human and environmental health [1-3]. The aim of the present study was to estimate the total (free and conjugated) levels of BPA, 4-t-OP, and 4-NP in 105 urine samples collected spontaneously from a non-occupationally exposed population aged from 2 to 51 in Mersin city province, to estimate baseline values concentration in Turkey. Identification and quantification of the target compounds were performed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The detection rates of BPA, 4-t-OP, and 4-NP were 100%, 100%, and 98%, respectively. The highest exposure levels of BPA, 4-t-OP, and 4-NP were 1.36, 0.164, and 0.082 µg/g creatinine in urine samples, respectively. In summary, despite the sample population's non-representativeness of the general Turkish population and relatively small size, this study provides the first reference range of human internal dose levels of BPA, 4-t-OP, and 4-NP in the Turkish population as a pilot study. Although our data suggest that exposure to BPA, 4-t-OP, and 4-NP is widespread in the Turkish population there are no health concerns according to the levels established by International Authorities (EFSA, FDA etc.).

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COMPARISON AND EVALUATION OF TOXICITIES OF BROMINATED AND ORGANOPHOSPHORUS FLAME RETARDANTS.

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Flame retardants (FRs) have been routinely used as additives in a number of consumer products such as TVs, electronics, polyurethane foams, textiles etc. for several decades in order to reduce the likelihood of ignition of these materials and/or decrease their rate of combustion. Until recently, polybrominated diphenyl ethers (PBDEs) accounted for a large proportion of FRs used in household products, including polyurethane foam and electronics. With respect to the human health effects, regulatory action and concern over the persistence, bioaccumulation, and toxicity of PBDEs, production and use of Penta and OctaBDE has been phased out in the US and banned in the EU. In 2009, these products were also listed as POPs. In the US, DecaBDE was subject to a voluntary phase-out at the end of 2013. It has also been restricted since 2008 in EU. Restrictions on the use of PBDEs have resulted in the increased use of alternate FRs, notably Organophosphate FRs (OPFRs). Despite the increased and varied uses of OPFRs, human data on the impacts of exposure to OPFRs are still limited. Some member of OPFRs (TDCPP and TPP) have been detected with high frequency in dust collected from homes and offices suggesting that the majority of the population receives chronic exposure to these compounds. Toxicological data suggests that certain OPFRs may be reproductive toxins and may also have carcinogenic and neurotoxic properties. The purpose of this study is to assess and compare the health risks of these two FR groups of people and the environment.

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ASSESSMENT OF TYPES OF SYNTHETIC CANNABINOIDS IN NARCOTIC CASES ASSESSED BY THE COUNCIL OF FORENSIC MEDICINE BETWEEN 2011-2015, ANKARA,TURKEY.

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Synthetic cannabinoids mimic the effects of cannabis and are the largest and fastest growing class of newly appearing designer drugs. Reports have revealed that various types of synthetic cannabinoids are mixed with herbal substances. The present study investigated the herbal substance cases involving synthetic cannabinoids in Ankara and nearby cities in Turkey. Data were collected from the reports of synthetic cannabinoids that were analyzed between January 01, 2011 and December 31, 2015 in the Ankara Narcotic Department of the Council of Forensic Medicine at the request of the judicial authorities. In all, 4610 narcotic reports were obtained and reviewed. Among these narcotic reports during the period, 370 reports (8%) were related to synthetic cannabinoids. 30 synthetic cannabinoid compounds could be identified in herbals: 5-F-AB-PINACA, 5-F-AKB-48, 5-F-NNEI, 5-F-PB-22, 5-F-PB-23, AB-CHMINACA, AB-FUBINACA, AB-PINACA, ADB-CHMINACA, ADB-FUBINACA, AKB-48, AM-2201, EAM-2201, JWH-018, JWH-022, JWH-031, JWH-122, JWH-201, JWH-210, JWH-250, JWH-251, JWH-307, MAM-2201, NM-2201, NM-2202, NPB-22, RCS-4, THJ-2201, UR-144, XLR-11. The amount of herbals was 30.72 g, 329.22 g, 665.89 g, 4844.7 g, and 5684.3 g in 2011, 2012, 2013, 2014, and 2015, respectively. Generally, herbals contained more than one synthetic cannabinoids. ADB-FUBINACA was the most common synthetic cannabinoid among the herbals determined in this study, which was 3132.43 g, excepting multi-synthetic cannabinoid herbals. The amount and diversity of synthetic cannabinoid compounds have increased dramatically between 2011 and 2015.

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TOXICITY RISK OF ARSENIC (AS) CONTAMINATION BASED ON HAIR AS CUT-OFF VALUE IN RESIDENTS OF NEVSEHIR PROVINCE, TURKEY

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Drinking water As levels in Nevsehir Province, Turkey are ranging from 11 to 500μgL^{-1} which is higher than the recommended level of 10μgL^{-1}. This study was carried out in three regions with different drinking water As levels in Nevşehir Province [>50μgL^{-1}(I) and 11-50μgL^{-1}(II) as exposed areas (E; n=420) and <10μgL^{-1}(III) as control area (C; n=185)]. Hair As levels of all subjects were measured by Atomic Fluorescence Spectroscopy and drinking water time weighted average As (TWA-As; μgL^{-1}) values were calculated. Individuals were examined for presence of skin lesions as indicators of early toxic effects. Median hair As level in E was found approximately 6 times higher than that of C (0.79 and 0.14μg/g respectively, $p<0.001$). Median hair As concentrations were significantly different among E and C (for the subjects from I, II and III; 1.06, 0.51, 0.14μg/g respectively, $p<0.001$). Positive correlation was found between hair As concentrations and TWA-As ($r=0.752$, $p<0.001$). Hair As concentration of 1.0μg/g is suggested as a cut-off value for As intoxication signs such as skin pathology. 91.7% of the hair samples of residents from I and 8.3% from II exceeded 1.0μg/g, while none of the hair samples of residents from C exceeded 1.0μg/g. The incidence of keratosis was higher in residents with >1μg/g hair As ($p<0.05$).In conclusion, residents with hair As level exceeding 1.0μg/g were found to have higher risk for As toxicity such as skin lesions.

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RAISING THE AWARENESS ON HEALTHY FOOD AND HEALTHY EATING AMONG CHILDREN

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Children consume more food and beverages per kilogram of body weight than do adults, and their dietary patterns are different and often less variable during different developmental stages. To define, explain and highlight important issues concerning healthy food and healthy eating habits, the project: “Raising the Awareness on Healthy Food and Healthy Eating Among Children“ was carried out. In the project realization, scientists from toxicology, food engineering, nutrition, food biotechnology, biology, education psychology and guidance, science education, curriculum development, measuring and evaluation, language scientists, web page design and documentary film production areas from Turkey (main leader of the project), Spain, Sweden, Serbia and Croatia were involved. The main goal of the project was to develope film for adolescents, a supplementary text book, and a manual for teachers. Mentioned educational tools are the achievements of the collaboration, communication and contribution of different professionals. The project content comprised; basic knowledge on nutrition sources and functions, healthy food choices and safety clues on food purchasing, storage, preparation steps, healthy food consumption, obesogenic environment and food contamination. The educational materials (originally developed on English) have been translated to Turkish, Spanish, Croatian and Catalan. An achievement test was used and administered in schools in Turkey, Spain and Croatia. Herewith the experiences and results of the testing in Croatian schools would be presented. It can be concluded that the movie led to a statistically significant increase in students’ awareness on healthy eating and healthy food choices in Croatia.
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AN EXTRAORDINARY APPROACH: DETECTION OF NEOPTERIN IN TEAR

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Neopterin, a non-conjugated pteridine derivative synthesized from guanosine triphosphate (GTP), is released from human macrophages and dendritic cells following stimulation of interferon gamma (IFN-γ) and it is considered as a sensitive biomarker of cellular immune response. It is known that detection of neopterin levels in biological samples provides information about the cellular immune condition and reflects the immune activation status. The aim of this study was to detect neopterin concentrations in human tear samples and to evaluate its potential correlation with serum neopterin levels. For this purpose, tear and serum neopterin levels were evaluated in 20 healthy individuals who had normal ophthalmological examinations, without any ocular inflammation. Both tear and serum samples were collected from each individual and enzyme-linked immunoassay (ELISA) was carried out to detect the quantity of neopterin in the samples. Tear neopterin levels were found significantly correlated with those in serum, even with three-fold differences. This is the first study to show neopterin levels in tear samples. It is thought that determination of biomarkers such as neopterin in tear might be useful for diagnosis and prognosis of disorders, especially immunopathological event-mediated ocular diseases.

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EFFECTS OF 2100 MHZ RADIO FREQUENCY RADIATION ON HEART, AORTA AND BLOOD PARAMETERS IN HYPERTENSIVE AND NORMAL RATS

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The aim was to investigate the possible effects of Radio Frequency Radiation (RFR) on heart, aorta and blood tissue in hypertensive and non-hypertensive rats with various parameters. Male Wistar Albino rats were divided into 4 groups:1.Control,2.Hypertension,3.RFR, 4.RFR+Hypertension. 60 mg/kg L-Nitroarginine Methyl Ester was administered 1 month by oral gavage to induce hypertension. Those with blood pressure greater than 140/90 mmHg were considered hypertensive. The rats were exposed to 2100 MHz RFR for 60 minutes/day, 5 days/week for 8 weeks.For RF fields; the root mean square value of electric field was found to be 17.25 V/m. (Specific absorption rate:0,23 W/kg). Blood hematocrit levels, blood and plasma viscosity, malondialdehyde, total nitric oxide (NOX), and glutathione levels in plasma and heart tissue were determined. Histological examination of the aortic wall and left ventricular muscle was performed. The results were analyzed with One Way Anova Tukey test. Those with $p<0.05$ were considered significant. RFR exposure resulted in a marked increase in cardiac and plasma malondialdehyde and NOX levels and blood viscosity, and a decrease in glutathione levels ($p<0.05$). There was an increase in left ventricular weight and number of muscle cells in hypertensive groups ($p<0.05$). The most pronounced aortic degeneration and left ventricular fibrosis were detected in the hypertensive group with RFR exposure. Our study suggests that exposure to RFR causes more negative effects on hypertension in terms of oxidative stress and antioxidants in the heart and plasma. However, RFR exposure showed more prominent left ventricular fibrosis and aortic degenerative effects in hypertensive groups.

P129

CLINICAL EVALUATION OF PATIENTS WHO STARTED TREATMENT WITH METHADONE AND A COMBINATION OF BUPRENORPHINE AND NALOXONE, BETWEEN THE YEARS 2008 AND 2012, IN THE UNIT OF ADDICTIVE BEHAVIORS OF LA VILA

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The aim of this study is to evaluate the effectiveness of maintenance programs with methadone and with a pharmaceutical drug, which contains buprenorphine and naloxone (Bup/Nal), and the influence of the consumption of benzodiazepines on the results.
Material and Methods: The study was designed as a retrospective observational study with 69 patients who came to the Unit of Addictive Behaviors to treat their addiction to heroin and opioids, between 2008 and 2012. For the data collection we used medical histories, SECAD sheets (an information system for the evaluation of the quality of the assistance in drug dependence) and ABUCASIS (a computer system for the management of the complete care process), as well as phone calls and interviews, always with patient authorization. Both physical pathology and psychopathology were described, as well as patterns of heroin use.
Results: A significant reduction in heroin, alcohol, cocaine and cannabis consumption was observed as a result of both treatments. No changes were observed in benzodiazepines or tobacco consumption. There was no significant dose-response relationship between the dose of methadone or Bup/Nal and the drug consumption at the end of the study. The consumption of benzodiazepines was associated to the presence of psychopathological diseases.
Conclusions: Remaining in maintenance programs with opioids is effective in reducing drugs consumption. The reduction of benzodiazepines use in clinical practice should be promoted, as long as it is not part of the treatment of the psychopathology of the patient.

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COMPARATIVE STUDY BETWEEN TREATMENT WITH METHADONE AND TREATMENT WITH A COMBINATION OF BUPRENORPHINE AND NALOXONE: ADVERSE EFFECTS, LIFE QUALITY AND STATE OF WELL-BEING AND HEALTH

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The study was aimed to study adverse effects of a drug containing Buprenorphine and Naloxone (Bup/Nal), in comparison with Methadone and to assess the life quality and the patient's perception of their state of health and well-being. This retrospective observational study was carried out in the Unit of Addictive Behaviors of La Vila with 35 patients. Of these patients, 14 were receiving treatment with Bup/Nal and 10 of them had received a previous treatment with methadone, the remaining 21 patients were treated with Methadone. Sociodemographic variables recorded were: gender, age, geographical origin, residence status, educational level, employment status. Clinical and toxicological variables recorded were: HBV infection, HCV infection, HIV infection, drugs consumption, first route of administration of heroin, parenteral administration, psychopathological diseases, existence of drug addicts in the family and relatives with psychopathological diseases. For the data collection, we used clinical histories, surveys and a Test for the Evaluation of Life Quality in Addicts to Psychoactive Substances (TECVASP). Drugs consumption was reduced from 100% of the patients at the beginning of the study, to 31% during the last month of the study. Also, the percentage of patients with legal problems was reduced, from 60% to 20% after the treatment. Bup/Nal induced less adverse effects than Methadone ($p<0.05$). Patients rated Bup/Nal treatment higher in terms of satisfaction with the treatment and scored higher in their health status. Furthermore, patients on Bup/Nal treatment were considered to have better life quality according to the TECVASP test.

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PREVALENCE OF ADVERSE EVENTS ASSOCIATED TO PSYCHOPHARMACOLOGICAL TREATMENT IN PATIENTS WITH INTELLECTUAL DISABILITIES

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The prevalence of psychoactive drugs in people with intellectual disabilities is very high. However, there is little evidence of their effectiveness and the percentage of the adverse events is high. This paper presents a summary of the adverse effects associated with the use of psychotropic drugs and other variables, as well as, the association between use of psychotropic drugs with ICAP (Inventory for service planning and individual programming) variables to assess conflicting and adaptive behavior. Data were obtained from the history, the UKU (Udvalg for Kliniske Undersogelser, a side effect rating scale) and the ICAP. Adverse events were identified in 33% of the subjects, but the attribution of causality due to the treatment was very difficult. Main significant associations were found in the use of antipsychotics and anticonvulsants according to UKU and ICAP variables. It was concluded that the detection of adverse events in people with severe or profound intellectual disabilities is very complicated. However, those adverse events can cause serious problems, for which it is important to reduce the use of psychotropic drugs and promote non-pharmacological methods.

P132

SUBJECTIVE PERCEPTION OF ANTIDEPRESSANT TREATMENT IN PHARMACY OFFICE USERS

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The objective of this study was to ascertain the patient's benefit and utility of the treatment with the antidepressants as there is literature questioning the effectiveness and safety of these treatments. The following information was recorded: demographic, occupational and medication data, patient's perception of antidepressant treatment, their subjective opinion about the benefit and usefulness of treatment, assessment of quality of life and possible side effects. A significant proportion of antidepressant users were reluctant to communicate their experiences with the drug at the pharmacy office. Although adverse effects are frequent, very few associate their discomfort with the use of the antidepressant. Many patients were not able to associate the contribution of antidepressant treatment to their wellbeing. While many claim that they take drug for not feeling worse, being in many cases the duration of the treatment for decades.

P133

INVESTIGATION OF EFFECTS OF NARINGENIN ON VANCOMYCIN-INDUCED NEPHROTOXICITY IN RATS

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Vancomycin (VCM) is a glycopeptide antibiotic which is used for the treatment of infectious diseases caused by methicillin-resistant Staphylococcus aureus (MRSA) [1]. Development of nephrotoxicity is the major limiting factor of VCM. Naringenin (NAR) is a flavanone classified under the group of flavonoids, is strong antioxidant. Anticancer, hepatoprotective, nephroprotective, antimutagenic activities, antiinflammatory, antidiarrheal, antiulcer and myocardial protective activities of NAR were reported in previous studies [2,3]. In this study, protective effects of NAR was determined by evaluating biochemical parameters on VCM-induced nephrotoxicity in rats. Experimental protocol was performed with the acceptance of Animal Experiments Local Ethics Committee of Mersin University (2016/HADYEK/E.98180). Adult male Wistar-Albino rats were randomly divided into five groups including: (i) saline (ii) Carboxymethyl cellulose (0.5% CMC), (iii) VCM (400 mg/kg/day), (iv) VCM+NAR25 (25 mg/kg/day for NAR), and (v) VCM+NAR50 (50 mg/kg/day for NAR) groups. After 8-day treatment, animals were sacrificed under anesthesia. Caspase 3, 8 and 9 were measured in the kidney tissue and histopathological examination was also carried out. Results of the study indicated that VCM administration caused marked changes in the kidney. The levels of caspase 3, 8 and 9 were elevated in the VCM group. On the other hand, these levels were decreased in the NAR treatment groups. This study suggests that NAR can be the potential protective effects of on VCM-induced nephrotoxicity in rats.

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DETERMINATION OF DICLOFENAC BY A NEW VALIDATED HPLC METHOD IN HUMAN PLASMA

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A simple, rapid and reliable high performance liquid chromatography method with ultraviolet detection was developed and validated according to ICH guidelines, for quantitative analysis and therapeutic drug monitoring of diclofenac sodium (DS) in human plasma. Plasma samples (0.7 mL) were acid hydrolysis by 100 µL, 1 M hydrochloric acid. Analytes were extracted by liquid–liquid extraction with 2 mL ethyl acetate (98.75%-99.32%). The separation was achieved by employing C_{18} analytical column under isocratic conditions using acetonitrile and NaH_2PO_4 mixture (42.5: 57.5, v/v) as mobile phase (pH:3.16) flow rate of 1.5 $mL.min^{-1}$. Naproxen (3 $\mu g.mL^{-1}$) was used as an internal standard (IS). The DS and IS were detected at 281 nm and eluted at 2.6 and 6.2 min, respectively. Run time was <7 min. Method showed linearity (r^2=0.999) over the concentration range of 50-1600 $ng.mL^{-1}$. Limits of detection (LOD) and quantification (LOQ) were 8.95 $ng.mL^{-1}$ and 27.12 $ng.mL^{-1}$, respectively. Intra-day precision and accuracy were between 0.93-5.27; 1.74-9.81, respectively. Inter-day precision and accuracy were between 2.71-6.64; 2.03-9.16, respectively. This method was successfully applied for determination of DS plasma concentrations during a pharmacokinetic study in healthy volunteers (n=12) after an oral administration of Voltaren® 75 mg/tablet and remarkable variations in DS levels were observed. In our study, on the contrary to equivalent doses of DS, the observed significant differences in plasma levels of DS, on 2nd, 4th and 6th hours, can be explained by pharmacokinetic differences, that arise from mainly polymorphisms of CYP2C9 and CYP3A4, which are major enzymes responsible for DS metabolism.

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DETERMINATION OF MIRTAZAPINE AND DESMETHYL MIRTAZAPINE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ULTRAVIOLET DETECTION

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A new, rapid and sensitive high-performance liquid chromatography method has been developed for the determination of mirtazapine (MIR) and its active desmethyl metabolite (D-MIR) in human plasma. The method is based on a liquid-liquid extraction and reversed-phase chromatography with ultraviolet detection. The separation was achieved on reverse phase C18 250x4.6mm column using a gradient elution. 20 mM phosphate buffer, triethylamine and acetonitrile (24.9:75.0:0.1:v/v/v) were used as mobile phase. Clozapine was used as an internal standard. Mobile phase flow rate was 1.2 $mL.min^{-1}$ and total run time was below the 10 minute. Calibration curves were linear in the 10-250$ng.mL^{-1}$ range for both compounds. The linearity of MIR and D-MIR (r^2) were found 0.9981 and 0.9987, respectively. Intra-day and interday assay precision (RSD%) were found as 1.6–9.3 and 1.0–8.1, respectively. Intra-day and interday accuracy of the method were calculated (RE%) as (-4.8)–5.2 and (-2.1)–7.9, respectively. The recoveries of MIR and D-MIR were 95.7%, 106.1%; respectively. Developed method was found robust according to mobile phase flow rate, UV detection, column oven temperature, and mobile phase pH. The method allows not only the therapeutic drug monitoring of the MIR which is the most prescribed tetracyclic antidepressant and its pharmacological active metabolite but also use in toxicological screening. MIR and D-MIR levels in plasma samples of 5 patients who were on treatment with MIR were successfully monitored by the developed method. Observed changes of drug and metabolite levels in human plasma, reflected the serious differences that activities of enzymes involved in MIR biotransformation.

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POSSIBLE GENOTOXIC EFFECTS OF PICLORAM, PLANT GROWTH REGULATOR AND HERBICIDE, ON ALLIUM CEPA ROOT TIP CELLS

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Picloram (4-amino-3,5,6-trichloro picolinic acid) is a synthetically produced plant growth regulator and used as a herbicides for weeds in agricultural applications. It is also used as synthetic auxin that regulates somatic embryogenesis as reported in plant biotechnology studies. Researchers conducted in birds, mammals and aquatic species have shown that low concentrations of picloram induce cell division by stimulating RNA, DNA, protein synthesis. However, higher concentrations of picloramcaused vascular tissue damage and also inhibit cell division and growth. On the other hand, no study has been conducted on the genotoxic effects of picloram using Allium ceparoot cells. In this study, potential genotoxic effect of picloramwas evaluated by using comet assay inAllium cepa root tip cells. For this purpose, 1.5-2 cm diameter, Allium cepa, which can works in tissue culture, was obtained. 0.61, 1.34, 2.01, 2.68, 3.35, 4.02, and 8.04 mg/L concentrations of picloram were used for 24 hoursin Alliumcepa root tips germinated in water.For each concentration, 25 cells were examined using a fluorescence microscope using specialized Image Analysis System. According to test results, comet tail intensity and tail moment significantly increased at 1.34, 4.02, and 8.04 mg/L compared to control group. These results indicate that especially high concentrations of piclorammay produce genotoxic effects in Allium cepa root tip cells. However, these results should also be evaluated in detail by other genotoxicity tests and other cell types.

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IN VITRO EVALUATION OF RHEUM RIBES (ROOTS) TOXICITY

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Rheum Ribes (Ribês, Rêwas, Rewes, Uçkun, Işkın) is a perennial herbaceous plant belonging to the family Polygonaceae that grows more in rocky and gravelly slopes in high altitude areas in Levant, Armenia, Northern Iraq, Iran and Turkey, especially Eastern Anatolia regions. In folk medicine R. Ribes is widely consumed against nausea and constipation.The roots, fruits and the stem part of R. Ribes are frequently used for different diseases, including diabetes, high blood pressure, high cholesterol, cirrhosis. Recently R. Ribes used againt leukemia and breast cancer. In our study, we used HepG2 cell line to evaluate the cytotoxic and genotoxic potential of R. Ribes dry roots water, methanol and chloroform extracts. Our results show the extracts cause cell death in a concentration dependent manner (IC_{50} 14.29 -33.67 mg/ml). Genotoxicity assay results indicate that only the maximum concentration of methanol extract causes a significant DNA damage. In conclusion the risk of R. Ribes is similar to a lot of plants used in the folk medicine, and most of them is still unknown. The uncontrolled use of this plant could harm the patients. There is a need for more in vivo and in vitro studies to evaluate the effects of R. Ribes.

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FROM BATCH TO CONTINUOUS FLOW CHEMISTRY: DOE-ASSISTED OPTIMIZATION OF BENZIMIDAZOL-2-ONE SYNTHESIS

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Until a decade ago, chemical synthesis in medicinal and organic chemistry has been performed mainly on batch using round-bottom flasks or reaction tubes. As the utilization of continuous flow technologies in chemistry has been increased, synthetic procedures have been altered in both academia and pharmaceutical industry to discover and develop new compounds in a most efficient way with respect to time, cost and safety [1]. Continuous flow chemistry systems offer several advantages [2] and a unique way to easily combine technologies, such as microwave irradiation, supported reagents or catalysts, electro/photochemistry and new solvent systems to improve conventional methods and process optimization [3]. In this communication, we report an efficient method for the continuous flow preparation of the benzimidazol-2-one scaffold, a well-established privileged structure in medicinal chemistry. Although several approaches have been reported for the preparation of this scaffold, most of them suffer from limitations including the use of hazardous reagents and conditions, and long reaction times. In this work, we have made use of statistical design of experiments (DoE) [3] for decision-making of experiments in evaluating the effect of temperature, flow rate and reagent stoichiometry on the reaction outcome. The results obtained have enabled to define a robust, reliable and versatile protocol for both large-scale preparation and substrate scope of benzimidazole derivatives.

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DISCOVERY OF NOVEL 5-LIPOXYGENASE ACTIVATING PROTEIN (FLAP) INHIBITORS BY VIRTUAL SCREENING

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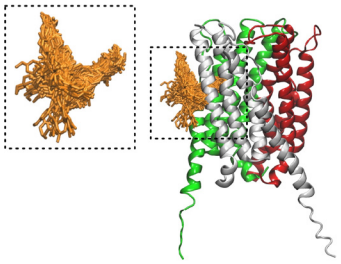
Leukotrienes (LTs) are proinflammatory metabolites of arachidonic acid (AA), which play significant roles in inflammation-related respiratory and cardiovascular diseases. 5-Lipoxygenase (5-LO) and 5-lipoxygenase-activating protein (FLAP) are both essential for the initial step of the LT biosynthesis in response to cellular activation. While there is only a single marketed inhibitor of 5-LO (zileuton) for the therapy of the patients, no FLAP inhibitor has reached the market yet. Hence, there is a growing number of studies aimed at developing novel inhibitors of both therapeutic targets. It is very important to better understand ligand protein interactions to develop novel drug candidates. The aim of this study is to discover novel and chemically diverse FLAP inhibitors by combination of computer-aided virtual screening methods for anti-leukotriene therapy. Ligand and structure-based approaches have been applied to explain the activities of previously known FLAP inhibitors in relation to their predicted binding modes against the refined crystal structure of FLAP [1,2]. We have performed a virtual screening study and the biological evaluation of the obtained compounds from this study resulted potent inhibition of FLAP-dependent cellular LT biosynthesis, IC_{50} values of the most potent compounds are at nanomolar range. This study may help to design and develop novel potential leads suitable for development of effective anti-inflammatory drugs.



Binding modes of the most potent FLAP inhibitors.

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P140

SYNTHESIS, ENZYME INHIBITION AND MOLECULAR DOCKING STUDIES OF NEW HYDRAZONE DERIVATIVES AS MONOAMINE OXIDASE INHIBITORS

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Hydrazones are a class of hydrazine analogues, which bear an azomethine -NHN=CH- group. A lot of studies indicate that various substitutions of hydrazone modulate the different biological evaluation including MAO enzymes inhibition [1-3]. This situation of hydrazine type inhibitors can be clarified by the structural similarity to MAO substrates, which usually carry an amino or imino group. Terminal nitrogen atom and C=N double bond are responsible for the physical and chemical qualities. Also, the C-atom is important for electrophilic and nucleophilic properties. Owing to mentioned these chemical properties of hydrazones, recent studies set light to several substituted hydrazones as MAO inhibitors.
In the light of above information, new hydrazone derivatives (2a-2i) were designed and synthesized for the aim of discovery of new MAO enzyme inhibition. Structures of gained compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, HRMS spectroscopic methods. Their inhibitory activity against MAO-A and MAO-B enzymes was elucidated by using in vitro Amplex Red® reagent based fluorometric methods. According to enzyme activity studies, 2a and 2b displayed significant MAO enzymes inhibition potential and good selectivity towards MAO-A isoform. In order to determine the type of inhibition, enzyme kinetic studies were performed for compounds 2a and 2b. Also, genotoxicity and cytotoxicity were performed and compounds 2a, 2b were found as non-cytotoxic and non-genotoxic. Moreover, docking studies were applied to explore the binding modes of these compounds to MAO-A enzyme active site and revealed that there is an interaction between MAO-A and compounds 2a, 2b.

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P141

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF THIOETHER AND OXIME DERIVATIVES OF IBUPROFEN-BASED 1,2,4-TRIAZOLES AS POTENTIAL ANTICANCER AGENTS

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Cancer continues to be the second leading health problem worldwide, after cardiovascular diseases. The metastatic ability of cancerous cells is also a condition that worsens most cases. Conventional drugs such as antimetabolites and alkylating agents used in cancer treatment have severe side effects. There is an urgent need for the discovery of new agents for effective and safe treatment of cancer. In the study conducted at our department by Kulabaş et.al. [1], N-(substituted phenyl)-2-(5-aryloxymethyl-4-benzyl-4H-1,2,4-triazole-3-ylthio)acetamide derivatives were synthesized and some of them exhibited anticancer activity for specific tumor cell lines at between 5.96-7.90 µM.

Over the past decades, nitric oxide (NO) has gained tremendous interest as an important physiological signaling molecule. This molecule seems to have a key role in reducing the side effects of non-steroidal anti-inflammatory drugs (NSAIDs). In addition, previous reports provide proofs about metabolic NO release mediates cytotoxic activities against different cancer cell lines. El-Din and co-workers, who synthesized a series of 1,2,4-triazole derivatives and their oxime analogues, reported that all of their molecules showed anti-inflammatory activity close to indomethacin. It was also noted that especially oxime derivatives showed lower ulserogenic activities than the parent ketone forms [2].

These findings [1, 2] led us to synthesize new thioether and oxime derivatives of ibuprofen-based 1,2,4-triazoles and to investigate their anticancer activity. The synthesized compounds were characterized by the elemental analysis and spectral data. Target compounds were evaluated for their anticancer activities against K562, A549 and PC-3 cell lines and for selectivity they were also investigated against NIH3T3 cells.

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P142

SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF CARBOHYDRAZIDES AND 1,3,4-OXADIAZOLE DERIVATIVES BEARING IMIDAZOLIDINE MOIETY AGAINST THE YELLOW FEVER AND DENGUE VECTOR, AEDES AEGYPTI

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Zika virus is an Aedes mosquito-borne flavivirus that has rapidly spread throughout in South America, Central America and the Caribbean [1]. Because vaccines or other spesific treatments are not available for Zika virus infection, chemical insecticides remain a major method for disease reduction by mosquito control [2]. 1,3,4-oxadiazole and imidazolidine rings are important heterocyclic compounds exhibiting a variety of biological activities [3]. In this study, a series of new oxadiazole derivatives containing 1,3,4-oxadiazole group bearing an imidazolidine moiety were synthesized and screened for insecticidal activities. The proposed structures of the 17 synthesized compounds were confirmed using elemental analysis, IR, ^{1}H-NMR, and mass spectroscopy. None of the compounds showed larvicidal activity at the tested concentrations against 1st instar Aedes aegypti larvae. However, nine compounds exhibited promising adulticidal activity with the mortality rates of ≥80% at 5 mg/mosquito. Further dose-response bioassays were undertaken to determine LD_{50} values. The structure-activity relationship of these compounds may lead to the development of new effective insecticides. A limited series of mode of action studies are tried to explain the lethality in mosquitoes by neurotoxic or mitochondrial respiratory mechanisms.

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P143

CYTOTOXICITY OF SOME NEW CARBOHYDRAZIDE AND UREA DERIVATIVES BEARING PYRIDINE RING

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Internal and external factors can damage DNA, the genetic material. Numerous repair mechanisms which can repair those damages exist in the normal cell. One of those is called Poly (ADP-ribose) polymerase-1 (PARP-1). If PARP-1 inhibition occurs, single strand DNA breaks can not be repaired and double strand DNA breaks can be formed. Eventually, cells undergo necrosis or apoptosis [1]. Urea and carbohydrazide derivatives are important compounds exhibiting anticancer activities [2,3]. In this study, a series of new urea and carbohydrazide derivatives containing an pyridine ring were synthesized. The proposed structures of the synthesized compounds were confirmed using elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. The cytotoxic potencies of synthesized compounds were determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) on BRCA mutant carrying HCC1937 and Capan-1 cell lines as well as on MCF7, HeLa and MRC5 cells. Cellular PARP inhibitory activity of compounds was assessed by measuring the inhibition of the H_2O_2-induced PARylation in HeLa cells using immunofluorometric assay system.

This study was supported by TUBITAK with 215S112 project number.

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P144

EVALUATION OF ANTI-PROLIFERATIVE EFFECT OF NOVEL SYNTHESIZED 4-(3-(4-FLUOROPHENYL)TRIAZ-1-EN-1-YL) BENZENESULFONAMIDE ON LUNG CANCER CELL LINE (A549)

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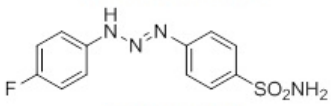
Abstract

Cancer is one of the most detrimental disease in the current century. Therefore, developing new anti-cancer agents for the treatment of cancer without or lesser side effects are still in demand. In this perspective, the present study was aimed to evaluate the anti-proliferative effect of newly synthesized triazene substituted compound 4-(3-(4- luorophenyl)triaz-1-en-1-yl)benzenesulfonamide on lung cancer cell line (A549). Dose-dependent (0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml) anti-proliferative activities of 4-(3-(4- luorophenyl)triaz-1-en-1-yl)benzenesulfonamide were determined by using the MTT method. 4-(3-(4-luorophenyl)triaz-1-en-1-yl)benzenesulfonamide showed dose-dependent anti-proliferative effect on A549 cells. Our preliminary results show that the newly synthesized compound 4-(3-(4- luorophenyl)triaz-1-en-1-yl)benzenesulfonamide have dose-dependent cytotoxic effects on A549 lung cancer cell lines. To make a more accurate assessment about anticancer activity of this compound, it is necessary to work the apoptosis induction and related molecular pathways in future studies.

Keywords: 4-(3-(4-luorophenyl)triaz-1-en-1-yl)benzenesulfonamide, Anti-proliferative effect, Lung cancer, A549.

Chemical Structure



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Chemical structure of 4-(3-(4-fluorophenyl)triaz-1-en-1-yl) benzenesulfonamide

P145

EVALUATION OF 2-AMINO-3-[(2-NITRO-1-PHENYLPROPYL)THIO]PROPANOIC ACID DERIVATIVES AS ANTIMICROBIAL AGENTS

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In recent years, the antimicrobial resistance (AMR) or multidrug resistance (MDR) has become a serious health concern and major challenging issue worldwide. The extensive and routine use of the first-line antibiotics to control infections imposed the development of multiple mechanisms of microbial resistance. Despite the fact that numerous advanced technologies enable the molecular design of new antibiotics, the MDR-associated infections remain a great challenge to modern medicine [1].
β-Methyl-β-nitrostyrenes are known for their various pharmacological activities, particularly antibacterial, antifungal, antineoplastic, antiseptic, antiplatelet and antitubercular activities. Furthermore, the addition products with a nitrostyrene derivatives have been recognized to have diverse biological activities, especially antimicrobial and anticancer effects [2]. Cysteine is a sulfur-containing amino acid and an important structural and functional component of proteins and enzymes. Thiol group of cystein is also nucleophilic and thus can undergo addition and substitution reactions [3].
In vitro antimicrobial activities of the 2-amıno-3-[(2-nitro-1-phenylpropyl)thio]propanoic acid derivatives 1-7 were tested against gram-positive (Bacillus subtilis subsp. subtilis, Enterococcus faecium, Staphylococcus aureus, Enterobacter hormaechei) and Gram negative (Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli O157H7, Stenotrophomonas maltophilia) and fungi (Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis) using the microdilution broth method [4,5]. Ampicilline.3H_2O and fluconazole were used as reference in antibacterial and antifungal activity test, respectively.
As a result of this study, all of the synthesized compounds might be taken into consideration as promising antimicrobial compounds.

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P146

SYNTHESIS AND IN VITRO ANTIMYCOBACTERIAL ACTIVITIES OF 6-SUBSTITUTED-3(2H)-PYRIDAZINONE-2-ACETYL-2-(SUBSTITUTED/NONSUBSTITUTED ACETOPHENONE)HYDRAZONE

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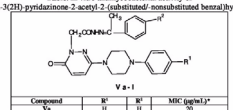
Tuberculosis (TB) is a major global health problem. The World Health Organization estimates that two billion people are infected with Mycobacterium tuberculosis. Multidrug-resistant TB (MDR-TB) is also an emerging issue in the world [1]. The past 20 years have seen the worldwide appearance of MDR-TB, followed by extensively drug-resistant TB and most recently, strains that are resistant to all anti-TB drugs [2]. Hydrazone derivatives are a considerable pharmacophore group for antimicrobial activity [3]. Hydrazones have been reported to possess, among others, antibacterial, antifungal, antitubercular, antiviral and antimalarial activities. In this context, we synthesized 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted/nonsubstitutedacetophenone)hydrazone Va-l in order to investigate their in vitro antimycobacterial activities by using the agar proportion method against M. tuberculosis H37Rv standard strain. [4]. Among the target compounds, Vb and Vf exhibited the best antimycobacterial activity, with a MIC value of 5 µg/mL.

Table. In vitro antimycobacterial activity of 6-Substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted/-nonsubstituted benzal)hydrazones **Va-l.**



V a - l

Compound	R^1	R^2	MIC (µg/mL)*
Va	H	H	20
Vb	H	Br	5
Vc	H	Cl	40
Vd	H	F	20
Ve	F	H	20
Vf	F	Br	5
Vg	F	Cl	N/A
Vh	F	F	N/A
Vi	Cl	H	N/A
Vj	Cl	Br	40
Vk	Cl	Cl	40
Vl	Cl	F	N/A
INH			0.2
EMB			1

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P147

NOVEL 6-SUBSTITUTED PIPERAZINE AND 9-TETRAHYDROPYRANYL PURINE DERIVATIVES: SYNTHESIS AND CYTOTOXIC ACTIVITY ON SELECTED HUMAN CANCER CELL LINES

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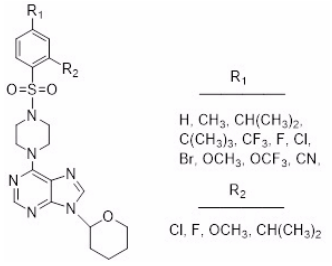
Purine derivatives, which are structurally, metabolically and pharmacodynamically similar, are known to have different biological activities. These diverse effects have been reported to be associated with anti-cancer, anti-viral, anti-fungal and antibacterial activities due inhibition of the enzymes involved in cell proliferation. Purine and purine nucleoside analogs are significant drugs used in chemotherapy for the treatment of solid tumors and hematological malignancies.

As a result of our ongoing investigations of purine and purine nucleoside derivatives, which have displayed promising cytotoxic activity, herein, we synthesized new series of substituted purines and characterized for their cytotoxicity in human cancer cell lines. The 6-(4-substituted piperazine)-9-(tetrahydropyran-2-yl)purine derivatives were obtained from commercially available 6-chloropurine. The in vitro cytotoxicity of purine compounds were initially analyzed on liver (Huh7), colon (HCT116) and breast (MCF7) carcinoma cell lines, using a sulforhodamine B (SRB) assay. The IC_{50} values for each molecule were also calculated in comparison with the nucleobase analogue 5-fluorouracil (5-FU), nucleoside analogues fludarabine, cladribine, pentostatine. N^6-(4-t-butylphenylsulfonyl) derivative displayed the best cytotoxic activity, with IC_{50} values of 1.2 µM against Huh7 cell line. We then tested the cytotoxic effect of the most potent purine derivatives on additional hepatocellular carcinoma (HCC) cell lines: Hep3B HepG2, PLC, Mahlavu, FOCUS, Snu475, Snu182, Snu387, Snu398, Snu423 and Snu449. We observed the most significant cell growth inhibition in the presence of 6-(4-t-butylphenylsulfonyl), 9- tetrahydropyranyl derivative, with IC_{50} values of 2.9-4.9 µM against Snu182, FOCUS, Snu398 cells.



P148

SYNTHESIS OF SOME NEW COMPOUNDS OF 1-(4-FLOROPHENYL)-2-(3,5-DIMETHYLPYRAZOLE1-YL)ETHANO N DERIVATIVES AND INVESTIGATION OF CYTOTOXIC EFFECTS

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Intensive studies are being conducted to develop effective anticancer compounds. In the clinic, alkylating agents, porphyrin drugs, inorganic metal complexes and azole compounds are used as anticancer drugs. Due to the lack of selectivity of the drugs; the high cytotoxicity, the serious side effects, development of resistance and the need for medical care has not been fully met. Suggesting that more effective and more selective new chemotherapeutic drugs for cancer treatment should be developed [1,2]. In this study 5 compounds are synthesized. The structures were elucidated by HRMS, IR, ^{1}H-NMR and ^{13}C-NMR spectral data analyzes. In vitro anticancer activities against the A549, HCT 116, HeLa, MCF7 and SH-SY5Y, cell lines of the compounds were evaluated with MTT tests. % Cell viability and IC_{50} values were calculated in the cell lines.

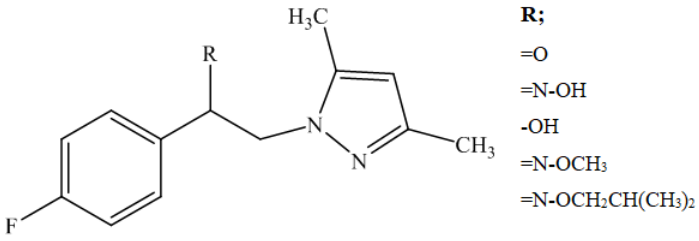

Figure 1. Structure of synthesized compounds

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P149

STUDIES ON QSAR AND ANTIMICROBIAL ACTIVITIES OF 2-ACETYLNAPHTALENE DERIVATIVES

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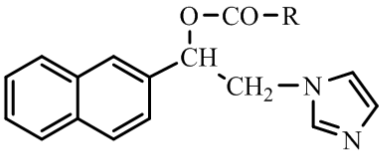
Antimicrobial activities of the compounds which has imidazole ring have also been determined due to the structural similarities of these compounds with 1-subtituted 1H-azole antifungal compounds. Antimicrobial activity of the compounds were tested against two Gr (+) (S. aureus, E. faecalis), two Gr (-) (E. coli, P. aeruginosa) bacteria and three yeast like fungi (C. albicans, C. parapsilosis ve C. krusei) by microdilution method. All of the compounds with imidazole ring were showen antibacterial activity against Gr (+) and Gr (-) bacteria, were also showed antifungal activity against fungi (C. krusei and C. parapsilosis) [1].

HOMO, LUMO, band gap, dipole moment, length, log P, molecular refractivity and polar surface area values estimated to be related to activity for physicochemical parameters of the molecules, have been theoretically calculated. As a result of these calculations, regression analyzes were carried out together with MIC which is the experimental data. As a result of the QSAR study equations were created to calculate MIC values.



R
-$CH_2CH_2CH_3$
-$CH(CH_3)_2$
-$CH_2(CH_2)_2CH_3$
-$CH_2CH(CH_3)_2$
-$CH(C_3H_7)_2$
-C_6H_5
-pClC_6H_4

Figure 1. Structure of compounds studied on QSAR

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P150

INVESTIGATION OF ANTICANCER PROPERTIES OF RU(II) AND OS(III) COMPLEXES: MOLECULAR DOCKING CALCULATIONS

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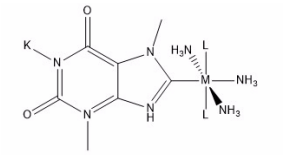
Investigation for new cancer drugs is important field for researchers. Searching of cancer drugs has been attractive for many years. The first study in this field has been done by Rosenberg et al. in 1965 [1]. Recently, ruthenium and osminium complexes has been investigated as anticancer drugs. There are limited computational studies in literature over complexes which have anticancer properties, such as caffeine and its derivatives. Computational researches has many advantages compared to the experimental studies. In this paper, there are two ruthenium complexes with caffeine and its derivative and osminium complexes with caffeine. Schematic diagram of mentioned complexes is represented in Scheme 1. The aim of this study, structural and spectral (IR, UV-VIS and NMR) analyses of mentioned complexes are performed at B3LYP/6-311++G(d,p)(LANL2DZ) level in gas phase. At same level, some quantum chemical descriptors which are mainly used by computational researcher are used to investigate anticancer activity. Additionally, the effect of complexation on anticancer properties is investigated by quantum chemical parameters which are energy of the highest occupied molecular orbital (E_{HOMO}), energy of the lowest unoccupied molecular orbital (E_{LUMO}), energy gap between LUMO and HOMO (E_{GAP}), absolute hardness (η), absolute softness (σ), absolute electronegativity (χ), chemical potential (CP), electrophilicity index (ω), nucleophilicity index (N), additional electronic charges (ΔN_{max}), global softness (S). Mentioned descriptors of caffeine and its derivate are calculated at B3LYP/6-311++G(d,p) level in gas phase. Interaction energies between vascular endothelial growth factor receptor 2 (VEGFRTK) (ID: 3WZE) and mentioned complexes are calculated by molecular docking calculations.



Complex **I**	M: Ru	K: H	L: Cl
Complex **II**	M: Ru	K: CH_3	L: Cl
Complex **III**	M: Os	K: CH_3	L: NH_3

Scheme 1. Schematic representation of mentioned complexes with atomic labelling.

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INVESTIGATIONS OF BIOLOGICAL PROPERTIES OF SOME METAL (MN, PD AND AG) COMPLEXES WITH PENTA-AMINE MACROCYCLIC SCHIFF BASE LIGAND: MOLECULAR DOCKING CALCULATIONS

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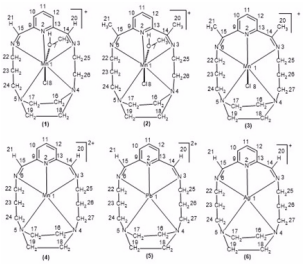
Huge endeavors have been toiled to synthesize the transition metal complexes with macrocyclic Schiff base ligand [1]. Macrocyclic Schiff base ligands and their metal complexes have significant properties such as anti-corrosion, anti-cancerous, anti-HIV, anti-bacterial and anti-fungal material and DNA cleavage. A lot of paper have been published related with synthesis and characterizations via experimental methods [2]. Quantum chemical calculations or computational investigations have been attractive in recent years.
In this study, six different metal complexes represented in Scheme 1 are investigated by computational techniques. The goals of this study are to complete the characterization of complexes and to perform the IR, UV-VIS and NMR analyses. Three different methods (HF, B3LYP and LANL2DZ) are used with 6-31G**(LANL2DZ) mix basis set in vacuo. Firstly, optimized structures of complex (2), (3) and (5) are calculated in each level and geometric parameter of them are compared with experimental results. The best calculation level is determined via these analyses. Then, the rest complexes are optimized by using the best level and geometric parameters are reported. Related compounds are interacted with 1BNA protein and they are compared with each other within themselves.

"This work is supported by the Scientific Research Project Fund of Cumhuriyet University under the project number F-489."



Scheme 1. Schematic representation of studied metal complexes with atomic labeling.

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P152

SYNTHESIS, CRYSTAL STRUCTURE AND ANTIMYCOBACTERIAL ACTIVITIES OF 4-INDOLYL-1,4-DIHYDROPYRIDINE DERIVATIVES CONTAINING DIFFERENT ESTER GROUPS

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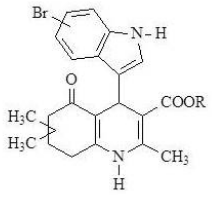
Tuberculosis (TB) is one of the most important infectious diseases caused by Mycobacterium tuberculosis. Human TB has existed for thousands of years and still remains a major global health problem. The recent emergence of multidrug resistance to the first-line drugs have made the treatment of TB more complicated and indicates clearly the urgent need for the development of new drugs with divergent structure and preferably novel mechanism of action for the successful clinical control of patients with TB.

Within the scope of new antitubercular drug design; preparation and antimycobacterial evaluation of some 1,4-dihydropyridines (DHP) with lipophilic groups have been reported to possess considerable antitubercular activity. The indole moiety is the most widely spread nitrogen heterocyclic structure found in numerous natural and synthetic compounds with a wide variety of biological activities including antitubercular effect.

In the present study, we aimed to synthesize twenty condensed 1,4-dihydropyridine derivatives with indole moiety as antimycobacterial agents. The compounds were obtained by an easy, very rapid and convenient method under microwave irradiation. Among them, seven compounds were found to be promising antitubercular agents with a good safety profile. It is important to note that the introduction of ethyl or isopropyl groups to the ester moiety significantly improved the preferential activity. Docking results presented that the compounds are likely to bind InhA as their possible target enzyme. Our data suggest that a condensed dihydropyridine-based scaffold with the indole ring may serve as a new pharmacophore for antimycobacterial activity.



R: CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $C(CH_3)_3$

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SYNTHESIS AND CYTOTOXIC ACTIVITY OF NOVEL 6,9-DISUBSTITUTED PURINE ANALOGS

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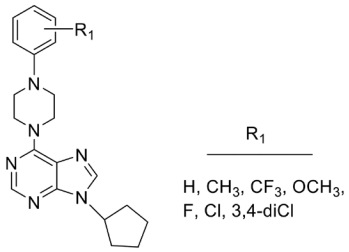
Cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Globally, nearly 1 in 6 deaths is due to cancer. Approximately 70% of deaths from cancer occur in low- and middle-income countries. A well-known pioneer fluorinated nucleobase analogue, 5-fluorouracil, is highly preferred in clinics for the treatment of various cancers. Purine derivatives, 6-mercaptopurine and 6-thioguanine have been used as an inhibitor of nucleic acid metabolism in paediatric acute lymphoblastic leukaemia. In this study we synthesized novel purine analogs containing a 4-(substituted phenyl)piperazine in the substituent at N6- and cyclopentyl group at 9-position as putative cytotoxic agents. The newly obtained compounds were first evaluated for their anti-tumor activities against human liver (Huh7), colon (HCT116) and breast (MCF7) carcinoma cell lines. The IC_{50} values were in micromolar concentrations with 6,9-disubstituted purine derivatives. Time-dependent IC_{50} values for each molecule were also calculated in comparison with known cytotoxic agents Camptothecin (CPT), 5-Fluorouracil (5-FU), Cladribine, Fludarabine and Pentostatine. N6-(3,4-dichlorophenyl) / N6-(4-trifluoromethyl phenyl) / N6-(4-chlorophenyl) derivatives 9, 5, 8 displayed the best cytotoxic activity with IC_{50} values of 0.04-0.16, 0.05-2.5, 0.1-4.1 µM against Huh7, HCT116 and MCF7 cell lines. The N6-(nonsubstitutedphenyl) analog 3 was also very active (IC_{50}= 0.1 µM) against MCF7 cell line. Furthermore, compound 9 had a better cytotoxic activity than the DNA topoisomerase inhibitor camptothecin (CPT) and the known cell growth inhibitors 5-FU, Cladribine, Fludarabine and Pentostatine on Huh7 and HCT116 cells.



P154

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL THIOETHER DERIVATIVES OF 1,2,4-TRIAZOLES AND 1,3,4-OXADIAZOLES AS POTENTIAL ANTICANCER AGENTS

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Cancer is a disease with more than 100 different types and it is the cause of 13% of all global deaths [1]. Plant-derived anticancer compounds such as taxol, vinblastine, vincristine, etoposide, camptothecin have been in clinical use for many years in spite of their serious side effects and high toxicity. Not only the undesirable side effects but also the exponentially increasing resistance to existing chemotherapeutics are the constant needs to develop new and effective anticancer agents [2]. Carvacrol [2-Methyl-5-(1-methylethyl)phenol] is a natural member of monoterpenic phenol derivatives which is present in the volatile oils of Thymus vulgaris, Carum copticum, origanum and oregano. Strong anticancer activity of carvacrol against various human cancer cell lines has been already reported as well as its antiproliferative and pro-apoptotic effects [3]. Therefore, carvacrol became the focus of many studies concerning on cancer. Within the context of our previous work [4], 2-{[3-[[5-methyl-2-(propan-2-yl)phenoxy]methyl)-4-benzyl-4,5-dihydro-1H-1,2,4-triazol-5-yl]sulfanyl}-N-(4-substituted phenyl) acetamide derivatives were synthesized and shown to exhibit anticancer activity against specific tumor cell lines at 5.96-7.90 µM. Our previous research [4] led us to synthesize new carvacrol based 1,2,4-triazoles/ 1,3,4-oxadiazoles and their thioether derivates to investigate their anticancer activity. Thioethers, synthesized from 4-alkyl-5-(aryloxymethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones or 5-(aryloxymethyl)-1,3,4-oxadiazole-2(3H)-thiones, have been characterized using elemental analysis and spectral data. Target compounds were evaluated for their anticancer activities against K562 (ATCC, CCL-243), A549 (ATCC, CCL-185) and PC-3 (ATCC, CRL-1435) cell lines. Cytotoxic properties of these compounds were also investigated on NIH3T3 (ATCC, CRL-1658) cells to determine their selectivity.

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SYNTHESIS OF SOME THIAZOLE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY EVALUATION

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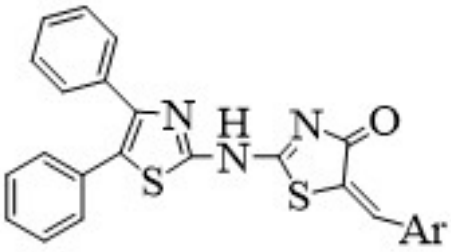
Antimicrobial resistance was first reported in the 1940s. Now antimicrobial resistance has become a world problem because travel and trade, especially, in the 20th and 21st centuries resistant organisms quickly spread to all the corners by humans or via the food chain [1]. The progression of drug-resistant strains is responsible for the incompetence of the straight antimicrobial therapy. Therewithal large-scale scrutiny for fungal infections has shown an increasing incidence of drug-resistant fungal opportunist microorganisms [2, 3]. Thiazoles and their derivatives have got many uses in medicinal chemistry because of their varied biological activities as such antifungal, antibacterial antiinflammatory, antitumor and as new inhibitors of bacterial DNA gyrase B [4,5].

In view of the aforementioned consequences, the aim of this study was to synthesize and investigate antimicrobial activity of some new thiazole derivatives. The structural elucidation of the compounds was performed by ^{1}H-NMR, ^{13}C-NMR and LC-MS/MS spectral data and elemental analyses. The title compounds were obtained by reacting 2-[(4,5-diphenylthiazol-2-yl)amino]thiazolidin-4-one with some heterocyclic aldehyde. Activity studies are still in progress.



Scheme 1. Synthesized compounds

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SYNTHESIS AND BIOLOGICAL EVALUATION OF ORTHO-PROPOXYPHENYL SUBSTITUTED 1H-BENZIMIDAZOLE DERIVATIVES AS BUCHE AGENTS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline and memory loss associated with various neuropsychiatric symptoms and behavioral disturbances. The prevalence of AD increases dramatically with age and doubles for every five year interval after the age of 65 [1]. Pathologically, AD is mainly characterized by extracellular deposition of beta amyloid fibrils and intracellular accumulation of neurofibrillary tangles, which results in the molecular and cellular abnormalities in AD brain [2]. Acetylcholine (ACh) is a significiant neurotransmitter in the brain and ACh deficiency is associated with AD [3]. The cholinesterase (ChE) enzymes catalysis the hydrolysis of neurotransmitter ACh. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are the two types of ChE enzyme [4]. BuChE which modulates cholinergic transmission and regulates brain ACh levels that may substitute for AChE [4-5]. ChE inhibitors provide more ACh available for neurotransmission [4]. Using of selective BuChE inhibitors or dual AChE-BuChE inhibitors can provide for an advantage for the treatment of AD [5]. In this study, a series of 1H-benzimidazole derivatives bearing o-propoxy side chain were synthesized and evaluated for their inhibitor activity against BuChE, also molecular docking studies were carried out.

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P157

MANNICH BASES OF BENZIMIDAZOLE DERIVATIVES AS METAL-CHELATING PROPERTIES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease and major cause of dementia in the elderly [1]. Dementia, including AD, affects over 35 million people worldwide and this number is expected to double by 2030 and more than triple by 2050 to 115 million [2]. AD is a multifactorial disease caused by various factors, such as several abnormal amyloid-β (Aβ) deposition and accumulation, tau hyperphosphorylation, oxidative stress, dyshomeostasis of biometals and deficits of acetylcholine (ACh) [3-5]. Mainly treatment strategies for AD are cholinesterase inhibitors, NMDA receptor antagonists, Aβ agregation inhibitors, antioxidants. The modulation of bio-metals in the brain has been considered as a valuable therapeutic strategy for the treatment of neurodegenerative diseases [6, 7]. Metal ions, notably copper, zinc and iron that play a crucial role in the pathogenesis of neurodegenerative disorders, affect the metabolic processes like protein aggregation, oxidative stress [8]. In our previous study, a series of Mannich bases of benzimidazole derivatives having a phenolic group were synthesized and evaluated for their anticholinesterase and antioxidant activities (Fig 1) [9]. In this study, the chelation abilities for biometals of the synthesized compounds were tested by spectrophotometric method. These studies suggest that the mannich bases of benzimidazole derivatives have metal ions-chelating potency as well as anticholinesterase and antioxidant activity potency. Therefore, these benzimidazole derivatives may represent promising lead compounds as design of multi-functional drugs for AD therapy.

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SYNTHESIS AND EVALUATION OF NEW THIAZOLE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS TARGETING AKT

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Akt pathway is one of the most frequently deregulated signaling pathways in human cancers. As a result, inhibition of Akt has long been an attractive therapeutic approach in oncology and extensive efforts have been devoted to the discovery of new potent anticancer drugs targeting Akt [1]. In an effort to develop potent anticancer agents targeting Akt, herein new thiazole derivatives were synthesized via the reaction of 4-(4-cyanophenoxy)benzaldehyde thiosemicarbazone with phenacyl bromides. MTT assay was performed to assess the cytotoxic effects of the compounds on A549 human lung adenocarcinoma, C6 rat glioma and NIH/3T3 (healthy) mouse embryonic fibroblast cell lines. The most potent compounds were also investigated for their effects on apoptosis and Akt enzyme. 2-[2-((4-(4-Cyanophenoxy)phenyl)methylene)hydrazinyl]-4-(4-cyanophenyl)thiazole (6) was found to be the most promising anticancer agent in this series against A549 and C6 cell lines due to its selective inhibitory effects on A549 and C6 cells with IC_{50} values of 12.0±1.73 µg/mL and 3.83±0.76 µg/mL, respectively. Compound 6 did not show any cytotoxic activity against NIH/3T3 cell line. Furthermore, compound 6 increased early and late apoptotic cell population (32.8%) in C6 cells more than cisplatin (28.8%) and significantly inhibited Akt enzyme (71.66±4.09%) in C6 cells similar to cisplatin (77.25±5.75%). The molecular docking study was performed to predict the possible binding modes of compound 6 inside the active site of Akt (PDB code: 4EJN) [2]. According to docking results, the cyanophenyl ring of compound 6 presented π-π stacking interaction with the conserved Tyr272 residue of the binding site of Akt (Fig. 1).

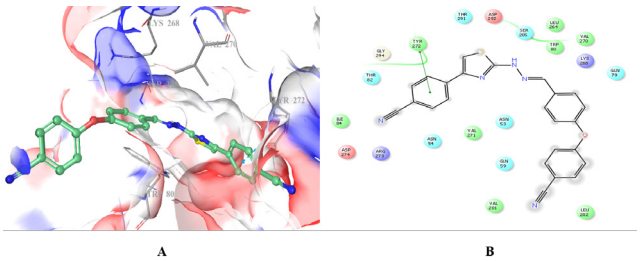

Fig. 1. Docking position (A) and docking interactions (B) of compound 6 at the active site of Akt

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SYNTHESIS OF SOME N-(2-(4-(TERT-BUTYL)PHENYL)BENZO[D]OXAZOL-5-YL)-P-SUB STITUTED BENZENESULFONAMIDES

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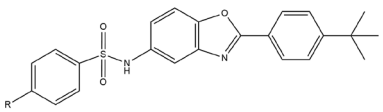
Heterocyclic compounds play an important role in designing a new class of structural entities of medicinal importance with new mechanisms of action. Benzoxazoles, structural isosteres of natural nucleotides that can interact with biopolymers, constitute an important class of heterocyclic compounds [1, 2]. So that benzoxazoles showed potential antitumor, antiviral and antibiotic activities as the new topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors. [3-5]. In this study, firstly, 5-Amino-2-(p-tert-butylphenyl)-benzoxazole (1) was synthesized by heating 2,4-diaminophenol with p-tert-butyl benzoic acid in polyphosphoric acid (PPA). Then compounds 2-8 were obtained by treating a solution of p-substituted-benzenesulfonyl chlorides with 5-amino-2-(p-tert-butylphenyl)-benzoxazole. All the results compounds (2-8) (Figure 1) were prepared as original products (except compound 2) with the hope of discovering new effective antimicrobial agents. The ^{1}H-NMR, ^{13}C-NMR and mass spectra and elemental analysis results agree with those of the proposed structures.



2-8 (R= H, Cl, F, Br, CH_3, OCH_3, NO_2)

Figure 1. Chemical structures of compound 2-8

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P160

DESIGN, SYNTHESIS AND EVALUATION OF NEW THIAZOLYL-PYRAZOLINE DERIVATIVES AS POTENTIAL ANTICANDIDAL AGENTS

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Candida species represent the main cause of opportunistic fungal infections worldwide, leading to high morbidity and mortality. Current antifungal therapy is limited due to the short arsenal of antifungal agents, the emergence of resistance and toxicity problems. Since fungi are eukaryotic pathogens, there is a paucity of targets that can be used for antifungal drug development [1,2]. In an effort to develop potent anticandidal agents, herein new thiazolyl-pyrazoline derivatives were synthesized via the ring closure reaction of 3-(4-nitrophenyl)-5-(4-chlorophenyl)-1-thiocarbamoyl-2-pyrazoline with phenacyl bromides. The synthesized compounds were evaluated for their in vitro inhibitory effects on pathogenic Candida species using a broth microdilution assay. Among these compounds, 2-[5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-methoxyphenyl)thiazole (4) was identified as the most potent antifungal agent against Candida albicans (ATCC® 90028™) with a MIC value of 25 µg/mL when compared with ketoconazole (MIC= 50 µg/mL). Compound 4 also did not show any cytotoxic activity against NIH/3T3 mouse embryonic fibroblast cell line. Molecular docking studies were also performed to explore the possible binding modes of compound 4 on the active site of lanosterol 14α-demethylase enzyme (CYP51) (PDB code: 5JLC) which was previously defined [3]. As shown in Figure 1, compound 4 showed good affinity into the active site of CYP51 through the formation of π-π interactions with His406 and Phe242 residues [3]. This outcome pointed out that compound 4 might inhibit ergosterol biosynthesis. Therefore, further in vitro studies are required to support molecular docking studies.

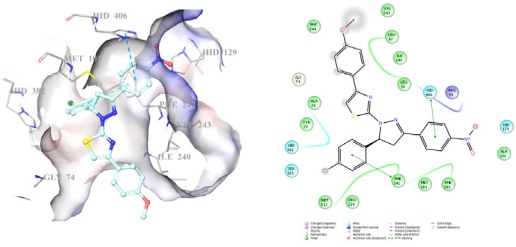

Figure 1. Docking position and interactions of compound 4 on the active site of CYP51 (PDB: 5JLC).

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SYNTHESIS AND INVESTIGATION OF ANTIOXIDANT ACTIVITIES AND CHOLINESTERASE INHIBITION EFFECTS OF SOME 4-ARYL-1,4-DIHYDROPYRIDINE DERIVATIVES

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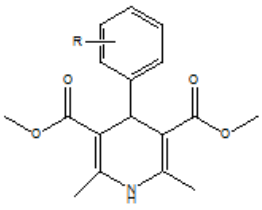
The 4-aryl-1,4-dihydropyridine (1,4-DHP) derivatives are widely used in the treatment of cardiovascular diseases in the clinic and also are becoming interesting due to the antihypertensive effects of calcium channel blockers and α-1a-antagonists [1]. Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is characterized by defects in memory and cognitive functions [2]. Although, cholinesterase inhibitors such as tacrine, donepezil, galantamine and rivastigmine are important in the treatment for AD, they have side effects containing gastrointestinal problems [3]. Therefore, It is important to find a new cholinesterase inhibitor with low toxicity and side effects. In this study, a series 1,4-DHP derivative; dimethyl 4- (phenyl / 4-substitutedphenyl) -2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate compounds were synthesized according to the Hantzsch reaction (Figure 1) [4]. The structure of the synthesized compounds, were elucidated by spectroscopic methods using IR, ^{1}H-NMR analysis. In addition, antioxidant activities of these compounds were investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl), PRAP (phosphomolibdenum-reducing antioxidant power) and metal chelating activities assays. At the last part of study, acetylcholinesterase and butyrylcholinesterase inhibitory effects of compounds were determined by Ellman's spectrophotometrical methods. The results of these experiments showed that Compound b was higher antioxidant activities than among tested compounds. Similarly, Compound b was higher cholinesterase inhibitory effect than tested other compounds with 34.05 ± 2.23% and 24.93 ± 0.68% at 250 µM.



R: a-H, **b**-4-Br, **c**-3-Br, **d**-3-Cl, **e**-4-CH_3, **f**-3-CH_3, **g**-4-OCH_3, **h**-3-OCH_3

Figure 1. 4-Aryl-1,4-dihydropyridine Derivative Compounds

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SYNTHESIS AND ELUCIDATION OF STRUCTURES OF NEW ISATINE SCHIFF BASE COMPOUNDS

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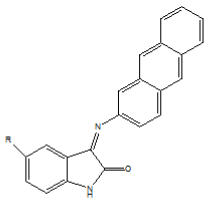
Indole, which has an important role in heterocyclic ring systems, has shown significant biological effects [1]. İsatine (2,3-dihydro-indole-2,3-dione) has been used as a reagent in a large number of synthesis due to its biological properties [2-4]. One of the most used methods is the Schiff base reactions [5]. In this study, we have synthesized two new Schiff base compounds (Figure 1), by the reaction of isatine and 5-nitro-isatine with 2-aminoantracene. The structure of the synthesized compounds, were elucidated by spectroscopic methods using IR, ^{1}H-NMR, ^{13}C-NMR spectra and Mass analysis. The activities of the new Schiff base isatine compounds ; 3-(anthracen-2-ylimino)indolin-/5-nitroindolin-2-one derivative compounds will be examined. New compounds will be synthesized with different isatine derivatives. And also the acidic proton in the structure is suitable for new derivatizations.



R: -H, -NO_2

Figure 1. Structure of two new Schiff base compounds

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P163

SYNTHESIS AND ENZYME ACTIVITY STUDIES OF NOVEL QUINOXALINE-HYDRAZONE DERIVATIVES AS MONOAMINE OXIDASE INHIBITORS

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Monoamine oxidase (MAO), having two isoforms (MAO-A and MAO-B), plays an important role in the oxidative metabolism of neurotransmitters. MAO-B isoform involved in dopamine metabolism. Dopamine is a neurotransmitter that responsible for transmitting signals between the substantia nigra and multiple brain regions. Thanks to these signals, smooth, purposeful movement can be performed. However, in case of dopamine shortage, it results in abnormal nerve-firing patterns and movement capability is disturbed. It is separately reported that quinoxaline and hydrazide derivative have monoamine oxidase inhibitory effects, then we aimed to combine two group in a scaffold and derivatize with different piperidine, morpholine and piperazine moieties Synthesized 13 final compounds were analyzed by spectroscopic methods of IR, NMR and MS. Biological activity were tested by a fluorometric method. After biological evaluation, compound 2d showed a significant inhibitory activity towards MAO-B enzyme. In order to determine the type of inhibition enzyme kinetic studies were performed. Also, docking studies were successfully done to obtain binding modes of this compound to MAO-B enzyme active region.

P164

COMPARATIVE STUDY OF MICROWAVE-ASSISTED AND CONVENTIONAL SYNTHESIS OF SOME ARYL ETHANONE OXIME DERIVATIVES BEARING IMIDAZOLE AND PYRAZOLE MOIETIES AND DETERMINATION OF THE E/Z ISOMER RATIOS

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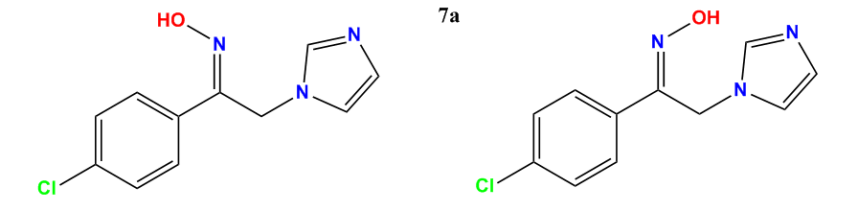
Microwave-assisted organic synthesis is being widely applied in the medicinal chemistry, particularly for developing compounds in the lead optimization phase of drug development. In this phase, chemists use diverse synthetic techniques to develop candidate drugs from lead compounds. From this point of view, in the present study some aryl ethanone oxime derivatives bearing imidazole and pyrazole moieties were designed, synthesized and characterized. The yields of the microwave-assisted and conventionally synthesized compounds were compared and the ratios of the E/Z isomers were determined for both methods. The structures of oxime compounds were confirmed by ^{13}C-NMR, ^{1}H-NMR and mass spectra. Furthermore, E/Z isomers of 7a were confirmed by two-dimensional nuclear magnetic resonance (2D-NMR) spectra.



(*E*)-1-(4-chlorophenyl)-2-(1*H*-imidazol-1-yl)ethan-1-one oxime

(*Z*)-1-(4-chlorophenyl)-2-(1*H*-imidazol-1-yl)ethan-1-one oxime

Compound 7a

P165

SYNTHESIS AND HUMAN MONOAMINE OXIDASE INHIBITORY ACTIVITY OF THIAZOLE-HYDRAZONE SCAFFOLD

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Mitochondrial monoamine oxidases (MAOs) are FAD-dependent enzymes that catalyze the oxidative deamination of neurotransmitters and exogenous arylalkylamines. In mammals, two different types of MAOs are present in most tissues, namely, MAO-A and MAO-B. Inhibition of MAOs, in fact, represents the rationale for the treatment of those pathologies in which there is an impairment of synaptic transmission, such as depressive disorders and neurodegenerative diseases [1]. Numerous compounds among the great variety of substituted hydrazines, hydrazides and hydrazones behave as MAO inhibitors. A common structural feature of substrates and inhibitors is an amino or imino group that is assumed to play an essential role in orientation and complex formation at the active site of the enzyme [2]. In this study, we have reported the synthesis and MAO inhibitory activities of thiazole-hydrazone derivatives. We were particularly interested in examining the effects of different substituents at C4 of the thiazole nucleus on MAO inhibitory activity and selectivity. Chemical structures of the synthesized compounds were identified by spectroscopic methods. Theoretical ADME predictions were calculated for final compounds. The cytotoxic activities of the final compounds were screened against healthy NIH3T3 cell line (mouse embryonic fibroblast cells). According to the biological studies, the compound 3j displayed a good MAO-A inhibition and low cytotoxicity.

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P166

SYNTHESIS AND EVALUATION OF NEW BENZOTHIAZOLE DERIVATIVES AS POTENTIAL ANTI-BREAST CANCER AGENTS

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Benzothiazole has emerged as a privileged scaffold for anticancer drug discovery. Antitumor effects of benzothiazole derivatives on different cancer cells have been extensively studied and these studies have led to the discovery of clinical candidates such as Phortress [1,2]. In the current work, N-(benzothiazol-2-yl)-2-[(5-arylamino-1,3,4-thiadiazol-2-yl)thio]acetamide derivatives were synthesized and evaluated for their anticancer effects on MCF-7 human breast adenocarcinoma and NIH/3T3 (healthy) mouse embryonic fibroblast cell lines. According to MTT assay, compounds 1, 2, 3, 5, 6 and 7 were determined as selective antitumor agents against MCF-7 cell line and showed low cytotoxicity against NIH/3T3 cell line (Fig. 1.). Moreover, the effects of these compounds on DNA synthesis and apoptosis were also investigated. According to the DNA synthesis inhibition assay, compounds 1, 2, 3, 5 and 7 significantly inhibited DNA synthesis in a concentration-dependent manner (Fig. 1.). Flow cytometric analysis also indicated that the ethoxy substituted compound (7) caused apoptosis in MCF-7 cell line (21.6%) more than cisplatin (16.6%) at IC_{50} values (Fig. 1.). Further studies are required to determine the in vivo anticancer activity of these compounds, particularly compound 7.

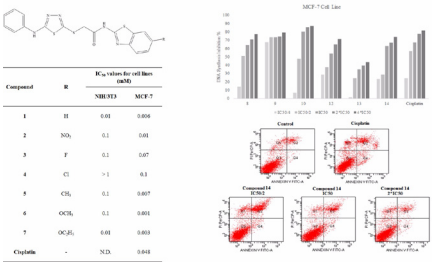

Fig. 1. The cytotoxic effects of the most potent agents

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P167

ANTICANCER EFFECTS OF NEW INDOLE-PYRAZOLE AND INDOLE-ISOXAZOLE HYBRIDS; DESIGN, SYNTHESIS AND EVALUATION OF ACTIVITY

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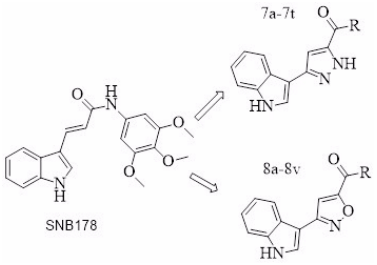
Cancer remains one of the leading causes of death worldwide and requires a pressing need for the development of novel and more effective treatments. By preliminary screening of indol-3-acrylamide type compounds synthesized by our group, SNB178 was identified as a lead compound. In this study, we aimed to design, synthesis and evaluate biological activities of indole-pyrazole and indole-isoxazole hybrid derivatives. These derivatives were investigated firstly by sulforhodamine B method to evaluate their cytotoxic activities. Later, the most active derivatives were evaluated by more advanced anticancer activity tests (inhibition of tubulin polymerization, colchicine binding assay, RT-CES, apoptosis and cell cycle analysis). Most of the derivatives owned anticancer activities equal to or more than 5-FU against cancer cell lines. We found that compound 8a possessed the highest activity against Huh7 cancer cell line (IC_{50} =0.1 µM). According to our results, the inhibition of tubulin polymerization for the chosen derivatives 7i (IC_{50}= 19 µM) and 8a (IC_{50}= 18 µM) were very close to the lead compound SNB178 (IC_{50}= 17 µM). We observed that compound 7i induced morphological changes in the cell nucleus. These morphological changes can confirm that this compound had potent activity and the main mechanism of action may be according to mitotic catastrophe. (This study was supported by TUBITAK research grant 113S973).



P168

HEPATOCELLULAR CARCINOMA TERAPEUTICS :SYNTHESIS AND BIOLOGICAL EVALUATION INDOLE-3-CARBOXAMIDES

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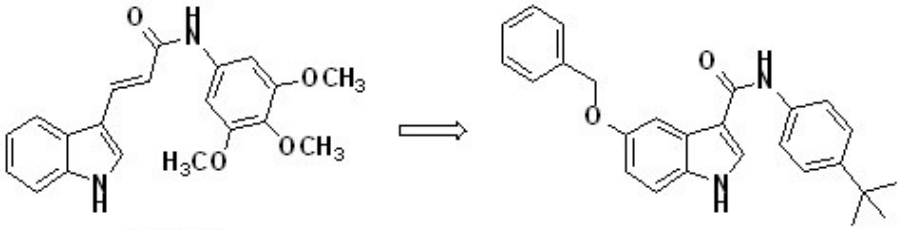
Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. At present, the multikinase inhibitors Sorafenib and Regorafenib are drugs approved by the FDA for the treatment of HCC. Since liver cancer cells show resistance to conventional chemotherapy and radiotherapy, it is very important to develop new therapeutic agents against HCC. After preliminary screening of indole-3-acrylamide type compounds synthesized by our group, SNB178 was identified as a lead compound. The linker between the indole and the phenyl ring at the terminal in SNB178 is 4 atoms in length. We have synthesized indol-3-yl-carboxamide, indol-3-yl-acetamide and indol-3-yl-propionamide derivatives by removing the unsaturation in the linker and ensuring 2, 3 and 4 atom lengths in the linker. These derivatives were investigated firstly by sulforhodamine B method to evaluate their cytotoxic activities. Synthesized indole-3-acetamide and indole-3-propionamide derivatives have low activity on cancer cells. Indole-3-carboxamide derivatives with high activity were selected for study in the liver cancer panel. Two of them were found to be highly effective in epithelial HepG2 and mesenchymal Mahlavu and SNU475 cell lines. The compounds caused apoptotic cell death, particularly on the Mahlavu and SNU475 cells. Cell cycle analysis showed that these compounds caused cell cycle arrest at sub-G1 or G1 phase.

This study was supported by TUBITAK research grant 113S973.



SNB178

P169

IN VITRO CYTOTOXIC ACTIVITIES OF PLATINUM(II) COMPLEX WITH 1-METHYL-2-(3′- HYDROXYPROPYL)BENZIMIDAZOLE AND 2-(3′-HYDROXYPROPYL)BENZIMIDAZOLIUM HEXA- AND TETRACHLOROPLATINATE SALTS

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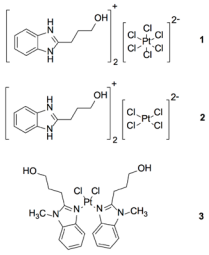
Two 2-(3′-Hydroxypropyl)benzimidazolium hexa- and tetrachloroplatinate salts $(hpb)_2[PtCl_6]$ (1) and $(hpb)_2[PtCl_4]$ (2) {hpb = 2-(3′-hydroxypropyl)benzimidazole} and one platinum(II) complex with the structure $(mhpb)_2[PtCl_2]$ (3) {mhpb = 1-methyl-2-(3′-hydroxypropyl)benzimidazole} were synthesized and evaluated for their in vitro cytotoxicities against human HeLa (ER-), MCF-7 (ER+), and MDA-MB-231 (ER-) cell lines. Compound 3 showed appreciable activity for all examined cell lines compared to that of reference compound cisplatin.



P170

CYTOTOXIC ACTIVITY STUDIES OF SOME PLATINUM (II) COMPLEXES WITH BENZIMIDAZOLE LIGANDS

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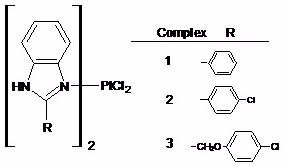
Platinum-based drugs cisplatin, carboplatin and oxaliplatin are widely used in the therapy of cancer. The intrinsic along with acquired resistance and side effects observed in some patients represent major limitations of the treatment of human tumors with the platinum drugs currently used in the clinic [1]. It is generally believed that biological activity of cisplatin is associated with the recognition of its DNA adducts by cellular poteins such as repair enzymes, transcription factors, histones, and high mobility group (HMG) domain proteins. Design of new platinum antitumor drugs has been introduced on the basis of preventing resistance by enhancing this mechanism using more hydrophobic platinium compounds [2]. In the present study, three Pt(II) complexes with 2-substitutedbenzimidazole were synthesized and evaluated for their in vitro cytotoxic activities on HeLa, MCF-7 cell lines. MTT test results showed that complex 2 which was found to be the more active than carboplatin was the most cytotoxic active compound against the HeLa cell line among the tested compounds.



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SYNTHESIS OF PLATINUM(II) COMPLEXES CONTAINING OPTICALLY ACTIVE BENZIMIDAZOLE LIGANDS

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Cisplatin is a clinically important antitumor drug used in the treatment of testicular, ovarian, bladder, and head and neck cancers [1]. Nowadays, cisplatin, carboplatin and oxaliplatin are used treatment of various cancer types worldwide [2]. Most notably, long-term survival for rates of testicular cancer patients using cisplatin improved from less than 10% to greater than 90% [3]. It is aimed to synthesize new platinum complexes containing optically active benzimidazole ring as “carrier-ligands” and chloro groups as “leaving-ligands”. The synthesis of the Pt(II) complexes was performed by using K_2PtCl_4 and the ligands. Chemical structure of the complexes was elucidated by their elemental analyses, IR, NMR, and Mass spectroscopy methods. Investigation on the in vitro cytotoxic activities of the complexes have been performing.

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P172

SYNTHESIS OF NEW BENZOTHIAZOLONE DERIVATIVES AND EVALUATION OF THEIR EFFECTS ON CHOLINESTERASE ENZYMES

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Alzheimer's disease (AD), neurodegenerative disorder of the brain, is the most common cause of dementia among the aged people. According to World Health Organization (WHO), 30 million people worldwide suffer from AD and the number of patients is expected to reach 100 million by 2050 [1]. Since the cause of disease are not fully elucidated, available treatments are insufficient. Pathophysiological changes of AD cover lack in cholinergic neurotransmission, faulty amyloid-β (Aβ) protein metabolism, formation of intracellular neurofibrillary tangles and the participation of inflammatory, oxidative and hormonal pathways. Based on these markers, several hypotheses have been proposed to explain the mechanism of AD, which are cholinergic, amyloid and tau hypotheses [2]. According to the cholinergic hypothesis, since the symptoms of AD was linked to a deficiency in the brain neurotransmitter acetylcholine (ACh) that mediates memory and learning functions, current treatment of AD focuses on increasing acetylcholine levels by inhibiting cholinesterase, the enzymes responsible for ACh hydrolysis. Because AChE is the main enzyme involved in the breakdown of ACh in the normal brain, cholinergic therapy for AD initially focused on AChE inhibition. Recently research shows that the enzyme has also non-cholinergic function in addition to catalytic activity of AChE [3]. On the other hand, as the disease progresses, AChE levels decrease while the levels of BuChE increase [4]. Therefore, concurrent inhibition of both enzymes should provide additional benefits in the treatment of AD.

In the present study, we designed and synthesized eight new benzothiazolone derivatives in order to investigate their acetylcholinesterase/butyrylcholinesterase inhibitory activities.

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DETERMINATION OF ACHE/BCHE INHIBITORY ACTIVITY OF SOME NEW SYNTHESIZED 3(2H)-PYRIDAZINONE DERIVATIVES WITH PROTEIN-LIGAND DOCKING APPROACH AND QSAR METHODS

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Two important enzymes from the group of serine hydrolases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are usually defined as cholinesterases (ChEs). AChE catalyse the hydrolysis of acetylcholine (ACh) in cholinergic synapses. The inhibition of these enzymes causes an increase in the concentration of ACh in cholinergic synapses and can subsequently affect a number of pathogenic processes. ChE inhibitors (ChEIs) are used in the treatment of various neuromuscular disorders and have provided the first generation of drugs for the treatment of Alzheimer's disease [1,2]. According to the studies, 3(2H)-pyridazinone derivatives are competitive, reversible and potent inhibitors of acetylcholinesterase (AChE) [3-5]. In present study, we investigated the role of 3(2H)-Pyridazinone derivatives for the AChE and BChE inhibitory activities by combined protein–ligand docking approach and QSAR methods. DRAGON software generated descriptors and quantum-chemical descriptors have been calculated and total of 1236 descriptors were utilized. The most predictive model is shown to be a 2-variable model. The GA-MLRA based model showed perfect results ($R^2_{training}$=0.850, Q^2=0.741, R^2_{test}=0.911) for AChE ($R^2_{training}$=0.916, Q^2=0.753, R^2_{test}=0.675) for BChE, with high internal and external correlation coefficients.

This study was supported financially with a grant from Research Foundation of İnönü University (2013/94).

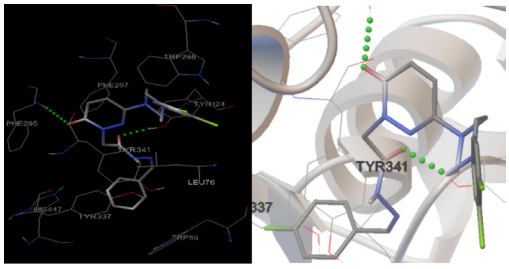

Figure 1. The H-bond interactions of the ligands 5a and 5e in the active site of the enzyme

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SYNTHESIS OF SOME NEW BENZALHYDRAZONE DERIVATIVES AND EVULATION OF THEIR ACHE INHIBITORY ACTIVITY IN VITRO

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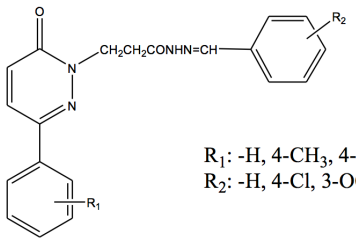
The hydrolysis of acetylcholine (ACh) which is a significant neurotransmitter for regulation of cognition in humans is the main task of AChE in cholinergic synapses. ACh level rises in the cholinergic synapses when these enzymes are inhibited [1-3]. Thus, cholinesterase inhibitors are used in the treatment of various neuromuscular disorders which occur as a result of reduced cortical and hippocampal levels of ACh, such as Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by synapse dysfunction, neuronal death, loss of memory and learning ability [4-6].Many researchers have synthesized hydrazone compounds as target structures and evaluated their biological activities. Hydrazones have been reported to possess, antibacterial, antifungal, antitubercular, antiviral and antimalarial activities [7-9]. In this context, we synthesized new 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(substituted/nonsubstituted benzalhydrazone) in order to investigate their in vitro AChE/BChE inhibitory activities by using the Ellman's method. Compounds have been synthesized starting from substitue/nonsubstitue acetophenon. 6-(4-substitue/nonsubstituephenyl)-3(2H)-pyridazinone was obtained from reaction of acetophenon with succinic anhydrate. Ester derivatives were obtained from the reaction of the resulting pyridazinone compound with propyl bromoacetate. After that acetohydrazide derivatives were contituted by reaxion of these ester derivative compounds with hydrazinehydrat and the acetohydrazides were changed to title compounds which have benzalhydrazone structure by using substitue/nonsubstitue benzaldehydes.



R_1: -H, 4-CH_3, 4-Cl
R_2: -H, 4-Cl, 3-OCH_3, 4-$N(CH_3)_2$

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SYNTHESIS AND ANTITUBERCULAR ACTIVITIES OF 1-(4-(1H-1,2,4-TRIAZOL-1-YL)BENZYLIDENE)-2-(4-SUBSTITUED PHENYL)HYDRAZINES

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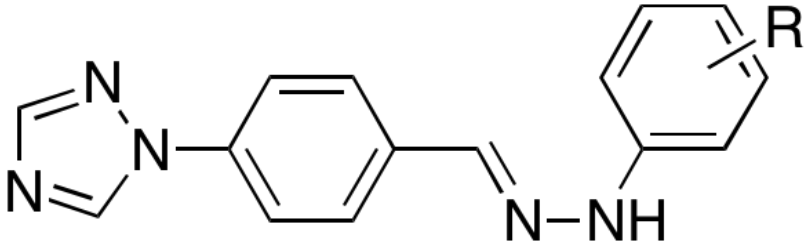
Tuberculosis is a chronic and infectious disease caused by Mycobacterium tuberculosis. An increase of multidrug-resistant tuberculosis qualifies this infectious disease as a serious global health problem [1]. Hydrazones are considered as interesting compounds in the treatment of tuberculosis [2, 3]. On the other hands, many studies showed that azole heterocycles such as triazole are useful pharmacophores for antimycobacterial activity [4-6]. In this study, a series of 1-(4-(1H-1,2,4-triazol-1-yl)benzylidene)-2-(4-substituedphenyl)hydrazines was synthesized. Structures of the synthesized compounds were elucidated by using spectral methods (IR, 1H-NMR and ESI-MS). The target compounds were tested for their antimycobacterial activity in vitro against Mycobacterium tuberculosis H37Rv using the agar dilution method.



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P176

N-ACETYL DERIVATIVES OF 2-PYRAZOLINES AS POTENT MULTI-TARGETING AGENTS FOR ALZHEIMER'S DISEASE

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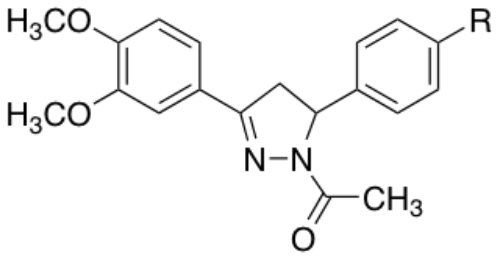
Alzheimer's disease (AD) depicted by memory loss and cognitive impairment is a neurodegenerative brain condition that currently affects more than 30 million people worldwide [1]. Although morphology of AD have not been elucidated, acetylcholine (ACh) deficiency, beta peptide (Ab) aggregation, tau protein hyperphosphorylation and oxidative stress are several pathogenesis associated with the disease [2-4]. Hence the complex nature of AD, the studies to reach 'multi-target-directed ligand' (MTDL) have been carried in recent years [5]. Pyrazoline derivatives have interesting biological activites including cholinesterase inhibition and Ab anti-aggregation [6-8]. In this study, we designed, synthesized and evaluated a new series of N-acetyl-2-pyrazoline derivatives as MTDL against AD. Structures of the synthesized compounds were elucidated by using spectral methods (IR, ^{1}H-NMR and ESI-MS). The pharmacological studies included in vitro tests for cholinesterase inhibition and Ab anti-aggregation activity.



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SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF SOME 5-CHLORO-2-BENZOXAZOLONE DERIVATES

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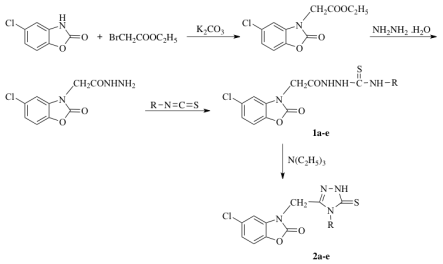
The multi-drug resistance against antimicrobial agents has been causing problems in the cases of infectious disease treatments. The evolution rate of bacterial resistance to antibiotics is evidently higher than the development rate for new classes of antibiotics. Compounds which have 1,4-disubstituted-3-thiosemicarbazide [1, 2] and 1,2,4-triazole structures have been synthesized and promising antimicrobial results have been gained [3, 4]. In this study, 1-[2-(5-chloro-2-oxobenzoxazol-3-yl)acetyl]-4-substituted thiosemicarbazide derivatives (1a-e) were synthesized by the reaction of 2-(5-chloro-2-oxobenzo[d]oxazol-3(2H)-yl)acetohydrazide with different aliphatic and aromatic isothiocyanates. Cyclization of these compounds with TEA resulted in 5-chloro-3-[(4-substituted-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]benzo[d]oxazol-2(3H)-one (2a-e). The structures of the compounds were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis. All newly synthesized compounds were screened for their antibacterial activities against laboratory strains of Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and antifungal activities against yeast strains which are Candida albicans ATCC 90028, C. krusei ATCC 6258 and C. parapsilosis ATCC 90018 by using microdilution method.



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SYNTHESIS AND CARBONIC ANHYDRASE INHIBITORY ACTIVITY OF SOME NOVEL SULFONAMIDES

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Carbonic anhydrase (CA) enzymes (EC 4.2.1.1) are current in many living systems and play a significant role in a variety of pathological and physiological effects containing fluid balance, neurological disorders, osteoporosis, bone resorption, pH regulation, carboxylation reactions, glaucoma, calcification, cancer, tumorigenicity and the synthesis of bicarbonate (HCO_3^-) [1]. These enzymes diverge in their catalytic activity, subcellular localization and susceptibility to different classes of inhibitors. Some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane bound (CA IV, CA IX, CA XII and CA XIV), two are mitochondrial (CA VA and CA VB), and one is buried in saliva (CA VI) [2]. Moreover, sulfonamides are the most studied class of compounds in order to develop novel CA inhibitors. These type compounds bind catalytic zinc ion through the deprotonated nitrogen of the sulfonamide moiety, blocking the CA enzymatic activity [3-5]. In present work new sulfonamide derivatives (3a-3n) were synthesized. The chemical structures of the compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectral data. The CA inhibitory activity was confirmed by using in vitro methods. The results indicated that compound 3c showed good inhibition against hCAI enzyme with an IC_{50} value of 0.176 µM.

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SYNTHESIS OF SOME NOVEL BIOLOGICALLY EFFECTIVE BENZIMIDAZOLE DERIVATIVES

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Benzimidazole compounds that have attracted attention due to their diverse biological and pharmacological properties, are important fragments in medicinal chemistry because of their wide range of biological activities such as antibacterial [1], antifungal [1], anthelmintic [2], antiviral [3], anti-inflammatory [4], analgesic [5] and anticancer agents [6] . Regarding these findings a new series of benzimidazole derivatives was designed and synthesized and the synthetic pathways for preparation of the target compounds are shown in Scheme with the hope of discovering new effective antimicrobial agents. The structures of them were supported by spectral data. The ^{1}H-NMR, ^{13}C-NMR and mass spectra and elemental analysis results agree with those of the proposed structures.

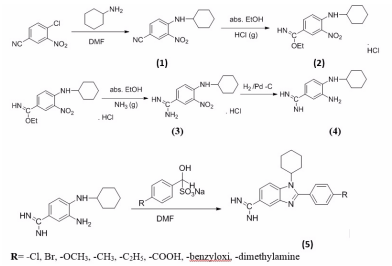

Figure 1 . Synthesis of compounds

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SYNTHESIS OF SOME THIADIAZOLE/TRIAZOLE LINKED 1H-BENZIMIDAZOLE DERIVATIVES AS INHIBITORS OF EGFR TYROSINE KINASE

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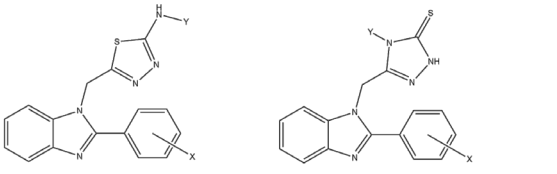
Benzimidazole derivatives have been found to possess a wide variety of biological activities including antiviral, anti-inflammatory, antihypertensive, antioxidant, antiulcer, antimicrobial and anticancer. There are several marketed benzimidazole based drug such as astemizole, omeprazole and albendazole. Particulary benzimidazole derivatives have been found as anticancer inhibitors including tyrosine kinases. For the purpose of developing novel EGFR tyrosine kinases inhibitors, we designed and synthesized some thiadiazole/triazole linked 1H-benzimidazole derivatives and evaluated of their EGFR kinase inhibitor activities comparing with erlotinib. For this purpose, firstly benzimidazole derivatives bearing phenyl/substituted phenyl ring at the 2nd position and acylhydrazide at the 1st position were obtained and reactions of these compounds with appropiate isothiocyanates thiosemicarbazide derivatives were synthesized. Finally, thiadiazoles in concentrated sulfuric acid and triazoles in sodium hydroxide were synthesized from the thiosemicarbazides, respectively. Following the structure elucidation, EGFR kinase inhibitor activities of synthesized compounds were measured by using ADP-Glo™ Kinase Assay. The compounds showed significant effects in the above test.



X: H, 4-Cl, 4-OCH_3 X: H, 4-Cl, 4-OCH_3, 3,4-dibenzyloxy

Y: methyl, ethyl Y: methyl, ethyl, 2-Piperidinoethyl, 2-(4-Morpholino)ethyl

General formula of the synthesized compounds

References

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SYNTHESIS AND ANTITUBERCULOSIS ACTIVITY OF INDOL-3-YL-ALKYL-2-(4-CHLOROPHENYL)-5-ALKYL-4,6-DIOXO OCTAHYDROPYRROLO[3,4-B]PYRROLE-3-CARBOXYLATE

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Pyrrolidine ring, also known as tetrahydropyrrole, is found in the structures of a large number of synthetic and bioactive compounds [1-4] Some pyrrolidine derivatives show important bioactive properties such as anti(myco)bacterial, antifungal and anticancer activity, and also some of them have acetylcholinesterase inhibitor activity [5]. yrrolidine derivatives have been extensively studied due to possessing such these properties [6]. Contuniation of our work related to such these ring possesing compounds [7]. the titled compounds were synthesized by ring rearrangement of prepared such hetereocycles in the presence of a base, the titled pyrrolidine derivatives were obtained in good yields. The structures of these compounds were elucidated by analytical techniques such as single crystal X-ray diffraction (single crystal-XRD) spectroscopy, nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR) and mass spectrometry (MS). Antimycobacterial activity of these novel compounds were tested by performing Microplate Alamar Blue assay [8] against M. tuberculosis H37Rv strain. The tested compounds showed moderate antimycobacterial activity when tested against M. tuberculosis H37Rv strains.

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SYNTHESIS, CHARACTERISATION OF AMINOCARBOTHIOL PYRROLIDINE- PT(II) COMPLEXES; THEIR ANTIBACTERIAL AND ANTITUBERCULOSIS ACTIVITY

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Platinum complexes are known to have important pharmacological and biological properties and attract much attention in synthesis for medicinal chemistry. Many of them displayed remarkable antitumoural activity [1, 2] and some Pt complexes found to be potentially bioactive such as antibacterial [3, 4], antifungal [5], antimalarial [6] and antituberculosis [7] activities. In this study, some novel Pt(II) complexes of cyclic and bicyclic aminocarbothiol pyrrolidine derivatives were synthesized and their characterization/structural determination were made by spectral analytical techniques. The biological activities of prepared compounds against M. tuberculosis H37Rv strain and Staphylococcus aureus (ATCC 25925), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 25923), Acinetobacter baumannii (ATCC 02026), and Aeromonas hydrophila (ATCC 95080) standard strains were tested.

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SYNTHESIS OF SOME NEW ACETAMIDE BEARING THIAZOLE RING DERIVATIVES AND THEIR ANTICANCER ACTIVITY EVALUATION

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All the cancer types are the most important diseases in the world and are estimated to become one of the the major cause of the death in recent years. Also resistance to chemotherapy and molecularly marked therapies is a major problem facing cancer research. Therefore the researchers focus on the specific mechanism of the cancer progression and they try to inhibit these mechanisms [1, 2]. Thiazole derivatives are one of the compounds used for this aim and affected by many pathways as lipid kinase inhibitors and protein kinase inhibitors, for example: inhibitors of phosphatidylinositol-3-kinase and tubulin polymerization inhibitors [3, 4, 5]. Due to the reasons mentioned above, we synthesized and investigated the anticancer activity of some new acetamide bearing thiazole ring derivatives. The title compounds were obtained by reacting 2-chloro-N-(4-(pyridin-4-yl)thiazol-2-yl)acetamide with some 4-substituted piperazine derivatives. The synthesised compounds were investigated for their anticancer activities against C6 rat glioma cell line and A549 human lung carcinoma cell line. Cisplatin is the reference drug. Compounds 3e, 3f and 3g displayed activity against A549 lung carcinoma cell. Especially compounds 3f (IC_{50}=32.67±6.43 µg/ml) and 3g (IC_{50}=31.33±5.77 µg/ml) has a half potency of cisplatin's IC_{50} (16.33± 1.53 µg/ml). On the other hand, compounds 3d, 3e, 3f and 3g showed strong activity against C6 glioma cells. In particular, compounds 3e (IC_{50}=9±1 µg/ml) indicated 3 times more active against cisplatin (IC_{50}=21±1 µg/ml). Also 3f (IC_{50}=18±5.29 µg/ml) and 3g (IC_{50}=16±2.83 µg/ml) exhibited equal strength against cisplatin (IC_{50}=21±1 µg/ml). Activity studies are still progressing.

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MOLECULAR MODELLING STUDIES ON GLYCOGEN SYNTHASE KINASE 3β INHIBITORS FOR ALZHEIMER'S DISEASES

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Glycogen Synthase Kinase 3β(GSK-3β) is a serine/threonine kinase which has essential roles diverse biological processes [1]. The activity of this receptor has been linked with several human diseases as diabetes, cancer and Alzheimer's Diseases (AD).
AD is a common disease in over the age of 60. Because of the decline in cognitive ability and severe behavioral abnormalities AD has a huge economic impact in health expenses. AD studies become more crucial owing to this economic burden. AD shows neuropathological markers as tau hyperphosphorylation and accumulation of amyloid β (Aβ) proteins. Aβ proteins are generated from sequential cleavages of amyloid precursor protein(APP) [2]. Recent studies show that inhibition of GSK-3β causes to decrease in the cleavage of APP. Also this stirs up to a decrease in β-site APP cleaving enzyme 1(BACE1) in the next phase. Thus the accumulation of Aβ was prevented by this process [2]. Due to the therapeutic benefit of the inhibition of GSK-3β it has been a favored target for scientists. In this study, our aim is to generate some hypothesis for the development of new lead GSK-3β inhibitors for Alzhemier's Diseases. For this purpose we investigated the tertiary structure of the GSK-3β by using homology modelling and the crystal structure. Additionally, pharmacophore analysis was obtained using HipHop module of Discovery Studio 3.5 Client [3]. Molecular docking studies were applied by using AutoDock vina v1.5.6 [4].

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PROTOBERBERINE ALKOLOIDS AS A DRUG CANDIDATE FOR ALZHEIMER'S DISEASES

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Alkaloids are secondary metabolites which are produced by a large variety of organisms as plants with diverse structures. The isoquinolines are one of the largest groups of alkaloids. Protoberberines form from isoquinoline skeleton and they are abounded in Berberidaceae family of plants. Alzheimer disease (AD) accounts for nearly 60–70% of all dementia cases and is a major socioeconomic health problem, affecting more than 44 million individuals worldwide [1]. Because of the prevalence and economic burden of the disease, drug development studies have picked up speed and scientists especially focused on natural products [2]. AD is basically characterized with tau hyperphosphorylation and accumulation of amyloid β (Aβ) proteins. Aβ proteins are generated from sequential cleavages of amyloid precursor protein(APP) by β and γ secretases, and β-site APP cleaving enzyme 1 (BACE1) is a β secretase essential for Aβ production. Although protoberberine alkaloids such as berberine, palmatine, jatrorrhizine, columbamine, magnoflorine were found to prevent a progressive neurodegenerative disorder as experimentally, the mechanisms of them are not absolutely clear. In this study, we have aimed to elucidate the binding and affect mechanism of these alkaloids on the BACE1 open and closed forms. For this purpose, molecular docking studies were applied for these natural products to the both forms of BACE1 by using AutoDock vina v1.5.6 [3] and it was subjected to explicit solvent simulations by AMBER [4] molecular dynamic package.

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5-PYRIMIDINYLFERROCENE: SYNTHESIS, CHARACTERIZATION, NOVEL PLATINUM(II) COMPLEXES AND DNA BINDING STUDIES

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Ferrocene and its derivatives are of importance in medicinal chemistry for their potential utility as bioorganometallic agents. The interest in ferrocene is due to its excellent stability in biological media, its lipophilicity and redox activity [1,2]. 5-Pyrimidinylferrocene was synthesized using Suzuki coupling reaction method [3]. After then 5-pyrimidinylferrrocene has been reacted with K_2PtCl_4 under ambient conditions to afford a series of complexes of ligands coordinated to platinum [4]. Their DNA binding ability has been studied by spectroscopic techniques and gel electrophoresis. As a potential anticancer compound, platinum ferrocenyl coordination molecule, new Pt(II) complexes were synthesized and characterized. The spectroscopic studies supported the promising anticancer activity of the molecules.

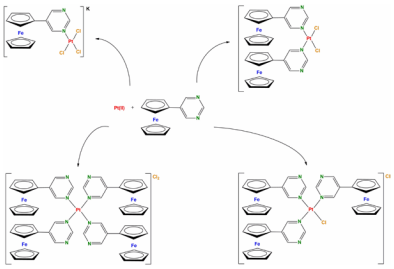

Figure 1. Synthesis diagram of new Pt(II) complexes containing 5-pyrimidinylferrocene

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SOME BISBENZAZOLE DERIVATIVES: DESIGN, SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY

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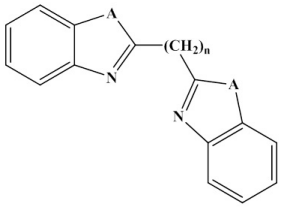
In recent years, the increasing uses of antimicrobial agents accelerate the development of the resistance. This is accompanied by a growing failure in the treatment of bacterial and fungal infections [1]. These developments require the new drug candidates with different mechanisms of action. Nowadays, benzazoles have been showing the promising activity in the treatment of several diseases. The structures of compounds are isosteres of DNA bases and are placed in the natural structure of vitamin B12 [2]. Although there are a number of studies on antimicrobial activities of benzazole core structures, comparative studies on bis structure with these constructs are rarely found in the literature. Structure-activity relationship studies on the compounds recorded in the literature have reached the conclusion that the compounds planned to be synthesized in this work may have antimicrobial activity. In this study, we aimed to find synthesis methods for bisbenzazole derivatives. The compounds in the title were prepared from 1,2-phenylenediamine, 2-aminophenol and 2-aminothiophenol and suitable carboxylic acid derivatives by comparative studies with conventional and microwave irradiation methods [3]. The structures of the synthesized compounds elucidated by IR, NMR, and mass spectroscopy methods. Then, we demonstrated in vitro antimicrobial activity of these synthesized compounds against various microorganisms. Most of the compounds showed moderate activity with MIC values in the range of 62.5-250 g/mL. Further analysis is therefore needed to determine the mode of action of active compounds.

Financial support was provided by grant from the Research Foundation of Mersin University (2017-2-TP2-2508)



n; 1, 2, 3
A; -NH-, -O-, -S-

The structure of compounds

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SYNTHESIS, STRUCTURAL EXPLANATION AND INVESTIGATION OF ANTIPROLIFERATIVE ACTIVITIES ON SOME BIS DERIVATIVE COMPOUNDS

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Despite the major advances in cancer chemotherapy during the recent years, its mortality rates are one of the highest in the world. As cancer chemotherapy has not yet reached the desired level, intensive studies are continue to develop more potent, more selective and less toxic novel anticancer drugs [1]. Bisbenzazoles such as bisbenzoxazole, bisbenzothiazole and bisbenzimidazole have shown special biological activities viz. antitumor, antiviral, antifungal, anti-inflammatory and treatment of physiological disorders. Structural modifications of the bisbenzazole structures are being carried out in several ways in order to anticipate enhanced biological activities. These structural modifications have given a large number of available benzazole compounds for pharmacological research [2-4]. In this study, we have synthesized a series of bisbenzazole derivatives and characterized their structure by spectral data. Then, antiproliferative effects of bisbenzazole compounds against human embryonic kidney cell line (HEK 293) and lung cancer cell line (A549), human kidney carcinoma cell line (A498), human cervical cancer cell line (HeLa), and human liver hepatocellular carcinoma cell line (HepG2) were investigated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) method.
Most of the compounds showed moderate to high activity with IC_{50} values in the range of 0.278-36.187 µM. Of all the synthesized compounds, RHE-131, RHE-139 and RHE-142 exhibited the most potent anti-proliferative activities against cancer cells, and RHE-142 was identified as the most promising compound. Further analysis is therefore needed to determine the mode of action of active compounds.

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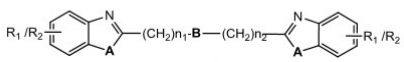

A ; B= -NH-, -O-, -S- n_1; n_2= 0, 1, 2, 3
R1 ; R2 = -H, -Cl

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DESIGN AND SYNTHESIS OF SOME AMID BRIDGE CARRIED BIS-BENZAZOLE COMPOUNDS AND INVESTIGATION ON THEIR ANTIFUNGAL AND ANTITUBERCULOS ACTIVITIES

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Increasing amount of misusage antifungal and antitubercular drugs causes to accelerate the development of multi drug resistance in recent years and available drugs in this area are going to became less effective. Therefore, development of drug molecules with new and different mechanisms of action is an urgent necessity [1,2]. Nowadays, since their important pharmacological effects in various therapeutic area, compounds with bis-structure attract attention [3]. By performed numerous studies, compounds contain bis structure have antimicrobial and antituberculos (anti-TB) activities were determined [4]. In the present study, the drugs used for the antifungal and antituberculosis activity are screened and then structures of them selected. Based on this knowledge, the temple structure of bis-benazaole compounds was designed by action of heterocyclic compounds. In this study, initially desired bis-benzazole structures were designed and synthesized and the structures of the synthesized compounds were elucidated by IR, NMR and mass spectroscopy methods. Then, in vitro antifungal and anti-TB activity of some amid linker bis-compounds determined by microdilution method and the agar proportion method, respectively. According to the present study, since the antifungal and antitubercular potency of the group of the compounds, the structures could be seen as promising lead compounds for further development of novel benzazoles structures.

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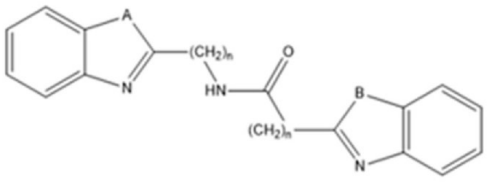

n; 0, 1, 2 A, B; -NH-, -O-, -S-

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SYNTHESIS AND SPECTORCOPIC STUDIES OF QUERCETIN-PHENYL BORONIC ACID AND ANTIOXIDANT ACTIVITIES

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Boron ore is characterized as an element which this is semiconducting between metal and nonmetallic and has above of 230 mineral [1]. Boron is a Lewis acid. Mostly it tends to form multicenter bonds by the way it has a great related to oxygen [2]. In our body after of certain age the collagen layer of skin is getting fall into ruin of the end of increasing free radicals and aging process starts. Antioxidant in plants effect on holding up this process. Oxidative stress, which causes free radical products to form on the skin, is one of the main causes of aging. To protect the skin from oxidative stress, cosmetics and skin care products containing antioxidants have been developed. Flavonoids found in the bodies of plants, leaves, shells and flowers are among the largest groups of polyphenols. It has antioxidant, antimicrobial, antiviral and antibacterial properties. Flavonoids have been found to have many positive effects on human health [3]. In this study, the substance synthesis with boron derivate was carried out quercetin which is type of flavonoid being synthesized phenyl boronic acid hydroxyl groups. Antioxidant properties of this synthesized compound was determined by DPPH free radical scavenging and ABTS radical cation decolonization methods.

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SYNTHESIS, CHARACTERIZATION AND ANTICANCER ACTIVITY STUDIES OF NEW HYDRAZIDES CARRYING AMIDE MOIETY

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Hydrazides have widely been used at pharmaceutical chemistry for several years. Not only being precursors for the synthesis of several organic compounds, but also they are known to possess various biological activities on their own [1]. Although firstly being associated with their anti-tubercular activity, they are also known for their anticancer, antileishmanial and antioxidant activities [2-5]. In the light of these considerations, a new series of hydrazides carrying amide moiety were synthesized and characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic methods. The newly synthesized compounds exhibited significant selectivity to different cancer cell lines (PC3 and MCF7) with IC_{50} values at micromolar concentrations via MTT assay.

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DESIGN AND SYNTHESIS OF SOME NOVEL FLUOROQUINOLONE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AND ANTITUBERCULOSIS AGENTS

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The Fluoroquinolones (FQs) are currently the most commonly used antibacterials to treat bacterial infections due to their broad-spectrum antimicrobial activity, good penetration and high safety profiles [1]. Tuberculosis (TB) and its causative agent Mycobacterium tuberculosis present a serious threat to global health. There are over 1 million deaths each year and approximately 9 million new cases [2]. The ideal strategy to such challenges is to discover novel agents that inhibit new targets in pathogens, but a more practical approach is to modify the structures of existing antibacterial agents to increase potency and to overcome resistance [3]. Development of novel antibacterial agents with little or no bacterial resistance is therefore an important topic of current research [4]. Accordingly, the purpose of our studies was to prevent drug resistance and enhance pharmacokinetic / pharmacodynamic properties of some fluoroquinolones. To accomplish this, some novel fluoroquinolone derivatives were designed and synthesized. Purity and identity of the synthesized compounds were confirmed by IR, 1H-NMR, 13C-NMR and high-resolution mass spectral data besides elemental analysis. Antibacterial activity of synthesized compounds was evaluated by using microdilution method. Compound KUC140132 was found active against Escherichia coli ATCC 25922, Yersinia pseudotuberculosis ATCC 911, Klebsiella pneumonia and Pseudomonas aeruginosa ATCC 43288 at <0.63 µg/ml. MABA assay was used to determine the MIC values of the synthesized compounds against M. tuberculosis H37Rv and compound KUC140137 was the most active compound with the MIC value of 1.56 µg/ml while MICs of other compounds of this series were found between >25 and 6.25 µg/ml.

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SYNTHESIS AND CHARACTERIZATION OF NEW GENERATION MICHEAL ADDITION PRODUCTS IN DEEP EUTECTIC SOLVENTS

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Deep eutectic solvents (DESs) as a solvent/organocatalyst have gained widespread attention in the last decade. DESs are simple and inexpensive to prepare, generate no waste, require no purification, and possess high atom economy aspects thus embrace the principles of Green Chemistry [1]. DES have emerged as preferred solvents due to them being an environmentally benign and sustainable alternative to conventional organic solvents in synthetic chemistry, and also because they increase efficiency in organic transformations [2]. The concept of DES was first introduced by Abbott et al [3] as a mixture of two or more components that forms a eutectic, the melting point of this eutectic mixture is lower than both of the individual components. The most popular component among all DESs is cholinechloride (ChCl) as a donor compound with components including urea, sugars, carboxylic acids, and ethyleneglycol or M^{2+}ions such as Sn^{2+}.In this study the combination of cholinechloride and urea as a deep eutectic solvent has been used for synthesis series of new generation Michael Addition products (Figure 1). The mechanism of formation of the synthesized compounds was discussed and the assigned structure was established via spectral data (IR, ^{1}H-NMR and Mass). In the later of the study the biological activities of these new products will be examined.

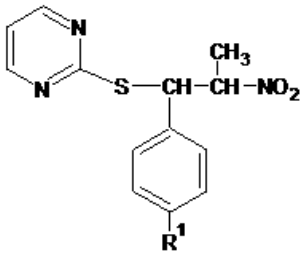

Figure 1

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SOME NOVEL DITHIOCARBAMATE DERIVATIVES BEARING BENZYLAMINE MOIETY AS MONOAMINE OXIDASE INHIBITORS

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Neurological and neurodegenerative disorders are closely related to the reduced levels of monoaminergic neurotransmitters in central nervous system (CNS) [1]. By blocking monoamine oxidase enzymes (MAOs), MAO inhibitors raise the levels of neurotransmitters in synaptic gaps. MAOs are flavin adenine dinucleotide (FAD)-containing enzymes and exist as two isoforms: MAO-A and MAO-B. MAO enzymes are responsible for the oxidative deamination of numerous endogenic and exogenic amines [2]. MAO inhibitors are of great importance, selective MAO-A inhibitors are employed in psychiatry for the therapy of depression, while selective MAO-B inhibitors are used for the symptomatic treatment of Parkinson's and Alzheimer's diseases [3,4]. First generation of MAO inhibitors were abandoned owing to adverse side-effects such as 'cheese effect' characterized by hypertensive crises [5]. Many MAO inhibitors possess arylalkyl amine moiety in their structures [6]. Therefore, we combined arylalkyl amine moiety with dithiocarbamate scaffold. Lipophilicity is a vital criterion to cross the blood-brain barrier (BBB) for a MAO inhibitor agent [7]. Dithiocarbamate combination may increase the lipophilicity, and thus the activity of the compound. To introduce more potent and selective MAO inhibitors, herein, we reported design, synthesis, MAO inhibitory activity and docking studies of some new dithiocarbamate derivatives containing benzylamine moiety. MAO inhibition assay revealed that compound 4j has the highest MAO inhibitory activity.

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DESIGN, SYNTHESIS AND ACTIVITY OF SOME NOVEL PHARMACOPHORIC PHENYLAMINOPYRIMIDINE CORE CONTAINING 5-ARYLIDENE-2-ARYLIMINO-4-THIAZOLIDINONE DERIVATIVES TARGETING NEUROBLASTOMA CANCER CELLS

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According to report published by World Health Organization in 2014, 14 million people suffered and 8.2 million people died from cancer in the year of 2012. The number of deaths caused by cancer annually is expected to reach 13 million by 2022 [1]. Since the launch of imatinib in 2001, kinase inhibitors in drug discovery remains to be one of the hot topics regarding cancer treatment in academia and in pharmaceutical industry [2]. The number of kinase inhibitor drugs approved by FDA is currently over 30 [3]. Imatinib is the pioneering kinase inhibitor drug which has phenylaminopyrimidine core structure. In literature, there are various data indicating that phenylaminopyrimidine derivatives are straight candidates for anticancer drug discovery [4, 5]. Similarly, the diversity in the biological response of 2-imino-4-thiazolidinones has attracted the attention of many researchers. 2-Imino-4-thiazolidinones were reported as potential anticancer agents [6, 7] as well as kinase inhibitors [8,9]. Besides, there is an urgent need in developing new chemotherapeutic compounds upon the limited number of selective anticancer agents and resistance mechanisms [10]. For this purpose, aseries of novel 2-imino-4-thiazolidinone derivativescontaining phenylaminopyrimidine core were designed and synthesized.Purity of the synthesized compounds was checked with TLC and HPLC. The structures of final compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data besides elemental analysis. Cytotoxic bioactivity of synthesized compounds was evaluated in vitro against neuroblastoma cell line. Molecular modeling studies were performed to simulate potential inhibition of different kinases by synthesized compounds.

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SYNTHESIS AND CYTOTOXIC PROPERTIES OF CONDENSED 1,4-DIHYDROPYRIDINE DERIVATIVES

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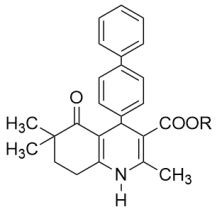
Calcium channels contribute to the slow depolarization phase of the excitable cells such as cardiomyocytes, skeletal muscle or smooth muscle cells. Calcium channel blockers block the influx of calcium by stabilizing the calcium channels on the membrane and are currently in use for the treatment of hypertension, cardiac arrhythmias and angina pectoris act on the L-type channels.The 1,4-dihydropiridine derivatives in this group are widely used in antihypertensive and antiarrhythmic therapy and angina pectoris treatment [1,2]. In this study, the series of compounds having condensed 1,4-dihydropyridine structure, alkyl 4-(1,1'-biphenyl)-2,6,6-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (Figure) were synthesized by Hantzsch reaction [2]. The structure of the compounds were proved by IR, ^{1}H-NMR, and elemental analysis. The synthesized compounds were investigated for growth inhibition and cytotoxicity on C2C12 mouse embryonic myoblasts cell line [3]. Among several concentrations tested, Compound I and Compound IV exhibited a significant growth promoting effect at 1 and 10 mM concentrations with the former exhibiting the highest impact. A significant toxicity was not observed in any of the compounds within the tested dose range.



R: CH_3, C_2H_5, $CH(CH_3)_2$, $CH_2CH(CH_3)_2$, $C(CH_3)_3$, $CH_2CH_2OCH_3$, $CH_2CH_2O_2CC(CH_3)=CH_2$, $CH_2C_6H_5$.

Figure: Structure of the synthesized compounds

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SYNTHESIS OF SOME BISBENZIMIDAZOLE COMPOUNDS AND INVESTIGATION OF THEIR ANTIBACTERIAL ACTIVITY WITH STRUCTURE-ACTIVITY RELATIONSHIP

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The rising of resistance against effective anti-bacterial agents', results in increased failure of antibacterial therapies today. Therefore, design and development of novel antibacterial active structures with different mechanisms of action is a never-ending area for drug developers. Benzimidazole and bisbenzimidazoles are an important pharmacophore groups in medicinal chemistry and the potential area of research. Extensive pharmacological studies have confirmed that bisbenzimidazole derivatives are effective against various microorganisms.[1] The reason for a special interest of researchers toward benzimidazole derivatives can be explained by their purine anti-metabolites properties as they are isoster for DNA bases, which facilitate recognition of the structures by living organisms. In addition, some drugs such as astemizole, mebendazole, enviroxim, and benomyl contain benzimidazole structure that interfere with bacterial growth.[2] In this study, we synthesized some bisbenzimidazole derivatives based on the structure of the lead benzimidazole compounds and their antibacterial activity with the establishment of their structure-activity relationship. The compounds were prepared from substituted 1,2-phenylendiamine with dicarboxylic acid derivatives by comparative studies with conventional and microwave irradiation methods.[3] All synthesized compounds were characterized by spectral data. Then, we demonstrated in vitro antibacterial activity of these synthesized compounds against various microorganisms using the microdilution method. Most of the compounds showed moderate to high activity with MIC values in the range of 3.9-500 µg/mL. These data suggest that bisbenzimidazole derivatives which have longer linker group might possess different antibacterial mechanism, which deserves further study.

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The Structure of Compounds

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SYNTHESIS AND CHOLINESTERASE INHIBITORY ACTIVITIES OF SOME 4-(3/4-NITROPHTHALIMIDO)BENZENESULFONAMIDE DERIVATIVES

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Phthalimide is a very important scaffold for medicinal chemists to prepare biologically active molecules [1]. To date, several phthalimide derivatives with various bioactivities such as anticancer, anti-inflammatory, anticonvulsant and acetylcholinesterase (AChE) inhibitory activity have been synthesized [2, 3]. On the other hand, sulfonamide derivatives are another important class of pharmacophores constituting various pharmacological acitivities such as diuretic [4], anticonvulsant [5], antihypertensive [6], antibacterial, anti-inflammatory, anticancer [7]. In the present study, we synthesized four 4-(3/4-nitrophthalimido)benzenesulfonamide derivatives bearing two pharmacophore groups (phthalimide and sulfonamide) and evaluated their cholinesterase inhibitory activities (Figure 1). Structure of the synthesized compounds were confirmed by spectral analyses. The cholinesterase inhibitory activities of the title compounds were evaluated by modified Ellman's method with rivastigmine as the reference compound [8]. It was observed that all compounds exhibited higher inhibitory activity against AChE. In addition, the compounds showed better AChE inhibition when compared with the reference compound rivastigmine. Compound 3 appeared as the most potent derivative against AChE with IC_{50}=2.22±0.05 μM in the tested compounds.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME QUINOXALINE DERIVATIVES AS CHOLINESTERASE INHIBITORS

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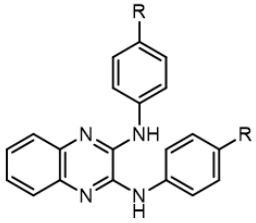
Alzheimer's disease (AD) is the most common age related neurodegenerative disease, characterized by memory loss and cognitive impairments [1]. Although the etiology of AD is not fully understood several factors, such as low levels of neurotransmitter acetylcholine, the aggregation of β-amyloid peptide, hyperphosphorylation of tau protein, and oxidative stress are considered to play important role in the pathophysiology of AD [2]. Inhibition of acetylcholinesterase (AChE), is currently the main pharmacological strategy for Alzheimer's disease [3]. At present, AChE inhibitors have successfully reached the market such as tacrine, donepezil, rivastigmine and galanthamine, but their efficacy is limited due to peripheral adverse effects [4]. Therefore, there is an urgent need to discover and develop new agents with enhanced activity profile.

Quinoxaline derivatives are an important class of heterocyclic compounds which exhibit various biological activities such as antibacterial, anti-inflammatory, anticancer , antitubercular, antileishmanial, antimalarial, antidepressant and anticholinesterase activities [5]. In this work, five compounds with quinoxaline core have been synthesized to evaluate their cholinesterase inhibitory activities compared to rivastigmine as reference compound by using Ellman's method (Figure 1) [6]. Structure of the compounds were confirmed by spectral analysis. According to the data, all compounds displayed higher AChE inhibitory activity than BuChE inhibitory activity. Among the tested compounds, compound 3 was found to be the most active derivative against AChE with IC_{50} value of 7.19±0.17 µM.



Compound No	R
1	OCH_3
2	Cl
3	NO_2
4	Br
5	COOH

Figure 1. Structure of the synthesized compounds

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SYNTHESIS AND EVALUATION OF A SERIES OF ARYLIDENE INDANONES AS NEW ANTITUMOR AGENTS

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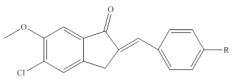
Arylidene indanones, the rigid cousins or homologs of chalcones, have attracted a great deal of interest owing to their outstanding therapeutic applications. In particular, considerable research on arylidene indanones in relation to their anticancer activity has been accomplished [1]. Due to their important role in anticancer drug discovery, new arylidene indanones (1-10) were synthesized via the base-catalyzed Claisen-Schmidt condensation of 5-chloro-6-methoxy-2,3-dihydro-1H-inden-1-one with p-substituted benzaldehyde derivatives (Figure 1). MTT assay was performed to assess the antitumor effects of the compounds on HeLa human cervix carcinoma and MCF-7 human breast adenocarcinoma cell lines. The cytotoxic effects of the compounds on NIH/3T3 mouse embryonic fibroblast cell line were investigated to determine the selectivity of the compounds. The most effective arylidene indanones were also evaluated for their effects on apoptosis and DNA synthesis inhibition. In addition, the most apoptotic agents were investigated for their effects on caspase-3 activation in HeLa cells. MTT assay indicated that compounds 2, 3, 4, 5, 6 and 7 showed selective anticancer activity against HeLa cell line. Among these compounds, compounds 2 and 3 were the most promising apoptotic agents against HeLa cells. Compound 2 was found to be the most potent compound on caspase-3 activation in HeLa cells, whereas compound 3 was identified as the most effective agent on DNA synthesis inhibition in HeLa cells. On the other hand, compounds 7 and 10 showed notable antitumor effects on MCF-7 cell line and induced apoptosis in MCF-7 cells. Compound 10 also exhibited significant DNA synthesis inhibitory activity against MCF-7 cells.



Compound	R
1	Pyrrolidin-1-yl
2	Dimethylamino
3	Diethylamino
4	Acetamido
5	Piperidin-1-yl
6	Morpholin-4-yl
7	4-Methyl-1-piperazin-1-yl
8	1*H*-Imidazol-1-yl
9	1*H*-1,2,4-Triazol-1-yl
10	2-Morpholinoethoxy

Figure 1. The synthesized arylidene indanones (1-10)

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SYNTHESIS AND EVALUATION OF A NEW SERIES OF THIAZOLES AS ANTICHOLINESTERASE AGENTS

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Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common cause of dementia worldwide. Although considerable research on AD has been carried out over a century, donepezil, galantamine, rivastigmine and memantine are the only drugs currently used for the management of AD. These drugs provide symptomatic treatment but do not alter the course of the disease [1]. As a result, extensive efforts have been devoted to the discovery of new potent cholinesterase inhibitors for the treatment of AD. In an attempt to develop potent cholinesterase inhibitors, herein new thiazolyl-pyrazoline derivatives were synthesized via the the ring closure reaction of 3-(2-furyl)-5-(1,3-benzodioxol-5-yl)-1-thiocarbamoyl-4,5-dihydro-1H-pyrazole with phenacyl bromides. The compounds were investigated for their inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) using a modification of Ellman's spectrophotometric method. 2-[5-(1,3-Benzodioxol-5-yl)-3-(2-furyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(naphthalen-2-yl)thiazole was found to be the most effective AChE inhibitor (38.5±2.85%), whereas 2-[5-(1,3-benzodioxol-5-yl)-3-(2-furyl)-4,5-dihydro-1H-pyrazol-1-yl]-4-(4-fluorophenyl)thiazole was found as the most potent BuChE inhibitor (43.02±2.71%) in this series. As a part of this study, the compliance of the compounds to the Lipinski's rule of five was evaluated. The physicochemical parameters (log P, TPSA, nrotb, molecular weight, number of hydrogen bond donors and acceptors, molecular volume) were calculated using Molinspiration software. These compounds only violated one parameter of Lipinski's rule of five. On the basis of Lipinski's rule, they were expected to have reasonable oral bioavailability. In the view of this study, further mechanistic studies and structural modification of identified compounds are on-going for the generation of new cholinesterase inhibitors with enhanced efficacy.

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SYNTHESIS OF SOME NEW MANNICH BASES WITH ANTI-TYROSINASE ACTIVITY

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Nowadays, tyrosinase inhibitors which can be used to prevent or treat melanin hyperpigmentation are gradually gaining importance in medicine, cosmetics and food industries. Several natural or synthetic tyrosinase inhibitors have been discovered until today. The well-known skin bleaching agents are hydroquinone, arbutin, kojic acid and azelaic acid. However, a few of them can be used in the market due to toxicity problems. Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one), the most intensively studied inhibitor of tyrosinase, has an important role in cosmetic industry for their skin lightening effect and depigmentation after sunburn. Unfortunately, unstability during storage limits its use and development of novel kojic acid derivatives is needed in cosmetics industry [1,2]. In our laboratory, some Mannich bases of kojic acid derivatives were synthesized and their extensive bioactivities were determined including anticonvulsant, antibacterial, antifungal, antiviral, antioxidant, anti-mycobacterium, anti-aging, anti-tyrosinase activities and cytotoxicity [3-7]. 2-[(3,4-Dichloro benzyl)piperazinylmethyl]-3-hydroxy-6-hydroxymethyl-4H-pyran-4-one was synthesized without by-product formation by means of a simple and efficient method that is conducted at room temperature. It has better permeability than kojic acid and is not irritative in high amounts. The result of the anti-tyrosinase activity of the compound was six time higher than kojic acid. This compound was covered by the Turkish and International Patent [7]. Hence, in the light of these findings, some new Mannich bases derivatives were synthesized and their anti-tyrosinase activities were evaluated. Anti-tyrosinase activity thereof is determined by spectrophotometric method using L-DOPA as a substrate.

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SYNTHESIS AND ANTICANCER EFFECTS OF SOME NOVEL BENZIMIDAZOLE-DERIVED PARP INHIBITOR MOLECULES

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PARP inhibitors are compounds that are used to treat various types of cancers which are among the leading health problems throughout the world. A series of novel potential PARP inhibitor substituted (piperazine-1-carbonyl)phenyl)-1H-benzo[d]imidazole-4-carboxamide derived compounds (Fig.1) were designed. After the synthesis [1-4] and structural elucidation of the compounds expected to be potentially PARP inhibitors, they were tested for their in vitro anticancer activities in leukemia cell line K562 [5]. Colorimetric MTT assay and western blot were used to determine the effects of novel compounds on cell viability and PARP fragmentation respectively. Our preliminary results showed that compound 6b (2-(3-(4-(1-adamantanecarbonyl)piperazine-1-carbonyl)phenyl)-1H-benzo[d]imidazole-4-carboxamide) inhibited cell viability and increased PARP fragmentation significantly and we will further examine the action mechanism of this compound in K562 cells. Since PARP inhibitors are widely used for their anticancer activities against cancers which carry mutations in genes that result in defective DNA repair, we also suggest testing the effects of these candidates on various cell lines with different mutations.

Figure1.

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STUDIES ON THE SYNTHESIS AND ANTICANCER ACTIVITIES OF SOME NOVEL CHROMONE DERIVATIVES

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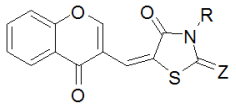
Cancer has been the second common cause of human death in the developed world [1]. The efficacy of current chemotherapeutics is low and undesirable side effects are still unacceptably high. Hence, the development of novel, efficient, and less toxic anticancer agents remains as an important and challenging goal of medicinal chemists worldwide [2]. The nature has always provided us a broad range of bioactive product for the treatment of life threatening human diseases. To our knowledge, compounds bearing chromone core have been reported to exhibit a large field of biological activities, including antitumor [3], antibacterial [4], and antiinflammatory [5] activities.

The objective of this study was to synthesize, characterize and examine anticancer activities of some novel chromone derivatives (Figure). Chromonyl-2,4-thiazolidinedione / rhodanine compounds were synthesized by the Knoevenagel condensation reaction of chromone moiety with 2,4-thiazolidinedione and rhodanine rings and their derivatives. Their structure was characterized by ^{1}H NMR, Mass and elementary analysis data. All ^{1}H NMR, Mass and elementary analysis data were in accordance with assumed structures. Further studies on biological activities about these derivatives are still underway and will be reported in the future.



R= H, CH_2COOH, CH_2COOEt
Z= O, S

Figure: Formula of the synthesized compounds

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 5-ARYLIDENE-2-IMINO-1,3-THIAZOLIDINONE DERIVATIVES AS ANTICANCER AGENTS

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Cancer is the second leading cause of death in the nation next to cardiovascular diseases. Side effects and drug resistance lead to major limitations during current cancer therapy, so the development of more potent and safer anticancer drugs still remains as an important research area. Earlier reports showed that 5-arylidene-2-imino-1,3-thiazolidin-4-one derivatives have a broad range of biological properties, including anticancer activity [1-3]. In the present study, we aimed at synthesis of new arylidene derivatives of 2-heteroarylimino-1,3-thiazolidin-4-one derivatives with potential anticancer effects. The target compounds were synthesized by Knoevenagel condensation of several aromatic aldehydes with 2-heteroarylimino-1,3-thiazolidin-4-one derivatives. The synthesized compounds were identified by the use of IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data while the purities of them were proved with TLC and elemental analysis. Synthesized compounds were evaluated for their anticancer activities against K562, MCF-7, HT-29, SJSA1, A549, PC-3 and HeLa cancer cell lines at 10 µM dose. Additionally, cytotoxic properties of these arylidene derivatives were investigated on mouse fibroblast cell line NIH3T3 at 10 µM dose to determine their selectivity.

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A STUDY ON SYNTHESIS OF NOVEL MANNICH BASES DERIVED FROM 1,2,4-TRIAZOLE-5-THIONE CONTAINING NAPROXEN MOIETY

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Inflammation is a protective attempt of healthy immune system against injury or infection. However, when inflammation becomes chronic and uncontrolled, it may cause to diseases, such as atherosclerosis and arthritis resulting in chronic pain, inability to do daily activities. Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be a widely prescribed categories of drugs worldwide for the treatment of pain, inflammation and inflammation-related disorders. It is well known that chronic NSAID treatment carries some gastrointestinal (GI) toxicity risk, ranging in severity from mild dyspepsia to GI hemorrhage and perforation. Therefore, the need for development of more effective NSAIDs endowed with an improved safety profile is still urgent. In our previous studies, we synthesized new compounds by fusing two biologically active structures into one molecule not only increased the anti-inflammatory/analgesic activity but also reduced the side effects of commercial NSAIDs. In these studies, compared with the starting materials, some of the compounds have shown higher analgesic/anti-inflammatory activity and lower ulcerogenic risk in the stomach [1-3]. The present study is an attempt to develop a new class of analgesic/anti-inflammatory agents with improved pharmaceutical profiles by Mannich reaction of 1,2,4-triazol-5-thione containing naproxen moiety. The starting compound, 3-[1-(6-methoxy-2-naphthyl)ethyl]-1,2,4-triazole-5-thione, was conveniently prepared via dicyclohexylcarbodiimide (DCC)-promoted amide formation reaction from naproxen to the method reported earlier [1]. The easily accessible triazolethione was then treated with secondary amines to obtain the Mannich bases [4]. The structures of the newly synthesized compounds were established by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data.

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HYDRAZONES: A VALID SCAFFOLD FOR MONOAMINE OXIDASES INHIBITORS

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Monoamine oxidases (MAOs) are FAD-containing enzymes catalyzing the oxidation of endogenous (e.g. norepinephrine, dopamine, serotonin) and exogenous amines to the corresponding aldehyde and ammonia [1]. Currently, two MAO isoforms have been identified based on substrate specificity [1-3]. MAO-A preferentially metabolizes serotonin and kynuramine, whereas MAO-B has a greater affinity for phenylethylamine and benzylamine [4]. The two MAO isoforms can be also differentiated according to their inhibition by synthetic compounds: clorgyline and moclobemide for MAO-A and selegiline and lazabemide for MAO-B [5]. Although MAOs are widely distributed in various organs, most of the studies concerning their functional properties and involvement in pathological processes have been mainly focused on the central nervous system. These studies showed that MAOs play a major role in regulating brain concentrations of biogenic amines, and their abnormalities have been involved in various psychiatric and neurodegenerative disorders [6]. Hydrazone and acylhydrazone derivatives have been a major source of inspiration for the development of novel compounds with anticonvulsant, antiinflammatory, antidepressant, analgesic, antithrombocyte and anti-cancer activities. There have been many reports on the antidepresant/MAO-inhibition activity of hydrazones derived from substituted hydrazides and their reduction products [7]. In this study, some new substituted benzoxazoline hydrazones were synthesized by reacting 5-substitutedbenzoxazolinone-3-carboxylic acid hydrazide with various aldehydes, their structures were confirmed using IR, ^{1}H-NMR, mass and elemental analysis and their MAO inhibition were evaluated by in vitro tests.

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INVESTIGATION OF POLYPHENOL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF LYCOPERDON MOLLE AND LYCOPERDON LIVIDUM SPECIES

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Mushrooms have been valued as edible and medicinal resources. Laboratory studies confirm that extracts of fungi contain many secondary compounds which have specific biological effects. These compounds which, could be found in fruit bodies, mycelium and broth, are verified to be phenolics, flavonoides, glycosides, polysaccharides, tocopherols, carotenoids and ascorbic acid. Some of the most recently isolated and identified compounds originating from the medicinal mushrooms have shown promising antiviral, antibacterial, antioxidant, antidiabetic, immunomodulatory, antitumor, cardiovascular, and hepatoprotective properties. In this study, the ethanol extracts of Lycoperdon molle and Lycoperdon lividum were studied for the polyphenolic contents using spectrophotometric method. Furthermore, the mushroom extracts effects were examined on the glutathione peroxidase (GPx) and catalase enzyme activities by kinetic assay. Total phenolic contents were determined by using the Folin-Ciocalteu's method. Also, the total concentration of flavonoids in extracts were determined by employing the aluminium chloride colorimetric method. According to the results, the highest phenolic and flavonoid contents were detected in the ethanol extract of L. molle, with 8.244±0.29 mg gallic acid equivalent/L and L. lividum, with 2.076±0.08 quercetin equivalent/L, respectively. The ethanol extracts of L. molle and L. lividium (0.625-10 mg/ml) were investigated for their activities on GPx and CAT enzymes in vitro; maximum concentrations of L. lividium extract effectively activated the GPx enzyme at around 28% and the CAT enzyme at 2%.

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IN VIVO ANTI-INFLAMMATORY ACTIVITIES OF CENTAUREA CALOLEPIS BOISS. AND CNICIN AGAINST VIPER VENOM INDUCED-INFLAMMATION

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In snake bite cases, general findings are various systemic and local effects including necrose, haemmorhage, pain and local edema. The Ottoman Viper, Montivipera xanthina (Gray, 1849) (Viperidae), whose venom could be haemolytic and cytotoxic, is distributed in Middle, South and West Anatolia [1].
Different Centaurea L. species (Asteraceae) are used for tonic, expectorant, antipyretic, antidiarrheal effects and treatment of snake bites in Anatolian traditional medicine [2,3]. C. calolepis Boiss. is an endemic species, distributed in West and South-West Anatolia. Chloroform extract of plant and its major compound cnicin, a sesquiterpene lactone, showed strong anti-inflammatory activity in-vitro and in-vivo experiments [4,5]. The aim of this study was to determine anti-inflammatory activities of C. calolepis and cnicin against Montivipera xanthina venom induced-edema in rat.
C. calolepis chloroform extract and cnicin were applied to Wistar rats per orally in different doses. Snake venom (37.5 µg/paw) was injected into hind paw, measured by plethysmometer at ½, 1, 2, 3 and 4 h and determined percent of edema increasing. Indomethacin was used as positive control. 10 mg/kg of cnicin (28.31%) and 25 mg/kg of chloroform extract (11.12%) showed stronger anti-inflammatory effect than standard agent indomethacin (38.27%) at ½ h.

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VOLATILE COMPONENTS OF VARIOUS PARTS OF PISTACIA TEREBINTHUS L. SPP. PALAESTINA (BOISS.)

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The genus Pistacia (Anacardiaceae) has known economical-cultural importance and its eight species belong to Turkey [1-5]. Pistacia terebinthus L. spp. palaestina (Boiss.) is used for diabetes in Turkey and generally known as 'menengiç', 'çıtımık' and 'çöğre' [5, 6]. This study identified the composition of essential oil of leaves, twigs, ripe and unripe fruits of P. terebinthus ssp. palaestina from Tokat. Essential oils obtained by micro distillation. Chemical composition of the oils identified using both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). As a result, 33 compounds representing 100% of the oil were characterized from the oil of ripe fruits with α-pinene (34.3%), (Z)-β-ocimene (24.8%), β-pinene (13.5) and (E)-β-ocimene (7.3%) as major ingredients. (Z)-β-ocimene (35.8%), α-pinene (22.4%), (E)-β-ocimene (11.1%) and cis-alloocimene (8.1%) were found as main compounds among the 29 constituents including 99.9% of the oil were determined in the oil of the unripe fruits. 59 components representing 99.6% of the oil were detected and main constituents were α-pinene (25.4%), β-pinene (16.7%) and α-phellandrene (10.0%) in the leaves. 25 compounds consisting of 99.9% of the oil were described and major constituents were determined as α-pinene (36.4%), α-phellandrene (15.0%), β-pinene (14.8%) and β-caryophyllene (8.9%) in the twigs. Flamini et al. found α-pinene (63.1%), myrcene (13.3%), and limonene (4.3%) in the leaves, (E)-ocimene (41.3%), sabinene (20.3%), terpinen-4-ol (6.4%), α-pinene (5.3%), and (Z)-ocimene (3.8%) in the unripe fruits, and (E)-ocimene (33.8%), sabinene (24.1%), (Z)-ocimene (13.0%), and α-pinene (6.5%) in the ripe fruits of Jordanian Pistacia palaestina Boiss. [7].

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CYTOTOXIC EFFECTS OF THE GENUS OF RHODODENDRON FLOWERS EXTRACT ON HUMAN EPITHELIAL COLORECTAL ADENOCARCINOMA CELL LINE (CACO-2)

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The genus Rhododendron used in traditional medicine for the treatment of inflammation, pain, cold, asthma, skin and gastro-intestinal disease, is distributed widely around the world. The genus Rhododendron, contains graytonotoxins with diterpene qualities. Increased incidence of cancer, treatment is costly and create serious side effects applied to people, it becomes necessary to investigate a scientific alternative treatment and supportive way. The aim of the study was to investigate in vitro cytotoxic effects of R. ponticum L, R. smirnovi L., R. ungennii L. and R. rufeum L. flower extracts on heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2). During the flowering period of common Rhododendrons gathered from the Altinordu District of Ordu (R. ponticum L. and R. rufeum L.) and from Artvin (R. smirnovi L. and R. ungennii L.) and dried under suitable conditions, extracted with distilled water and lyophilized. In our study, cytotoxic activity of different concentrations of the extract of Rhododendrons; through mitochondrial (MTT) activity was evaluated in heterogeneous human epithelial colorectal adenocarcinoma cell line (Caco-2). IC_{50} were found for R. ponticum L. 4637 µg/ml, for R. rufeum L. 761,3 µg/ml , for R. smirnovi L 3825 µg/ml and for R. ungennii L. 927,9 µg/ml, respectively. Cytotoxicity in glioma indicate that Rhododendron species are expected to be a potential anticarsinogentic activity. Our research on Rhododendron species continues on other cancer cell lines with cytotoxicity tests.

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ANATOMICAL INVESTIGATION OF INULA VISCOSA (L.) AITON (ASTERACEAE) USED AS FOLK MEDICINE IN WESTERN ANATOLIA (TURKEY)

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The genus Inula L. belongs to the family Asteraceae and comprises about 120 species, distributed mainly in Europe, Africa and Asia [1]. In Turkey, there are about 28 species, 33 subspecies and varieties respectively of Inula and 8 of them are endemic [2]. Inula species are commonly used in the treatmant of cancer, ulcer, diabetes, wound healing, fever, rheumatism and asthma [3-5]. It is related to the presence of biologically active compounds, such as flavonoids, azulens, essential oils and sesquiterpenes [5]. The Plant specimens were collected at the flowering stage from Manisa (Turkey). Voucher specimens were deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University. Samples for anatomical studies were fixed in 70 % alcohol. Cross sections of the plant leaves and stem, and surface sections of leaves taken by free-hand and stained with Sartur solution. Each structure preparations were observed and the well-staining sections were photographed on Philips digital color camera type LTC 0600/50 and Olympus BH-2 light microscope. The stem epidermis consist of a single layered and oval cells. It is surrounded by a thick cuticle layer. The surface of stem epidermis was covered with long or multicellular nonglandular hairs and glandular hairs. The leaves are amphistomatic and isolateral. The anomocytic stomata occur on the both surfaces with 4 neighboring cells. The upper epidermal cells are almost straight but the lower epidermel cells are distinctly undulate. There is short stalked, big head and multicellular glandular hairs and multicellular nonglandular hairs.

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ANTI-INFLAMMATORY ACTIVITIES, PHENOLIC AND FLAVONOID CONTENTS OF AQUEOUS, ETHANOL AND METHANOL EXTRACTS OF CAPPARIS OVATA VAR. PALAESTINA

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Phenolic and flavonoid compounds are recognized as secondary metabolites which are formed in plants [1]. It's reported that phenolic and flavonoid compounds possess various biological properties such as anti-inflammatory and antioxidant activities [2]. In this study, anti-inflammatory activities of aqueous, methanol and ethanol extracts of Capparis ovata var. palaestina Zoh.'s buds and fruits were investigated and also their phenolic and flavonoid contents were measured. Phenolic and flavonoid contents were estimated by Folin–Ciocalteu and aluminium chloride methods, respectively. In vitro anti-inflammatory activities of the C. ovata var. palaestina extracts were evaluated by using HRBC (human red blood cells) membrane stability method. Due to phenolic content test results, the lowest value (179,92 ±3,30 mgGAE/100g) was obtained from Fruit-Aqueous (FA) extract and the highest value (1017,42 ±44,18 mgGAE/100g) was obtained from Fruit-Methanol (FM) extract. Beside this, in a similar manner the lowest flavonoid content (1228,33 ±9,45 mgQE/100g) was seen in FA extract and highest flavonoid content (2990 ±21,21 mgQE/100g) was seen in FM extract. On the other hand results which were obtained from anti-inflammatory test showed that all extracts indicate anti-inflammatory function as compared to acetylsalicylic acid ($p<0.05$). FM extract of C. ovata var. palaestina (0,5394 ±0,0145 mg/ml) and FA extract of C. ovata var. palaestina (1,0081 ±0,0077 mg/ml) demonstrated highest and lowest anti-inflammatory activities, respectively (Table 3). The results of this study showed that flavonoid and phenolic contents which are shown in extracts, may be associated with anti-inflammatory activity of C. ovata var. palaestina fruits and buds.

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BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL STUDIES ON JUNIPERUS DRUPACEA

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Juniperus drupacea Labill. is one of the Juniper species growing in Turkey. It is used for medicinal purposes and to obtain a traditional food product “pekmez” for centuries. We aimed to evaluate in-vitro antidiabetic and antioxidant activities of the extracts prepared from fruits, leaves and branches of J. drupacea. Therefore, α-amylase and α-glucosidase inhibitory effects and antioxidant activities of the water, ethyl acetate, and methanol extracts of the plant were evaluated. Additionally, total phenol and flavonoid contents of the extracts were investigated. Qualitative and quantitative analysis of amentoflavone and umbelliferone were performed using a RP-HPLC-DAD method in the methanol extracts. All extracts showed excellent and dose dependent inhibitory effect on α-glucosidase enzyme. Moreover, fruit (99.92± 0.30%) and leaf methanol extracts (99.44 ± 1.78%) were more effective than Acarbose (98.88 ± 0.07%) at 1 mg/ml. Additionally, extracts rich in flavonoids and phenolics showed remarkable antioxidant activity. Results of HPLC analysis revealed that amentoflavone was detected in high amount especially in the leaves as 0.148±0.001 g/100 g dry weight. Umbelliferone was determined as minor compound in both leaves and branches.

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IN VITRO ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL STUDIES ON TWO CISTUS SPECIES OF TURKEY

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In Turkish folk medicine, the tea prepared from the leaves of Cistus species is used to decrease symptoms of diabetes. Our previous studies have shown that the ethanol extract of C. laurifolius possesses a potent antidiabetic activity in in-vivo and in vitro experiments. Thus, we aimed to evaluate antidiabetic potential of C. creticus L. and C. salviifolius L. in this study. First, α-glucosidase and α-amylase inhibitory activities of the aqueous, ethyl acetate, and methanol extracts of leaves, branches flowers and fruits of the Cistus species were evaluated. Additionally, antioxidant activities by using metal chelating capacity, ferric-reducing antioxidant power, total antioxidant activity, superoxide anion, ABTS radical cation and DPPH radical scavenging activity tests, total phenolic and flavonoid contents of the extracts were evaluated. All extracts showed strong inhibitory effect on α-glucosidase enzyme ranging between 100.00-14.71%. C. salviifolius leaf water (99.33± 0.78%) and methanol extracts (99.91 ± 0.34%) were more effective than Acarbose (98.88 ± 0.07%) at 1 mg/ml. C. salviifolius fruit water extract had the highest total phenolic content (321.64 ± 2.21 mg gallic acid equivalent/g extract) and C. salviifolius leaf ethyl acetate extract had the highest flavonoid content (118.66 ± 4.61 mg quercetin equivalent/g extract). High performance liquid chromatography was used to determine phenolic acids, flavonoids and catechins in the methanol extracts.

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IN VITRO ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF THIRTEEN MARINE ORGANISMS FROM MEDITERRANEAN SEA

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Aim of the study is to evaluate the in vitro antidiabetic and antioxidant potential of marine organisms collected from Mediterranean coast. Methanol extracts of one soft coral (Eunicella singularis) and twelve sponge species (Agelas oroides, Aplysina aerophoba, Axinella cannabina, A. polypoides, Cliona viridis, Dictyonella incisa, Dysidea avara, Ircinia incisa, I. oros, I. variabilis, Petrosia ficiformis, Sarcotragus spinulosa) were investigated for their α-amylase and α-glucosidase inhibitory activities. On the other hand, total phenolic content, total antioxidant capacity, ferric reducing antioxidant power, metal chelating, and [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation scavenging activities of all extracts were determined. Dysidea avara was found to be the most active extract on α-glucosidase enzyme (94.66-4.87% for 3000-100 µg/ml). Therefore, α-glucosidase inhibitory activity of its major compounds avarol and avarone was tested and exerted 86.18 ± 1.76% and 78.94 ± 1.38% inhibitory effect at 10 µM, respectively. The present study indicated that the sponge D. avara found in Mediterranean coasts will be evaluated as a new natural source in the treatment of diabetes mellitus.

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SECONDARY METABOLITE ISOLATION OF ETHANOL EXTRACT OF SALVIA ROSIFOLIA

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Members of Salvia genus produce many useful secondary metabolites including terpenes and phenolics and their derivatives that have been reported in the pharmacopoeias of many countries. Antioxidant activities of the many members of the genus Salvia were reported elsewhere. Additionally, previous reports concerning the biological activities of Salvia species native to the Turkish flora confirm that this genus has great potential, especially in antioxidant systems, for the food and cosmetic industries [1]. In this study, dried samples of the aerial parts and the subsoil root parts of S. rosifolia were pulverized and chloroform and ethanol extracts were prepared. Ethanol extracts were found more active in antioxidant activity tests therefore we decided to fractionate the ethanol extract and isolate the pure substance by the preparative thin layer chromatography method. As yet, we have been isolated nine pure compounds. These pure compounds are Taraxasterol, Ursolic acid, Oleanolik acid, Salvigenin, Lupeol, 3-Acetyl lupeol, Beta-sitosterol, 4-Hydroxybenzaldehyde and Bis(2-ethylhexyl) benzene-1,2-dicarboxylate, which some of them shown below. The identification of these compounds has been determined by comparing the HRMS, ^{1}H-NMR, ^{13}C-NMR and 2D NMR values of these compounds with those in the literature.

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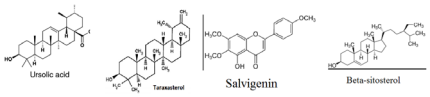

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IN VITRO CYTOTOXIC ACTIVITY OF CHLOROFORM AND ETHANOL EXTRACTS OF SALVIA CERINO-PRUINOSA VAR. CERINO-PRUINOSA

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Salvia species (Labiatae) have been used as medicinal plants throughout the world. They possess antifungal and antibacterial activities and are used against tuberculosis, as anticancer agents and for the treatment of heart disease [1]. Anticancer agents induce apoptosis, so that disruption of apoptotic cell death reduces treatment sensitivity. Extensive varieties of natural compounds possess significant cytotoxic as well as chemopreventive activity, which act via apoptosis. Extracts of plants used in traditional medicine also have a similar property [2]. In this study, the cytotoxic activity of chloroform and ethanol extracts of Salvia cerino-pruinosa var. cerino-pruinosa was determined by MTT method. Chloroform extracts of S. cerino-pruinosa var. cerino-pruinosa (SCC-KK) showed cytotoxic effect on healthy fibroblast cell line at above a specific concentration. SCC-KK showed cytotoxic activity against HT29 cell lines at above 100 µg/ml concentration. Consequences of 72-hours incubation is better than 48-hours incubation. The chloroform extracts of this species was found to have cytotoxic activity against MCF7 cells in 48-hour incubation at 500 µg/ml concentration and in 72-hour incubation at 250 µg/ml concentration. Ethanol extracts of S. cerino-pruinosa var. cerino-pruinosa (SCC-KE) did not show any activity againist HT29 cell lines after 48-hours incubation. However, SCC-KE showed moderate activity in 72-hour incubation at 500 µg/ml concentration. The ethanol extracts of this species showed no cytotoxic activity against MCF7 cells at any concentrations.

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ANTIOXIDANT AND ANTI-TYROSINASE ACTIVITIES AND TOTAL PHENOLIC CONTENT OF PRUNUS SPINOSA L. GROWING IN TURKEY

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Prunus spinosa L. (Rosaceae), also known as blackthorn, is a therapeutic plant known for many years. It's native to Europe, western Asia and northwest Africa [1]. For treatment; the fruits, flowers, leaves and young branches of P. spinosa have been used [2]. Fruits of P. spinosa is laxative, astringent, diuretic and purgative properties [1]. It is also known that P. spinosa is used in cosmetic products with high protective factor against free radicals [3].
In this study, fruits of P. spinosa, collected from Turkey, were extracted with methanol and distilled water. Antioxidant activity of methanol and distilled water extracts were expressed as percentage of DPPH radicals inhibition and ABTS free radical scavenging. Values in percentage ranged from 0.072 to 0,691% and 0,189 to 0,595%, respectively. Anti-tyrosinase activity of methanol and distilled water extracts were 0,312 and 0,538 mg/ml, respectively, expressed as ascorbic acid. The total phenolic contents of methanol and distilled water extracts were 2,548 and 1,278 mg/g, respectively, expressed as gallic acid equivalents. Highest value of antioxidant activity, anti-tyrosinase activity and total phenolic content were determined in methanol extract.

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TRIGONELLA SPICATA SIBTH. & SM.; DETERMINATION OF FATTY ACID COMPOSITION, STEROL, TOCOPHEROL AND AMINO ACID CONTENTS

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The genus Trigonella L. (Fabaceae) comprises of over 135 species distributed all through the Mediterranean regions, Southeastern Europe, Western Asia, North and South Africa [1,2]. Trigonella foenum-graecum L. commonly called fenugreek, is the most widely used species in traditional medicine for many years due to their wide range of biological activities and nutritional values. According to the literature the chemical constituents of this genus had generally been reported as flavonoids, alkaloids, saponins, fixed oil, polysaccharides, minerals and proteins. The seeds used in many traditional systems as aromatic, carminative, galactogogue, antibacterial, antidiabetic, hypocholesterolemic, diuretic and analgesic agent [1-3].
In Turkey, the genus Trigonella represented by 13 sections, 8 groups and 54 taxa [4,5]. The aim of this study was to determine fatty acid compositions, sterol, tocopherol and aminoacid contents of T. spicata Sibth. & Sm. for the first time. The seed oil content of the studied species was found to be 1.71±0.09 g/100 g. Linoleic acid (31.78±0.91 %), palmitic acid (21.11±0.72 %) and oleic acid (16.04±0.73) were the main fatty acids. The total sterol content was 1736.79±0.56 mg/kg, which consisted high amounts of β-sitosterol (39.79±0.82 %). The dominant tocopherol was found to be α-tocopherol (342.56±1.84 mg/100 g). Aspartic acid (4936±0.13 mg/100 g) was the main aminoacid. The results of the present study revealed that this species is important source of essential fatty acids, tocopherols and aminoacids.

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PROFILING AUSPICIOUS BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY OF HYUGANIN C

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Cholinergic therapy based on cholinesterase (ChE) inhibitory drugs is the mainstay for the treatment of Alzheimer's disease (AD). For this purpose, an extensive research has been continuing for the discovery of novel drug candidates as inhibitors of ChEs including acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). In this study, hyuganin C, a naturally occurring dihydropyranocoumarins, was tested in vitro for its AChE and BChE inhibitory potential using microtiter assays. The compound was isolated in pure form from the petroleum ether extract of Mutellina purpurea (Poir.) Reduron, Charpin & Pimenov (Apiaceae) fruits through high performance counter-current chromatography (HPCCC). The structure elucidation was performed with NMR and MS experiments. Hyuganin C was ineffective against AChE, whereas it was found to be the highly active (IC_{50}= 38.86 ± 1.69 µM) inhibitor of BChE as compared to that of galanthamine as the reference drug (IC_{50}= 46.58 ± 0.91 µM). Further molecular docking experiments by in silico methods indicated that hyuganin C is able to block the access to key residues in the catalytic triad of the enzyme, while it complements some of the hydrophobic residues of the cavity. Consequently, hyuganin C is a highly promising BChE inhibitory natural molecule towards AD therapy.

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CHOLINESTERASE INHIBITORY ACTIVITY AND LC-MS ANALYSIS OF THE BULBS OF LILIUM L. SPECIES GROWING IN TURKEY

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Medicinal plants represent an important source of bioactive compounds that could be used for new drugs development. Turkey is one of the home country of many bulbous plants. It is worth to mention that a good number of the geophytes are considered as medicinal plants in addition to their ornamental values.Therefore, geophytes are well-known to possess economic and medicinal prominence. For this purpose, we have initiated a huge project from different scientific disciplines relevant to geophytes growing all over the country in order to search and collect all data relevant to their botany, cultivation as well as chemotaxonomy and some biological activity(1). We have now aimed to screen the extracts of seven Lilium L. species (Liliaceae) for cholinesterase inhibitory activity that were not studied with respect to their neuroprotective potentiality. It should be also noted that oxidative stress plays a remarkable role in neurodegeneration and antioxidant function of drug candidates used in the treatment of Alzheimer's disease is desired. Our results indicated that the dichloromethane and ethyl acetate extracts of Lilium ponticum subsp. ponticum (C. Koch) Davis and Hender. exerted high to moderate BChE inhibition between 85.17±0.64 % and 65.76 ± 3.89 %, respectively at 200 µg/mL (for galanthamine hydrobromide 72.76±0.82 %) with no AChE inhibition. Further study is ongoing for the isolation of active compounds. This is the first report on the cholinesterase inhibitory activity of the bulbs of Lilium species growing in Turkey.

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INHIBITORY EFFECT OF NEPETA POGONOSPERMA EXTRACTS ON KEY ENZYMES LINKED TO DIABETES MELLITUS AND ALZHEIMER'S DISEASE

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The genus Nepeta (Lamiaceae) consisting of about 400 species globally, and 75 species in Iran, 53 % of which are endemic [1]. Most of the species have been used in folk medicine for treating various disorders including nervous, gastrointestinal, common colds, rheumatism and digestive diseases [2]. One of the endemic Nepeta plants of Iran is Nepeta pogonosperma, that in the present study the inhibitory effect of its decoction, hydroalcoholic (80:20 ethanol:water) and hexane extracts on α-amylase, α-glucosidase, (diabetes mellitus linked key enzymes) acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Alzheimer's disease linked key enzymes) were evaluated. All the extracts were prepared from aerial parts of the plant, collected at flowering stage. According to the obtained results the highest α-amylase and α-glucosidase inhibitory activities were found in hydroalcoholic extract with 1.02 and 1.89 mg acarbose per g plant extract, respectively. Decoction and hexane extract of Nepeta pogonosperma illustrate moderate α-amylase and α-glucosidase inhibitory potential. Also, hydroalcoholic extract and decoction of the plant exhibited the highest inhibition against ACh (1.9 and 1.87 mg galantamine per g extract, respectively). The same preparations showed the highest BCh inhibition with the values of 2.97 and 2.51 mg galantamine per g extract, respectively. The hexane extract of the plant showed moderate to low ACh and BCh inhibitory potential (1.18 and 1.51 mg galantamine per g extract, respectively). These results demonstrate that Nepeta pogonosperma can be considered as a new source of antidiabetic and anticholinesterase in pharmaceutical industry.

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PHENOLIC CONTENT, α-AMYLASE AND α-GLUCOSIDASE INHIBITORY EFFECT OF PHLOMIS HERBA-VENTI AND PHLOMIS OLIVIERI

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The genus Phlomis belonging to Lamiaceae consist of about 100 species in the world. This genus is represented by 17 species in Iran [1]. According to the ethnopharmacological studies several Phlomis species can be consumed as stimulants, tonics and diuretics [2]. Also, antidiabetic, antinociceptive, antiulcerogenic, anti-inflammatory, antiallergenic, antioxidant and antimicrobial properties of Phlomis species have been scientifically demonstrated [3]. In this study total phenolic content, α-amylase and α-glucosidase inhibitory potential of methanolic extracts of Phlomis herba-venti and Phlomis olivieri were evaluated. According to the obtained results methanolic extracts of P. herba-venti and P. olivieri showed 50.3 and 42.1 mg gallic acid equivalents per g extract. Both of the plant species exhibited similar α-amylase inhibition activity, with the value of 0.41 mmol acarbose equivalents per g extract. Also, α-glucosidase inhibition assay showed that P. olivieri has higher antidiabetic properties with the value of 1.69 mmol acarbose equivalents per g extract, than P. herba-venti (1.39 mmol acarbose equivalents per g extract). These findings suggest that Phlomis species (especially P. olivieri) may be useful in the development of an alternative agent for diabetes mellitus.

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THE BIOLOGICAL ACTIVITIES AND ESSENTIAL OIL COMPOSITON OF HYPERICUM TERNATUM

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The genus Hypericum a member of Hypericaceae family, is represented by 100 taxa, 45 being endemic to Turkey [1]. In Turkish folk medicine, the genus Hypericum is known as “sarı kantaron, kantaron, binbirdelik otu, mayasıl otu” and most of them, especially H. perforatum, have been used for the treatment of burns, wounds, hemorroids, diarrhea and ulcers. Aqueous extracts of the flowering aerial parts of the Hypericum species are used in the treatment of neuralgia, anxiety, neurosis and depression [2-3]. In this study, the essential oil content of H. ternatum was analyzed by GC-MS/FID. Additionally, the essential oil H. ternatum was tested for antioxidant (CUPRAC, DPPH free radical scavenging activity and ABTS cation radical decolorisation) and anticholinesterase activities in this study. The dried aerial parts of species were cut into small pieces and subjected to hydro- distillation with water for 4 h, using a Clevenger-type apparatus to produce essential oils which were dried over anhydrous sodium sulphate and stored at +4°C until required. The essential oils were diluted by dichloromethane (1:3, v/v) before the GC run. Identification of the compounds was based on the comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data. The major components of the essential oil were identified as α –pinen, caryophyllene and germacrene D for H. ternatum.The anticholinesterase effect H.ternatum essential oil showed good activity, but it showed low antioxidant activity in all methods.

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ANTI-INFLAMMATORY ACTIVITY OF ALCHEMILLA MOLLIS (BUSER) ROTHM., ALCHEMILLA PERSICA ROTHM. AND THEIR CONSTITUENTS; ELLAGIC ACID AND QUERCETIN-3-GLUCURONIDE

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The genus Alchemilla L. belongs to Rosaceae family and A.vulgaris is the most studied species in this genus. This plant is used for menopausal complaints, gynaecological and gastrointestinal disorders, inflammation, weight loss internally in folk medicine.In addition its used as a gargle for mouth and throat inflammation as well as for wounds and skin disorders such as eczema and rashes, externally [1-3].
Present study is aimed to evaluate anti-inflammatory activities of Alchemilla mollis, Alchemilla persica and their constituents; ellagic acid and quercetin-3-glucuronide (miquelianin).
Extracts of roots and aerial parts of plants were prepared with methanol:water (80:20) solvent system. Anti-inflammatory activity of the extracts and compounds were tested by measuring their human red blood cell (HRBC) membrane stabilizing effects. This results will be presented.

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ASSESSMENT OF ANTI-INFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF NEPETA ISAURICA BOISS. & HELDR. USING IN VIVO MODELS AND ANALYSIS OF ITS PHYTOCHEMICAL COMPOSITION WITH LC-MS/MS

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The genus Nepeta L. (Lamiaceae) is composed of about 400 species which is widely distributed within Central and Southern Europe, North Africa, and Central and Southern Asia [1,2]. In Turkey, the genus Nepeta is represented by 44 taxa and 22 of them are endemic to Turkey [3]. Nepeta (catmint) belongs to the subfamily Nepetoideae, tribe Mentheae and is one of the largest and economically important genera in the Nepetoideae [1,4]. Nepeta species are used in folk medicine as antispasmodic, expectorant, diuretic, antiseptic, emmenagogue, antitussive, antiasthmatic, disinfectant, anticonvulsant, sedative, blood depurative, diaphoretic, analgesic, febrifuge, antifungal and antiviral [2-6]. Nepeta species contain essential oils, monoterpenes, sesquiterpenes, triterpenoids, iridoids, flavonoids, phenolic acids, steroids and their biological properties attributed to their terpenoids, flavonoids and iridoids content [1,7].

Nepeta isaurica Boiss. & Heldr. is one of the endemic species of Turkey which there aren't any comprehensive studies about its phytochemical composition and biological properties [4]. So, we aim to determine the anti-inflammatory and antinociceptive effects of N. isaurica using in vivo models, and phytochemical composition of N. isaurica with LC-MS/MS analysis. For this reason, the MeOH extract obtained from the whole plant was fractionated through subsequent solvent extractions in increasing polarity with n-hexane, chloroform and n-butanol. The MeOH extract and all fractions of N. isaurica were tested for their anti-inflammatory activity using carrageenan-induced hind paw edema model and antinociceptive activity using p-benzoquinone induced abdominal contractions model. Results will be discussed in the presentation.

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ANTIOXIDANT ACTIVITIES AND ESSENTIAL OIL COMPOSITIONS OF ANGELICA SYLVESTRIS VAR. SYLVESTRIS

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Adequate knowledge on the volatile compounds of Angelica sylvestris var. sylvestris (Apiaceae) growing wild in our floristic-rich country is lack. In this study, we aimed to determine the essential oil compositions of roots, leaves, flowers and fruits of the plant by Gas Chromatography (GC) and Gas Chromatography-Mass Spectroscopy (GC/MS), simultaneously. Each essential oil was obtained by hydrodistillation for 3 hours. The possible antioxidant activities of each essential oil were determined by in vitro methods. The major volatile compounds were identified as spathulenol (12.4%), germacrene D (10.6%) and α-humulene (7.6%) in the leaves; α-pinene (23.2%) and β-phellandrene (34.5%) in the fruits; α-pinene (42.0%) and β-phellandrene (25.5%) in the flowers; elemol (5.4%), 10-epi-ɣ-eudesmol (5.4%) and spathulenol (4.8%) in the roots. Among essential oils, the fruit essential oil was found to show radical scavenging activity against ABTS radical which was lower than gallic acid as positive control.

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ANTIOXIDANT, ANTIMICROBIAL AND CYTOTOXIC ACTIVITY OF ESSENTIAL OIL FROM TANACETUM AGROPHYLLUM VAR. AGROPHYLLUM

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The chemical composition of essential oils isolated from the aerial parts by hydrodistillation of Turkish Tanacetum agrophyllum var. agrophyllum was analyzed by GC–MS. The present study is to determine the in-vitro antioxidant, antimicrobial and cytotoxicity activities of essential oils obtained from the aerial parts of T. agrophyllum var. agrophyllum. The antioxidant activity of the essential oil was evaluated by DPPH and ABTS radical scavenging activity. The antimicrobial activity towards five bacterial strains including two gram positive, two gram negative and one yeast was determined using micro-dilution method. Cytotoxicity activity was investigated by MTT assay against breast cancer cell lines (MCF-7, MDA-MB-231). The results show that the EC_{50} values for DPPH and ABTS radical scavenging activity of the essential oil were 2.22 ± 0.03 mg/mL and 2.19 ± 0.009 mg/mL respectively. The essential oil of T. agrophyllum exhibited antibacterial activity against all of the tested microorganisms with MIC value of 5 mg/mL. These results demonstrate that the essential oil prepared from aerial part of T. agrophyllum possesses moderate antioxidant activity and antimicrobial activity. The results of MTT assay showed essential oil of T. agrophyllum var. agrophyllum have in a dose dependent manner cytotoxic effect on breast cancer cell lines, MCF-7 and MDA-MB-231.

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BIOLOGICAL ACTIVITIES AND LC/MS-MS PROFILE OF VERBASCUM ESKISEHIRENSIS ENDEMIC TO ESKISEHIR, TURKEY

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In this study, the aerial parts of Verbascum eskisehirensis were dried in the shade and air-flowed area. The dried samples were macerated with 70% methanol in water (250 mL) for 3 three times during three days. The extract was freeze-dried after methanol removal. The yield of the extract was calculated as 24 g/100 g of the aerial parts. The antioxidant activities (DPPH and TEAC) and antibacterial effects (microdilution) of the extract were evaluated by in vitro. In the same time, the phenolic composition of the extract was determined by LC-ESI-MS-MS system. According to results, the extract was found rich in luteolin derivatives. The major compound was identified as luteolin-pentosyl-hexoside. This compound has never identified in Verbascum species before, but it was recorded in Thymus genus. Other luteolin derivatives identified in the extract were found in Verbascum species by different studies. The antioxidant activities of the extract were lower than Vitamin C. TEAC value of the extract was calculated as 0.184±0.08 mM at 5.6 mg/mL concentration while Vitamin C had TEAC value of 0.869±0.01 mM. The IC_{50} values of the extract and Vitamin C were 176.7 µg/mL and 6.3 µg/mL, respectively. The antibacterial effect against Salmonella typhimurium ATCC 13311 was calculated as 2.5 mg/mL (MIC). The MIC values of the extract against Staphylococcus aureus ATCC 6538 and Staphylococcus aureus ATCC 700699 were >5.0 mg/mL. The extract showed weak antibacterial activity against tested bacteria strains.

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IN VITRO CYTOTOXIC ACTIVITY OF SOME COMPOUNDS OBTAINED FROM SALVIA SPECIES

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The Salvia genus belongs to the subfamily Nepetoideae in Lamiaceae family. The genus consists of about 900 species. Many Salvia species are used as herbal tea and for food flavoring, as well as in the cosmetic and pharmaceutical industries throughout world. Medicinal plants are biologically active materials traditionally used in the treatment of a variety of diseases since ancient times [1]. Salvia species are commonly used in traditional medicine for treatment of more than sixty diseases such as headhache, cough, colds, stomachache, antipyretic, anti-inflammatory [2]. The aim of the our study was to determine the cytotoxic activity of some compounds (Acetyl royleanon, Vanillic acid, Fumaric acid, Ferrulic acid, Ascorbic acid and Salvianolic acid A) obtained from Salvia species by the MTT assay. In this study, human-derived cancer cell series and the Primary Dermal Fibroblasts series were used. For this purpose, the breast cancer cell line (MCF-7), the colon cancer series (HT-29) and the Primary Dermal Fibroblast Series (PDF) were provided. For each cell series, the number of cells to be placed on the platelets was optimized. It was measured the absorbance of the samples using a microplate (ELISA) reader at 570 and 690 nm. It was generally observed that cell viability decreases with increasing concentration.Among the studied six compounds only acetyl royleanone and salvianolic acid A have the cytotoxic activity.

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OBSERVING THE ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENT OF KITAIBELIA BALANSAE BOISS. WITH DIFFERENT EXTRACTION METHODS

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Kitaibelia, is one of the small genera of Malvaceae family represented with 2 species: Kitaibelia vitifolia Willd. and Kitaibelia balansae Boiss. K. balansae is endemic to Turkey and K. vitifolia is endemic to Yugoslavia [1].
In this study, K.balansae was examined for its phenolic content and antioxidant activity. Therefore we decided to study the effects of extraction methods on antioxidant activity. This study has shown the effect of the different extraction methods on antioxidant activity and phenolic content. Air-dried K.balansae herb material was powdered and extracted with 70 % methanol with maceration and soxhlet apparatus. The methanol extracts were than fractioned with ethyl acetate and butanol. All the extracts were investigated using in vitro antioxidant assays. 1,1diphenylpicrylhydrazyl ($DPPH^{\cdot}$), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+\cdot}$) radical scavenging activity and β-carotene bleaching methods were used. Total phenolic and flavonoid content of the extracts were also analyzed [2]. The highest total phenolic (259.780±15.07 mgGAE/gextract)and flavonoid (90.693±1.56 mgCAT/gextract) contents were observed in ethyl acetate fraction of the 70% methanol extract prepared with maceration. This fraction also has shown the highest antioxidant activity in DPPH radical scavenging activity. All the extracts showed the highest level of activity at 1 mg/mL in ABTS radical scavenging activity and no extracts showed as much activity as BHA. In β-carotene bleaching assay no extract was found as active as BHA. As a result the maceration extracts were found to exhibit more potent antioxidant activity than the by Soxhlet extracts.

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IN VITRO ANTILEISHMANIAL ACTIVITY OF BERBERIS VULGARIS GROWING WILDLY IN TURKEY

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The aim of the present study was to determine the in vitro antileishmanial efficacy of Berberis vulgaris collected from Spil Mountain, Manisa, Turkey. The air dried and ground aerial parts of B. vulgaris were extracted with ethanol under stirring at room temperature. The consecutive concentrations of the plant extract (25-100µg/ml) were prepared for in vitro assays. In addition to in vitro antileishmanial activity against Leishmania tropica promastigotes, cytotoxic activity of the plant extract was also measured using WST-1 Cell proliferation assay (1,2). The percentages of parasite inhibition in the presence of B. vulgaris ethanol extract in comparison with glucantime reference group at time interval of 12-72 hours were observed between 88,0 and 100,0 %. The plant extract was found to have cytotoxic activity with 444,81±2,12 µg/ml IC_{50} value. This is the first study that involves the assessment in vitro antileishmanial activity of B. vulgaris which is wildly growing in Turkey. Initial results demonstrated that the ethanol extract of B. vulgaris gave promising results and it could be used as an potential source for isolation of new antileishmanial agents in future.

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IN VITRO ANTIBACTERIAL ACTIVITIES OF VARIOUS PLANT EXTRACTS

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Nowadays, there is an emerging crisis of antimicrobial resistance for microbial pathogens throughout the world [1,2]. According to the results of ethnobotanical investigation in Savaştepe and Kepsut districts (Balıkesir), the plants species which are commonly used among the public were selected for this study [3]. The aim of this study was to examine the antimicrobial activities of seventeen different extracts of fourteen different plants with microbroth dilutions technique against several bacteria. Antimicrobial activities of seventeen extracts against Staphylococcus aureus ATCC 29213, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumonia ATCC 4352, Pseudomonas aeruginosa ATCC 27853 and Proteus mirabilis ATCC 14153 were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations [4]. According to our results most of the compounds showed antimicrobial activity against at least one microorganism with MIC values of 9.75-1250 µg/mL. Aetheorhiza bulbosa (L.) Cass. was showed antimicrobial activity against E. faecalis with MIC values of 9.75 µg/mL. Nevertheless it was found that none of the extracts were showed antibacterial activity against E. coli and P. mirabilis. Overall our study highlighted that plant extracts showed better antimicrobial activity against Gram positive bacteria rather than Gram negative bacteria. The most active extracts to Gram negative bacteria were found Cistus salviifolius L. and Paliurus spina-christi Mill. The obtained results clearly revealed that Aetheorhiza bulbosa exhibited better antimicrobial activity against E. faecalis. Thus, the effects of these extracts represent a promising option for the future development of antimicrobial agents.

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IN VITRO ANTIFUNGAL ACTIVITIES OF VARIOUS PLANT EXTRACTS AGAINST CANDIDA ALBICANS

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Candida species especially Candida albicans, are the leading cause of invasive fungal infections in hospitalized patients and the fourth most common isolates recovered from cases of nosocomial bloodstream infections [1]. Although there are different antifungal drugs used in clinical treatments, resistance against them is growing [2]. In this regard there has been strong interest in the development of effective antimicrobical molecules, especially natural extracts or newly synthesed chemical molecules. According to the results of ethnobotanical investigation in Savaştepe and Kepsut districts (Balıkesir), the plants species commonly used among the public were selected for this study [3]. The aim of this study was to examine the antimicrobial activities of seventeen different extracts of fourteen different plants with microbroth dilutions technique against C. albicans. The plant samples were collected in company with local people in Savaştepe and Kepsut districts of Balıkesir (Turkey). Antimicrobial activities of seventeen different extracts against C. albicans ATCC 10231 was determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations [4]. According to our results except Ballota nigra L. and Aetheorhiza bulbosa (L.) Cass. (tuber) all plant extracts showed antifungal activity with the MIC values of 78-625 µg/mL. against C. albicans. The obtained results clearly revealed that Primula vulgaris Huds. (root) exhibited better antimicrobial activity against C. albicans among the other tested compounds. Therefore, the effects of these extracts represent a promising option for the future development of antimicrobial agents.

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CYTOTOXIC AND ANTILEISHMANIAL ACTIVITIES OF CHRYSOPHTHALMUM DICHOTOMUM BOISS. & HELDR.

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Chrysophthalmum dichotomum Boiss. & Heldr. (Asteraceae), an endemic species to Turkey, is a perennial herb with thickened peduncles and elliptic to oblanceolate leaves that grows in wooded or shrubby valley beds [1]. Until now, no phytochemical data has been reported on this plant. In our previous study, we firstly reported the cytotoxicity of the plant against selected cancer cell lines by Sulforhodamine B assay [2]. Our continuing researches on C. dichotomum, we now aimed to evaluate cytotoxic activity against different cancer cell lines and antileishmanial effects. After extraction with 80 % MeOH of the whole plant, we further fractionated by successive solvent extractions with n-hexane, chloroform and n-butanol. All fractions and MeOH extract were tested on cytotoxic activity against HeLa, H-460, MCF-7, PC-3 and 3T3 by using MTT assay as well as leishmanicidal effects on promastigotes of Leishmania major by in vitro bioassay. MTT assay revealed that the chloroform fraction had significant activities against the tested cancer cells with IC_{50} values ranging from 5.63 to 11.89 µg/ml. According to our leishmanicidal activity results, the chloroform fraction displayed significant activity with IC_{50} value of 8.86 µg/ml. Moreover, the methanol extract and remaining water fractions had moderate activities with IC_{50} values of 55.91 and 56.75 µg/ml. Our results showed that the chloroform fraction of C. dichotomum has significant cytotoxic and antileishmanial effects. This data strongly supports that extensive research should be conducted to isolate and characterize phytochemical constituents responsible for the biological activities.

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PHYTOTOXIC, TOXIC AND INSECTICIDAL ACTIVITIES OF CHRYSOPHTHALMUM GUENERI AYTAC & ANDERB.

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Chrysophthalmum gueneri Aytac & Anderb. (Asteraceae) is an endemic herbaceous plant with linear-lanceolate leaves and slender peduncles that grows around Cirlasun bridge Alanya, Turkey [1]. Up to date, no phytochemical data has been reported on C. gueneri. In our continuing investigation on the genus Chrysophthalmum, the cytotoxic activity of C. gueneri was tested against some cancer cell lines by Sulforhodamine B assay for the first time [2]. Following our studies on C. gueneri, we now aimed to evaluate in vitro phytotoxic, toxic and insecticidal activities of the plant. For this reason, the MeOH extract obtained from the whole plant was fractionated through subsequent solvent extractions in increasing polarity with n-hexane, chloroform and n-butanol. The MeOH extract and all fractions of C. gueneri were evaluated for their biological acitivities using in vitro screening bioassays such as toxicity on brine shrimp lethality and phytotoxicity against Lemna minor as well as insecticidal activity against Rhyzopertha dominica and Tribolium castaneum. According to our results, the n-hexane and chloroform fractions showed significant phytotoxic activities (100 % growth inhibition) at 1000 µg/ml against Lemna minor. Moreover, the MeOH extract and n-butanol fraction have moderate phytotoxic activities with 53 and 46 % of growth inhibition at 1000 µg/ml, respectively. Otherwise, all samples had no toxicity against the brine shrimp. In addition, the remaining water fraction had low insecticidal activity with 20 % of mortality against Tribolium castaneum. Our result exerted that C. gueneri had potential biological activities such as toxicity against brine shrimp, phytotoxic and insecticidal effects.

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IN VITRO ANTIPROTOZOAL EFFECT OF ARTEMISIA LUDOVICIANA NUTT. (ASTERACEAE) ESSENTIAL OIL AGAINST ACANTHAMOEBA CASTELLANII, LEISHMANIA INFANTUM AND TRICHOMONAS VAGINALIS

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Artemisia ludoviciana Nutt. (Asteraceae) is an aromatic, herbaceous, perennial plant and known commonly name as "White Sage", "Black Sage", "Prairie Sage" or "Cudweed Sagewort" [1,2]. A. ludoviciana is traditionally used as an antispasmodic, anthelminthic, antidiarrhoeal, stomachic, hepatic colic, appetizer, regulator of menstruation, antimalaric and antiparasitic efficiancy [3,4]. The chemical composition of the hydrodistilled essential oil obtained from the herb with flowered of A. ludoviciana was analyzed by GC-FID and GC-MS. Results showed that the major components of oil were camphor (40.57±0.77%), 1,8-cineole (25.53±0.21%) and camphene (4.73±0.05 %), among 74 identified compounds, comprising 98.47±0.41 of the total oil. Also, the oil was tested against Acanthamoeba castellanii, Leishmania infantum and Trichomonas vaginalis. In this study, it was demonstrated that A. ludoviciana oil is effective against three important parasites as the first time.

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THE FRUIT ANATOMY OF THE GENUS SCALIGERIA DC. (APIACEAE) IN TURKEY

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The genus Scaligeria DC. is represented with 7 species of which two are endemic in Turkey. These species recorded in Turkish Flora are listed as follows: S. napiformis (Sprengel) Grande, S. tripartita (Kalen.) Tamamsch, S. lazica Boiss. (endemic), S. meifolia (Fenzl) Boiss., S. glaucescens (DC.) Boiss., S. hermonis Post, S. capillifolia Post (endemic) [1]. Traditionally Turkish Scaligera species have been associated with anise, and their local names were given inspired from this similarity both from the morphology and aromatic properties. Thus, Scaligeria species are commonly named using “Kıl anason", "Puslu anason", "Laz anasonu", "Uzun anason" etc. referring to anise [2]. In this research, fruits of Scaligeria species were anatomically studied for the first time. The fruits are globose, smooth; valleculae with 1-3 large vittae in the middle of pericarp; commissures with 2 or numerous, slender, interrupted vittae. Mericarps are two, generally homomorphic, elliptic, half-round, pentagonal or almost round in transverse section. Druse crystals were determined in the endosperms of some Scaligeria species. In addition, sclerenchymatic tissue is found in the commissural surface of the S. napiformis and S. tripartita. Epidermal surface of only S. lazica was pubescent. Interestingly, Turkish Scaligeria species are differ from terms of many aspects of fruits characteristics. Based on fruit anatomical results, we are able to identify and classify the species of this genus.

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ANTIOXIDANT AND CYTOTOXIC PROPERTIES OF MARRUBIUM ROTUNDIFOLIUM BOISS.

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Marrubium L. (Lamiaceae) has 40 taxons in the world. It is represented by 21 taxons, the better part of which are endemic [1,2]. Different Marrubium species, known as “bozotu, bozkaşık, karaderma”, are used traditionally as diuretic, carminative, expectorant in Anatolia [3]. Antioxidant, cyctotoxic, analgesic and anti-inflammatory effects of various Marrubium sp. were reported previously [4,5,6].Marrubium rotundifolium Boiss. is an endemic species and distributed in West Anatolia [7].

By this study total phenolic and flavonoid components, total antioxidant capacity, OH and DPPH radical scavenging activity of methanol, ethyl acetate and n-hexane extracts prepared from aerial parts of plant extracts were investigated. Besides, cytotoxic activities of the same extracts was established by MTT assay.

The highest total phenolic and flavonoid compounds were determined in methanol (2,62 µg/ml GAE) and n-hexane extracts (168,63 µg/ml QAE), respectively. The highest effect was observed with Methanol extract by all of OH and DPPH radical scavenging activities and TEAC assay (EC_{50}: 0.277 mg/ml and 0.033 mg/ml and 3.21 mg/ml, respectively). As to MTT assay, methanol extract showed cytotoxic effect against PC-3 cell line (IC_{50}: 0.173 mg/ml), whereas ethyl acetate had cytotoxicity against CaCo-2 cells (IC_{50}:0.262 mg/ml).

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DETERMINATION OF ISOORIENTIN IN THE LEAVES OF ASPHODELUS RAMOSUS L. BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Reversed-phase high-performance liquid chromatography with a photo-diode array detector (RP-HPLC-DAD) was used for the determination of isoorientin in Asphodelus ramosus L. [1]. Four similar flavonoids [i.e., (+)-catechin, isovitexin, luteolin and Apigenin] which are commonly found in some Asphodelus species were also separated within the same run. Optimum chromatographic conditions were achieved on a Zorbax SB-Aq column (4.6 mm × 150 mm, 5 µm), a linear gradient system starting with 5:95 to 90:10% (%, v/v) ACN/H_2O in 12 min, at a flow rate of 1.0 mL min^{-1}, 25°C, and a sample injection volume of 5 µL. Isoorientin was monitored at 280 nm maximum wavelength. Ultrasound-assisted solid-liquid extraction was performed using 1.0 g of the dried leave samples with 50/50 (%, v/v) MeOH/H_2O within 20 min. The extract was filtered and diluted three times with 45/55 (%, v/v) ACN/H_2O before being injected into the HPLC. Isoorientin was calibrated with its standard prior to its quantitation while the other four flavonoids were not found in the studied plant leaves. Calculations showed that the sample contained 1.24± 0.05% (g/g) of isoorientin. Limit of detection (LOD) and limit of quantitation (LOQ) were found as 0.10 and 0.34% (g/g), respectively. The calibration curve was linear over the dynamic range of 0.34–5.0% (g/g), %RSD (n=7) was lower than 3.3 and coefficient of determination (R^2) was 0.9974. The method was proven to be fast, cheap, inexhaustible and efficient for the extraction of isoorientin from Asphodelus ramosus L.

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A STUDY ON THE BIOLOGICAL ACTIVITIES AND PHYTOCHEMISTRY OF TURKISH MOLTKIA SPECIES

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The genus Moltkia Lehm., is represented by two species; Moltkia aurea Boiss., and M. coerulea (Willd.) Lehm. in Turkey. They are used as food and for medicinal purposes. Hence, we aimed to investigate the in vitro antidiabetic and antioxidant activities of the aqueous, ethyl acetate, and methanol extracts of flowers, leaves and roots of the Moltkia species. α-Glucosidase and α-amylase inhibitory activities, antioxidant activities, total phenolic and flavonoid contents of the extracts were evaluated. Especially, α-glucosidase inhibitory activities of the ethyl acetate extracts were higher than the others'. M. aurea root ethyl acetate extract had the highest total phenolic content (376.53 ± 34.19 mg gallic acid equivalent/g extract) and M. coerulea leaf ethyl acetate extract had the highest flavonoid content (127.46 ± 4.33 mg quercetin equivalent/g extract). High performance liquid chromatography (HPLC) was used to identify the phenolic compounds in the extracts and rosmarinic acid contents were also determined by using HPLC.

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CAN BLACK CURRANT (RIBES NIGRUM L.) BE A POTENTIAL AGENT FOR WOUND MANAGEMENT?

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Ribes nigrum L., usually known as black currant, is an aromatic shrub, evergreen and unarmed. This species naturally grows in East Anatolia [1]. It is widely cultivated because of its fruits which are very popular as food all over the world [2]. According to the IUCN Red Data Book, R. nigrum is evaluated in the vulnerable category in Turkey [3]. Its leaves have been used internally as diuretic and diaphoretic and externally for healing of skin wounds [2, 4, 5]. According to our previous study, methanol extract of R. nigrum leaves was determined to be the most active species among the other eight Ribes species growing in Turkey. Therefore, in the present study we investigated the wound healing potential of the fractions of the methanol extract of R. nigrum leaves. The methanol extract was successively fractionated with dichloromethane, ethyl acetate and n-butanol to obtain three fractions in different polarity. The ointments containing 1% fractions were prepared in order to be applied onto the excision and incision wounds created in the dorsal parts of the mice and rats, respectively [6]. The herbal ointments were topically applied once daily to the animals. The results were compared with the base ointment treated animals. The skin tissues were histopathologically analysed along with the determination of antioxidant status. Among the fractions, ethyl acetate fraction was found to possess healing effect onto skin wounds and can be considered as a potential wound healing agent.

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INVESTIGATION OF WOUND HEALING EFFECT OF EUPHORBIA NICAEENSIS ALL. SSP. GLAREOSA (PALL. EX BIEB) A.R. SMITH VAR. LASIOCARPA BOISS. EXTRACT WITH IN VITRO CELL CULTURE MODEL

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Euphorbiaceae has more than 2000 species and have been used for the treatment of skin diseases, migraine, and intestinal parasites and as wart cures. Some Euphorbia species have effects to treat skin diseases and wounds in Turkey [1-3]. The cytotoxicity test was done with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The wound healing effect of Euphorbia nicaeensis all. ssp. glareosa A.R. Smith var. lasiocarpa Boiss. extract was studied with in vitro wound healing cell culture model on Adult human dermal fibroblast (HDFa). The Euphorbia nicaeensis all. ssp. glareosa A.R. Smith var. lasiocarpa Boiss. extract (7.5, 12.5, 25, 37.5, 50, 65.5 µg/ml) was dissolved in DMSO. Vehicle control group, negative control group, placebo control group and extract group are studied. Cells were fixed in % 4 paraformaldehyde and stained by DAPI at 0 and 24th hours. The apoptotic percentages and viabilities of the cells were determined with the Muse ™ Cell Analyzer [4]. EC_{50} value was found 18.324 ±1.09 µg/ml for leaf and flower extract. When 30 µg extract was applied it was observed wound healing was reached optimum value with 98.94% cell viability. The extract group was significantly decreased the wounds size compared to placebo and vehicle control groups. ($p < 0.001$) at 24th hour.

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CHEMICAL COMPOSITION AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OIL FROM T. AGROPHYLLUM VAR. AGROPHYLLUM ON PROSTATE CANCER CELLS

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The genus Tanacetum is belonged to Asteraceae family, and widespread in Europe and western Asia, comprised of about 200 species. These species traditionally have been used as condiment, salad and for food because of its contained various terpenic compounds which have the biological activiy [1]. In Turkish flora, the genus represented by 44 species and 59 taxa, it contains high amount of essential oils, sesquiterpen lactones as well as small amount of bitter substances [2]. In this study, the essential oil of T. agrophyllum was obtained by hydrodistillation and analyzed by GC (Gas Chromatography) and GC-MS (Gas Chromatography-Mass Spectrometer). According to the GC data, the oil of TA essential oil was dominated by 1,8-cineol (24.4%), α-pinene (10.2%), linalool (9.9%), and linalilacetate (9.9%) as major components. The oil was assessed for its cytotoxicity activity on prostate cancer cell lines (PC-3, DU-145) using the MTT assay [3]. The results demonstrated that, the essential oil prepared from T. agrophyllum var. agrophyllum possesses cytotoxic capacity. Thus, it allows the use of this oil in the pharmaceutical preparation and food industry. According to cell viability assays it was observed that the increasing doses of essential oil reduced the viability of PC-3 and DU-145 cells.

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CHEMICAL PROFILE OF SALVIA SUFFRUTICOSA ETHANOL EXTRACT BY LC-MS/MS

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Salvia L. is one of the largest and the most important aromatic and medicinal genus of the Lamiaceae family which exists approximately 900 species widespread throughout the world. Salvia L. genus is represented with about 98 species in Turkey and half of them is endemic [1]. Salvia species are commonly used in traditional medicine for treatment of more than sixty diseases such as headhache, cough, colds, stomachache, antipyretic, anti-inflammatory. Salvia species are possess high amount rosmarinic acid which has antioxidative, anti-inflammatory, antimutajenic, antimicrobial, antibacterial, antiviral effects [2-4]. Root and aerial parts (stem, leave,flower and seed) of Salvia suffruticosa were collected from Van in flowering period. Phenolic components of the methanol extract was quantified by LC-MS/MS. LC-MS/MS analysis of the phenolic compounds was performed by using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. In the current study, twenty-four phenolic compounds (flavonoids, flavonoid glycosides, phenolic acids, phenolic aldehyde, coumarin) and three non-phenolic organic acids which are widespread in plant materials were qualified and quantified in Salvia suffruticosa. Among 27 compounds, LC-MS/MS study showed that rosmarinic acid, caffeic acid, protocatechuic acid, fumaric acid and malic acid were found to be the more abundant compounds in Salvia suffruticosa. All of the extracts of the studied species showed that fenolic acid contents were higher than flavonoid contents. Traditionally this species have been used as tea depends on the high amount of rosmarinic and caffeic acids as antioxidant potential. So, this species may have positive effects on oxidative stress and neurodegenerative diseases.

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SECONDARY METABOLITES ISOLATION OF SALVIA CERINO PRUINOSA RECH VAR. ELAZIGENSIS

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The Lamiaceae family comprises about 200 genera and 3000 species. One of the largest genus of the family, Salvia L., is represented by over 900 species, and is widely distributed in various regions of the world. Salvia species have been used since ancient times for more than sixty different ailments ranging from aches to epilepsy, and mainly to treat colds, bronchitis, tuberculosis, haemorrhage, and menstrual disorders [1,2]. The main constituents of Salvia species are terpenoids and flavonoids. Their aerial parts contain flavonoids, triterpenoids, and monoterpenes, particularly in the flowers and leaves, while diterpenoids are found mostly in the roots. Ethanol and chloroform extracts of the root and aerial parts of the Salvia cerino-pruinosa Rech var. elazigensis were prepared for isolation studies. Ethanol extracts were found more active in antioxidant activity tests therefore we decided to fractionate the ethanol extract and to isolate the pure secondary metabolites by the preparative layer chromatography method. As yet, we have been isolated five pure secondary metabolites. These pure secondary metabolites are Salvigenin, Beta-sitosterol, 4-OH Benzoic acid, Rosmarinic acid and inuroyleanol. The identification of these compounds has been determined by comparing the ^{1}H-NMR, ^{13}C-NMR and 2D-NMR values of these compounds with those in the literature.

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MORPHOLOGICAL AND ANATOMICAL INVESTIGATIONS OF PIMPINELLA CYPRIA BOISS. (APIACEAE)

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The genus Pimpinella is one of the largest genera within Apiaceae, with approximately 170-180 species [1]. Also the genus is represented by 4 species in Cyprus [2-4]. The seeds of Pimpinella species especially P. anisum, mainly due to its essential oil attributes. This species was cultivated by Romans, Greeks, and Egyptians for their aromatic uses. Many Pimpinella species also have been used in folk medicine as carminative, appetizers, sedative, and agents to increase milk secretion [5]. In this detailed study we aimed to find out morphological and anatomical fruit features of endemic Pimpinella cypria. Carpological characters are very important for the delimitation of genera or species [6]. Diagnostic characters of the fruits of P. cypria can be distinguished morphologycally by the narrowly ovoid-ellipsoid shape, white pillose surface, 3 x c.1 mm striate-ruminate micromorphological feature and anatomically by ovat-depressed shape, two big and 2 small vittae on the commissural side; 20-24 dorsal vittae and 4-6 valecular vittae. The observations were examined using light and scanning electron microscopy.

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ANTIMICROBIAL ACTIVITIES OF POLYGONATUM MILL. SPECIES (ASPARAGACEAE) IN TURKEY

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Polygonatum Mill. (King Solomon's-seal, Solomon's Seal) a genus of ca. 60 species belongs to family Liliaceae. It is distributed in the temperate regions of the East Asia mainly in China and Japan [1]. But recent phylogenetic studies put it in Asparagaceae family [2].
Solomon's seal has been used for thousands of years in herbal medicine. The rhizomes are adaptogenic, antioxidant, cardiotonic, demulcent, diuretic, energizer, hypoglycemic and tonics are used in the treatment of dry coughs and pulmonary problems [4-6]. Also, the antibacterial and antifungal activity of Polygonatum has been reported [7,8].
This study aims to investigate the antimicrobial activities of the several extracts obtained from 5 Polygonatum species (P. glaberrimum K. Koch, Polygonatum latifolium Desf. , P. multiflorum (L.) All. , P. orientale Desf., P. verticillatum (L.) All.) distributed in Turkey. Antimicrobial activity was analyzed using a microdilution assay against several microorganisms [9,10]. According to our results, it is observed that P. verticillatum exhibited significant antifungal activity against C. albicans. Also, P. verticillatum and P. multiflorum showed antibacterial activity against S. epidermidis. To the best our knowledge, evaluation of the some of these Polygonatum species antimicrobial activity is the first of its kind.

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ANTICHOLINESTERASE ACTIVITIES OF ESSENTIAL OILS FROM SOME HYPERICUM SPECIES

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The genus Hypericum a member of Hypericaceae family, is represented by 100 taxa, 45 being endemic to Turkey. Hypericum is known as “sarı kantaron, kantaron, binbirdelik otu, mayasıl otu” and most of them, especially H. perforatum, have been used for the treatment of burns, wounds, hemorroids, diarrhea and ulcers. Aqueous extracts of the flowering aerial parts of the Hypericum species are used in the treatment of neuralgia, anxiety, neurosis and depression [1-3]. In this research, anticholinesterase activities of essential oils obtained from some Hypericum species (H. scabrum, H. lydium, H.pruinatum H.lysimachioides var. spathulatum and H. hyssopifolium var. elongatum) were compared. A spectrophotometric method developed by Ellman et al. was established to indicate the acetyl- and butyryl-cholinesterase inhibitory effects [4]. Aliquots of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL BChE (or AChE) solution were stirred and incubated for 15 min at 25 ºC, then DTNB (10 µL) is added to mixture. In the next step, by the addition of butyrylthiocholine iodide (or acetylthiocholine iodide) (10 µL) the reaction was started. At the end, final concentration of the tested solutions was 200 µg/mL. BioTek Power Wave XS at 412 nm was used to monitor the hydrolysis of these substrates. The experiments were carried out in triplicate. Galanthamine was used as a reference compound. It was determinated that studied species showed high acetylcholinesterase and butrylcholinesterase activity.Among the studied species especially H. scabrum showed high activity than the others in the methods.

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ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF THE EXTRACTS FROM DIFFERENT PARTS OF EUPHORBIA SEGUIERIANA SUBS. SEGUIERIANA

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Euphorbia species are commonly used in Turkish folk medicine for the treatment of rheumatism, swelling as well as a wart remover. However, inflammation and diarrhoea are the two potential side effects that might occur during the treatment [1]. The genus Euphorbia is the largest in the spurge family, comprising about 1100 species in the world [1]. Most of the representative Euphorbia species are characterized by the occurrence of highly irritant latex [1]. Euphorbia species are named as “Sütleğen” and “Xaşîl” [2]. The genus Euphorbia has numbers of biologically active compounds. An increasing attention has been paid to Euphorbia diterpenes because of their diverse structures and therapeutical importance [3]. Root and aerial parts (stem, leave, flower and seed) of E. aleppica and E. Eriophora were collected from Diyarbakır in flowering period. β-Carotene method, ABTS cation radical decolorisation method, cupric reducing antioxidant capacity assay and DPPH free radical scavenging activity were carried out to determine their antioxidant activities. Additionally, the methanol extract these Euphorbia species were tested for anticholinesterase (Acetyl- and butyrylcholinesterase enzymes) activities. The methanol extracts of E. aleppica and E. eriophora showed significant antioxidant and weak butyrylcholinesterase activities.

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CHOLINESTERASE AND TYROSINASE INHIBITORY ACTIVITIES OF THE SECONDARY METABOLITES FROM PHLOMIS FLOCCOSA D. DON.

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The genus Phlomis L. (Lamiaceae) is represented by over 100 species distributed through Europe, Asia, and North Africa and by one species in the flora of Libya, Phlomis floccosa D. Don. Many Phlomis species are used to promote health by protecting especially gastrointestinal and cardiovascular systems in various countries. Moreover, some species of this genus are also consumed for culinary purpose. In this study, the aerial parts of P. floccosa have been investigated phytochemically and isolated compounds were subjected to high-throughput screening against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (TYR), the fundamental enzymes associated with the pathology of Alzheimer's and Parkinson's diseases. P. floccosa was collected during flowering stage from El-Marj of Libya in March 30th, 2016. Employing a series of chromatographic studies, three iridoid glycosides, e.g. lamiide (PF-1 & PF-9), ipolamide (PF-5), and auroside (PF-6), three phenylethanoid glycosides, e.g. verbascoside (PF-4 & PF-8), forsythoside B (PF-3), alyssonoside (PF-7), and one flavonoid glycoside, e.g. luteolin-7-O-glucuronide (PF-2 & PF-10) were isolated from the ethanol extract of this plant. Structures of the isolated compounds were elucidated by means of spectroscopic methods, UV, 1D NMR (^{1}H NMR and^{13}C NMR, DEPT-135) and 2D NMR (COSY, HSQC, and HMBC). Cholinesterase and TYR inhibitory potential of the isolated compounds was tested at 1 mg mL^{-1} stock concentration using ELISA microplate reader. The compounds were found to have low inhibition against BChE (between 8.77±0.97 % - 28.05±3.48 %), while no inhibition was observed against AChE and TYR.

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SEASONAL VARIATIONS OF PHENYLETHANOID GLYCOSIDES IN SCUTELLARIA SALVIIFOLIA

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Scutellaria salviifolia Bentham (Lamiaceae) is one of the endemic plants of Turkey [1]. Some of Scutellaria species are used as tonic, wound healing and hemostatic in Anatolia [2]. Phytochemical investigations on the genus were resulted isolation of flavonoids, iridoids, phenylethanoid glycosides, diterpenoids, triterpenoids, alkaloids and essential oils [3]. These herbaceous, greenish herbs flower in May-August [1]. In our previous study, S. salviifolia was collected from Ankara, Beynam Forest in October when plant is in fruiting period (Sample A). As a result of repeated column chromatography on phenylethanoid-rich fractions of aqueous extract, acteoside, teucrioside, leucosceptoside A and martynoside were isolated as main phenylethanoid glycosides [4].
To investigate the composition variation of phenylethanoid glycosides in different vegetation time, the plant sample was also collected from Ankara, Mamak/Kıbrıs Village in June, when the plant is in flowering period (Sample B). The same extraction and separation procedures were applied to the sample B and only martynoside was isolated as main phenylethanoid glycoside. Isolated pure compounds from sample A were used as standards in HPLC-DAD system for further comparison. The comparative studies showed the presence of trace amounts of acteoside and leucosceptoside A, while martynoside as a major component of Sample B. This result indicated that the diversity and amounts of phenylethanoid glycosides showed alteration, depended on the vegetation time in Scutellaria salviifolia and they have reached the maximum level during the fruiting period.

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ANTIOXIDANT AND ANTI-LIPOXYGENASE ACTIVITIES OF DIFFERENT EXTRACTS FROM AERIAL PARTS OF CENTAUREA SALICIFOLIA GROWN IN TURKEY

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In this study, total phenolic content, antioxidant and anti-inflammatory activities of hexane, chloroform, and aqueous methanol fractions of methanol extract of Centaurea salicifolia were evaluated by Folin-Ciocalteu, DPPH/ABTS and lipoxygenase inhibition assays, respectively.
Aqueous methanol fraction of methanol extract showed the highest antioxidant activity in DPPH and ABTS assays with IC_{50} values of 0.20 and 0,07 mg/mL, followed by chloroform fraction (0,27 and 0,10 mg/mL), and hexane fraction (13,90 and 0,28 mg/mL), respectively.
The highest total phenol content was found in the chloroform fraction of methanol extract from Centaurea salicifolia (167,6 mg of GAE/g of dried extract).
Chloroform fraction at a concentration of 156 µg/mL showed the highest anti-lipoxygenase activity with inhibition rate of 48,06%, followed by aqueous methanol fraction (38,22%), and hexane fraction (29,67%).
The results obtained in the present study indicate that chloroform fraction and aqueous methanol fraction can be a potential source of anti-inflammatory and antioxidant agents.

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COMPARISON OF ANTIOXIDANT ACTIVITIES OF ETHANOL EXTRACTS FROM CERASUS MICROCARPA FRUITS AT DIFFERENT RIPENING STAGES

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The genus Cerasus belonging to the Rosaceae family is represented by 15 taxa in Turkey and are known with the local names “kiraz”, “visne”, “mahlep”, “yabani kiraz”, and “dag kirazi”.In traditional medicine,they are used for the treatment of menstrual disorders,prostate diseases,urinary tract infections,kidney disease (kidney sand,kidney stone,nephritis),respiratory tract diseases (dyspnea,cold and cough), also as antipyretic,tonic,diüretic,antihypertensive,antidiabetic,expectorant.

In this study,antioxidant activities of ethanol extracts of Cerasus microcarpa fruits collected at different ripening stages, with different colours (yellow, red and purple) were investigated against DPPH and ABTS radicals.Their total phenolic contents were determined by Folin-Ciocalteu method and the results were expressed as mg of gallic acid equivalent per g dry extract.Yellow fruits had the highest DPPH radical scavenging activity with a IC_{50} value of 0,97 mg/mL, followed by purple fruits (1,02 mg/mL), and red fruits (1,23 mg/mL).Yellow fruits had the highest ABTS radical scavenging activity with a IC_{50} value of 0,17 mg/mL followed by purple fruits (0,28 mg/mL), and red fruits (0,32 mg/mL). When the total phenol contents of the extracts are compared, the highest amount of phenolic content was found in yellow and purple fruits (18,29 and 18,49 mg/g, respectively), followed by red fruits (12,48 mg/g).

The results indicate that the ripening stage plays an important role on antioxidant activity and total phenolic content of C. microcarpa fruits. Also, these results show that phenolic compounds may be responsible for the activity of the yellow fruits with high antioxidant activity.

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INHIBITORY EFFECTS OF THE EXTRACTS AND FRACTIONS OBTAINED FROM THYMELAEA MICROPHYLLA ON THE GROWTH OF RAT BRAIN C6 GLIOMA AND HUMAN CERVICAL CARCINOMA CELLS

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Cancer is one of the major diseases of our time. It is estimated that by 2030, there will be 22.2 million new cases of cancer and 12.7 million cancer-related deaths worldwide [1]. The complicacy of this disease necessitates the development of new therapeutic agents. Plants have been used in the treatment of cancer for years and have been a useful source of approved anti-cancer drugs. In this study, the extracts and fractions of Thymelaea microphylla Coss. et Dur. were tested for their inhibitory effect on rat brain C6 glioma and human cervical carcinoma (HeLa) cell lines by BrdU ELISA assay at the concentration of 5-100 µg/mL. In general, the fractions obtained from the 50% CH_2Cl_2-MeOH extract exhibited remarkable inhibitory effect against C6 cell line and dose dependent effect on HeLa cells. Notably, F6 (IC_{50}= 17.7 ± 0.1 µg/mL) was found to be more potent than 5-Fluorouracil (5-FU, an anticancer drug) at 100 and 75 µg/mL against C6 cell line. On the other hand, 70% MeOH:H_2O extract and its fractions exhibited higher inhibitory effect against HeLa cells than that of 50% CH_2Cl_2-MeOH and its fractions. B-W4 (IC_{50}= 17.7 ± 0.1 µg/mL) exhibited a powerful effect against HeLa cells. In this study, we have determined the biologically active fractions especially F6 and B-W4 on the growth of C6 and HeLa cell lines. Further studies are needed to isolate and identify the compounds from these active fractions and also to evaluate in vivo biological activities of the isolated compounds.

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THE EFFECT OF BOILING TIME ON THE ANTIOXIDANT ACTIVITY OF THE VARIOUS EXTRACTS FROM ORIGANUM ACUTIDENS

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In this study, the effect of boiling time on the antioxidant activities of four extracts from Origanum acutidens (Hand.–Mazz.) Ietsw. was evaluated. The aerial parts of O. acutidens were dried and powdered. The sample was boiled with distilled water (1 L) for 1 and 2 hours, filtered, and extracted with ethyl acetate (EA) and n-butanol (n-BuOH). Finally, the plant residue was extracted with 1:1 (v/v) methanol/chloroform (MeCh). According to the results, the boiling time has affected the antioxidants activities of the extracts. Total phenolics contents of the extracts increased by two hours boiling time. The extracts prepared by boiling for 2h showed higher radical scavenging effect than that of 1h. The order of EA (2h) > EA (1h) > n-BuOH (2h) > n-BuOH (1h) > water (2h) > water (1h) > MeCh (2h) > MeCH (1h). The reason for this increase in radical scavenging ability could be due to the higher phenolic content in the 2h boiled extracts. On the other hand, in metal chelating activity assay, 2h boiled extracts showed lower chelating activity than that of 1h. This could be due to antioxidants other than polyphenols may be defective upon boiling.

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ANTIOXIDANT ACTIVITIES OF THE FRACTIONS FROM BUTANOLIC EXTRACT OF BERBERIS HISPANICA

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Berberis hispanica Boiss. & Reuter is a deciduous shrub belonging to the family Berberidaceae. The infusion of the stem bark of this plant has been used in traditional medicine to treat the gastro-intestinal affections, inflammation, liver and biliary disorders [1]. In this study, antioxidant activities of six fractions from n-butanol extract of B. hispanica were evaluated by DPPH scavenging, metal chelating and total phenolic content assays. The highest free radical scavenging was observed for fraction T36 (IC_{50}<5 µg/mL) and this fraction exhibited higher DPPH scavenging activity than the synthetic antioxidant BHT (IC_{50}=23.14 ± 0.16 µg/mL). Among the fractions, T26 (IC_{50}= 2.36 ± 0.06 mg/mL) showed the highest metal chelating effect. On the other hand, the highest total phenolic content was found in fraction T36 (442.5 µg/mg). The content of total phenolic compounds in the tested fractions ranged between 14.3 to 442.5 µg/mg. A significant correlation (R=0.945, p<0.01) was observed between total phenolic content and DPPH scavenging activity of the fractions that indicating phenolic compounds were primarily responsible for this activity. This study supports the documented medicinal effects of B. hispanica and opens up the possibilities of pharmaceutical applications.

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EVALUATION OF ANTIOXIDANT PROPERTIES OF THE EXTRACTS AND FRACTIONS FROM ENDEMIC DESERT SPECIES THYMELAEA MICROPHYLLA COSS. ET DUR.

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Thymelaea microphylla Coss. et Dur. is an endemic plant of Algeria desert. This species has been used in folk medicine for the treatment of wounds and various cutaneous conditions such as erysipelas, abscess, and pimples [1]. The aim of this study was to investigate the antioxidant activities of the extracts and fractions from T. microphylla by using DPPH radical scavenging, metal chelating, and total phenolic contents assays. Two extracts were prepared from the aerial parts of T. microphylla by sequentially extracting the dried plant material with a mixture of 50% (v:v) CH_2Cl_2:MeOH and 70% (v:v) MeOH:H_2O as solvent systems. Chromatographic fractionation of the extracts on silica gel column afforded the tested fractions. The fractions obtained from the 50% CH_2Cl_2-MeOH extract are exhibited higher radical scavenging activity than the fractions obtained from the 70% MeOH-H_2O extract. Moreover, fraction F5 from 50% CH_2Cl_2-MeOH (IC_{50} = 12.4 ± 0.1 µg/mL) had higher radical scavenging ability than the synthetic agent BHT (IC_{50} = 22.7 ± 0.6 µg/mL). F5 also showed the highest phenolic content (293.67 ± 0.12 µg/mg). On the other hand, 70% (v:v) MeOH:H_2O extract and its fractions exhibited remarkable metal chelating effects. There was no significant ($p > 0.01$) difference in the chelating activities between synthetic chelating agent ethylenediaminetetraacetic acid (EDTA) (93.1 ± 0.4%) and B-W6 (93.3 ± 0.4%) obtained from 70% MeOH-H_2O extract at the concentration of 1 mg/mL. The results showed that T. microphylla could be used as a natural antioxidant agent in food and pharmaceuticals industries.

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CYTOTOXIC COUMARINS FROM THE ROOTS OF PETROEDMONDIA SYRIACA (BOISS.) TAMAMSCH.

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Petroedmondia syriaca (Boiss.) Tamamsch. (Apiaceae) is a recently described monotypic genus that has close relation to both genus Heptaptera and genus Smyrniopsis [1,2]. While genus Heptaptera is a rich source of sesquiterpenoid coumarins genus Smyrniopsis mainly yields furanocoumarin esters [3,4]. These compounds have important biological activities such as antibacterial, antiviral, antileishmanial, anti-inflammatory, P-glycoprotein inhibitory and cytotoxic activities [5].
Previously we have reported cytotoxic activity of the roots of P. syriaca on MCF-7, COLO205, KM12 cell lines and the compounds namely; scoparone, psoralen, bergapten, marmesin, marmesin acetate, 4'-acetyl-3'-isobutyroyloxymarmesin, deltoin, smyrnioridin, colladonin and 14-acetoxybadrakemin from the active dichloromethane extract [6,7].
The roots of P. syriaca were collected from Şanlıurfa province of Turkey in June 2013. Coarsely powdered roots of the plant were sequentially extracted at room temperature with dichloromethane and methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to dryness. Methanol extract was dissolved in methanol/water (10:90) and then partitioned with ethyl acetate, the resulting extracts were separately concentrated in vacuo to dryness. Cytotoxic activities of the extracts and isolated compounds were investigated using the MTT assay on 3T3 cell line.
The highest activity was found in the dichloromethane extract of the roots. Among the isolated compounds colladonin and deltoin showed the highest activity with IC_{50} values of 8.8 and 15.6 uM respectively.

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ISOLATION AND PURIFICATION OF COUMARIN DERIVATIVES FROM FRUITS OF SESELI DEVENYENSE SIMONKAI. BY HIGH-PERFORMANCE COUNTER-CURRENT CHROMATOGRAPHY

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The genus Seseli (Apiaceae) is a well-known source of the coumarin derivatives, and it contains numerous species that have been used in folk medicine since ancient times. Many biologically active compounds have been identified in different members of the genus and coumarin derivatives seem to be the most important.
One of the representative - Seseli devenyense Simonkai, is a herbaceous perennial plant widespread in Eastern and Central Europe. It grows on rocks, sunny, rocky hillsides in shallow skeletal, nutrient-rich soils or in rock crevices, mostly on limestone and Tertiary igneous rocks. As rare coumarin derivatives have been identified so far, it is important to elaborate the efficient method of their isolation. Thus a preparative high-performance counter-current chromatography (HPCCC) method was successfully applied for isolation of natural coumarin derivatives. Compounds were obtained from methanolic extract of fruits and their identification was performed with NMR and MS methods. Different mixtures of heptane, ethyl acetate, methanol and water were tested. After injection of crude extract, devenyol, (+)-octanoyllomatin, (+)-cis-khellactone, laserpitin and disenecionyl cis-khellactone were obtained in the pure form in single step separations in less than 40 minutes. Optimal conditions of separation were established using analytical coil and then they were transferred to semi-preparative 137 ml. This development makes an opportunity towards large scale isolation of biologically active compounds from S. devenyense or other plant species abundant in coumarin derivatives.
The work was financed from grant No 4/POLTUR-1/2016.

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THE EFFECT OF PORTULACA OLERACEA L. ON IMMUNE PARAMETERS AND ANTIOXIDANT SYSTEM

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Goal of this research is the study of influence of the Portulaca oleracea L. (purslane)tea on immune status and on antioxidant system of an organism. The research was held in a private clinic 'Nargiz' in Baku, which specialized in herbal treatment of different pathological diseases. In this research, bloods of 52 patients were examined: 33 women and 19 men aged from 16 to 58 years old. Changes in immune parameters were seen in all patients. All patients were given three times a day 100 ml of Portulaca tea during one month. Venous blood of patients was taken before and after treatment for analysis. Immune parameters like leucocytes, neutrophils, eosinophils, lymphocytes, monocytes, erythrocyte sedimentation rate, phagocytic activity of leucocytes, level of immune complexes, level of Ig A, M, G and Ig E were examined. Amount and activity of components of antioxidant system – thiol status, activity of glutathione reductase, glutathione peroxidase were studied by immunoassay method with the aim of determination of antioxidant activity of Portulaca oleracea. As a result of study noticeable positive improvement in immune parameters – decrease of level of leucocytes by 11%, increase of phagocytic activity by 23%, increase of amount of immunoglobulins (A, M, G) respectively by 4, 12 and 16% was observed. Decrease of level of Ig E by 8% was seen. Also noticeable increase of potency of antioxidant system was determined: thiol status increased by 42 % and activity of glutathione reductase increased by 17%. Thus, results show noticeable antioxidant and immunomodulating effect of portulaca.

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CHARACTERIZATION OF THE BIOLOGICAL ACTIVITY AND PHENOLICS IN ACHILLEA NOBILIS L. SUBSP. NEILREICHII (KERNER) FORMANEK

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The genus Achillea L., belongs to family Asteraceae is widely found in Europe, temperate areas of Asia and in North America. It contains phenolic compounds (terpenoids and flavonoids) responsible for biological activity. Achillea nobilis L. subsp. neilreichii (Kerner) Formanek is a member of the genus Achillea and is used in the treatment of diabetes, hemorrhoids and eczema in Anatolia [1,2].

The aim of this study was to investigate phenolic composition and compare biological activities of different extraction methods and solvents of A. nobilis subsp. neilreichii aerial parts. The total phenolic contents, antioxidant, anti-urease and antimicrobial activities of A. nobilis subsp. neilreichii extracts obtained by ultrasonic bath, Soxhlet and maceration extraction techniques were compared. The extracts were quantitatively analyzed for total phenolic contents using spectrophotometric methods. Antioxidant activities were measured using the DPPH, ABTS and FRAP assays. The urease inhibitory activity was determined according to a reported method [3] and antimicrobial activities were investigated using the disc diffusion and microdilution methods [4].

The ethanol and ethyl acetate extracts obtained by maceration extraction method exhibited the strongest DPPH, ABTS scavenging and FRAP activities. The n-hexan Soxhlet (30.54%) and ethyl acetate ultrasonic bath (28.05%) extracts exhibited the strongest anti-urease activity, respectively. The ethyl acetate and chloroform extracts by Soxhlet extraction method have stronger antimicrobial activity than other extracts. Subsequently, we tried to identify the phenolic compounds in the ethanol and ethyl acetate extracts by HPLC-DAD/ESI-Q-TOF LC/MS.

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COMPOSITION OF THE ESSENTIAL OILS OF SALVIA XANTHOCHEILA AND S. TRICHOCLADA FROM TURKEY

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The Salvia L. genus belongs to the subfamily Nepetoideae in Lamiaceae family. The genus consists of about 900 species [1]. Many Salvia species are used as herbal tea and for food flavoring, as well as in cosmetic, perfumery and the pharmaceutical industries throughout World [2]. Salvia species are generally known for their multiple pharmacological effects including their antibacterial, antiviral, antioxidative, antimalarial, anti-inflammatory, antidiabetic, cardiovascular, antitumor and anticancer activities [3]. Also, some studies showed that a part of these activities depend on their essential oil composition [4,5]. In this study, composition of the essential oils of Salvia xanthocheila and S. trichoclada were analyzed by GC-MS/FID. The dried aerial parts of species were cut into small pieces and subjected to hydro- distillation with water for 4 h, using a Clevenger-type apparatus to produce essential oils which were dried over anhydrous sodium sulphate and stored at +4°C until required. The essential oils were diluted by dichloromethane (1:3, v/v) before the GC run. Identification of the compounds was based on the comparison of their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as the literature data. The major components of the essential oils were identified as caryophyllene, germacrene D and (-) spathulenol for S. xanthocheila and caryophyllene, caryophyllene oxide and bornyl acetate for S. trichoclada.

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CHARACTERIZATIONS OF ESSENTIAL OIL OF TWO SMYRNIUM SPECIES

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Smyrnium genus, plants family name Apiaceae, is biennal plant growning in western and southern Europe, in coastal areas of British Islands and Mediterranean region. Smyrnium which has 38 species in world distribution, includes 6 taxa in the Turkish flora [1,2]. Smyrnium olusatrum L. roots, also known as Alexander, were used in medicine as antiscorbutic, appetite stimulant, diuretic and laxative properties, the fruits as stomachic and ashtmatic and stems as depurative, clearing the blood and preventing scurvy [3]. In the present work, essential oils (EOs) of Smyrnium olusatrum and S. connatum were obtained by hydrodistillation and chemical compositions were analyzed by GC-MS and GC-FID. S. olusatrum EO contained in 1-β-acetoxy-8,12-epoksi-4,7,11-eudesmatrien (40.0%), β-phellanderene (13.4%), α-pinene (12.1%), myrcene (6.3%), isofuranogermacrene (5.2%), β-pinene (2.8%), limonene (2.4%), furano-4(15)-eudesmen-1-one (2.1%), α-cadinol (1.0%). 1-β-Acetoxy-8,12-epoksi-4,7,11- eudesmatrien (68.9%), germacrane D (3.7%), spathulenol (2.1%) and 1,5-epoksi-spathulenol-(14)-ene (1.7%) were found as main constituents of S. connatum EO.

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ISOLATION AND PURIFICATION STUDIES ON HYPECOUM PROCUMBENS L. SUBSP. ATROPUNCTATUM A.E.DAHL (HH) AND HYPECOUM PSEUDOGRANDIFLORUM PETROVIC TAXA DISTRIBUTION IN WESTERN ANATOLIA

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Hypecoum L. belongs to the Papaveraceae family and is represented in 7 taxa in Turkey and most of them are spreaded in Western Anatolia. There have been some chemical and activity studies on the members of this family, and it has been seen that there are no extensive studies on this subject. Hypecoum species include alkaloids (isoquinoline, benzylisoquinoline, spirobenzylisoquinoline and benzophenanthridine groups), flavonoids and terpenic compounds as active ingredients [1-5]. In this work, Hypecoum procumbens L. subsp. atropunctatum A.E.Dahl (HH) which is collected from Izmir Homeros Valley in March 2016 and Hypecoum pseudograndiflorum Petrovic (HE) which is collected from Selçuk in April 2016, are investigated. Ethanol, methanol and chloroform extracts of these taxa were obtained by ultrasonic water bath and maceration methods. The chemical profiles of the extracts were investigated by TLC. As a result of further purification and isolation studies of HE with extract (HEM-ON) obtained with methanol with maceration; 20-35 fractions of the RP-11 (86-350) fraction (HP-1, 9mg), RP-12/14 150-160 fraction (HP-2, 10 mg), RP-3/5 (44-58) fraction of RP-11 (86-350) 70-84 fractions (HP-3, 11 mg) and RP-3/5 250-260 fractions (HP-4, 20 mg) were obtained in pure form and characterized by NMR and LC/MS-MS. A phenyl propanoate, a triterpene, a phytosterol and a phenylethanoyl glycoside have been described. In the next steps of our continuing studies, it is planned to carry out bioactivity studies by identifying bioactive groups and following advanced purification steps by performing purification and identification procedures with device combinations.

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ANTIDIABETIC ACTIVITY OF PRUNUS MAHALEB L. KERNELS IN VITRO

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In Turkey, kernels of Prunus mahaleb L. (syn. Cerasus mahaleb L. Mill.) are widely used as herbal medicine for diabetes and hyperlipidemia in Central Anatolia [1]. Unfortunately the scientific studies on this area are not sufficient to prove these activities. According to the former data of in vitro cytotoxicity assay, the kernels are known to be safe [2]. This study is aimed to investigate the effect of the extracts on diabetes related enzymes to determine antidiabetic activity. After obtaining the methanolic extract by continuous extraction with methanol of the kernels, it is prefractionated by n-hexane, n-buthanol, ethyl acetate. These fractions were separately used to assess any in vitro antidiabetic activity by measuring their effect on α-amylase and α-glucosidase enzymes. The ethyl acetate extract of Prunus mahaleb L. kernels exhibited a dose-dependent increase in the percentage and had the most potent inhibitory activity on α-glucosidase enzymes (IC_{50} 75.53 ± 1.35 µg/ml) . Acarbose was used as a positive control. Due to the phytochemical studies the compounds isolated from the P. mahaleb kernels were determined as cis-melilotoside and 4-O-β-glucopyranozyldihydroferulic acid and their structures were elucidated on the basis of NMR and MS analyses. These compounds were also first reported from the genus Prunus.

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ANTIOXIDANT AND WOUND HEALING POTENTIALS OF PLANTAGO HOLOSTEUM SCOP.

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The genus Plantago (Plantaginaceae) is represented by 21 species in Turkey [1]. Several effects are described for the genus Plantago such as antitumoral, anti-inflammatory, antifungal, antibacterial, analgesic, antispasmodic, antiviral and hepatoprotective [2]. Plantago species are known not only as a food plant, but also an old medicinal plant that has been used for wound healing, treatment of diabetes, urinary infections, constipation and hemorrhoids in Anatolia [2]. Wound healing consists of different stages of inflammation, proliferation and remodeling stage. In this study; antioxidant, proliferative and protective effects of aqueous extract were investigated against H_2O_2 damaged L929 murine fibroblasts to understand wound healing properties of P. holosteum. As a result of our study, aqueous extract of P. holosteum (PHE) showed radical scavenging activity against DPPH, NO, SO and ABTS radicals comparable to that of known antioxidants BHA and ascorbic acid while PHE did not increase the proliferation of the fibroblast in the concentration range of 10-200 µg/mL. In the case of protective effect of PHE against H_2O_2 injury, cytotoxicity of H_2O_2 was found dose-dependent in L929 fibroblasts. While pre-incubation with the extract protects fibroblasts against oxidative damage of H_2O_2, incubation with the extract after H_2O_2 application did not repair the oxidative injury. These results show correlation with the antioxidant potential of PHE. Our study on P. holosteum will continue to investigate other parameters of wound healing in different cell lines.

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ANTIOXIDANT PROPERTIES OF SOME COUSINIA SPECIES

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Asteraceae is an exceedingly large and widespread family of flowering plants. Cousinia, is one of the largest in central and south west Asia, with 600- 700 species [1]. There are 38 species and 6 section of Cousinia genus in Turkey. In this study C. davisiana Hub.-Mor., C. foliosa Boiss. & Bal., C. ramosissima DC., C. stenocephala Boiss, C. ermenekensis Hub.-Mor., C. aintabensis Boiss. et Hausskn. were examined. In this study the phenolics and flavonoid content and in vitro antiradical and antioxidant activities of methanol, butanol, water and ethyl acetate extracts of C. davisiana, methanol extracts of C. ramosissima, Cousinia ermenekensis, Cousinia aintabensis were determined by using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), 2,2-diphenyl-1-picrylhydrazyl radical scavenging and inhibition of lipid peroxidation using the beta-carotene bleaching method. The highest total phenolics content was observed in butanol extract of C. davisiana (242,811±12,89 mgGAE/g) and the highest flavonoid content was observed in ethyl acetate extract of C. davisiana (131,265±2,14 mgGAE/g). The methanol extract of C. ramosissima has shown the highest antioxidant activity (IC_{50} of 0,1305 mg/mL) for DPPH. The butanol extract of C. davisiana has shown the highest antioxidant activity (IC_{50} = 0,7947 mg/mL) for ABTS and inhibition of lipid peroxidation. To conclude, this research can be considered as the first detailed document regarding the in vitro antioxidant activity of Cousinia species. The results showed that the butanol extract of C. davisiana had the highest level of antioxidant activity than other Cousinia extracts and none of extracts is not active as BHA.

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SECONDARY METABOLITES ISOLATION OF ETHANOL EXTRACT OF SALVIA CERINO PRUINOSA RECH. VAR. CERINO PRUINOSA

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Higher plants are solar powered biochemical factories of extractable bioactive secondary metabolites [1]. Studies on plant secondary metabolites have been increasing over the last 50 years. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals [2]. Salvia is an important genus consisting of 900 species in the family Lamiaceae and some species of Salvia have been cultivated worldwide for use in folk medicine and for culinary purposes [3]. Members of Salvia genus produce many useful secondary metabolites including terpenes and phenolics and their derivatives that have been in the center of pharmacopoeias of many countries [4] Ethanol and chloroform extracts of the root and aerial parts of the Salvia cerino pruinosa Rech var. cerino pruinosa species were prepared for isolation studies. Ethanol extracts were found more active in antioxidant activity tests therefore we decided to fractionate the ethanol extract and isolate the pure secondary metabolites by the preparative thin layer chromatography method. As yet, we have been isolated thirteen pure secondary metabolites. These pure secondary metabolites are Stigmasterol, Benzoic acid, Apigenin, Caffeic Acid, Lupenone, Savianolic acid A, Salvianolic acid B, 4-OH Benzoic acid, Ferruginol, Ursolic acid, Oleanolic acid, Beta-sitosterol, and Bis(2-ethylhexyl) benzene-1,2-dicarboxylate.

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ANTI-INFLAMMATORY EFFECT OF CENTAUREA CALOLEPIS BOISS. AGAINST CARRAGEENAN-INDUCED PAW EDEMA IN RATS

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Centaurea L. (Asteraceae) is represented by 158 species in Turkish Flora, 94 of which are endemic [1]. It is used for expectorant, digestive, antidiarrheal, antipyretic and wound healing effects in Anatolian traditional medicine [2]. Antioxidant, cytotoxic and anti-inflammatory activities of different Centaurea species have been reported previously [3,4]. C. calolepis Boiss. is an endemic taxon, distributed in West and South-West Anatolia. In a previous study, chloroform extract of plant showed strong anti-inflammatory activity by in-vitro assays [4]. By this work; in-vivo anti-inflammatory activity of Centaurea calolepis chloroform extract (12.5, 25 ve 50 mg/kg) was investigated by carrageenan induced-paw edema assay.

Edema increasing was 10.59 % and 7.82 % in 12.5 and 50 mg/kg of extract, respectively at 1/2 hour (Indomethacin: 10.03 %). In first hour, paw volume increased 9.97 % at 12.5 mg/kg of extract, while 19.17% was established for indomethacin. At the end of 4 h, edema disappeared completely in all doses, whereas edema increasing by standard agent indomethacin was 16.01 %.

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BIOLOGICAL ACTIVITY STUDIES ON THE AQUEOUS METHANOL EXTRACT OF ANCHUSA UNDULATA L. SUBSP. HYBRIDA (TEN.) COUTINHO

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The genus Anchusa L. (Boraginaceae) is represented by 15 species in the flora of Turkey. Anchusa species are used traditionally as wound healing and diuretic agent. In addition, some Anchusa species are used as demulcent, expectorant, analgesic, sedative, diaphoretic and antihypertensive purposes [1]. Carminative, antidiabetic, hemostatic and antirheumatical usage is recorded also [2]. The aim of this study is to determine in vitro antioxidant, anti-AChE, anti-BuChE and α-glucosidase activity of methanol extract (Methanol: water = 80:20, v/v) prepared from roots and aerial parts of Anchusa undulata L. subsp. hybrida (Ten.) Coutinho. Antioxidant activity was evaluated using ABTS and DPPH radical scavenging assay, total phenolic and total flavonoid content. IC_{50} values for DPPH were 239.47 and 292.04 µg/mL and for ABTS were calculated as 41.15 and 32.3 µg/ml. The total phenolic content of root and herbal extract were found as 130.39 and 122.94 mg/g, while flavonoid content as 65.25 and 117.5 mg/g. In the enzyme inhibition studies, the methanol extract showed cholinesterase and α-glucosidase enzyme inhibition activities with the increasing concentrations. The significant enzyme inhibition activities are depended on higher amount of phenolic compounds.

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SCREENING OF SELECTED PLANT EXTRACTS, FATTY OILS AND VOLATILE OILS FOR THEIR NEURODEGENERATIVE DISORDERS

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The aim of this study is to take attention to oral or topically used medicinal plants and plant oils in terms of alzheimer and demantia disorders. Moreover this study will help us to create resources for the discovering new drugs or drug components for neurodegenerative disorders. The acetylcholinesterase (AChE)/butyrylcholinesterase (BChE) inhibition assays of nonpolar/polar plant extracts, fixed and essential oils were evaluated by Ellman method [1, 2]. Galanthamine hydrobromide was used as positive control. Determination was conducted in triplicates and the IC_{50} values were obtained from dose-effect curves by linear regression. The percentage inhibition of enzyme activity was calculated. It was found that Thymi aetheroleum has the highest AChE inhibition activity (92.31 µg/mL), and grape seed oil has the highest BChE inhibition activity (232.18 µg/mL).The most effective plant extract was found to be methanolic and aqueous extract of Lycium barbarum (AChE IC_{50} 256.56 and 281.412 µg/mL; BChE IC_{50} 338.01 µg/mL) and aqueous extract of Cuscuta arvensis (AChE IC_{50} 295.77 µg/mL; BChE IC_{50} 323.81 µg/mL) compared to galanthamine (AChE IC_{50} 33.27 µg/mL; BChE IC_{50} 32.6 µg/mL) as a standart. On the other hand, the methanolic extract of Achillea wilhelmsii has the lowest AchE inhibition (714.81 µg/mL) and, the garlic-lemon extract has the lowest BChE inhibition activity (783.81 µg/mL). Although there are some anti-alzheimer and anti-neurodegenerative preparations in the world, there is few plant-derived drug like galanthamine. This study can be a guide for phytochemical studies and to discovery of novel effective extracts or compounds for the treatment of neurological diseases.

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THE EFFECTS ON TYROSINASE ENZYME OF THREE ASTERACEAE SPECIES GROWING IN AKKUS (ORDU)

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Asteraceae is the largest family of flowering plants and contains about 900 genera and 13000 species. Many species belong to this family grow in Akkuş district (Ordu Province). Some plants belong to Asteraceae have been using by people living in Akkuş for various purposes for many years. Different dermatological disorders are the result of irregular distribution of pigments. Melanin is the most important factor affecting the skin and hair color of mammals. Tyrosinase is a key enzyme in the synthesis of melanin in plants, microorganisms and mammalian cells. In this study, the effects on tyrosinase enzyme of 3 species (Anthemis cotula, Anthemis tinctoria var. tinctoria and Tanacetum parthenium) belong to Asteraceae family were studied. The methanolic extracts prepared from aerial parts of these 3 species were investigated in respect of tyrosinase enzyme inhibition. The method developed by Masuda et al. was used for tyrosinase enzyme inhibition using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as a substrate [1]. Consequently, the inhibitor activity on tyrosinase enzyme of T. parthenium was determined as the best among the other species. IC_{50} value of T. parthenium were calculated as 712,95 µg/mL. In the light of the results, T. parthenium can be used as a alternative drug source for the treatment of skin diseases.

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THE EFFECTS ON TYROSINASE ENZYME OF THREE ROSACEAE SPECIES GROWING IN AKKUS (ORDU)

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Rosaceae family includes about 100 genera and 2000 species of herbs, shrubs and trees. Many species belong to this family have grown in Akkuş district (Ordu Province). Some plants belong to Rosaceae have been used by people living in Akkuş as a folk medicine in various diseases for many years. Tyrosinase is an oxidoreductase that is very important in medicine and cosmetics because the excessive production of melanin causes hyperpigmentation [1]. In this study, studies on tyrosinase enzyme inhibition of 3 species of Rosaceae family (Agrimonia eupatoria, Mespilus germanica, and Rubus ideaus) were investigated. Studies of tyrosinase enzyme inhibition were practised the method which was developed by Masuda et al. using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as a substrate. Consequently, the inhibitor activity on tyrosinase enzyme of methanolic extract of the aerial parts of R. ideaus was determined as the best among the other species. IC_{50} values of R. ideaus were calculated as 642,01 µg/mL. R. ideaus may serve as structural templates for the design and development of novel tyrosinase inhibitors as effective anti-browning agents in cosmetics.

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EFFECTIVE ERADICATION OF PINWORMS (SYPHACIA OBVELATA AND ASPICULURIS TETRAPTERA) WITH POLYGONUM COGNATUM MEISSN.

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Polygonum cognatum Meissn. (Polygonaceae) is a wild plant locally named as “solucanotu” in Turkey. In Turkish folk medicine it has been used for various purposes, such as diuretic effect and for the treatment of diabetes mellitus, as well to treat oxyuris and worms internally [1]. This study was designed to evaluate the anthelmintic activity of n-hexane, ethyl acetate and methanol extracts prepared from the aerial parts of P. cognatum. Mice infected with Syphacia obvelata and Aspiculuris tetraptera were used in this study. According to the results, it has been found that the methanol extract displayed significant anthelminthic activity against pinworms, Syphacia obvelata and Aspiculuris tetraptera, in mice. According to our results, it was concluded that P. cognatum contains potent anthelmintic compounds. In this study, in vivo experimental results have also supported the folk medicinal utilization of Polygonum cognatum.
Key words: Anthelmintic activity, Aspiculuris tetraptera, Syphacia obvelata, Polygonum cognatum, Polygonaceae

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P277

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF COTINUS COGGYGRIA SCOP.

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Cotinus coggygria Scop, which grows in our country, known as 'smoke tree' and it is widely used in the oral wound healing among the people. For this reason in this study, antioxidant, antiinflammatory activities and chemical composition of the dried leaves of Cotinus coggygria Scop. (Anacardiaceae) were investigated. The plant was extracted with 70% methanol for 3 days, solvent was removed in vacuo (40°C) and the exctract liyophilized. Chemical composition of the extract was analysed by spectrophotometric (total phenol, total flavonoids, total flavonols) and chromatographic/spectrometric (LC- MS/MS) techniques. For the purpose of determining the antioxidant activity of the extract; 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+\bullet}$) radical scavenging effect, preventing peroxidation of the phospholipids, β-carotene-linolenic acid co-oxidation assay, iron(II) chelate formation, reduction, 2-deoxy-D-ribose degradation by site and non site specific OH radical activity assays were investigated. The effects of the extract on IL-1β, IL-6, IL-10, TNF-α and nitric oxide were measured in lipopolysaccharide (LPS) treated RAW 264.7 cells to measure anti-inflammatory activity. The extract is found to be rich in phenolic compounds, especially flavonoids, phenolic acids and tannins. The results showed that these compounds might be responsible from the antioxidant and antiinflammatory activity.

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CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACTS OF THE ROOTS OF SOME FERULAGO W. KOCH SPECIES AGAINST SW480, PC-3 AND MCF-7 CANCER CELL LINES

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Cancer has become an important reason of morbidity and mortality throughout the world [1] and recently herbal resources gained popularity since they have a very important potential in respect to their anticancer effects. As a result of recent studies, some Ferulago species have revealed to possess anticancer effect. For example F. angulata has been shown to inhibit the proliferation of lymphoma and leukemic cells [2]. Therefore, we decided to study the cytotoxic activity of the roots of some Ferulago species against some different cancer cell lines.

For this purpose, ethanolic extracts of the roots of 5 Ferulago species (Ferulago syriaca, F. longistylis, F. isaurica, F. setifolia and F. cassia) were prepared and tested for their cytotoxic activities against SW480 (colorectal carcinoma), PC-3 (prostate carcinoma) and MCF-7 (breast carcinoma) cell lines in concentrations of 1, 0.1, 0.05, 0.025, 0.01 mg/ml via (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) test. Cell viabilities were measured with spectrophotometry (at 540 nm) while taking the cells in the control group as reference. The cytotoxic activities of the ethanolic extracts were determined and compared with each other along with their graphics. As a result we can conclude that the extracts have cytotoxic effects against the above mentioned cell lines in varying degrees.

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DECREASED EXPRESSION LEVELS OF ATAXIA TELANGIECTASIA MUTATED (ATM) GENE IN SQUAMOUS CELL LARYNX CANCER

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Larynx cancer (LC) is estimated to be the second most common malignancy of the head and neck region. The most significant risk factors playing roles in the development of LC are tobacco, alcohol consumption and exposure to radiation. Ataxia Telangiectasia Mutated (ATM) gene is located on 11q22.3 chromosome and encoded product by this gene belongs to the PI3/PI4-kinase family. This protein is an important cell cycle checkpoint kinase that regulates the activity of some tumor suppressor and DNA repair proteins. In the literature, the expression of ATM was frequently reported as downregulated in various types of cancer such as gastic, pancreatic, lung, breast, laryngeal, pharyngeal cancers, lymphoma and leukemia. In our study, we investigated the association of differentially expressed levels of ATM with LC carcinogenesis. The expression status of ATM was analyzed in tumor and matched-normal tissue samples of 50 patients with LC by the quantitative real-time polymerase chain reaction method (QRT-PCR). ATM and the reference gene expression status were analyzed by calculating the threshold cycle numbers (Ct) as fold changes using the $2^{-\Delta\Delta Ct}$ method. After evaluation of the expression levels, we selected the ratio of >=2 as the threshold for differentially expressed ATM. The decreased expression ratio of ATM was observed as 48% (24/50) in tumors. Our study suggests that there is an association between decreased expression levels of the ATM gene and LC as concordant with the literature.
This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project number:I.U.BAP-ONAP-42152).

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ALTERATIONS IN GAMMA-GLUTAMYL TRANSFERASE LEVELS IN OBSTRUCTIVE SLEEP APNEA PATIENTS WITH AND WITHOUT METABOLIC SYNDROME

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Gamma-glutamyl transferase (GGT) is a glycoprotein enzyme which is located on the outer surface of the plasma membranes of cells. Measurement of GGT activity in serum has been found useful in showing tissue damages. In addition to being a sensitive marker of hepatic diseases, correlation between increases in serum GGT levels, and cardiac mortality, and nonfatal myocardial infarction has been shown in several studies. Also, increased serum GGT activity has been proposed as a potentially useful marker of oxidative stress. Because patients with obstructive sleep apnea (OSA) and metabolic syndrome (MS) are under risk in terms of development of cardiovascular diseases, we aimed to investigate the serum GGT levels in patients with OSA with/without MS. Fasting blood samples were collected from 157 patients with OSA, 20 patients with OSA+coronary artery disease (CAD), 23 people with MS and 31 healthy controls. Serum GGT levels were measured by a spectrophotometry method. When evaluated separately for both gender, mean GGT levels were significantly higher in patients with OSA and OSA+CAD than those in healthy controls ($p<0.001$ and $p<0.05$ for males and $p<0.05$ and $p<0.05$ for females, respectively). When compared with controls, no statistically significant differences in GGT levels were found in metabolic syndrome group. Even if gamma-glutamyl transferase levels were within normal ranges, they were higher than in healthy controls and may be considered as an early diagnosis determinant for cardiovascular diseases or an independent risk factor.

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ENHANCED UNFOLDED PROTEIN RESPONSE SIGNALING IN OBESITY

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Phenylbutyric acid (PBA) has chaperone-like activities that alleviate ER stress. Under physiological conditions, all cells undergo some degree of ER stress and activate the unfolded protein response (UPR) as a cellular survival program. However, sustained ER stress and prolonged UPR activation can lead to cell death. Professional secretory cells are susceptible to ER stress because of physiological variations and also other conditions that can cause ER stress such as metabolic dysregulation associated with obesity, excess nutrients and inflammatory cytokines. This study was designed to examine the UPR activation in obesity and the effect of PBA treatment on UPR signaling in pancreatic islets. In the study, both male twenty-four wild-type mice (WT) and twenty-four ob/ob mice were divided into two groups with and without PBA administration for 30 days. Expressions of UPR mediators including CHOP promoting cell death and XBP1s targeting various genes encoding ER-related proteins and also SOCS3 being upregulated in response to cytokine stimulation were analyzed by RT-PCR. The results indicated that XBP1s and CHOP were markedly increased but SOCS3 was decreased in the islets of ob/ob controls compared to others. According to the expression levels of XBP1s, CHOP and SOCS3, there were no significant differences among the islets of PBA-treated ob/ob and WT mice. Results demonstrated that mouse models of obesity exhibit increased UPR activation and administration of the chemical chaperone PBA to ob/ob mice reduced ER stress and attenuate the activation of the UPR.

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CHANGES OF SERUM OMENTIN AND OBESTATIN LEVELS IN PATIENTS RECEIVING PERITONEAL DIALYSIS

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End stage renal disease (ESRD) incidence has been dramatically increasing worldwide. Chronic kidney disease leads to kidney failure and ESRD. Inflammatory and other metabolic factors may play critical roles in chronic kidney disease development and pathogenesis. Adipokines contribute to renal complications and are involved in kidney damage by mediating endothelial dysfunction, oxidative stress and inflammation. Omentin which is a recently identified novel adipocytokine, has taken on interest due to its favorable effects on inflammation. Omentin inhibits inflammation via several cellular signaling pathways. Obestatin has an opposite impact on appetite and little is known about its levels and its effect in dialysis patients. The aim of this study was to investigate the changes in circulating levels of omentin and obestatin in patients receiving peritoneal dialysis (PD). Study participants included were thirty patients undergoing PD therapy and thirty four healthy subjects. Serum omentin and obestatin levels were measured by using ELISA kits. Our results showed that the values for omentin levels in PD patients were higher than those in the healthy controls and anorexigenic hormone obestatin levels were lower in PD patients compared to healthy subjects ($p<0.05$). Based on the present observational data, patients on PD exhibited low serum obestatin levels and increased omentin levels. The results suggest that omentin levels may be associated with inflammatory response and also obestatin may be implicated in ESRD patients with receiving PD. These results should be further elucidated and further studies with larger sample sizes are needed to evaluate these parameters in patients receiving PD.

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THE RELATIONSHIP BETWEEN URIC ACID AND SLEEP PARAMETERS IN OBSTRUCTIVE SLEEP APNEA PATIENTS WITH AND WITHOUT METABOLIC SYNDROME

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Oxidative stress is one of the pathophysiological pathways suggested for the development of cardiovascular diseases (CVD) in obstructive sleep apnea. The recurrent nocturnal episodes of hypoxia/reoxygenation observed in patients with obstructive sleep apnea (OSA) appear to be partly responsible for the increased oxidative stress. During ischemia/reperfusion, a number of cell types, including cardiac myocytes, endothelial cells and neutrophils, may have the ability of producing reactive oxygen species (ROS). Circulating UA was proposed to be one of the major antioxidants of the plasma. The antioxidant properties of UA have been attributed to its ability to chelate transition metal ions and to react with potent biological oxidants. In this study, we measured the serum UA levels by a gas chromatography–mass spectrometry method in 67 OSA patients with/without metabolic syndrome (MS), 21 people with only metabolic syndrome and 22 healthy controls. The mean UA levels in total OSA patients, patients with MS, and patients without MS than in healthy controls ($p=0.003$, $p=0.006$, $p=0.013$, respectively). UA was significantly correlated with all OSA criteria, such as AHI, T% $SpO_2<90$, T% $SpO_2<85$, SaO_2I, lowest SaO_2, and mean SaO_2 in total patients and patients with MS. Also, elevated levels of UA were associated with OSA severity, as defined by AHI. Due to the increases in UA levels relative to the severity of apnea in this study and the well-known association of uric acid with CVDs, hyperuricemia can be considered a marker of CVD development in OSA patients.

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THE ANTIPROLIFERATIVE EFFECT OF EGCG IN F98 CELL CULTURE

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Epigallocatechin-3-gallate (EGCG) is the most abundant and biologically active tea polyphenol, representing 50-80 % of the total tea catechin. Epigallocatechin-3-gallate (EGCG) is well studied one of the most consumed antioxidant. The aim of this study was to evaluate the possible cytotoxic effect of EGCG on F-98 glioblastoma cells. F-98 glioblastoma cell line was used F-98 cells were seeded in 96 well plates in 100 µl medium DMEM. Cells treated with EGCG (200,100,50,25,12.5,6.2,3.3µM) for 24 h and 48 hour incubation. Measurement of EGCG treated and control groups cell proliferation performed with MTT assay and Wound Healing assay was employed to show migration capacity. LDH activity and glucose levels were determined by using a commercial kit. MTT Assay data shown are there was no significant change in cell viability in 24 hour. However 6,25 and 200 µM were able to cause multi-fold decreases in 48 EGCG on F-98 cells. In the present study, cytotoxic effect of EGCG were observed via wound healing assay. The increase glucose level of medium show that EGCG has antiproliferative effect in F-98 cancer cells at higher concentration. Our results here show that EGCG maybe a possible avenue for brain cancer treatment.

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METHYLATION STATUS OF THE TUMOR NECROSIS FACTOR-ALPHA (TNFA) GENE IN SQUAMOUS CELL LARYNX CANCER

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Larynx cancer (LC) is the most common cancer type seen among the head and neck cancers. In the etiology of LC, environmental factors such as exposure to harmful effects of smoking, physical and chemical carcinogens are defined and they also suppress of cellular immunity. There is no reliable biomarkers in LC to predicting in early diagnosis/prognosis. In our study, the methylation status in the promoter region of the TNFA gene was examined in patients with LC. The methylation status of TNFA was analyzed in tumor tissue samples of 50 patients with LC by DNA methylation analysis using restriction enzyme digestion method. After the enzyme digestion, PCR-amplified samples were visualized on gel electrophoresis. The promoter region of the TNFA gene was methylated in 84% (42/50) of the primary tumor samples of LC patients. We investigated the association between clinicopathological parameters of the patients and methylation status of TNFA ($P<0.05$). We found that methylation presence was associated with overall late stage. This study indicate that the methylation status of TNFA gene can be a prognostic/diagnostic marker for LC patients.

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EFFECTS OF VITAMINS TO THE OXIDATION OF THE RATS ESTABLISHED EXPERIMENTAL DIABETES

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In this study, the effects of vitamin C and vitamin E on lipid peroxidation and antioxidant enzyme activities in kidney tissue of streptozotocin-induced diabetic rats were investigated. Male wistar rats, were grouped into five each consisting 8 rats as non-diabetic (K), diabetic (D), diabetic Vitamin E (E), diabetic Vitamin C (C), and diabetic Vitamin E and C (EC). Diabetes mellitus was induced in rats by intraperitoneal injection of 55 mg/kg STZ. After the injection vitamins were administrated for four weeks. As an indicator of lipid peroxidation malondialdehyde levels, antioxidant enzymes as superoxide dismutase, catalase and glutothione peroxides activities were measured in kidney homogenates. In the study, MDA level of group D was significantly higher than groups K (p=0,004), E (p=0,008), C p=0,032) and EC (p=0,008). Statistically significant difference was not observed in CAT levels among the groups (p>0,05). SOD enzyme levels in group D was higher compared to group K (p=0,004), and lower in group EC compared to groups C (p=0,032), and D(p=0,008). GSH-Px enzyme levels in group D (p=0,004) and group C (p=0,017) were significantly higher compared to group K. Group D GSH-Px levels were significantly lower when compared to group E (p=0,008), C (p=0,008) and EC (p=0,008), GSH-Px levels of EC group was lower (p=0,008) compared to goup C. As a result, in this study it was demonstrated that vitamin C and vitamin E have positive effect on lipid peroxidation and as a result decreased the higher levels of MDA, SOD, GSH-Px levels of diabetic rat kidney tissues.

Table 1.1. Avarage CAT, SOD, GSH-Px, MDA values

Group	*Control*	*Diabetes*	*Diabetes + Vitamin E*	*Diabetes + Vitamin C*	*Diabetes + Vitamin E+C*	*p value*
MDA [a,b,c,d] *(µM/gr tissue)*	7,07±0,75	9,08±0,64	6,93±0,89	7,95±0,69	7,04±0,27	0,007
CAT (nmol/min/mL /gr tissue)	27,71±2,26	29,75±1,94	27,04±3,4	26,5±3,8	26,41±1,88	0,238
SOD [e,d,g] *(U/ml/gr tissue)*	1,8±0,23	2,34±0,14	2,29±0,5	2,14±0,49	1,54±0,13	0,02
GSH-Px [a,b,c,d,e,i,g,m] *(nmol/min/ml /gr tissue)*	226,57±13,98	317,22±13,98	232,66±22,89	256,35±8,71	227,06±12,34	0,001

(a: difference between K and D p=0,004, b: difference between D and E p=0,008, c: difference between D and C p=0,032, d: difference between D and EC p=0,008, g: difference between C and EC p=0,032, i: difference between K and C p=0,017, m: difference between C and EC p=0,008)

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INVESTIGATION OF NOVEL PLATELET RECEPTOR GPVI INHIBITOR CANDIDATES

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Platelets are highly reactive effectors of hemostasis and thrombosis in circulatory system. Its unwanted activation and aggregation are crucial process in the pathogenesis of cardiovascular diseases which are the leading cause of death in the world. Antiplatelet drugs are central in the treatment and prevention of the initial and subsequent vascular events, but all current antiplatelet therapies carry an increasing bleeding risk. Glycoprotein VI (GPVI), which is the primary platelet collagen receptor and only expressed in platelets, is an attractive new target as an antiplatelet reagent. Inhibition of GPVI signalling has demonstrated efficient antithrombotic potency in the experimental models of thrombosis without enhancing pathological bleeding. To discover new GPVI inhibitors, a commercial database which contains 6.5 million compounds, is pointed as a source of ready-to-order ligands. An e-pharmacophore model, based on published GPVI inhibitor; losartan, is used to screen the database (Figure 1). The results are evaluated by taking into account on docking scores and structural interactions. The selected ones in the compound library will be tested in vitro their binding affinity to GPVI to be able to detect the therapeutic candidates.

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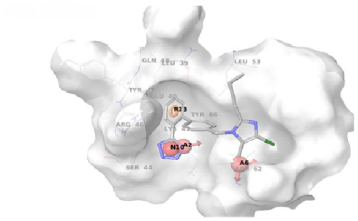

Figure 1. E-pharmacophore model for Losartan

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CAN ER STRESS BE A POTENTIAL REGULATOR OF RESISTIN AND CASPASE 3 LEVELS IN OBESITY?

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Endoplasmic reticulum (ER) stress activates the cytoprotective unfolded protein response (UPR), but it can also contribute to cell death via apoptosis in particular conditions. Most adipokines are upregulated in the obese state, leading to metabolic abnormalities. The relationship between ER stress and metabolism has been suggested to underlie the pathogenesis of obesity-related complications, especially insulin resistance. We aimed to investigate the effect of PBA on plasma insulin, adipokine resistin and caspase 3 levels in ob/ob mice. This study was comprised with fourteen wild-type (WT) controls and ob/ob mice which administered with either vehicle or PBA for 30 days. Bloods were taken at day 0, 20 and 30. All parameters were determined by ELISA. Our findings showed that all parameters in ob/ob mice markedly were higher compared to WT-controls at all days but in ob/ob mice gradually decreased with PBA administration. Compared to ob/ob controls, PBA-treated ob/ob group showed significantly lower concentrations of all parameters at day 30 and also resistin levels at day 20. However, insulin levels in PBA-treated ob/ob mice remain higher at all days, resistin levels remain higher at day 0 and 20, caspase 3 levels remain higher at day 0 compared to WT groups. Our results demonstrated that PBA lead to reduction in levels of these parameters. According to our findings, ER stress may be a potential regulator of these parameters in obesity. Further studies are necessary to understand the molecular links between them, it is crucial for the development of effective treatments for obesity.

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THE EFFECTS OF LONIDAMINE AND QUERCETIN COMBINATION ON CELL GROWTH AND APOPTOSIS OF MCF-7 BREAST CANCER CELLS

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Phytochemicals have long been in the center of cancer studies and are still being researched extensively for their anticancer properties. One of these compounds known as quercetin is a major member of flavanoids and has proapoptotic effects on tumor cells as well as blocking cell cycle at different phases and inhibiting angiogenesis[1,2]. Quercetin has also become a subject of combination-based experiments. When administered with a number of chemotherapeutic agents, it has been observed to potentiate their anticancer activity. Lonidamine (LND) was first introduced as an antispermatogenic agent but later found to be effective on cancer cells as well [3]. LND affects a wide range of solid tumors via interfering with energy metabolism by inhibiting glycolysis[4], altering the plasma and mitochondrial membranes [5,6] and affecting DNA repair [7] as well as cellular acidification[8]. While it exhibits limited anticancer activity when administered alone, it shows a remarkable potential in combination therapies[9,10]. The purpose of this study is to determine the cytotoxic and apoptotic activities of quercetin in combination with LND on MCF-7 human breast cancer cells. Cell viability analysis was performed using MTT method. Apoptotic activity was evaluated through performing Annexin V assay and total caspase levels. The results showed LND-quercetin combination decreased cell viability of MCF-7 cells when compared to control ($p<0.05$). The combined treatment has also induced apoptosis via increasing the total amount of caspase levels and Annexin V binding. Our results suggest that LND-quercetin combination has more favourable effect on MCF-7 cells when compared to the effects of compounds alone.

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THE EFFECTS OF ß-ELATERIN AND METFORMIN COMBINATION ON CELL CYCLE AND APOPTOSIS IN MCF-7 HUMAN BREAST CANCER CELLS

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Conventional chemotherapeutic regimens are commonly used to inhibit tumor growth, but there is great variability in clinical responses of cancer patients. Cancer cells often develop resistance to chemotherapeutics which results in tumor recurrence and further progression [1,2]. In recent years, combined therapy is found to be more effective than monotherapy [3]. Metformin is an oral anti-diabetic drug prescribed for type 2 diabetes [4,5]. Recent studies have shown that metformin reduces the risk of developing some types of cancers, including breast cancer [6-8]. β-elaterin, a tetracyclic triterpene, is isolated from plants in the Cucurbitaceae family and it's been used in traditional medicine for centuries [9-10]. In vivo and in vitro studies have shown that β-elaterin has potential anti-cancer agent [10]. At the present study, we aimed to evaluate the cytotoxic and apoptotic activities of metformin in combination with β-Elaterin on MCF-7 human breast cancer cells. In this concept, we performed MTT test for evaluating cell growth. Additionally, apoptotic profiled was determined by Annexin V binding assay and caspase assay. The results showed that, metformin and β-elaterin combination at 33.65 mM and 1 µM concentrations, respectively inhibited MCF-7 cell growth when compared to control and compounds alone ($p<0.05$). This treated combination has also induced apoptosis via increasing the binding of Annexin V and total caspase levels significantly ($p<0.05$). In this context, the present study is an important step towards the combined usability of the compounds in question. Further studies are needed to investigate the anti-cancer effect mechanisms of MCF-7 cells on combinations of mentioned compounds.

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CYTOTOXIC EFFECTS OF HSP70 INHIBITORS MKT-077 AND VER-155008 ON BREAST CANCER CELLS MCF-7

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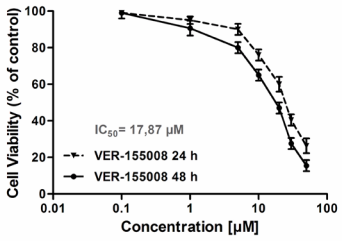
Hsp70 is a chaperone that possess several physiological roles such as folding of nascent proteins, refolding of misfolded proteins, cell-cycle control, inhibition of protein aggregation, and intracellular protein transport [1]. This protein is expressed at low levels under physiological conditions but is greatly overexpressed in tumor cells, contributing to resistance to therapy and tumor cell survival [2]. Therefore, specific inhibition of Hsp70 in tumor cells is a promising strategy for the treatment of many types of cancer [3]. The present study aimed to evaluate the cytotoxic effect and inducible Hsp70 (Hsp70i) protein expression change of Hsp70 inhibitors MKT-077 and VER-155008 on MCF-7 cells. Human breast cancer MCF-7 cells were cultured and treated with different concentrations of inhibitors (0,1-50 µM). Cell viability and Hsp70i protein levels were evaluated by XTT assay and ELISA method respectively. Interactions between the inhibitors and Hsp70i were determined by using docking programs. Our findings showed that inhibitors treatment significantly inhibit MCF-7 cell proliferation and increased the amount of Hsp70i in MCF-7 cells compared to the control cells ($p<0.05$). IC_{50} values of inhibitors for MKT-077 and VER-155008 calculated as 8,63 µM and 17,87 µM respectively. Docking results also revealed key interactions between the inhibitors and Hsp70i. In this study, it was shown that the inhibitors do not suppress the level of Hsp70i protein expression, but directly binding to the nukleotide binding region of the protein and blocking its essential functions.



Cytotoxic effect of VER-155008 on MCF-7 cells for 24 and 48 hours.

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SITE-DIRECTED MUTATIONS ON HSP70 NBD DECREASE THE PROTEIN FOLDING FUNCTION

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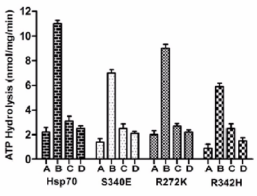
The nucleotide binding domain of Hsp70 (NBD), also known as ATPase domain, hydrolyzes ATP and hydrolysis is one of the most important step in the chaperone activity of HSP70 [1]. Since, Hsp70 is an ATP-dependent molecular chaperone and energy is essential for this chaperone function, ATP analogs such as VER-155008 and MKT-077 are widely used to inhibit the functions of this protein [2, 3]. In the present study, we aimed to show how the protein function alters by point mutation in some critical amino acids on HSP70 NBD presence or absence of VER-155008 and MKT-077. Initially; VER-155008, MKT-077, ATP, and AMP-PNP were docked with Hsp70 NBD structure in slico in order to identify common amino acids that interact with these four ligands. Site-directed mutagenesis of the determined critical amino acids in Hsp70 NBD was performed in vivo by using Quick Change II Site-Directed Mutagenesis Kit according to manufacturer's instructions. Finally, ATP hydrolysis and luciferase folding experiments were carried out to determine the change of the wild type protein functions of purified Hsp70 and of mutant Hsp70 proteins presence or in the absence of inhibitors. Our findings showed that mutant proteins displayed significant reduction in ATP hydrolysis and luciferase folding activities compared to the control wild type group. In particular, the presence of the inhibitors further reduced protein functions ($p<0,05$). This study identified a number of amino acid residues that may be important for protein function on Hsp70 NBD.



ATP hydrolysis graph of Hsp70 and mutant Hsp70 proteins in presence and absence of inhibitors.

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APOPTOTIC EFFECTS OF SOME NEW BENZOTHIAZOLE BASED IMIDAZOLE DERIVATIVES ON LUNG ADENOCARCINOMA AND FIBROBLAST CELL LINES

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Cancer is a disease in which the control of growth is lost in one or more cells and one of the widespread reason of death [1,2]. In many types of cancer, lung cancer is a significant reason of cancer-related mortality in the world [3]. At the same time; lung cancer is difficult to treat ,so it is important to develop new strategies to struggle for this disease in the clinic [1,2,4]. For this reason, in this work some novel N-(6-substituted-benzothiazol-2-yl)-2-[[4,5-dimethyl-1-((p-tolyl/4-nitrophenyl) amino)-1H-imidazol-2-yl]thio]acetamide derivatives were synthesized and searched for their cytotoxic activities against human lung adenocarcinoma (A549) and NIH/3T3 mouse embryonic fibroblast cell lines. To find cytotoxic activity of the compounds against A549 and NIH/3T3 cell lines, MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed. According to assay results; the most effective and selective cytotoxic agents against A549 were found as compounds 3 and 8 (IC_{50} values were; 16.00±5.292 and 35.00±5.00 µg/mL). After 24h incubation period, the apoptotic effects of compounds 3,8 were analyzed for A549 cell line based on Annexin V-PI binding capacities in flow cytometry according to their IC_{50} values. Early and late apoptotic effects of compounds 3 and 8 were determined as percentage of 35.3 and 43.8 for A549 human lung adenocarcinoma cells. Both of the effective compounds (3 and 8) contain 6-methoxy substitution on benzothiazole ring. Methoxy group can be responsible for anticancer avtivity. Findings about antiproliferative potential of these compounds could provide a base for the acquirement of new similar anticancer molecules in undergoing studies.

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THE NORMAL AND TUMOUR TISSUE MRNA EXPRESSIONS OF HINT1 AND SOD1 IN SEVERAL TYPES OF CANCER

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Histidine triad nucleotide-binding protein (HINT1) -an important tumour suppressor gene- is ubiquitously expressed in diverse species, including the mammalian tissues. The main purpose of this study was to investigate the changes of HINT1 mRNA expression rates in various types of cancer. We also aimed to study its antioxidant properties by comparing with SOD1, a well-known antioxidant. The mRNA expression levels of HINT1 and SOD1 were measured by RT-PCR in both normal and tumour tissues of the patients with colon, gastric, and breast cancers. HINT1 expression values were observed to be lower in tumour tissues of 14 of the 26 patients in total (53.85%), 8 of 13 patients with colorectal cancer (61.54%), and 3 of the 7 patients with breast cancer (42.86%), and 3 of 6 patients with gastric cancer (50.00%) than in normal tissues. We observed high positive correlations between HINT1 and SOD1 mRNA expressions in normal tissues ($R=0.768$, $p<0.0001$) of total cancer patients, in both normal ($R=0.753$, $p<0.005$) and tumour tissues ($R=0.919$, $p<0.0001$) of colorectal cancer patients, and in normal tissues ($R=0.945$, $p<0.005$) of gastric cancer patients. There was a weak positive correlation between HINT1 and SOD1 mRNA expressions in tumour tissue ($R=0.758$, $p<0.05$) of patients with breast cancer. According to the results obtained from this study, decreased levels of expression of HINT1 in tumour tissues, especially in the patients with colorectal cancer, may conform to the studies suggesting its tumour suppressor activity. In addition, HINT1 can be considered as an antioxidant due to its high correlation with SOD1.

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SPECTROSCOPIC AND QUANTUM CHEMICAL STUDIES ON SOME β-LACTAM INHIBITORS

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Amoxicillin (Amox) and ampicillin (Amp) are investigated by using quantum mechanical methods. This compounds was confirmed by XRD analysis and optimized bond parameters were calculated by density functional (DFT) at B3LYP/6-31G(d) level. The optimized geometrical parameters are in good agreement with crystal data. The experimentally observed FT-IR and NMR picks were assigned to calculated modes for the molecules. Some molecular descriptors are calculated with density functional theory (DFT/B3LYP) 6-31G(d) level in the gas phase. The highest occupied molecular orbital energy (EHOMO), the lowest unoccupied molecular orbital energy (ELUMO), the energy difference (ΔE), hardness (η), softness (σ), electronegativity (χ), chemical potential (μ), electrophilicty index (ω) and nucleophilicty index (ε) are calculated in the this level and associated with inhibition efficiencies of the mentioned β-lactam inhibitors. Molecular Electrostatic Potantial (MEP) maps was investigated and predicted the reactive sites. Some quantum chemical descriptors which are total static dipole moment (μ), the average linear polarizability (α), the anisotropy of the polarizability (Δα) and first hyperpolarizability (β) were evaluated for explaining the NLO properties in studies molecules. The inhibition activities [1] were studied using molecular docking studies. The antibiotics were docked into the cocrystallized structure of PXR with SR12813 (PDB ID: 1NRL) [2]. Docking results and order of inhibition activity associated with quantum chemical parameters was the same as that of experimental inhibition activity.

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SURFACE MOLECULARLY IMPRINTED INORGANIC POLYMER CAPPED MN-DOPED ZNS QUANTUM DOTS AS A PHOSPHORESCENT SENSOR FOR DETECTING TOBRAMYCIN

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This study focuses on molecularly imprinted inorganic polymer (IMIP) based room temperature phosphorescent (RTP) sensor developed by anchoring the inorganic MIP shell on the surface of Mn-doped ZnS quantum dots (QDs) via surface molecular imprinting technique. Firstly, surface of Mn-doped QDs was modified using 3-(mercaptopropyl)triethoxy silane (MPTS) as described by Wang et al. [1]. In the synthesis of inorganic MIP shell, tobramycin (TOB), 3-aminopropyltrimethoxy silane (APTES), and tetraethoxy silane (TEOS) were used as template, functional monomer, and crosslinker, respectively. Non-imprinted inorganic control polymer (INIP) was prepared by following the same procedure but in the absence of TOB. In this sensor design, QDs and MIP shell act as a phosphorescence signal transmitter and recognition element that prevents interfering molecules from coming into contact with the sensor, respectively. Thus, synergistic combination of RTP property of QDs and surface molecularly imprinted polymer not only improves selectivity of the sensor but also makes detection of non-phosphorescent TOB possible. The resultant phosphorescence sensor was characterized using FT-IR, X-ray photoelectron spectroscopy (XPS) and transmission electron microscope (TEM).

TOB quenched the RTP of MIP-capped Mn-doped ZnS QDs in a concentration dependent manner and obeyed Stern-Volmer equation. Higher degree of quenching for MIP-QDs than NIP-QDs proved the success of molecular imprinting. Thus, the developed RTP sensor is planned to apply to the determination of TOB in both organic and aqueous media.

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DEVELOPMENT OF HYBRID ORGANIC-INORGANIC SURFACE IMPRINTED MN-DOPED ZNS QDS AS A PHOSPHORESCENT SENSOR FOR THE RECOGNITION AND DETECTION OF TOBRAMYCIN

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Tobramycin (TOB) is an aminoglcoside antibiotic widely used against treatment of gram negative bacterial infections both in humans and animals. In the present study, a phosphorescence sensor based on Mn-doped ZnS quantum dots whose surface was coated with a molecularly imprinted polymer have been developed and then have been applied to the determination of TOB. Firstly, Mn-doped ZnS quantum dots have been synthesized and their surfaces were modified using 3-(mercaptopropyl)triethoxy silane (MPTS) and 3-(methacryloxy) propyl trimethoxy silane (MPS). Thus, a polymerizable vinyl group containing grafting agent (MPS) was chemically bonded on the surface of quantum dots through siloxane bonds. Functional monomer, 2-(trifluoromethyl) acrylic acid (TFMAA), and template, TOB, were dissolved in a porogen (dimethylesulphoxide) and then a certain amount of modified ZnS quantum dots have been added to this mixture. Then, crosslinker, ethylene glycol dimethacrylate (EGDMA), initiator, N, N'-azobisisobutyronitrile, (AIBN) have been added. Surface grafting copolymerization was conducted through free radical polymerization mechanism. . The resultant phosphorescence sensor was characterized using FT-IR, X-ray photoelectron spectroscopy (XPS) and transmission electron microscope (TEM). After extraction of template molecule from the imprinted sites on the surface of quantum dots, binding studies will be performed both in organic and aqueous media for the generation of calibration curves by considering the quenching in phosphorescence signal. Thus, the developed room temperature phosphorescent sensor will be applied to the determination of TOB in both organic and aqueous media.

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INHIBITOR-ASSISTED SYNTHESIS OF MOLECULARLY IMPRINTED CORE-SHELL MICROBEADS FOR PEPSIN BINDING

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In this study, a core-shell type synthetic protein scavenger material was developed for the selective removal of pepsin using molecularly imprinted polymers. For inhibitor-assisted molecular imprinting of pepsin, pepstatin-an inhibitor of pepsin-was immobilized onto the surface of amino polystyrene (APS) microbeads via a carbodiimide linker reaction mechanism. 3-aminophenylboronic acid (APBA) was used as a functional monomer, and poly(3-aminophenylboronic acid) (pAPBA) prepared in the presence (molecularly imprinted polymer; MIP) and absence (non-imprinted polymer; NIP) of the template pepsin were then grafted onto the surface of pepstatin immobilized APS microbeads via aromatic ring electron-pairing interaction in an aqueous solution. Ammonium persulfate was used as an initiator and imprinting was conducted in aqueous solution. The obtained core-shell microbeads were characterized using X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM). The results showed that pepsin-imprinted pAPBA shells were successfully grafted onto the surface of APS microbeads.
After the extraction of the template pepsin, pepsin binding sites at or close to the surface of the microbeads were generated. Batch rebinding studies using both pepsin-imprinted (MIP) and non-imprinted (NIP) microbeads were performed, and effects of functional monomer concentration, and the adsorption time on the binding affinities were analyzed. High imprinting factor, IF=1.90, and high binding capacity clearly showed that pepsin was effectively imprinted onto the surface of APS microbeads.

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PREPARATION OF MAGNETIC MOLECULARLY IMPRINTED NANOPARTICLES FOR RECOGNITION AND REMOVAL OF CARVEDILOL

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In this study, magnetic molecularly imprinted nanoparticles (MMIPs) have been prepared via sol-gel mechanism for the selective removal of carvedilol (CRV). CRV is used for the treatment of hypertension, ischemic heart disease, and congestive heart failure. Firstly, Fe_3O_4 was prepared and then coated with tetraethyl orthosilicate (TEOS) [1]. During the molecular imprinting, CRV was used as a template, 3-aminopropyltriethoxysilane (APTES) as a functional monomer, and TEOS as a crosslinker. Non-imprinted magnetic nanoparticles (NMIPs) were synthesized through the same protocol but in the absence of template, CRV. The resultant MMIPs were characterized by transmission electron microscopy (TEM) and x-ray photoelectron spectroscopy (XPS). After the extraction of CRV molecules from the inorganic shell part of the MMIPs, equilibrium binding studies will be performed and binding capacity of the MMIPs will be determined at various organic solvents and buffer solutions. The magnetic particles were easily separated from the solution by applying an external magnet and amount of bound CRV was determined spectrofluorimetrically (slit widths, excitation and emission wavelengths, and PMT voltage were set to 10.0 nm, 285.0 nm, and 358.0 nm, 600.0 V, respectively).

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DETERMINATION OF ROSUVASTATIN CALCIUM-BOVIN SERUM ALBUMIN COMPLEX FORMATION CONSTANT USING UV-VISIBLE SPECTROSCOPY

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Drugs are transported within the body by forming complexes that are reversible with proteins in the plasma. Drug-protein affects the size of the interaction and the bioactivity of the natural drug [1-3]. Drug-protein interactions are an important parameter in examining the pharmacological properties of drugs and in preparing drug dosage forms [4]. Bovine serum albumin (BSA) is the largest protein to which drugs can bind and is present in large amounts in plasma. Therefore, BSA:drug interactions are studied in vitro studies [5]. Several analytical methods have been developed to observe complex formation and calculate complex constant [6]. Rosuvastatin Calcium (RC) is a member of the statin group of drugs used to protect against cardiovascular diseases. RC is used as a synthetic lipid lowering agent and inhibits 3-hydroxy-3-metylglutaryl-coenzyme A (HMG-CoA). In this study, interaction of RC with BSA and complex formation were investigated using UV-Visible region spectroscopy. 40 ppm RC was titrated with BSA at the molar ratios of 1:8, 1:4 and 1:2.6 and 1:2 for BSA:RC. The UV absorption intensity of complex formed between RC and BSA increased parallel to that of BSA ratio and these absorbance changes were recorded at 240 nm wavelength. Complex constant was determined from three repeated analyses as $7.98 \times 10^{2} \pm 4.86\ M^{-1}$.

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RAPID DETECTION OF ACRYLAMIDE IN FOOD USING MN-DOPED ZNS QUANTUM DOTS AS A ROOM TEMPERATURE PHOSPHORESCENT PROBE

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A wide variety of analytes such as ions, pharmaceuticals, DNA, food additives, contaminants, and proteins in different sample matrix could successfully determine by room temperature phosphorescence (RTP) quantum dots (QDs) sensors [1, 2]. Acrylamide (ACR) is a potential carcinogen and is found in thermally processed foods such as potato chips, biscuits, baby foods, and coffee. In this paper, L-cysteine capped Mn-doped ZnS QDs as phosphorescent probes were used for the determination of ACR. The developed method was used to determine ACR in ten different food samples, i.e., coffee, potato chips, biscuits, bread, and coffee cream. This method based on quenching of the phosphorescence intensity of the QDs with the interaction of ACR. Room temperature phosphorescence intensity of QDs was quenched rapidly upon the addition of the quencher. The quenching mechanism of Mn-doped ZnS QDs by ACR was dynamic and the quenching constant was found as $3´10^4$ M^{-1}. A linear response was observed from 2 to 20 µg mL^{-1} of ACR with a limit of detection of 0.56 µg mL^{-1}. No interfering peaks were observed from any of the ingredients of the assayed samples. Obtained results from samples showed that the procedure had a suitable sensitivity for the determination of ACR. The results showed that the proposed method is sensitive, selective, fast, and does not require a derivatization step.

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P302

MN-DOPED ZNS QUANTUM DOTS AS A ROOM-TEMPERATURE PHOSPHORESCENT PROBE FOR ANALYSIS OF GLUTAMIC ACID IN FOODSTUFFS

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Glutamic acid (GLU) is produced in the body and binds with other amino acids to form a structural protein [1]. It is present in every food that contains proteins, such as cheese, soups, sauces, and meat [2]. GLU is generally determined by spectrophotometric, luminescence, and chromatographic techniques after a derivatization step, which is necessary to enhance the detection signals [3]. In this study, L-cysteine-capped Mn-doped ZnS quantum dots (QDs) were used for the determination of glutamic acid in foodstuffs, chicken cubes, beef cubes, and chicken soup. This method is based on measurement of the quenching of the phosphorescence intensity of the QDs after interacting with glutamic acid. A linear response was observed from 50 to 500 ng mL^{-1} glutamic acid with a limit of detection of 6.79 ng mL^{-1}. Room temperature phosphorescence (RTP) intensity of the QDs was quenched rapidly upon the addition of the quencher and the reaction reached equilibrium within 2 min. The quenching mechanism of phosphorescence of Mn-doped ZnS QDs by glutamic acid is dynamic and the quenching constant was found as 1.9×10^5 M^{-1}. The procedure showed suitable sensitivity for the determination of GLU and the concentrations were below the acceptable values (10 g kg^{-1}). No interfering peaks were observed from any of the ingredients of the assayed samples. The developed method has some advantages such as freeness of interference from autofluorescence or common cations. The results showed that the proposed method is sensitive, selective, and fast, and does not require a derivatization step.

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P303

A CORE-SHELL COLUMN APPROACH TO FAST DETERMINATION OF SYNTHETIC DYES IN FOODSTUFFS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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A novel and fast analytical method for determination of eight synthetic food dyes (Tartrazine, Amaranth, Indigo carmine, Ponceau 4r, Sunset yellow, Brilliant black BN, Allura red and Acid red 1) in foods was developed and validated using high-performance liquid chromatography coupled to photodiode array detection. Chromatographic system was equipped with C_{18} core-shell column (7.5 × 4.6 mm, 2.7 µm), using 100 mM ammonium acetate buffer (pH: 7) and acetonitrile mobile phase in the gradient elution mode. Parameters affecting separation, such as mobile phase composition, pH, column oven temperature and flow rate, were assessed and optimized by employing a step-by-step strategy. Using a core-shell column, the separation of eight synthetic dyes was achieved in 5.5 min with a run-to-run analysis time of 8 min. To demonstrate the applicability of proposed method, validation experiments were conducted in accordance with the recommendations of the International Conference on Harmonization [1]. Under optimum conditions, developed method was linear in the range of 0.25-10 µg/mL. The limits of detection were in the range of 58.0-69.1 ng/mL, whereas limits of quantification were ranged from 175.8-209.3 ng/mL. The relative standard deviation at three concentration levels (1, 5 and 9 µg/mL) was less than 3% with accuracy in the range of 97.95 to 102.89%. The method was applied to the determination of synthetic dyes in 7 different foods. The results of this study demonstrate that the use of core-shell column can be considered as a desirable alternative for the routine monitoring analysis of synthetic dyes.

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P304

SIMULTANEOUS DETERMINATION OF ACTIVE SUBSTANCES, IMPURITIES AND FATTY ACID ESTERS IN CREAM FORMULATION PHARMACEUTICAL PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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The purpose of this study is to develop a rapid, sensvitive, and reliable method to determine active substances, preservatives, fatty acid esters and impurities in semi-solid pharmaceutical products.
Shimadzu LC-2010A HT with Thermo MOS-1 Hypersil (5 µ x 125 mm x 4,6 mm) column was used. The mobile phase was 10 mM sodium acetate and methanol in gradient elution. The wavelengths were 258 and 286 nm. The column temperature was 40°C, flow rate was 2.0 mL/min, the injection volume was 10 µL and run time was 15 min. The retention times for etofenamate, benzyl alcohol, benzyl nicotinate, etofenamate myristate, etofenamate palmitate, etofenamate stearate, benzaldehyde, flufenamic acid, decarboxyl, diethylene glycol diflufenamate, and flufenamic acid butyl ester were 7.647, 3.366, 5.703, 9.794, 10.417, 11.270, 3.951, 4.823, 7.379, 8.816 and 8.609 min, respectively. Method validation was initiated according to ICH Q2 (R1) guideline. Resolution was greater than 1.5. The calibration graphs plotted with five different concentrations were linear with a correlation coefficient, $r > 0.997$. Limit of quantitaion is $6.2x10^{-5}$ mg/mL for etofenamate, $4.7x10^{-4}$ mg/mL for benzyl alcohol, $5.4x10^{-5}$ mg/mL for benzyl nicotinate, $4.7x10^{-5}$ mg/mL for etofenamate myristate, $6.5x10^{-5}$ mg/mL for etofenamate palmitate, $6.5x10^{-5}$ mg/mL for etofenamate stearate, $4.8x10^{-5}$ mg/mL for fenzaldehyde, $6.5x10^{-5}$ mg/mL for flufenamic acid, $3.5x10^{-5}$ mg/mL for decarboxyl, $5.6x10^{-5}$ mg/mL for diethylene glycol diflufenamate, and $8.8x10^{-5}$ mg/mL for flufenamic acid butyl ester. According to the validation results, the proposed method was a rapid, selective, precise and sensitive method to determine etofenamate, benzyl nicotinate and benzyl alcohol in semi-solid pharmaceutical products.

P305

EVALUATION OF ANTICANCER DRUG AND DNA INTERACTION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Temozolomide, a member of the DNA interactive agents group, is an alkylating agent with antitumor activity leading to the alkylation of DNA bases in the minor and major grooves. The aim of this study was evaluation of interaction mechanism of anticancer drug Temozolomide and DNA by using high performance liquid chromatography and identified the drug-DNA interaction mechanism easily, rapidly, more selectively, and safely. In addition, Uv-vis and fluorescence spectroscopic techniques were also used to investigate the interaction. In terms of the results obtained by using chromatographic and spectroscopic techniques, the binding coefficients of the drug and DNA were calculated $3,7\times10^4$ M^{-1} ve $2,6\times10^4$ M^{-1}, respectively. The type of interaction is complex including intercalative and groove binding. Moreover, degradation product of Temozlomide was identified by using chromatographic and spectroscopic methods. Degradation product,AIC, was also inteacrts with DNA.

P306

QUANTUM CHEMICAL SIMULATION STUDIES ON INHIBITION PERFORMANCES OF SOME THIAZOLE AND THIADIAZOLE DERIVATIVES AGAINST CORROSION OF IRON

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The prevention of corrosion using various methods is an important issue for industrial applications of materials. To prevent the corrosion of the materials, organic compounds having π-bonds and heteroatoms such O, N and S have been widely used[1]. In the present study, to predict corrosion inhibition performances of 2-amino-4-(4-chlorophenyl)-thiazole (Inh1), 2-amino-4-(4-bromophenyl)-thiazole (Inh2), 4-(2-aminothiazole-4-yl)-phenol (Inh3), 5,5′-(ethane-1, 2-diyldisulfanediyl) bis-(1,3,4-thiadiazole-2-amine) (Inh4), 5,5′-(propane-1,3-diyldisulfanediyl) bis-(1,3,4- thiadiazole-2-amine) (Inh5) against corrosion of Fe metal, density functional theory (DFT) calculations was performed on these mentioned molecules. Quantum chemical parameters such as the highest occupied molecular orbital energy (EHOMO), lowest unoccupied molecular orbital energy (ELUMO), the energy gap between ELUMO and EHOMO (ΔE), chemical hardness, softness, electronegativity, proton affinity, global electrophilicity, global nucleophilicity and total energy (sum of electronic and zeropoint energies) were calculated and discussed with the help of HF/SDD, HF/6-311G, HF/6-31++G, B3LYP/SDD, B3LYP/6-311G and B3LYP/6-31++G methods.

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P307

A SIGNAL PROCESSING TECHNIQUE APPROACH FOR THE SIMULTANEOUS DETERMINATION OF A BINARY MIXTURE IN A PHARMACEUTICAL DOSAGE FORM

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In this work, a continuous wavelet transform method [1, 2] was developed and applied to the simultaneous spectral quantification of irbesartan (IRB) and hydrochlorothiazide (HCT) in their synthetic mixtures and tablets. This signal processing method do not require any separation step for the analysis of both compounds having the strong overlapping spectra in the same spectral region [3, 4, 5]. After applying many wavelet functions, the family consisting of bior1.3 was found to be suitable for the quantitative determination of the mentioned drugs. After the name (RS-bior1.3-CWT) was given, the calibration equations were obtained by measuring the CWT-amplitudes at 235.9 nm for the IRB determination and at 262.8 nm for the HCT determination, respectively. The proposed methods were successfully applied to the analysis of the IRB- HCT in the pharmaceutical tablets. The combined use of the CWT method with zero-crossing technique is new and promising approaches for the quantification of the related compounds. In order to demonstrate the validity and applicability of the methods, the optimal control processes of the proposed methods was performed by using the analytical validation parameters.

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P308

QUANTITATIVE ESTIMATION OF ACTIVE COMPOUNDS IN A PHARMACEUTICAL TABLET BY USING RATIO SPECTRA – DERIVATIVE SPECTROPHOTOMETRY AND UPLC TECHNIQUE

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In this context, a hyphenated signal processing approach, ratio spectra – derivative (RD) spectrophotometric method was developed and applied to the simultaneous quantification of candesartan (CAN) and hydrochlorothiazide (HCT) in their synthetic mixtures and tablets [3]. Also an ultra performance liquid chromatography (UPLC) method was developed as a comparison technique. This mentioned spectrophotometric method does not require any separation step for the analysis of both compounds having the strong overlapping spectra in the same spectral region [1,2]. The RD method was applied to the UV spectra of the CAN and HCT. The calibration equations were obtained by measuring the amplitudes at 243.1 nm for the CAN determination and at 262.7 nm for the HCT determination, respectively. In order to demonstrate the validity and applicability of the methods, the optimal control processes of the proposed methods was performed by using the analytical validation parameters.

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P309

SIMULTANEOUS DETERMINATION OF PROCAINE, ISOXSUPRINE, NIKETHAMIDE, CHLORPROMAZINE AND FLUFENAMIC ACID IN HORSE URINE BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

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A new, efficient and quick method has been developed and optimized for the determination of some acidic and basic doping substances (Procaine, Isoxsuprine, Nikethamide, Chlorpromazine and Flufenamic acid) in horse urine by dispersive liquid-liquid microextraction in combination with high-performance liquid chromatograpy tandem mass spectrometry. The reversed-phase chromatographic separation was obtained with a column Zorbax SB-C18 extended (150x3 mm; 3,5 µm; 80 Å) in gradient elution mode using distilled water-0.1% formic acid (v/v) and methanol-0.1% formic acid (v/v). The total run time was 9 minutes. Horse urine samples were analyzed by the multiple reaction monitoring (MRM) in the positive ion mode for Procaine, Isoxsuprine, Nikethamide, Chlorpromazine and negative ion mode for Flufenamic acid. Best results were obtained by use of 110 V for Isoxsuprine and Chlorpromazine, 80 V for Procaine and Nikethamide, 100 V for Flufenamic acid as fragmentor voltage. Under optimum conditions, pH of the horse urine samples (1 mL) was adjusted 6. Acetonitril (900.0 µL) and sodium chloride (20%) were added. Chloroform (80.0 µL) was used as extraction solvent. The method presented linear range of 0-100.0 ng mL^{-1} for all these substances. LOD values were found as 0.07 ng mL^{-1} for Procaine, 0.06 ng mL^{-1} for Isoxsuprine, 0.08 ng mL^{-1} for Nikethamide, 0.093 ng mL^{-1} for Chlorpromazine and 0.075 ng mL^{-1} for Flufenamic acid. It is expected that this method, which was the first to combine the use of DLLME and LC/MS-MS for the simultaneous determination of these substances in horse urine.

P310

DISSOCIATION CONSTANTS DETERMINATION OF SOME NOVEL AMINOCARBOTHIOL-PYRROLIDINE DERIVATIVES BY POTENTIOMETRIC TITRATION METHOD

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Acid or base dissociation constants are important physicochemical parameters, which give some useful information about pharmaceutically active compounds such as solubility, absorption, distribution, metabolism, and elimination. Hence, the relationship between the acid dissociation constants and structural diversity in drug design studies is crucial. Several analytical methods have been used to determine acid dissociation constant such as potentiometry, chromatography spectroscopy and electrophoresis. Among these techniques, potentiometry is one of the convenient method to determine acid dissociation constants of biologically active hetereocycles and drug molecules [1]

Aminocarbothiol pyrrolidine derivatives have been extensively studied during the last decades due to possessing biological and pharmacological properties such as antifungal, antibacterial, and antimalarial activity [2]. Pyrrolidine ring also have important place in drug research and this structure is found in many alkaloids and drug molecules [3,4].

As part of our ongoing research work [5] acid ionization constants for $2x10^{-4}$ M of the synthesized aminocarbothiol pyrrolidine derivatives were determined in acetonitrile-water (20:80, (v/v)) hydroorganic solvent system at 25 ± 0.1 °C by using potentiometric titration method in the presence of sodium chloride and hydrochloric acid, and sodium hydroxide were used as titrant. Acid ionization constants were determined potentiometric titration method from obtained data by Molspin Titration System using HYPERQUAD computer program.

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A NEW APPROACH FOR EXTRACTION OF PHENOLIC SPECIES FROM OLIVE

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Toxic solvents and chemicals are commonly used for extraction methods in analytical chemistry.These chemicals pose serious risks for those who use it and the environment. Recently, deep eutectic solvents (DESs) were introduced as a green alternative to conventional solvents for sample preparation and extraction procedures. DESs are biodegradable, non-toxic, non-volatile, non-flammable, inexpensive, and easily accessible and environmentally friendly [1,2]. In this study, we aim to develop a new procedure to extract fenolic species from olive using deep eutectic solvents. For this purpose, different eutectic mixtures have been prepared for extraction of phenolic compounds, choline chloride -urea/choline chloride-lactic acid combinations were tried in the first step. The synthesized DESs were characterized by Fourier transform infrared spectroscopy (FT-IR). After extraction of the olives which planted in mersin region, phenolic substances were identified by High-Performance Liquid Chromatography with Diode-Array Detection (HPLC/DAD).

This study was supported by the Scientific Research Projects of Mersin University (Project number:2017-1-TP2-2223)

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P312

PROTONATION CONSTANTS OF GAMMA-AMINOBUTYRIC ACID, BETA-ALANINE AND THEIR METHYL ESTERS IN WATER-ACETONITRILE MIXTURES

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Amino acids are biologically important substances and have great significance as “building block” of living systems. In biological systems, one of the considerable clarifications of various processes in the systems may frequently be possible only by physical and chemical properties, such as macro and micro protonation constants of amino acids in water and water-solvent mixtures [1-3].
Acetonitrile (ACN) is one of the important dipolar aprotic solvents. Its capability of hydrogen-bonding is weaker than that of water. Consequently, the most suitable mediums for the determinations of macro and micro protonation constants are water and ACN (or another polar aprotic solvent of the close properties) mixture metabolically [4 -5].
In this work, the protonation constants of γ-aminobutyric acid (GABA), methyl 4-aminobutyric acid (GABAMe), β-alanine (βAla) and β-alanine methyl ester (βAlaMe) systems in different solvent mixtures of ACN in water (0 – 50% (v/v)) are examined at 20.0 °C and at constant ionic strength (0.1 mol L^{-1} $NaClO_4$). These systems are studied by potentiometric pH titration method in order to determine the protonation constants. The solutions used in the titrations are prepared and the calculations are made by the method of Irving and Rossotti [6]. For now, some of our results are as follows;

	%0 ACN		%10 ACN		%25 ACN		%50 ACN	
	pK_O	pK_N	pK_O	pK_N	pK_O	pK_N	pK_O	pK_N
GABA	4.0	10.7	4.2	10.8	4.4	10.8	4.9	10.7
GABAMe	-	10.2	-	10.3	-	10.2	-	10.1
βAla	3.5	10.7	3.6	10.6	3.9	10.7	4.3	10.7
βAlaMe	-	9.8	-	9.6	-	9.5	-	9.3

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ACIDITY DISSOCIATION CONSTANT DETERMINATION OF GABAPENTIN BY POTENTIOMETRIC METHODS

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Acidic dissociation constant (pKa) is a physicochemical parameter important in absorbtion, dissosiation and elimination mechanisms of drugs in human body. Depending on Henderson-Hasselbach [1] equation, ionized and non ionized forms of drugs are significant in terms of crossing through the cell membranes, binding to plasma proteins and tissue penetration. Ionization degrees at different pH values effects drugs crossing capability through biological membranes. Therefore, pKa determining is necessary for examining pharmacodynamic/pharmacokinetic due to solubility and permeation of drug substances through biological membranes [2]. Different analytical methods including potentiometric, spectrophotometric and chromatographic are used for the determination of pKa values of pharmaceutical active ingredients. Gabapentin (Fig. 1) (1-(aminomethyl) cyclohexaneacetic acid) is an anticonvulsant drug used in the treatment of epilepsy and neuropathic pain [3,4]. It is a hydrophilic analogue of the inhibitory neurotransmitter y-aminobutyric acid (GABA), and was designed to act as a GABA A receptor agonist that could freely cross the blood-brain barrier [5]. Any experimental value could not be find in the current literature for the pKa value of gabapentin. Therefore; the aim of this study is to determine gabapentin's pKa value by using potentiometric and derivative techniques. Gabapentin solution which contained 0.05 M KCl for ionic strength was titrated with 0.01 M NaOH. First and second derivative techniques were used to evaluate equivalence point of titration.

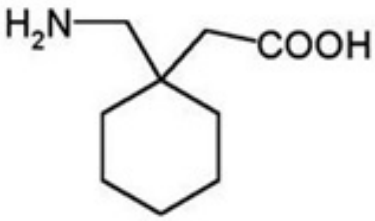

Fig. 1. Gabapentin

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SIMULTANEOUS DETERMINATION OF DISSOLVED SILVER AND SILVER NANOPARTICLES IN WATER SAMPLES BY SP-ICP-MS

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The use of engineered nanoparticles (NP) in consumer products such as textile, food additives and cosmetics is constantly increasing and still there is a concern about the toxic effects of nanoparticles on environment or living organisms. In general, NP characterization is performed by imaging techniques such as Scanning Electron Microscopy or Transmission Electron Microscopy. The sensitivity of these methods, especially determining NPs at environmentally relevant concentrations is insufficient [1]. Thus, analytical methods are needed for determination and characterization of NPs within these concentration levels. It has been turned up in recent years that ICP-MS could also be used for NP determination and this highly new method called as Single Particle ICP-MS (SP-ICPMS) [2]. In contrast to tranditional ICP-MS, thousands of individual intensity readings are acquired, each with a millisecond dwell time. Besides identifying the silver nanoparticle size, in this study it was aimed to determine simultaneously dissolved silver and silver nanoparticles in water samples at pg/ml level. In order to obtain the best sensitivity; nebulizer flow rate, dwell time and total acquisition time was optimized. Under optimized conditions; silver NP size distribution, NP number concentration, element mass concentration of NP and the dissolved silver concentration was determined in water samples. The main disadvantage is, if the mean diameter of NP is less than 20 nm, the particle size cannot be determined with the proposed method.

This study was supported by Gazi University Research Fund, Project No: 02/2017-09.

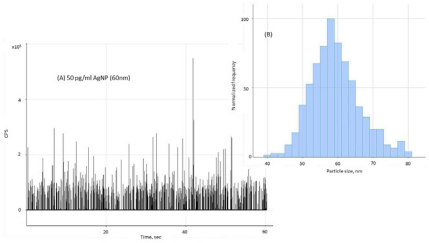

Figure 1. (A) Signal profile gathered from SP-ICP-MS, (B) Particle size distribution

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DETERMINATION OF PARABENS IN PHARMACEUTICALS AND PERSONAL CARE PRODUCTS BY DLLME-HPLC

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Dispersive liquid-liquid microextraction (DLLME) [1] with back-extraction was used prior to high-performance liquid chromatography (HPLC) for the extraction of the four most commonly used parabens [i.e, methyl- (MP), ethyl- (EP), propyl- (PP) and butylparaben (BP)]. Optimum extraction conditions were achieved using 225 µL chloroform (as an extraction solvent) and 750 µL ethanol (as a disperser solvent) at an extraction time of 15 s. Back-extraction of parabens into 100 µL of 50 mM NaOH solution within 20 s facilitated direct injection of the final extract into HPLC. The analytes were separated at 20 °C using a reversed-phase column, i.e., ACE-C18 (3 mm ID × 12.5 cm (5 µm), mobile phase MeOH:H_2O, 60:40 (%, v/v) at pH 7.0, flow rate 1.0 mL min^{-1} and injection volume of 20 µL. The analytes were monitored using a diode-array detector at 258 nm. Enrichment factors were up to 6.1 resulting in limits of detection (LOD) as low as 0.07 mg L^{-1}. Calibration graphs showed good linearity with coefficient of determination (R^2) higher than 0.995. DLLME-HPLC was demonstrated to be a simple and rapid method for the determination of parabens in pharmaceuticals and personal care products with percentage relative recoveries in the range of 89.3-110.7%.

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STRUCTURAL, SPECTROSCOPIC AND QUANTUM CHEMICAL CALCULATIONS ON CYCLOMETALATED IRIDIUM (III) COMPLEXES WITH ANTICANCER PROPERTIES

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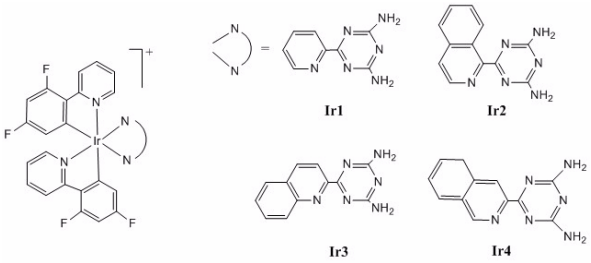
Organometallic complexes of the platinum-group metals ruthenium, osmium, rhodium and iridium have recently been indicated to be promising anticancer agents. In current literature, organometallic iridium complexes have found as alternatives to platinum-based anticancer agents. These complexes may find clinical use in the treatment of Pt-resistant cancers with fewer side effects and a wider spectrum of activity [1,2]. In this work, Some calculations for the four complexes $[Ir(dfppy)_2(L)]^+$ (dfppy=2-(2,4-difluorophenyl)pyridine, L=6-(pyridin-2-yl)-1,3,5-triazine-2,4-diamine, Ir1; 6-(isoquinolin-1-yl)-1,3,5-triazine-2,4-diamine, Ir2; 6-(quinolin-2-yl)-1,3,5-triazine-2,4 diamine, Ir3; 6-(isoquinolin-3-yl)-1,3,5-triazine-2,4-diamine, Ir4; will be made in gas phase. The structures of the complexes will be determined by some spectroscopic calculations (IR, NMR and UV-VIS spectra etc.). Molecular structure descriptors which are HOMO energy, LUMO energy, LUMO-HOMO energy gap, hardness, softness, electronegative and chemical potential will be examined. These descriptors will be associated with the anticancer properties of the complexes. The complexes will be subjected to molecular docking studies against the respective cancer cells. The anticancer properties of the complexes will be elucidated by the structure-activity relationship.



Chemical structures of Ir1-Ir4 complexes

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VALIDATED SQUARE-WAVE VOLTAMMETRIC METHOD FOR DETERMINATION OF THIOCOLCHICOSIDE IN PHARMACEUTICAL PREPARATIONS

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Thiocolchicoside (THIO) is (s)-N-[3-(BD-glucopyranoxyloxy)- 5,6,7,9-tetrahydro-1,2-dimethoxy-10- methylthio)9-oxobenzo- [a]heptalen-7yl]acetamide, sulfur derivative of cochicoside and possesses non-sedating muscle relaxant action [1-2]. There is no literature available about the electro reduction of THIO. The aim of the present study is to develop and validate simple, sensitive, accurate, precise and reproducible square-wave voltammetry (SWV) method for determination of THIO in pharmaceutical dosage forms and to investigate the reduction behavior of THIO on hanging mercury drop electrode (HMDE). The effect of supporting electrolyte pH and voltammetric parameters were investigated. Current type and reversibility of the electrode reaction were investigated by using cyclic voltammetry. The relationship between reduction peak current and concentration of THIO was investigated by using SWV. A well-defined cathodic peak was observed at -1050 mV versus Ag/AgCl reference electrode in pH 7 phosphate borate (FB) buffer. For SWV method LOD, LOQ and linearity range were found 0.29 µM, 0.41 µM and 0.41-22.56 µM, respectively. Validation parameters, such as sensitivity, accuracy, precision, ruggedness, robustness and recovery were evaluated. Developed and validated method was employed for quantification of THIO in pharmaceutical formulations.

Keywords: Thiocolchicoside, voltammetric methods, electrochemical behaviour, validation, pharmaceutical preparations.

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QUANTITATIVE ANALYSIS OF ROPINIROLE FROM TABLETS AND SERUM SAMPLES BY VOLTAMMETRIC TECHNIQUES

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Parkinson's disease is a neurodegenerative and progressive disorder and the treatment procedures generally aim to increase dopamine levels in patients' brains. Ropinirole, which is one of the non-ergoline dopamine agonists, binds specifically to D2 and D3 receptors; this binding is almost equally selective as dopamine [1].

In this study, voltammetric measurements were performed for ropinirole at glassy carbon electrode using differential pulse voltammetry (DPV) and square wave voltammetry (SWV) in order to develop rapid, simple and repeatable determination method. In order to analyze the oxidation behavior of this compound, different supporting electrolytes between pH 0.3 – 12.0 are used and cyclic voltammetry, DPV and SWV are employed. Two different supporting electrolyte (0.1 M H_2SO_4 and pH 7.0 Britton-Robinson buffer solutions) solutions are used for studying regression and the required validation parameters. Linearity was found between 8×10^{-6} M and 2×10^{-4} M for DPV and 4×10^{-6} M and 2×10^{-4} M for SWV in 0.1 M H_2SO_4 with detection limits of 1.06×10^{-6} M and 1.04×10^{-6} M, respectively. Linear range was found 2×10^{-6} – 6×10^{-5} M for DPV and 4×10^{-6} – 6×10^{-5} M for SWV in pH 7.0 Britton Robinson buffer solution with detection limits of 4.73×10^{-7} M and 2.65×10^{-7} M, respectively. Analysis from pharmaceutical dosage forms and serum samples are performed as well to demonstrate the applicability of developed techniques. Average recovery values were found between 99.65 % – 100.55 % which showed the accuracy of the developed techniques.

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ULTRAFILTRATION-BASED EXTRACTION AND LC-MS/MS ANALYSIS OF PHENYLALANINE IN HUMAN PLASMA SAMPLES

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Phenylketonuria (PKU) is an inherited disorder that increases the levels of a substance called phenylalanine in the blood. If PKU is not treated, phenylalanine can build up to harmful levels in the body, causing intellectual disability and other serious health problems. Therefore, an efficient screening test is required and must be performed as early as possible [1-2].

In our study, simple and sensitive LC-ESI-MS/MS method has been developed and validated for quantification of phenylalanine in human plasma using $^{13}C_6$ phenylalanine heavy isotope. Phenylalanine in human plasma samples were extracted by using ultrafiltration techniques. The instrument was set in the multiple-reaction-monitoring (MRM) mode. Parameters affecting LC separation and MS/MS detection were systematically investigated and optimized. Chromatographic separation was achieved on a Merck SeQuant ZIC-HILIC (100x4.6 mm, 5 µm) at a column temperature of 40 °C using a mobile phase of mixture of acetonitrile containing 0.1% formic acid and water containing 0.1% formic acid at a flow rate of 0.35 mL/min. The eluent from the HPLC column was introduced directly to the using the electrospray ionization interface in the positive ion mode. The transitions m/z 167.1→121.1 for $^{13}C_6$ phenylalanine, m/z 166.0→120.0 for phenylalanine itself were monitored using the MRM mode. The assay was linear concentration range of 2.5 ng/mL to 1000 ng/mL (r^2=0.999). The developed method was validated according to FDA guidelines [3]. The method was found linear, sensitive, precise, accurate, and selective.

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NEW APPROACH FOR DETERMINATION OF ESCITALOPRAM OXALATE IN HUMAN URINE BY CAPILLARY ELECTROPHORESIS

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Capillary electrophoresis has become an effective analytical method for determination of pharmaceuticals in biological fluids [1]. In this regard, reliable and sensitive method has been developed for quantification of escitalopram oxalate (ESC-OX) in human urine samples after a simple liquid-liquid extraction. Variation in analysis parameters was avoided by using metoprolol as an internal standard (IS). Separation was accomplished by a fused silica capillary with 40 cm effective (48.5 cm total, 75 µm i.d.) length and detection at 200 nm by using diode-array detector. The background electrolyte was consisted of 15 mM phosphate buffer (pH 2.5). The applied potential was 22.5 kV and the samples were injected at 50 mbar pressure for 10 s. The overall analysis time for both ESC-OX and IS was less than 8 min. The method was validated according to the ICH Q2(R1) guidelines [2]. The current method exhibited a linearity over 0.0269- 4.351 µg mL-1 with regression coefficient of 0.9987 and a satisfactory sensitivity with LOD of 0.00517 µg mL-1.

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EFFECT OF DIFFERENT TYPES OF FLUORINATED STATIONARY PHASES ON THE LIQUID CHROMATOGRAPHIC SEPARATION OF ESCITALOPRAM OXALATE AND RELATED IMPURITIES

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Variations on the liquid chromatographic separation of the fluorine atom containing antidepressant drug escitalopram oxalate (ESC-OX) [1] and its six impurities (namely oxalic acid, CIT A, CIT B, CIT C, CIT D and CIT E) were studied by utilizing different types of fluorinated stationary phases. The effect of particle structure (i.e. core-shell or fully porous), retention characteristics, main chromatographic system suitability parameters (i.e., number of theoretical plates, resolution, asymmetry, tailing, capacity factors) were examined, and the results were compared with each other for the performance evaluation. Acceptable chromatographic separation was achieved under isocratic conditions; the analytes were eluted using acetonitrile: methanol: water: phosphate buffer solution (pH 3.5, 50 mM) (25:5:20:50, v/v/v/v) mixture as the mobile phase and monitored at 210 nm via an UV- visible detector. The highest separation efficiency with good resolution and reasonable retention was obtained when Kinetex® PFP (2.6 µm, 150 × 4.6 mm, Core-Shell Particles, Phenomenex) column was used, which was followed by Fluophase RP® (3 µm, 10 cm × 4.6mm, Fully –Porous Particles, Thermo Scientific), Ascentis® Express F5 (2.7 µm, 10 cm × 4.6mm, Core-Shell Particles, Supelco) and Luna® PFP (2.6 µm, 150 × 4.6 mm, Fully –Porous Particles, Phenomenex). Moderate differences in the system suitability results were observed for the examined columns, which were inferred to be associated to the differentiations in the ligand type and diffusion pathway. (Autors would like to acknowledge the financial support of Anadolu University Scientific Research Projects Commission - Project No: 1502S091)

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PHOSPHORESCENT DETECTION OF DNA- DRUG INTERACTION BASED ON EMISSION QUENCHING OF ZNS QUANTUM DOTS VIA PHOTOINDUCED ELECTRON TRANSFER

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The interaction between epirubicin (EPI) and double-stranded deoxyribonucleic acid (ds-DNA) was investigated by quantum dots (QDs)/EPI nanohybrids as novel room temperature phosphorescence (RTP) sensor. L-cysteine caped Mn-doped ZnS QDs were synthesized using zinc sulfate, manganese chloride, and sodium sulfide in aqueous solution. The method based on the quenching effect of EPI on the phosphorescence emission of Mn-doped ZnS QDs via photoinduced electron-transfer (PIET) mechanism. EPI adsorbed on the surface of QDs and quenched the photoluminescence of QDs through the photoinduced electron-transfer process. The addition of DNA caused the restoration of QDs emission intensity, due to removing of EPI from the surface. The quantum dots were synthesized in an aqueous medium and characterized. Morphologies of QDs were shown to have a spherical shape and nearly a uniform size with a diameter of about 3.5 nm. The quenching mechanism of Mn-doped ZnS QDs by EPI is a combined dynamic and static. The static and dynamic quenching constants were found as $5.36x10^5$ M^{-1} and $3.19x10^4$ M^{-1}, respectively. In addition to this method, UV-Vis spectrophotometry and fluorescence spectroscopy were used to evaluate DNA/drug interaction and calculate the binding constant (K), which was $3.83x10^5$ M^{-1}. This method is simple, low cost and avoids interferences from autofluorescence and scattering light.

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OPTIMIZATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF PHENOLIC COMPOUNDS FROM DIFFERENT FOOD SAMPLES USING TAGUCHI DESIGN

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A reverse phase high performance liquid chromatography method has been developed and validated for the simultaneous determination of five phenolic compounds, namely, rutin, naringin, morin, quercetin and p-coumaric acid. Optimization was carried out by using Taguchi Design. Set of 32 experiments helped to optimize the parameters such as pH, concentration and percentage of buffer and temperature of the column. Analytes were separated on C8 column within 19 min using isocratic system of 0.1% phosphoric acid and acetonitrile (75: 25 v/v) as mobile phase with flow rate of 0.1 mL/min. Linearity of all five analytes were observed in the concentration ranges of 0.5-70 µg/mL. Limit of detection (LOD) and limit of quantification (LOQ) were found to be in the range of 0.024 to 0.081 and 0.081 to 0.269 µg/mL, respectively. Recoveries and relative standard deviations (%RSD) were found to be in the range of 96.7-105.3% and 0.05-1.68%, respectively. This developed method has been successfully applied for the quantitative determination of analyzed components from different food samples which are important sources of these compounds.

This study was supported by to TUBITAK (The Scientific and Technological Research Council of Turkey) as TÜBİTAK 2216 –RESEARCH FELLOWSHIP PROGRAMME FOR INTERNATIONAL RESEARCHERS

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SIMULTANEOUS DETERMINATION OF SEVEN PARABEN DERIVATIVES IN PHARMACEUTICALS AND COSMETICS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Paraben derivatives are widely used as antimicrobial preservatives in foods, cosmetics and pharmaceuticals [1,2]. Due to official regulations on the use of these compounds, their analysis is essential for the estimation of their exposure. On this basis, the presented study was realized to develop a simple, selective and cheap high-performance liquid chromatographic method for quantitative determination of methylparaben (MP), ethylparaben (EP), n-propylparaben (NPP), i-propylparaben (IPP), n-butylparaben (NBP), i-butyl paraben (IBP) and benzylparaben (BP) in pharmaceuticals and cosmetic products. The chromatographic separation of the analytes was achieved under flow rate gradient elution conditions using a C18-bonded core-shell silica particle column (2.6 µm particle size, 150 x 3.0 mm, Phenomenex Co.) (Figure 1). Samples were injected into the system as aliquots of 1.0 µl and the compounds were detected by using a photo-diode array detector set at 254 nm wavelength. The proposed method was fully validated and successfully applied on different pharmaceutical and cosmetic samples (n=27) including syrups, suspensions, oral sprays, gels, etc. (The authors would like to thank Anadolu University Scientific Research Projects Commission for the financial support – Project No: 1606S567)

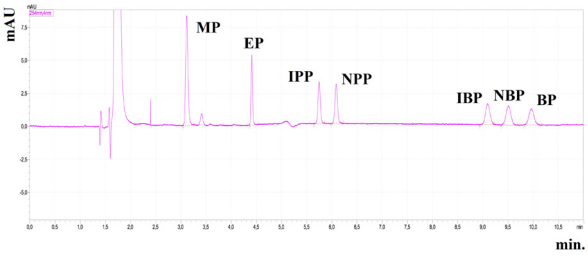

Figure 1. A typical chromatogram of MP, EP, NPP, IPP, NBP, IBP and BP.

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DETERMINATION OF CORROSION INHIBITION EFFECTS OF AMINO ACIDS: QUANTUM CHEMICAL AND MOLECULAR DYNAMIC SIMULATION STUDY

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Amino acids [1] are biomolecules that have vital significance to all organism and they are the building blocks of proteins and many essential substances like neurotransmitters, hormones and nucleic acids. Amino acids are biologically important organic compounds in the human body which contain two important functional groups namely: –NH2 (amine) and –COOH (carboxylic acid) in their structures. In the present work, corrosion inhibitive performance amino acids such alanine (Ala), methionine (Met), aspartate (Asp), asparagine (Asn), lysine (Lys), arginine (Arg) and histidine (His) were investigated. All quantum chemical calculations related to these amino acids at the B3LYP/6-31G++(d, p) HF/6-31G++(d,p) methods were performed. Corrosion inhibition effects of the subject amino acids were discussed not only in the gas phase but also in the water phase, acetic acid and formic acid. Furthermore, molecular dynamic simulations employing Monte Carlo sampling approach were applied to search for the most stable configuration and adsorption energies for the interaction of the amino acid corrosion inhibitors on Cu (111)/50 H2O interface. A good correlation between theoretical data and experimental data has been obtained. Moreover, arginine that is a basic amino acid the best corrosion inhibitor among amino acids, considered in this study

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IONIC LIQUID BASED ION-PAIRING MICROEXTRACTION COMBINED WITH SPECTROPHOTOMETRY FOR PRECONCENTRATION AND QUANTITATION OF MELAMINE IN MILK AND MILK-BASED PRODUCTS

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Melamine is a chemical species that can be supplied very cheaply and easily. Since melamine has high nitrogen content, it has been added to some food products such as milk, infant formula, yogurt, wheat gluten and biscuits in order to increase the apparent protein content by unethical manufacturers [1]. A simple, rapid and economical ultrasound-assisted green microextraction (UA-ME) procedure was developed for extraction and determination of trace amounts of melamine in milk products, followed at 310 nm by UV-Visible spectrophotometry. In the study, 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [C6mim][Tf2N] as ionic liquid (IL) was used in presence of sodium dodecyl sulfate (SDS) as both sensitivity enhancer and an oppositely charged auxiliary ligand at pH 4.0 for extraction of melamine, and methanol was selected as a disperser solvent. Under the optimal conditions, the linear working range for model solutions was 5–250 µg L−1 with a correlation coefficient of 0.9955 while it ranged from 5 to 210 µg L-1 for matrix-matched solutions. The limit of detection (LOD, 3Sb/m) and precocentration factor (PF) from preconcentration of sample of 50 mL were 1.6 µg L−1 and 125 with a sensitivity enhancement factor (EF) of 75 and a lower relative standard deviation (RSD) than 3.1%, respectively. The accuracy and precision were also checked by repeatability/reproducibility studies and recovery experiments, respectively. After the validity test, the method was applied successfully to the determination of melamine in milk products. The satisfactory recoveries in range of 94.2-103.7% were obtained for two different melamine concentrations (10 and 50 µg L−1).

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P327

A NEW SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NITRITE IN MEAT PRODUCTS AFTER ULTRASONIC ASSISTED IONIC LIQUID ASSISTED MICROEXTRACTION

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The importance of monitoring residual nitrate and nitrite in processed foods and beverages has long been recognized, but the reasons for this have changed in recent years. There was initial concern about the harmful effects of nitrate and nitrite on health, especially regarding the risk of methemoglobinemia and formation of carcinogenic N-nitroso compounds in infants [1]. A simple method for the sensitive determination of trace amounts of nitrite in meat products has been developed by combination of ultrasonic assisted ionic liquid assisted microextraction (UA-ILME) and spectrophotometre. Nitrite was extracted from aqueous solution using 1-butyl-3-methylimidazolium bis (trifluorosulfonyl) imide, [BMIM] [Tf2N] as extracting solvent. The effects of main parameters such as pH, sample volume, and salt concentration, ultrasonic time and temperature, ligand amount were investigated and optimized. Under the optimum conditions, a linear calibration graph was obtained in the range of 4–270 ng mL−1 of nitrite with limit of detection (LOD) of 1.5 ng mL−1. The relative standard deviation (RSD) for determination of 10 and 50 ng mL−1 of nitrite was 2.6 and 3.1%, respectively. The proposed method was successfully applied for the determination of nitrite in the meat products with recoveries of 91.3–104.2% using standard addition method.

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EXTRACTION AND DETERMINATION OF QUERCETIN IN DIFFERENT DRINKS BY ULTRASONIC ASSISTED MICROEXTRACTION BASED ON SPECTROPHOTOMETRY

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Quercetin is a powerful antioxidant which is regularly consumed by humans. The quercetin content of foodstuffs is not yet precisely known but the available data display a range of 2–250 mg L-1 in fruits, 4–16 mg L-1 in red wine, 10–25 mg L-1 in tea, 3–13 mg L-1 in fruit juices [1]. A new and simple ultrasonic assisted microextraction with combine spectrophotometry method for the separation and preconcentration of trace amounts of quercetin was developed. 1-butyl-3-methylimidazolium tetrafluoroborate, [Bmim][BF4] and ethanol was used as the extraction and disperser solvent, respectively. The factors influencing the extraction by UAE such as the volume of the extraction and disperser solvents, pH and concentration of salt were optimized. The optimal conditions were found to be; amount of the extraction solvent, 50 mg; the volume of the disperser solvent, 125 µL; and the pH of the sample, 8. Under the optimum conditions, the linear range and detection limit were 1.5 to 350 µg L-1 and 0.3 µg L-1, respectively. The relative standard deviation (R.S.D.) at 10, 50 and 200 µg L-1 levels of quercetin (n = 10) was in range of 2.1-4.5%. Product recoveries ranged from 90.4 to 102.9%. The method was successfully applied to the determination of quercetin in the different drink samples.Therefore, the method was simple and reliable, with potential application in beverage samples analysis of quercetin.

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EVALUATION OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA FOR APPLICATIONS IN FOOD AND PHARMACEUTICAL INDUSTRIES

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Bacteriocins are bioactive peptides with antimicrobial activity produced by many Gram-positive and Gram-negative bacteria. The potential applications of bacteriocins as natural preservatives represent an alternative antimicrobial strategy for the increasing antibiotic resistance problem. The aim of this study was to investigate the bacteriocin production characteristics of lactic acid bacteria (LAB) isolated from human microbiota (vaginal secretion, breast milk, healthy breast-fed infant) and traditional food sources. Pediococcus pentosaceus 36 isolated from sausage and Pediococcus acidilactici 25 from vaginal secretion of healthy woman were selected through the 50 LAB strains according to their bacteriocin activity (46.000 AU/mL). The highest activity was obtained following the 18hr incubation at 37°C on MRS medium. While the activity was inhibited by proteolytic enzymes, it was not affected by detergents and organic solvents. Activity was also maintained at high temperature and wide pH range. Inhibition was observed against important Gram-positive bacteria, including Listeria monocytogenes as an important food-borne pathogen and methicillin-resistant Staphylococcus aureus as a clinical pathogen. The molecular weight of the semi-purified bacteriocin was estimated as 4.000Da by SDS-PAGE. In conclusion, bacteriocins produced by Pediococcus species isolated from two different sources have been partially characterized within the scope of the study. Bacteriocin produced by Pediococcus pentosaceus 36, a food isolate may be used in the food industry as well as bacteriocin produced by Pediococcus acidilactici 25 isolated from human microbiota, may be used in pharmaceutical and cosmetics industry as bio-preservatives in the future. Additional studies will be based on purification, enhancement and improvement of the stability.

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THE INVESTIGATION OF ESCHERICHIA COLI O157:H7 SEROTYPE IN DRINKING AND USING WATERS IN SIVAS

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Enterohemorragic E.coli O157:H7 strain which has been causing many severe infections in humans is known to be a food-borne pathogen especially recent years. This study aimed to determine the presence of E.coli O157:H7 strain in drinking-using waters of Sivas and the presence of coliform bacteria and fecal E.coli bacteria in the water. In this research;"TS EN ISO 9308-1 Water quality: Enumeration of E.coli and Coliform bacteria: Membrane Filtration System" was used according to the method. Collected from Gemerek, Sarkısla, Ulas, Yıldızeli and Zara counties of Sivas, 200 samples of drinking-using water filtered by membrane filtration method in TTC Tergitol. Suspected colonies were planted on TSA agar for Oxidase test. Oxidase(-) bacterias were planted on LST+MUG broth.UV light was checked and this bacterias were planted on EMB agar. Confirmed E.coli were planted on SMAC agar. Latex agglutination test was done. 80 of the samples were identified as coliform bacteria. 66 of these bacteria were identified as E.coli. Because of 3 bacterias showed sorbitol(-); these 3 bacterias were commented as suspicious about E.coli O157:H7.After latex agglutination test result, E.coli O157:H7 serotype was not found. The presence of E.coli in drinking-using water shows us fecal contamination. Therefore the results we obtained show that the pathogenic factors that occur in the places where the drinking-using water are inadequate in the control studies with chlorination which threaten public health.

Our work was supported by the Cumhuriyet University Scientific Research Projects Office with the project number T-590.

	Gemerek	Sarkisla	Ulas	Yildizeli	Zara	Total
Samples Numbers (n)	30	49	20	62	39	200
E. coli (n)	11	16	5	21	13	66
E. coli (%)	36,67	32,70	25	33,90	33,30	33
Coliform Bacterias (n)	12	20	8	24	16	80
Coliform Bacterias (%)	40	40,82	40	38,71	41,02	40

E. coli and Coliform bacterias isolation results

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EXTRACTION AND RELIABLE DETERMINATION OF ACRYLAMIDE IN THERMALLY PROCESSED FOODS USING IL-ME COMBINED WITH SPECTROPHOTOMETRY

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Acrylamide (AAm) is a carcinogenic chemical that can form in thermally processed foods by the Maillard reaction of glucose with asparagine [1]. The AAm can easily be formed especially in frequently consumed chips and cereal-based foods depending on processing conditions. Considering these properties of AAm, our study group has developed a new, simple and green method using ultrasound-assisted microextraction for the extraction of AAm from thermally processed food samples, and then its analysis at 530 nm by spectrophotometry. In the study, ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate, [Bmim][BF4]) as extractant was used in the presence of cationic phenazine group dye, 3,7-Diamino-5-phenylphenazinium chloride (PSH+, phenosafranine) at pH 7.5 for the extraction of AAm as ion-pair complex from selected samples. Ultrasonic power was utilized in the sample preparation step because it is more eco-friendly. The experimental variables that could affect microextraction efficiency were optimized in detail. Under optimum conditions, the analytical values obtained for the proposed method were as follows; linear working range, the limits of detection (LOD, 3Sb/m) and quantification (LOQ, 10Sb/m), preconcentration factor, sensitivity enhancement factor, sample volume and recovery% were 2.2–350 µg kg−1, 0.7 µg kg−1, 2.3 µg kg−1, 120, 95, 50 mL and 94.1-102.7%, respectively. The validity of the method was verified by the analysis of certified reference materials (CRMs) and intra-day and inter-day precision studies, and the meaningful results were obtained. Finally, the method was successfully applied to the determination of AAm levels in thermally processed foods using the standard addition method.

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P332

THE EFFECTS OF NUTRITION STATUS AND GENERAL CHARACTERISTICS ON KIDNEY STONE FORMATION AND RECURRENCE OF PATIENTS APPLICATED TO GAZI UNIVERSITY HOSPITAL UROLOGY CLINIC KIDNEY STONE BREAKING CENTER

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Stone disease is a complex, interrelated and has multi factor pathogenesis [1]. In this research, the investigation of the effects of general characteristics and nutritional status of patients treated in the Gazi University Hospital Urology Clinic Kidney Stone Breaking Center on kidney stone formation and recurrence, and to raise awareness about this subject are aimed. For this purpose Eighty-six patients who had kidney stone participated in the investigation. Data were collected with the questionnaire method. The 70.9% of the participant is male and 29.1% is female. The 50% of the patients had a kidney stone in their family history. Stone recurrence is found higher (67.4%) in participants who have kidney stone history in their family. The 46.5% of the patients are overweight and 18.6% are obese. In overweight and obese group stone recurrence ratio is found higher (65% - 62.5%). Patients are generally carrying on an inactive lifestyle. The 46.5% of the patients consume insufficient water (1500 ml and <). The 5.8% of patient consumed milk, 38.4% of patient consumed yogurt, 64% of patient consumed cheese, and 10.5% of patient consumed vegetable every day. The recurrence rate of people who consumed fruit and vegetables every day was lower than others who consumed 3-5 times in a week. The recurrence rate of patients who consumed nuts every day was found highest (100%). Drinking tea every meal also caused high stone recurrence rate (61.3%). It was determined that general characteristics of patients and their nutritional habits are effective for kidney stone formation.

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QUORUM SENSING ACTIVITY AND QS-DEPENDENT VIRULENCE OF CLINICAL PSEUDOMONAS AERUGINOSA SPECIMENS.

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It has been indicated in literature studies that bacteria are social communities that use intercellular communication mechanisms called quorum sensing to adapt to changing environmental conditions. These mechanisms are based on the synthesis of extracellular signal molecules, which allow bacteria to perceive the population density in the environment and change the general order of gene expression. QS plays a significant role in the regulation of many virulence factors such as bacterial motility, antibiotic biosynthesis, biofilm formation, bacterial plasmid conjugation, etc. Virulence mechanisms of Pseudomonas aeruginosa, which causes various types of infections in humans, are also regulated by quorum sensing and has importance in the pathogenity formation. In this study, our aim was to investigate the QS signal molecules and QS-dependent virulence of clinical P. aeruginosa isolates. Short (C4-HSL) and long chain (C12-HSL) signal molecules were determined by Chromobacterium violaceum CV026 and Rhizobacterium radiobacter BAA 2240 biosensor strains and QS dependent swarming-swimming-twitching motility, elastase, protease, pyocyanin, rhamnolipid and biofilm production were evaluated. As a result, it was found that 100% of the 141 clinical P. aeruginosa specimens were produced C12-HSL and 59.5% were produced C4-HSL. It was determined that depending on the C4-HSL production; rhamnolipid, elastase, pyocyanin and based on C12-HSL production; bacterial motility, alkaline protease, elastase and biofilm production were shown in different values.

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IMMUNOMODULATORY EFFECT OF PEDIOCOCCUS PENTOSACEUS OZF DERIVED EXOPOLYSACCHARIDES (EPS) AND EPS/CPG-ODN COMPLEXES

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The aim of this study was to examine the immunomodulatory potential of exopolysaccharides (EPS) derived from Pediococcus pentosaceus OZF to establish whether EPS may become a good candidate for clinical use. The strain was isolated from healthy human breast milk and exhibited potential probiotic properties. The highest production of released form of EPS (eps-r: 1.533 mg/L) was detected following 12h incubation at 34°C in lactose containing medium. Cell-bound EPS (eps-b) was also extracted with the amount of 600 mg/L. We investigated the effect of eps-r and eps-b on the production of inflammatory mediators by mouse spleen cells and compared with the effect of lipopolysaccharide (LPS) and peptidoglycan (PGN) by ELISA following 24hr stimulation period. Our data demonstrate that eps-r had no significant effect, while eps-b was found as a weak stimulant in terms of pro-inflammatory (TNF-α, IL-6, IL-12) cytokines. We also examined the effect of EPS/CpG-ODN (D35 and K23) complexes within the similar aim. The effect of EPS/CpG-ODN complexes was found depended on type of EPS and their doses (3, 1, 0.3, 0.1 µM). A significant reduction on IL-6 production ($p<0.05$) was observed with the stimulation of eps-b:K23-ODN complexes (3 µM and 1 µM). From the point of IL-12 production, suppression was also observed by high doses of both polymer ($p<0.05$). In conclusion, these data provided a new and different perspective on bacterial EPSs in the literature. To predict whether EPS could be clinically useful as an immunomodulatory agent, further in vivo studies with highly purified EPSs are necessary.

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ANTIMICROBIAL ACTIVITY OF THE COMMERCIAL ORIGANUM ONITES L. OIL AGAINST NOSOCOMIAL ESBL PRODUCER ESCHERICHIA COLI ISOLATES AND ITS CHEMICAL COMPOSITION

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In recent years rapidly growing antibiotic resistance has increased interest to natural products especially essential oils because of their various effects. The aim of this study was to determine the antimicrobial activity of the commercial Origanum onites essential oil (EO), against ten different ATCC strains including eight bacteria (Escherichia coli ATCC 25922, Escherichia coli ATCC 35218, Staphylococcus aureus ATCC 29213, Staphylococcus aureus ATCC 43300, Staphylococcus epidermidis ATCC 35984, Staphylococcus epidermidis ATCC 12228, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633), two fungi (Candida albicans ATCC 10231, Candida albicans ATCC 033) and 79 clinical nosocomial E. coli isolates that produce extended spectrum beta lactamase (ESBL), and identify the chemical composition of the oil. The antimicrobial activity of O. onites oil against standard strains was determined by disc diffusion method. Agar dilution and micro-dilution methods were used to determine the microbial growth inhibition of bacteria at various concentrations of O. onites oil for clinical isolates. O. onites oil has antimicrobial activity against all standard strains and inhibited microbial growth of extended spectrum beta lactamase (ESBL) positive E. coli isolates. O. onites EO was found most effective against E. coli ATCC 25922 and E.coli ATCC 35218 with 40 mm. MIC value's ranges are 1.56-25 µL/mL and 3.12-25 µL/mL for micro-dilution and agar dilution methods, respectively for clinical E. coli isolates. O. onites oil may be an alternative in combination with the other antibiotics for treatment of infection caused by multidrug resistant bacteria after further tests.

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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF UREAPLASMA UREALYTICUM WITH DIFFERENT METHODS IN SEXUALLY ACTIVE WOMEN.

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Ureaplasma urealyticum is potentially pathogenic bacterial species that is sexually transmitted and responsible of infertility, prematurity, recurrent abortus and newborn respiratory distress. Since the infections caused by these microorganisms exhibit importance, proper isolation and identification of these bacteria are required to make proper and reliable. The aim of this study was to determine the prevelance of U. urealyticum and their antimicrobial susceptibility with using two different commercial kits and Mycoplasma agar plates. For this purpose a total of 220 vaginal and urinary clinical samples were taken from 110 sexually active women in Obstetrics Clinic of Başkent University Ankara Hospital. We assessed the laboratory performances of Mycoplasma IES (IES) and Mycoplasma IST 2 (IST2) compared to Mycoplasma Agar plates for the detection of U. urealyticum in clinical samples. U. urealyticum was detected positive in 82 (%74,5) patients with Mycoplasma agar plates. U. urealyticum was determined with Mycoplasma IST 2 kit as 46,8 % (103/220) and with Mycoplasma IES kit as 53,6 % (118/220). The following rates of resistance were determined using the Mycoplasma IES assay: levofloxacin, 3%, erythromycin, 19% , tetracycline 20% and ciprofloxacin 6%. Mycoplasma IST 2 assay; tetracycline 17%, ciprofloxacin 9%, erythromycin, 11%. A isolate was susceptible to pristinamycin, josamycin, and doxycycline. The Mycoplasma IES kit was found to be equivalent or superior compared to other commercial culture-based assays for a rapid and accurate identification of U.urealyticum and detection of resistance. Also in determining U.urealyticum made with the kit, vaginal samples was found to be significantly higher than urine samples.

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DETERMINING ANTIBACTERIAL ACTIVITIES OF IN VITRO REGENERATED LEGUME INDIGOFERA ZOLLINGERIANA

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Indonesia is home to more than 10 percent of the world's known plant species. Indigofera zollingeriana with high protein content is an important forage crop that grows throughout Indonesia. There is no callus induced antibacterial study reported in I. zollingeriana. The leaves, hypocotyl and cotyledons explants of the plant were cultured on different concentrations of BAP + NAA for calli induction. Their methanol extracts were tested for antibacterial activity using disc diffusion method of Staphylococcus aureus ATTCC25923 ($Gram^+$) and Pseudomonas aeruginosa ATCC25823 ($Gram^-$). Gentamicin, Erythromycin and Penicillin were used as positive control. Antibacterial activity on calli extracts was positively affected by the type of phytohormonal combinations and the explants; with the best results noted from the callus extracts of hypocotyl explants. The Staphylococcus aureus ATTCC25923 showed visible and equally effective inhibition due to Erythromycin and callus extracts from hypocotyl regenerated on MS medium containing either of 0.5 mg/L BAP with 0.15 mg/L NAA, 1 mg/L BAP with 0.1 mg/L NAA and 2 mg/L BAP with 0.15 mg/L NAA. This activity was higher to the activity shown by Gentamicin and Penicillin. The Pseudomonas aeruginosa showed more inhibition using callus extract obtained on 0.5 mg/L BAP with 0.1 mg/L NAA compared to the antibacterial activity shown by any of the three antibiotics used as control. It was concluded that the different BAP+NAA concentrations used in tissue culture could result in fluctuation of the antibacterial ability of extracts obtained from the respective calli.

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ANTIMICROBIAL ACTIVITIES OF SOME HERBAL MEDICINAL PRODUCTS AGAINST VARIOUS PATHOGENIC BACTERIA

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Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of nosocomial infections, causing a variety of life-threatening syndromes; such as bacteraemia, endocarditis, wound infections and pneumonia. For many years, MRSA infections were acquired in hospitals and other healthcare facilities; however, more recently, new MRSA strains have emerged in the community, causing infections in patients without previous healthcare contact. Recently, there has been an increasing interest in the discovery of new natural antimicrobials, because of an increase in risk in the rate of infections with antibiotic resistant microorganisms. The antimicrobial activity of methanolic extracts of 23 Turkish medicinal plants will be tested against Methicillin-resistant Staphylococcus aureus, Gram-negative and Gram-positive bacteria by using disc diffusion method and minimum inhibitory concentrations (MICs) will be determined using broth microdilution method.

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ANTIMICROBIAL ACTIVITY OF CORIANDER COLD PRESSED OIL

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Medicinal plants have been used for their therapeutic potentials for centuries. Coriander as a medicinal plant in the Mediterranean area has a long history for many therapeutic and culinary purposes especially as a spice in the food court. Coriandrum sativum L. (Coriander/Kişniş) belonging to the Apiaceae family is a glabrous aromatic, herbaceous annual plant, represented by two species as C. sativum and C. tordylium in Turkey. The species has many important bioactivities and medicinal importance. The beneficial effects of this species on health and the healing properties can be attributed to its exceptional phytoconstituents like phenolic acids, flavonoids, anthocyanins, coumarins, lignans, catechins, and terpenoids. The plant contains two exceptional part in the oil as essential oil, and fatty oil attributed aromatic and nutrient values. The aim of this work was to study the in vitro antimicrobial effect of coriander cold press oil which contains essential and fatty oils against Gram (+) and Gram (-) bacteria. Our results showed that coriander oil has no effect against all bacteria tested, but the oil exhibited notable antifungal activity for the Candida albicans. Therefore, the oil has been tested for the other Candida strains. As a conclusion, the oil was demonstrated the antifungal activity is having MIC values of 1/4-1/8-1/16 against C. albicans, C. parapsilosis, C. krusei, respectively. The extraction procedure of the oil is the most important one in this study. The aim of the present work was to screen the antimicrobial activity of the coriander cold press oil.

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INFLUENCE OF DIFFERENT GROWTH CONDITIONS DUE TO NITROGEN SOURCES ON EXOPOLYSACCHARIDE PRODUCTION BY 3 LACTOBACILLUS SALIVARIUS STRAINS

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EPSs produced by lactic acid bacteria (LAB) are the subject of an increasing number of studies. LAB can produce EPSs that are potentially useful as safe additives to improve texture. In this study, the influence of nitrogen sources (0.5% Peptone, 0.5% Beef Extract, 1% Tryptone for 3 Lactobacillus salivarius strains) on EPS production was determined. Medium composition values for 3 L. salivarius strains (BIS312, BIS722, ZDM2132) 449, 349, and 339 mg/L EPS, respectively. When 0.5% peptone is used as the nitrogen source, the BIS312, BIS722, and ZDM2132 strains produced 455, 413, and 400 mg/L EPS, respectively. The BIS312, BIS722, and ZDM2132 produced 369, 359, and 346 mg/L EPS, respectively, while 0.5% beef extract is used as the nitrogen source. When 1% tryptone is used as the nitrogen source, the BIS312, BIS722, and ZDM2132 produced 487, 460, and 410 mg/L EPS, respectively. In addition, in this study, the influence of different development times (12, 24, 36, and 48h) on EPS production was determined. The results indicated that in three strains, for EPS production was the most efficient development times 48 hours. 48 hours of development the BIS312, BIS722, and ZDM2132 strains produced 450, 350, and 339 mg/L EPS, respectively. The results indicated that in three strains, for EPS production was the most efficient nitrogen source tryptone. The yield of EPS produced by the lactobacilli might be influenced by changing the medium composition.

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EFFECT OF CARBON SOURCES ON EXOPOLYSACCHARIDE PRODUCTION BY LACTOBACILLUS SALIVARIUS BIS312, BIS722, AND ZDM2132 STRAINS

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Lactic acid bacteria (LAB) are usually accepted as safe and exopolysaccharides (EPSs) isolated from LAB provide alternative source of microbial polysaccharides for wider use in food formulations. EPSs can exert functional effects on foods, improve the rheology of fermented milk products, and have beneficial health effects in gastrointestinal systems. Lactobacillus salivarius strains are probiotic microorganisms, which secrete significant amounts of extracellular polysaccharides. In this study, as a source of carbon, different ratio of lactose, sucrose, and glucose (2%glucose, 3%glucose, 2%glucose + 1%lactose, 2% glucose + 2%lactose, 1%glucose + 1%lactose, 2%lactose, 2%glucose + 1%sucrose, 2%glucose + 2%sucrose, 1%Glucose + 1%sucrose, and 2%sucrose) were added in Lactobacillus salivarius BIS312, BIS722, and ZDM2132 strains growth medium where it is cultivated in order to increase EPS production capacity and EPS production quantities were determined. As a result of these studies, L. salivarius strains were compared with the control groups and the best EPS production was found in medium containing 2% lactose and 2% glucose + 2% sucrose. It has been determined that carbon source 2% lactose was increased EPS production by 30% and 2% glucose + 2% sucrose was increased EPS production by 33%. In conclusion, L. salivarius strains were able to produce EPS in the presence of various sugars, and that different sugar concentrations have a significant effect on EPS production.

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ANTIMICROBIAL EVALUATION OF SOME HETEROCYCLIC COMPOUNDS

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The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of microbial infectious and by drug side effects [1,2]. Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed toward the design of new agents. Benzoxazoles are the structural isosters of natural nucleotides and interact easily with the biopolymers. A benzoxazole derivative; calcimycin (Figure 1) is a carboxylic polyether antibiotic from a strain of Streptomyces chartreusis (NRRL 3882). It was found to very active against gram-positive bacteria including some Bacillus, Micrococcus strains. So that benzoxazoles constitute an important class of heterocyclic compounds with antimicrobial and antibiotic activities [3-5].

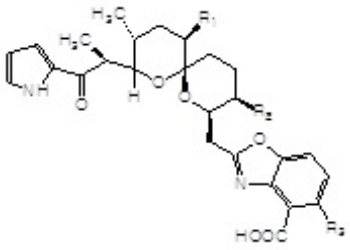

Figure 1. Calcimycin

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THE ANTIMICROBIAL ACTIVITIES OF SOME COMMERCIAL ESSENTIAL OILS

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Recently, increasing prevalence of antimicrobial drug resistance has led researchers to search for novel antimicrobial molecules to treat various human pathogens. Essential oils have a great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens. In this study, we evaluated and compared the antimicrobial effects of commercially avaible avocado, cinnamon, coconut, calendula and olive oil over the standard strains of some clinically important microorganisms by disc diffusion and agar well diffusion methods. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300 (MRSA), Staphylococcus epidermidis ATCC 12228 (biofilm-negative), Staphylococcus epidermidis ATCC 35948 (biofilm-positive) and Candida albicans ATCC 10231 standard strains were used in this study. As a result, we found that cinnamon oil has a remarkable inhibitory activity against to all bacteriae and the yeast. Other essential oils had no inhibitory activity against tested microorganisms.The results were found to be compatible with each other in both methods. Consequently, cinnamon oil shows significant inhibitory activity and this results indicated that this oil has a potential for the development of alternative agents and can be used in pharmaceutical industry depending on the results will be obtained in future studies.

Test microorganisms	Agar well diffusion zone diameters (mm)	Disc diffusion zone diameters (mm)
Pseudomonas aeruginosa ATCC 27853	18	18
Escherichia coli ATCC 25922	34	34
Staphylococcus epidermidis ATCC 12228 (biofilm-negative)	43	42
Staphylococcus epidermidis ATCC 35948 (biofilm-positive)	42	40
Staphylococcus aureus ATCC 43300 (MRSA)	35	32
Staphylococcus aureus ATCC 25923	34	32
Candida albicans ATCC 10231	65	62

Inhibition zone diameters of cinnamon oil

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DETERMINE OF POTENTIAL ANTIVIRAL RESISTANCE MUTATION PROFILES WITHIN THE HBV REVERSE TRANSCRIPTASE NUCLEOS(T)IDE ANALOGUE IN UNTREATED AND -TREATED PATIENTS WITH CHRONIC HEPATITIS B

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Chronic hepatitis B virus (HBV) that conducive a major health problem worldwide it may cause serious complications such as fibrosis, cirrhosis and/or hepatocellular carcinoma. In recent years, oral nucleos(t)ide analogue (NA) drugs including lamivudine, adefovir, entecavir, tenofovir, and telbivudine have revolutionized the management of HBV infection. However prolonged use of NAs that directly inhibit reverse transcriptase activity of the HBV polymerase may lead to the development of drug resistance. Have the possibility of drug resistant virus may be transmitted from patient to healthy individuals. Therefore, there is a possibility of infection with drug-resistant HBV before the first treatment. A total of 124 patients who 72 received treatments and 52 untreatment (naive) were studied. By isolating DNA from plasma samples of these patients, the sequencing reaction was performed by PCR-DNA sequence analysis. In the region between the codon rt150-293 of the studied reverse transcriptase gene, different types of mutations were detected in 13 (18,05 %) of 72 treated patients and in 18 (34,61 %) of 52 untreated patients ($p<0.05$). Primary drug resistance mutations were not detected in any patient samples however, potential mutations to be associated with drug resistance such as rtR164R, rtG165D/A, rtG172Q, rtS176N, rtF178V, rtA181G, rtS185N/G/C, rtV207M, rtQ215H/S, rtL231V, rtI233K, rtN238S, rtV253T, rtC256G/S and rtI266R/V were detected.

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