GAZİ INTERNATIONAL GPSS SYMPOSIUM SERIES
GAZİ PHARMA SYMPOSIUM SERIES
PHARMA

GAZİ UNIVERSITY FACULTY OF PHARMACY
1982

INTERNATIONAL

GAZİ PHARMA SYMPOSIUM

November 12-15, 2015
Port Nature Luxury Resort Hotel & Spa
Belek - ANTALYA / TURKEY

ABSTRACT BOOK

Dear Colleagues,

It is a great honor and pleasure to welcome you all to the 1st International Gazi Pharma Symposium Series (GPSS-2015), which is organized by the Gazi University, Faculty of Pharmacy.

Contributions of Pharmaceutical sciences in various steps of Drug Discovery and Development process lie at the heart of almost every developed drug starting from bench to clinic. Pharmaceutical sciences cover a wide range of topics on all aspects of the development, manufacture and study of medicinal compounds. GPSS2015 symposium will provide a broad but thorough background of the main science subjects such as chemical, biological, natural and pharmacological as well as technological disciplines including dosage formulation, biopharmaceuticals, pharmaceutical manufacturing, quality systems, validation and pharmaceutical legislation, as they all relate to the study of medicinal products.

Our Symposium intends to bring together scientists involved in various disciplines related to all aspects of drug discovery, development and optimization. The scientific programme includes plenary and main lectures by invited speakers and senior scientists, as well as oral presentations and poster communications by young researchers.

We sincerely hope that you will enjoy the science along with the rich Turkish Culture in one of the best touristic place of Turkey.

On behalf of the Organizing Committee,

Prof. Dr. Tuncer Değim
Symposium Chairman

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PROGRAMME OVERVIEW

November 12, 2015 (Thursday)

Opening Remarks	
17:00	Prof. Dr. Tuncer Değim Symposium Chairman and Dean Gazi University, Faculty of Pharmacy, Turkey
17:15	Prof. Dr. Süleyman Büyükberber Rector Gazi University, Turkey
17:30	**Plenary Lecture** ***'Aziz Sancar: DNA Damage and Cancer'*** Prof. Dr. Mehmet Öztürk İzmir Biomedicine and Genome Center, Turkey
19:00	**Welcome Reception**

November 13, 2015 (Friday)

	1A	*Biosimilars: Challenges and Opportunities*	1B	*Cosmetics and Safety Issues*
08:45	**Session Chairs** Prof. Dr. Nevin Çelebi, Gazi University, Turkey Dr. Sundar Ramanan, Amgen, USA		**08:45**	**Session Chairs** Prof. Dr. Sema Burgaz, Gazi University, Turkey Prof. Dr. Vera Rogiers, Vrije Universiteit Brussel, Belgium
08:50	**L1**	***'Biosimilars: Characterization and Comparability'*** Asst. Prof. Dr. Devrim Demir Dora Akdeniz University, Turkey	**08:50**	**L4** ***'Nanomaterials in Cosmetics-Recent Trends'*** Prof. Dr. Figen Tırnaksız Gazi University, Turkey
09:20	**L2**	***'Clinical Study Considerations in Biosimilars Development'*** Dr. Sundar Ramanan Amgen, USA	**09:10**	**L5** ***'Safety Assesment of Cosmetic Products-General Principles'*** Prof. Dr. Sema Burgaz Gazi University, Turkey
10:00	**L3**	***'Program for Biopharmaceuticals at Izmir Biomedicine and Genome Center'*** Prof. Dr. Mehmet Öztürk İzmir Biomedicine and Genome Center, Turkey	**09:35**	**L6** ***'Safety of Cosmetics; Non-animal Tests and Future Perspectives'*** Prof. Dr. Vera Rogiers Vrije Universiteit Brussel, Belgium
			10:10	**L7** ***'Safety Related Issues and Legislation'*** Assoc. Prof. Dr. Evren Algın Yapar Turkish Ministry of Health, Turkey
10:30 Exhibition & Coffee Break				

2A	*Panel 10:45 - 12:45*	2B	*Epigenetics and Omics*
Biotechnological Products/Biosimilars: Studies and Investment Opportunities in Turkey **Moderators:** Prof. Dr. Nevin Çelebi, Gazi University, Turkey Dr. Hakkı Gürsöz, Turkish Ministry of Health, Turkey • **Prof. Dr. Şaban Tekin,** TÜBİTAK-MAM, Turkey • **Dr. Devrim Satık,** AIFD, Turkey • **Dr. İrem Yenice,** Arven Pharmaceuticals, Turkey • **Dr. Hasan Zeytin,** Nobel Pharmaceuticals, Turkey • **Dr. Cem Koçak,** TISD, Turkey • **Dr. Ferhat Farshi,** IEIS, Abdi İbrahim Pharmaceuticals, Turkey • **Dr. Hakkı Gürsöz,** Turkish Ministry of Health, Turkey	**11:00**		**Session Chairs** Prof. Dr. Jean Rosenbaum, Université de Bordeaux, France Assoc. Prof. Dr. Semra Demokan, İstanbul University, Turkey
	11:05	**L8**	***'The Related ATPases Pontin and Reptin in Liver Carcinogenesis: Role and Targeting'*** Prof. Dr. Jean Rosenbaum Université de Bordeaux, France
	11:40	**L9**	***'The Role of Epigenetics in Basic and Clinical Cancer Research'*** Assoc. Prof. Dr. Semra Demokan İstanbul University, Turkey
	12:20	**O1**	***'Expressions of GGT1, GGT5 and GGT6 Genes in Different Types of Cancers'*** Ece Miser-Salihoğlu Gazi University, Turkey
12:45 Lunch & Posters (P1-P75)			

	14:00 - 15:00 Satellite Presentations		
14:00	**Pharmacoinformatics in Drug R&D Process** Dr. Andrea Carotti, University of Perugia, Italy Abdurrahman Olğaç, Gazi University, Turkey The Presentation on Pharmacoinformatics is organised especially for young researchers to explain the evolving role of Pharmacoinformatics as a tool for drug discovery	**14:00**	**Accreditation of Pharmacy Education and Benefits of Smart Pharmacy** Michael Rouse, B. Pharm (Hons), MPS ACPE, Chicago, USA
14:40	**LC/MS based Solutions for Characterizing Therapeutic Proteins** Georges Tsoupras Agilent Technologies, Switzerland	**14:30**	**Smart Pharmacy Experience: Pros and Cons** Dr. sc. Arijana Meštrovic MPharm, Zagreb, Croatia These presentations will discuss key principles behind accreditation of pharmacy education and describe how such principles and quality indicators - as used in the SMART Pharmacy Project - can drive positive changes in education and practice.

15:00 Exhibition & Coffee Break

	3A	*Implementation of Quality by Design (QbD): A Regulatory Perspective*		3B	*Analytical Method Development: New Approaches*
15:30	**Session Chairs** Prof. Dr. Sevgi Takka, Gazi University, Turkey Prof. Dr. James E. Polli, University of Maryland, USA		**15:30**	**Session Chairs** Prof. Dr. Uğur Tamer, Gazi University, Turkey Prof. Dr. Arunas Ramanavicius, Vilnius University, Lithuania	
15:35	**L10**	***'The Pharmaceutical Industry Benefits of QbD'*** Dr. Buket Aksu Santa Farma Pharmaceuticals, Turkey	**15:35**	**L15**	***'BioMEMS and Microfluidic Devices for Lab-on-a-Chip Applications'*** Prof. Dr. Haluk Külah Middle East Technical University, Turkey
15:55	**L11**	***'Impact of Excipient Variability on QbD & BASF's Support on QbD'*** Cihan Sancaktaroğlu BASF, Turkey	**16:05**	**L16**	***'Formation and Evaluation of Molecularly Imprinted Polypyrrole'*** Prof. Dr. Arunas Ramanavicius Vilnius University, Lithuania
16:15	**L12**	***'Integration of Biopharmaceutics and Quality by Design: The Biopharmaceutics Risk Assessment Roadmap (BioRAM) Building in Clinical Relevance for the Patient Benefit'*** Dr. Arzu Selen, FDA, CDER, USA	**16:35**	**L17**	***'Oligonucleotide Aptamers: Emerging Affinity Probes for Bioanalytical Mass Spectrometry and Biomarker Discovery'*** Asst. Prof. Dr. Basri Gülbakan Hacettepe University, Turkey
16:45	**L13**	***'QbD Implementation in Pharmaceutical Industry'*** Vijay Kshirsagar TRAC Pharma Consulting, India	**17:05**	**L18**	***'Exploring the Elution Order in Chiral Chromatography'*** Prof. Dr. Benedetto Natalini University of Perugia, Italy
17:15	**L14**	***'Biopharmaceutic Risk Assessment of Brand and Generic Lamotrigine Tablets: In Vitro and In Vivo'*** Prof. Dr. James E. Polli University of Maryland, USA			

November 14, 2015 (Saturday)

	4A	*Biomarkers for Predicting Disease and Toxicity of Drugs*		4B	*Natural Products Chemistry*
08:45	**Session Chairs** Prof. Dr. Bensu Karahalil, Gazi University, Turkey Prof. Dr. Helga Stopper, University of Wuerzburg, Germany		**08:45**	**Session Chairs** Prof. Dr. Ayla Kaya, Anadolu University, Turkey Dr. Milen Georgiev, Bulgarian Academy of Sciences, Bulgaria	

08:50	**L19**	***'Genetic Polymorphisms can be Prognostic and Predictive Biomarkers for Cancer Risk'*** Prof. Dr. Bensu Karahalil Gazi University, Turkey	**08:50**	**L22**	***'Towards Accelerated Lead Finding: NMR-based Metabolomics'*** Dr. Milen Georgiev Bulgarian Academy of Sciences, Bulgaria
09:25	**L20**	***'Drug-induced Liver Injury'*** Prof. Dr. Ahmet Aydın Yeditepe University, Turkey	**09:25**	**L23**	***'Improving the Pharmacokinetic Disposition and Pharmacology of the Bioactive Herbal Molecules through Pharmaceutical Formulation'*** Prof. Dr. Kit-Lam Chan Universiti Sains Malaysia, Malaysia
10:00	**L21**	***'Biomarkers of Insulin-mediated DNA Damage'*** Prof. Dr. Helga Stopper University of Wuerzburg, Germany	**10:00**	**L24**	***'Modification Studies on Cycloartane-Type Sapogenols'*** Prof. Dr. Erdal Bedir Ege University, Turkey
10:30 Exhibition & Coffee Break					
	5A	***Chronopharmacology***		**5B**	***Signaling Pathways as Drug Target***
11:00	**Session Chairs** Prof. Dr. Nurettin Abacıoğlu Gazi University, Turkey		**11:00**	**Session Chairs** Prof. Dr. Yeşim Özkan, Gazi University, Turkey Assoc. Prof. Dr. Okay Saydam, Medical University of Vienna, Austria	
11:05	**L25**	***'Systems Medicine to Deliver Personalised Cancer Chronotherapy'*** Prof. Dr. Francis Lévi Warwick University, United Kingdom	**11:05**	**L28**	***'PI3K/AKT/mTOR Cell Survival Pathway: Target for Cancer Drug Discovery'*** Assoc. Prof. Dr. Rengül Çetin-Atalay Middle East Technical University, Turkey
11:35	**L26**	***'Drug Transporters and Cancer: Circadian Rhythms and Therapeutic Implications'*** Assoc. Prof. Dr. Alper Okyar Istanbul University, Turkey	**11:35**	**L29**	***'Medulloblastoma Extracellular Vesicles Carry OTX2 that Promotes Tumor Growth and Serves as A Diagnostic and Prognostic Biomarker'*** Assoc. Prof. Dr. Okay Saydam Medical University of Vienna, Austria
12:05	**L27**	***'Drug Development in Oncology Yesterday, Today & Tomorrow'*** Dr. Sedat Altuğ Lilly, Turkey	**12:05**	**L30**	***'Robust Network Modeling Identifies Personalized Therapeutic Strategies in Glioblastoma'*** Dr. Nurcan Tuncbağ Middle East Technical University, Turkey
12:30 Lunch & Posters (P76 - P155)					
14:00 Key Lecture ***'Targeted Drug Delivery through VIP Receptors is Safe and Effective'*** Prof. Dr. Hayat Alkan Önyüksel University of Illinois at Chicago, USA					
15:00 Exhibition & Coffee Break					

	6A	*Drug Delivery and Targeting*		6B	*New Strategies on Lead Generation & Development*
15:30	**Session Chairs** Prof. Dr. Füsun Acartürk, Gazi University, Turkey Prof. Dr. Ir. W.E. Hennink, Utrecht University, The Netherlands		15:30	**Session Chairs** Prof. Dr. Fatma Gümüş, Gazi University, Turkey Prof. Dr. Gabriele Costantino, University of Parma, Italy	
15:35	L31	***'Endogenous-Inspired Hydrophobic Drug Delivery to Cancers: LDL-like Nano Particles Designed to 'Put the Drug in the Cancer's Food'*** Prof. Dr. David Needham University of Southern, Denmark	15:35	L34	***'Targeting a Disease Tolerance Defense Pathway for Novel Therapeutic Opportunities'*** Prof. Dr. Antonio Macchiarulo University of Perugia, Italy
16:05	L32	***'Polymeric Nanoparticles for Targeted Drug Delivery'*** Prof. Dr. Ir. W.E. Hennink Utrecht University, The Netherlands	16:10	L35	***'Exploiting the Sulfur Assimilation Pathway in Search for Novel Antibacterials. Design and Synthesis of Cyclopropane-based Inhibitors of O-Acetylserine Sulfidrilase (OASS)'*** Prof. Dr. Gabriele Costantino University of Parma, Italy
16:35	L33	***'Advances in Gene Delivery'*** Prof. Dr. Jülide Akbuğa Marmara University, Turkey	16:45	L36	***'Structure- and Computer-Based Design of Epigenetic Inhibitors for Anti-Parasitic Therapy'*** Prof. Dr. Wolfgang Sippl Martin-Luther-Universität Halle-Wittenberg, Germany
			17:20	O2	***'Cyclohexadienones as Mutant Specific Inhibitors of K-Ras G12c'*** Shaista Aziz University of London, United Kingdom
20:00 GALA DINNER					

November 15, 2015 (Sunday)

	7A	*Clinical Pharmacy*		7B	*General Orals-I*
09:00	**Session Chairs** Prof. Dr. Nurettin Abacıoğlu, Gazi University, Turkey Assoc. Prof. Dr. Mesut Sancar, Marmara University, Turkey		09:00	**Session Chairs** Prof. Dr. Deniz Songül Doğruer, Gazi University, Turkey	
09:05	L37	***'Given the New Situation in Professional Terms: Clinical Pharmacy'*** Prof. Dr. Nurettin Abacıoğlu Gazi University, Turkey	09:05	O3	***'E-Cadherin and TGF-β Levels in Patients with Endometrial and Ovarian Cancers'*** Taylan Turan Gazi University, Turkey

09:30	**L38**	***'Clinical Based Education in Clinical Pharmacy Graduate Programs'*** Assoc. Prof. Dr. Mesut Sancar Marmara University, Turkey	**09:25**	**O4**	***'Surface Enhanced Raman Scattering Based Detection of Group a Beta-Hemolytic Streptococcus'*** Merve Eryılmaz Gazi University, Turkey
09:55	**L39**	***'Emerging Face of Clinical Pharmacy - Oncology Pharmacy'*** Ahmet Sami Boşnak, MSci. Pharm. Gaziantep University, Turkey	**09:45**	**O5**	***'Genetically Modified Animals, Current Products and Regulatory Approaches'*** Onur Kenan Ulutaş Gazi University, Turkey
10:20	**L40**	***'Clinical Pharmacy in Turkish Republic of Northern Cyprus: Education and Services'*** Assoc. Prof. Dr. Bilgen Başgut Near East University, TRNC, Northern Cyprus	**10:05**	**O6**	***'Determination of the Wound Healing Effect of Calendula officinalis L. Using In Vivo and In Vitro Models'*** Mert İlhan Gazi University, Turkey
10:45 Coffee Break					
	8A	***Phytopharmacy***		**8B**	***General Orals-II***
11:00	**Session Chairs** Prof. Dr. Fatma Tosun, Gazi University, Turkey		**11:00**	**Session Chairs** Prof. Dr. Gülderen Yentür, Gazi University, Turkey	
11:05	**L41**	***'Herbal Medicinal Products - Requirements on Manufacture and Quality Assurance'*** Dr. Gudrun Abel BIONORICA SE, Germany	**11:05**	**O7**	***'Investigations on Transplantable Bioartificial Pancreas Device'*** Prof. Dr. Nefise Özlen Şahin Mersin University, Turkey
11:45	**L42**	***'Intentional Adulteration and Counterfeiting Issues in Phytopharmaceuticals: Safe or Risky?'*** Prof. Dr. İlkay Erdoğan Orhan Gazi University, Turkey	**11:25**	**O8**	***'Direct Analysis of Drugs in Biological Fluids by On-line Chromatography'*** Assist. Prof. Dr. Sena Çağlar Andaç İstanbul University, Turkey
12:15	**O10**	***'Ability of Viburnum opulus L. to Inhibit the Development of Endometrial Implants in Rats: Surgically-Induced Endometriosis Model'*** Assoc. Prof. Dr. İpek Süntar Gazi University, Turkey	**11:45**	**O9**	***'In Situ Niosome Forming Proniosomal Tablets: In Vitro and In Vivo Evaluation'*** Zerrin Sezgin-Bayındır Ankara University, Turkey
12:30 Closing					

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LECTURES

AZIZ SANCAR: DNA DAMAGE AND CANCER

Mehmet Öztürk

Dokuz Eylül University, Izmir International Biomedicine and Genome Institute, Balçova, İzmir, Turkey

Aziz Sancar was born at Mardin-Savur in 1946. He graduated from İstanbul University Faculty of Medicine in 1969. Aziz Sancar who lives in USA since 1974 received 2015 Nobel Award in Chemistry for his discovery of mechanisms of nucleotide excision repair. He shared this award with **Tomas Lindahl** and **Paul Modrich** who discovered respectively **"base excision repair"** and **"DNA mismatch repair"** mechanisms**.** If the mechanism discovered by Aziz Sancar does not work, a disease called "*Xeroderma Pigmentosum*" is observed in humans. These people develop first skin lesions and then cancers when exposed to sun light, UV light more precisely. Increased susceptibility to cancer is also observed in most diseases associated to impaired DNA repair. This is why, DNA repair genes are considered as cancer preventing genes. However, the inactivation of a DNA repair gene is not necessary for the development of most cancers. Moreover, it is well established that DNA repair genes work more effectively in cancer cells as compared to normal cells. Therefore, we may also assume that DNA repair genes facilitate the survival of cancer cells. This intriguing crosstalk between cancer and DNA repair may be explained by the notion of "*cancer cell evolution*".

TARGETED DRUG DELIVERY THROUGH VIP RECEPTORS IS SAFE AND EFFECTIVE

Hayat Onyuksel, PhD

University of Illinois at Chicago, USA

Vasoactive intestinal peptide (VIP) is a neuropeptide with several biological activities such as vasodilator, immunomodulator and growth factor. Receptors (R) of VIP are widely expressed in the body, but over-expressed in some pathologic conditions like cancer. Most cancer cells, including cancer stem cells express VIP-R five- fold more than normal cells. However, VIP-R express only at the extra vascular space and VIP in the blood must extravasate out of circulation, in order to interact with its receptors to show an effect. When VIP is attached on the surface of a drug carrier as a targeting agent, it cannot extravasate normal vasculature but only extravasates at the leaky vasculatures of the diseased tissues, like cancer. Therefore off target and side effects are eliminated. Furthermore, VIP-R are internalizing receptors, so cause more effective uptake of the drug in the carrier, into cancer cells. Moreover, by this receptor internalization mechanism, drug resistance can be overcome. All these properties clearly indicate that VIP-R are ideal targets to deliver anti-cancer drugs as nanomedicines.

BIOSIMILARS: CHARACTERIZATION AND COMPARABILITY

Devrim Demir Dora

Department of Medical Pharmacology, Faculty of Medicine, Akdeniz University,07070, Antalya, Turkey

The regulatory body for the approval of medicines in the EU is the European Medicines Agency (EMA); and defines biosimilar as a biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product) in the EEA [1]. Comparability exercise should be established for demonstrating similarity in terms of quality characteristics, biological activity, safety and efficacy. Biosimilar medicinal products are regulated in Turkey according to; 'The Regulation on the Registration of Medicinal Products for Human Use [2]' and 'Biosimilar Medicinal Products Guideline [3].' According to the Turkish Guideline on Biosimilar Medicinal Products a biosimilar drug is a medicinal product that shows similarity to a licensed biological reference product in terms of quality, safety and efficacy. The active substance of a biosimilar product should show molecular and biological similarity to the active substance of the reference medicinal product. The pharmaceutical form, potency and route of administration must be same as of the reference biological product [1,3]. Standard generic approach is not appropriate for biosimilar drugs. Comparing the biosimilar and the reference biological product starts from a comparison of the quality of the two products both at the active substance and at the drug product level. Comparability exercise for a biosimilar product versus the reference medicinal product is needed beside a full quality dossier (CTD Module 3). In order to show the similarity in terms of quality, validated state-of-the-art analytical tests should be performed that are sensitive enough to detect the possibilities of changes to the product. Head-to-head characterization studies are required to compare the biosimilar and the reference product at both drug substance and drug product levels. For the development of the biosimilar product and its manufacturing process the quality target product profile (QTPP) should be created [4]. Characterization studies include basically, physicochemical properties, biological activity, immunochemical properties, purity, contamination, strength, content and should be performed by the ICH Q6B guideline. Due to the complexity and heterogenity of proteins, it is recommended that more than one analytical methods apply to each item of quality [5]. Differences between the biosimilar and the reference product should be evaluated for their potential impact on safety and efficacy of the biosimilar. When determining the final comparability, non-clinical and clinical data are required to consider together [6].

References

[1] 'Guideline on similar biological medicinal products' - EMA/CHMP/437/04 Rev 1
[2] 'The Regulation on the Registration of Medicinal Products for Human Use' - Official Gazette #25705 of 19.01.2005
[3] 'Biosimilar Medicinal Products Guideline' - Ministry Approval No:5285, Date: 07.08.2008
[4] 'Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (revision 1)' - EMA/CHMP/BWP/247713/2012 Rev 1
[5] ICH Guideline Q6B: 'Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products'
[6] 'Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issue' - EMEA/CHMP/BMWP/42832/2005 Rev 1

CLINICAL CONSIDERATIONS IN BIOSIMILAR DEVELOPMENT

Sundar Ramanan

Director, Global Biosimilars Regulatory Policy, Amgen, Inc

Clinical elements are an important step in the “step-wise” process of biosimilar development. In this talk I will address the role of clinical pharmacology and clinical studies in building the “totality of evidence” toward biosimilar approval. Additionally, the type and nature of clinical studies that would be needed to address questions such as “extrapolation” and “interchangeability” will also be discussed.

PROGRAM FOR BIOPHARMACEUTICALS AT IZMIR INTERNATIONAL BIOMEDICINE AND GENOME INSTITUTE

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The Izmir Biomedicine and Genome Center (İBG-izmir) is a recently created institute devoted to translational research, core services and post-graduate education in the fields of biomedicine, genomics and health biotechnologies including a program on biopharmaceuticals. Our biopharmaceuticals program is focusing on translational research aiming at developing targeted therapies against cancer using recombinant proteins such as monoclonal antibodies. In addition to innovative therapeutics, we are also investing on know-how and facilities to provide service to pharmaceutical firms interested in producing and marketing “biosimilars”. Our portfolio includes R&D labs for vector construction, cell line development and master cell banking. We are also in the process of constructing a pilot production facility with an initial capacity of 200 L mammalian cell bioprocessing. Downstream processes such as chromatography and filtration under aseptic conditions are also included. We are also constructing GMP laboratories for in vitro and in vivo quality control, safety, preclinical toxicology and pharmacology testing of biopharmaceuticals. The program has already started. We expect that we would reach to full capacity for these services within two years.

NANOMATERIALS IN COSMETIC PRODUCTS - RECENT TRENDS

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Nanomaterials used in cosmetics can be basically categorized into two main groups: *i)* nanocarriers; and *ii)* insoluble nanomaterials). Nanocarriers have important advantages such as increased chemical stability of active substances or improved product efficacy. During application onto the skin, nanocarriers breakdown into their molecular components and these systems are known as soluble nanomaterials. Insoluble nanomaterials have also a very wide range of uses. They are found in most cosmetic products used for personal hygiene, eye and face make-up, hair and skin care [1].

It is absolutely essential for certain fundamental properties to be identified in nanomaterials used in cosmetic products. Chemical properties, particle size, the distribution of particles sizes, surface area, surface properties, dustiness, density, pH, viscosity, morphological characteristics, catalytic activity and concentration within the product are all considered as the fundamental characteristics that need to be identified for nanomaterials. During the production of cosmetics, significant changes may occur in the fundamental properties of the nanomaterial. It is very well known that, in case the necessary precautions are not taken, nanomaterials between 1 to 100 nm in size can form aggregates larger than 100 nm. This situation naturally can affect the stability and shelf life of the product. Another important aspect for the nanomaterials used in cosmetics is their absorption probability. Various materials that increase penetration and absorption of nanomaterial through the skin are commonly used in the formulation of cosmetic products. Therefore, there is the risk that the insoluble nanomaterial might be absorbed from the area where the product was applied. Thus, differences might be observed between a macro-sized material and a nanomaterial, particularly with respect to toxicity [2]. For this reason, risk assessment protocols also need to take into consideration properties such as particle size and surface properties. In conclusion, it is necessary to conduct specific studies in order to eliminate concerns and suspicions regarding nanomaterials.

It appears that, owing to the advantages they provide, the use of insoluble nanomaterials in cosmetic products will gradually increase over time. In this context, the main subjects that need to be considered are the identification of the various properties of nanomaterials within cosmetic products, determination of whether absorption takes place following application, and the investigation of whether the use of the cosmetic product has any adverse effects on human health and the environment.

References

[1] C. Shelley-Egan, D.M. Bowman, The Challenge of Distributing Regulatory Responsibilities for Unknown Risks: 'Nano'-Cosmetics and the EU Cosmetics Regulation as a Case Study, *J Clinic Res Bioeth*, 6, 212, 2015.
[2] P. Singh, A. Nanda, Nanotechnology in Cosmetics: a Boon or Bane? *Toxicol.Environ.Chem.,* 94, 1467-1479, 2012.

SAFETY ASSESMENT OF COSMETIC PRODUCTS-GENERAL PRINCIPLES

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Cosmetics are a category of consumer products marketed worldwide. There is high global demand for cosmetics and Turkey's cosmetics market is the second fastest growing beauty and personal care products region in the world for the past six years [1].More than 10.000 chemicals are used in cosmetics [2]. Since cosmetic products may be used over a large part of human life span and sensitive population may be involved, short- as well as long-term safety aspects for consumers have to be considered. Safety of cosmetics products is in the European Union based on the safety of ingredients and based upon the principles and practice of the chemical risk assessment process [3,4]. This lecture describes the key components of this methodological safety process including hazard identification, dose-response analysis, exposure assessment and risk characterization as major steps.

References

[1] Unless otherwise stated, all stats Euromonitor International,2014.
[2] FDA (U.S. Food and Drug Administration). 2007. Compliance Program Guidance Manual. Program 7329.001. Chapter 29 - Colors and Cosmetics Technology.
[3] Scientific Committee on Consumer Safety, SCCS, The SCCS's Notes of Guidance for the testing of cosmetic substances and their safety evaluation, 8 th revision,2012.
[4] A.M.Fan, L.W.Chan (eds): Toxicology and Risk Assessment: Principles, Methods and Application, Marcel Dekker,NewYork,1996.

SAFETY OF COSMETICS: NON-ANIMAL TESTS AND FUTURE PERSPECTIVES

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The safety of cosmetics in the EU is today based on Regulation 1223/2009. The basic principles remain largely comparable with those of the previous Directive 76/768/EEC. They can be summarized as follows: (i) a cosmetic product must be safe for the consumer, (ii) its safety is based on safe ingredients (chemical structure, toxicological profile and exposure), (iii) safety must be obtained through the use of validated alternative, non-animal methods and the safety of the finished product is guaranteed through quantitative risk assessment Two channels are in place: the SCCS (Scientific Committee on Consumer Safety) functions at the EU level under DG SANTE and performs the safety evaluation (opinions) of the Annex substances. The second safety layer is obtained by safety assessment at the industrial level by an independent safety assessor. This is done on the final product and all its ingredients (taken up in a Product Information File, PIF) under the final responsibility of a EU-based Responsible Person (RP). For both, risk characterization is composed of 3 essential pillars namely hazard identification, dose response assessment and exposure assessment, described in detail in the SCCS's Notes of Guidance. A 9th revision in which the new Regulation is fully implemented will become available at the end of 2015. As expected, the animal testing and marketing bans are at the basis of major challenges in the safety evaluation process of new cosmetic ingredients since for local toxicity and short-term exposure validated alternatives exist, but not for long-term exposure. Realistic *in vitro* test proposals for repeated dose toxicity and reproductive toxicity still are in their infancy. Both animal-consuming tests are essential for the determination of the no observed adverse effect level (NOAEL) and the margin of safety (MoS) calculation on which risk characterization for regulatory purposes is actually based. The situation for cosmetics, allowing only validated replacement alternatives, is indeed unique in the world and has as well negative as positive consequences. There are new methodologies under development, but validation according to the running schemes are time-consuming and expensive. It is necessary that a shift takes place in our way of looking to the toxicity of cosmetic ingredients. New strategies need to be built up which go away from the actual replacing of test by test, but rather an integrated strategy needs to be developed based on the combination of mechanistic tests and new emerging technologies using human cells and tissues whenever possible. Promising data are being produced based on a number of new developments including human stem cells differentiation, 3D-printing and-culture technology and, as a solid basis, applying the concept of adverse outcome strategy (AOP) to develop robust human-relevant *in vitro* methods.

SAFETY RELATED ISSUES AND LEGISLATION

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In Turkey Cosmetic Legislation harmonised within the scope of EU Legislation and EU Acquis, a cosmetic product made available on the market must be safe for human health when used under normal or reasonably foreseeable conditions of use, taking account presentation, labelling, instructions for use and disposal [1-3]. To ensure the safety of cosmetic products placed on the market, they should be produced according to Good Manufacturing Practices (GMP). GMP is all the planned and systematic activities during the production, control, storage and transportation of cosmetic products in order to meet the quality standards [4]. Cosmetic Product Information File (PIF) should include a cosmetic product safety report documenting that a safety assessment has been conducted by the safety assesor defined in legislation. The PIF should be kept for a period of ten years following the date on which the last batch of the cosmetic product was placed on the market. In terms of safety, PIF should contain the safety assessment and a description of the method of manufacturing and a statement on compliance with GMP. Before placing a cosmetic product on the market, it shall be ensured that the cosmetic product has undergone a safety assessment on the basis of the relevant information and that a cosmetic product safety report is set up in accordance with Annex I/B [5]. The safety of finished cosmetic products can be ensured on the basis of knowledge of the safety of the ingredients that they contain. For every cosmetic product that contains nanomaterials, a high level of protection of human health shall be ensured. Cosmetic products containing nanomaterials shall be notified to competent autority before six months being placed on the market. Safety assessment of nanomaterials must be made in accordance with “Guidance On The Safety Assessment Of Cosmetics Containing Nanomaterials” [6]. According to cosmetic legislations of EU and Turkey animal testing is forbidden for safety assessment and efficacy test of finished cosmetic products and ingredients or combinations of ingredients used in cosmetic products [7]. But there are exceptional circumstances, where serious concerns arise as regards the safety of an existing cosmetic ingredient [3]. Briefly safety related issues in cosmetics need to be evaluated in the scope of regulations, standards and scientific views.

References

[1] Republic of Turkey Ministry of Health Cosmetic Directive (Official Gazette dated 23/05/2005 and numbered 25823).
[2] Republic of Turkey Ministry of Health Amending Cosmetic Directive (Official Gazette dated 15/07/2015 and numbered 29417 2).
[3] Regulation (EC) No 1223/2009 of The European Parliament And of The Council.
[4] International Organization for Standardization (ISO) standard for cosmetic GMPs (ISO 22716:2007)
[5] The Sccs’s Notes of Guidance For The Testing of Cosmetic Substances And Their Safety Evaluation
[6] SCCS Guidance on The Safety Assessment of Nanomaterials In Cosmetics.
[7] Turkish Medicines and Medical Devices Agency, Guideline on Alternative Test Methods to Animal Testing in Cosmetics dated 30/04/2014 numbered 1282615.

THE RELATED ATPASES PONTIN AND REPTIN IN LIVER CARCINOGENESIS: ROLE AND TARGETING

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Hepatocellular carcinoma (HCC), the main primary cancer of the liver, is one of the deadliest cancers worldwide. In the aim of finding new mechanisms of liver carcinogenesis, we performed a proteomic screen comparing HCC with non-tumor liver, which led us to the discovery of an overexpression of Reptin, and its homologous protein Pontin in HCC.

Pontin (or RUVBL1) and Reptin (RUVBL2) are members of the AAA+ family (ATPases Associated with various cellular Activities). They are highly conserved and essential proteins, involved in many cellular functions through their participation to several protein complexes. For instance, they have a chaperone activity for members of the PIKK family (mTOR, ATM, ATR etc.), they regulate gene transcription via several mechanisms, and they are involved in the remodeling of chromatin.

We found that Pontin and Reptin are significantly overexpressed in a large series of human HCC and that their level of expression was correlated with the prognosis of patients. Experimentally, we have demonstrated that Pontin or Reptin silencing with RNA interference *in vitro* led to HCC cell growth arrest and loss of viability, which may be explained in part by the role of Reptin on DNA damage sensing and repair. We further showed that silencing Reptin *in vivo* in established HCC xenografts in mice induced the regression of tumors.

Using mutants of Reptin devoid of ATPase activity, we demonstrated that the ATPase activity is required for the effect of Reptin on HCC cell growth and viability. We thus embarked on the search for small molecules inhibitors of the ATPase activity and discovered several Pontin ATPase inhibitors. This lead has been followed by pharma companies that have now developed highly specific inhibitors that will hopefully be turned into anti-cancer drugs.

THE ROLE OF EPIGENETICS IN BASIC AND CLINICAL CANCER RESEARCH

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Alterations in methylation patterns are frequent events observed in many human tumor types. Silencing of tumor supressor genes (TSGs) by aberrant hypermethylation in the promoter region of some known or putative TSGs occurs frequently during the carcinogenesis of various cancers including head and neck cancer (HNC). HNC is a highly heterogenous group of malignant diseases and the sixth most frequently observed cancer type in developing countries. Both environmental and genetic factors play a role in the development of the disease, but the underlying mechanism is still far from clear. Tobacco and/or alcohol use are the main etiological factors and play an important role in oral cavity, pharynx, and larynx cancer. The prognosis of the disease varies according to tumor size, local invasion, histology, and grade as well as ethnic origin. These diverse varieties reflect the versatile pathogenesis of the disease. In last three decades, there was not much improvement in the overall survival rates of HNC patients. Epigenetic biomarkers predicting clinical response, tumor recurrence, or patient survival are not available for HNC. Identification of novel therapeutic targets and new and specific biomarkers for the early detection of HNC could greatly increase the survival rate and might also help as prognostic indicators. We have shown that TSGs were expressed in normal tissues whereas they were supressed in tumor cells, and then we focused on biomarker discovery studies which may be used in early detection or screening, and define terapeutical approaches or prognosis, in the body fluids by methylation-based, non-invasive methods. Molecular detection of HNC in bodily fluids has the potential to improve post-treatment surveillance, provide prognostic information, and may influence therapy. In previous studies, bodily fluids such as sputum for lung cancer, urine for urologic tumors, salivary rinses for HNC, and breast fluid for breast cancer have been used in multiple detection strategies. Development of DNA methylation-based, non-invasive biomarkers offers the possibility of using rapid diagnostic assays for screening and diagnosis. In our recent work, we have demonstrated the diagnostic utility of methylation analysis of a panel of genes in HNC in the North American population [1-3]. We demonstrated the utility of promoter hypermethylation in a panel of four genes – Deleted in Colorectal Carcinoma (*DCC*), Endothelin receptor type B (*EDNRB*), *p16INK4a* and Kinesin chain member 1A (*KIF1A*)- in pre- and post saliva samples from HNC patients. Patients displaying methylation in surveillance salivary rinses are at significant risk for recurrence. Our data show that quantitative measurement of salivary methylated DNA may have promise for surveillance and early detection of recurrence. Thus, we suggest that gene silencing by hypermethylation is an important mechanism that has great promise for therapy and for the discovery of new biomarkers.

References

[1] Demokan et al., *Int J Cancer* 127, 2351-59, 2010.
[2] Kaur et al., *Int J Cancer* 127, 2367-73, 2010.
[3] Pattani et al., *Cancer Prev Res* 3, 1093-103, 2010.

BUSINESS BENEFITS OF QbD IN TERMS OF LICENSING

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Due to promote an harmonised quality system which applicable across the lifecycle of the pharmaceutical product and accents an integrated approach to quality risk management and science, the ICH instituted a series of quality guidances like Q8, Q9, Q10 and Q11, all emphasizing the adoption of systematic principles of QbD (Quality by Design) and PAT (Process Analytical Techniques) as its 21st century quality initiatives. QbD as implemented in ICH Q8: A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management. The holistic QbD-based philosophy for systematic development of nanostructured systemsinvolves defining the QTPP (Quality Target Product Profile), CQAs (Critical Quality Attributes), identification of CMAs (Critical Material Attributes) and CPPs (Critical Process Parameters), selection of experimental designs, demarcation of design and control spaces to embark upon the optimum formulation, postulation of control strategy for continuous improvement [1,2]. Added values by QbD are Improved the decision making process, ensuring of identifing how the best benefit is assured for the patient, working science based and acting with concrete data, minimization of subjective approaches, classifiying and prioritizing risks, ensuring better utilization of resources. A study presents the results of a survey about actual experiences, examples and candid industry opinions on business benefits of QbD of companies which use QbD approach at different levels was conducted by the ISPE United Kingdom/Ireland PAT COP [3]. As study shows for pharma industry in particular, QbD execution leads to improved time to market, enhanced knowledge sharing, limited product recalls and rejects, reduced consumer skepticism towards generics, decreased post-approval changes and efficient regulatory oversight.

References

[1] B. Aksu, S. Beg, B. Garg, R. Kapil, B. Singh, Quality By Design (QBD) And Its Applications In Development Of Nanostructured Drug Delivery Systems. Chapter in *Drug Nano-carriers* Vol. III, p. 1-30.
[2] R. A. Lionberger, S. L. Lee, L. Lee, A. Raw, L. X. Yu, Quality by design: concepts for ANDAs. *AAPS J.* 10, 268-76, 2008.
[3] T. Kourti, B. Davis, The Business Benefits of Quality by Design (QbD), *The Official Magazine of ISPE,* 32(4), 2012.

IMPACT OF EXCIPIENT VARIABILITY ON QbD & BASF'S SUPPORT ON QbD

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A systematic approach to develop pharmaceutical dosage forms, that begins with predefined objectives (a "Target Product Profile")and emphasizes ingredient, product and process understanding and process control based on sound science, permanent improvement and quality risk management. QbD is about building robust formulations and processes which can adapt to normal expected product.To understand that developing a "QbD Medicine" may also impact the excipient manufacturers.The message is well received and understood by excipient manufacturers. Pharma industry will turn to excipient suppliers with questions and demandsdepending on actual drug product under development /in life cycle management which will lead to requirements on better understanding of: Excipient CQA/FRCs, Excipient batch uniformity, Batch-to-Batch Consistency, Suitable analytical test methods. It will also lead to requests of excipient batches from normal range productionat the low, mid and max of the agreedspecification ranges for the identified critical properties. Manufacturers understand that there is no "one size fits all" approach exists: design space for each drug product will be different and this may put otherrequirements on excipient's CQA/ FRCs.A lot of pharmaceutical excipients (different grades) are framed by technical or cosmetic products (e.g. poloxamers, PVP). These products can be used for QbD considerations as well, for investigating the impact of variations in the excipient's quality onto the final product performance. Pharmaceutical excipient application labs have comprehensive experience in a huge variety of pharmaceutical technologies. Consultation can help to define the Critical Process Parameters, to evaluate the relevance of batch to batch variations, to decide for the best manufacturing technology, to define a proper Design Space.

INTEGRATION OF BIOPHARMACEUTICS AND QUALITY BY DESIGN: THE BIOPHARMACEUTICS RISK ASSESSMENT ROADMAP (BIORAM) BUILDING IN CLINICAL RELEVANCE FOR THE PATIENT BENEFIT

Arzu Selen

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QbD IMPLEMENTATION IN PHARMACEUTICAL INDUSTRY

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Product Quality cannot be tested but it has to be built in. Historically regulators found that mostly the end product quality is 6 Sigma but when it comes to built in quality it was just 2 or 3 Sigma, accepting the fact that there could be some exceptions. This was the guiding spirit behind ICH Q8 guideline on product development adopted by EU in June 2009introduced in 2006 & ICH Q11 on API development introduced in May 2012. Further it became US FDA's priority & expectation in 2013. European authorities too are found to raise lot of queries for which the root cause is non-implementation of QbD during development stages. So one can file the dossier without QbD but can hardly succeed today in getting it approved in todays changed scenario.

The presentation is aimed at explaining this paradigm shift undertaken by regulators all over the globe & explain practical approach through case studies towards successful implementation of QbD for both product & analytical method development. How to set Quality Target Product Profile (QTTP) & link Critical Quality Attributes (CQA's) of material, method & process to it, how to plan Design of Experiments (DoE's) & analyse the output shall be covered. Lot is being talked about Life Cycle Management approach which shall be explained as applicable to QbD. What & how of information which needs to be provided to authorities shall also be the part of discussion.

BIOPHARMACEUTIC RISK ASSESSMENT OF BRAND AND GENERIC LAMOTRIGINE TABLETS: IN VITRO AND IN VIVO

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The goal of this presentation is to discuss the biopharmaceutic risk of tablets of the anti-epileptic drug lamotrigine. This assessment will consider the physiochemical characteristics of the drug, which as is BCS class 2b drug (i.e. low soluble, high permeability, weakly basic), and QC market surveillance. This assessment will also consider results from a novel BE study, denoted "BioEquivalence in Epilepsy Patients" or BEEP, where BE was determined in "generic brittle" epilepsy patients.

References

[1] Vaithianathan S, Raman, S, Jiang W, Ting TY, Kane MA, and Polli JE. (2015): Biopharmaceutic Risk Assessment of Brand and Generic Lamotrigine Tablets. DOI: 10.1021/acs.molpharmaceut.5b00154. *Mol Pharmaceutics* 12: 2436−2443.
[2] Ting, T.Y., Jiang, W., Lionberger, R., Wong, J., Jones, J.W., Kane, M.A., Krumholz, A., Temple, R., and Polli, J.E. (2015): Generic lamotrigine versus brand-name LAMICTAL bioequivalence in epilepsy patients: a field test of the FDA bioequivalence standard. DOI: 10.1111/epi.13095. *Epilepsia* 56:1415-24.

BioMEMS AND MICROFLUIDIC DEVICES FOR LAB-ON-A-CHIP APPLICATIONS

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This presentation introduces MEMS and microfluidics technology for biomedical applications, including their applications with examples from the literature. BioMEMS projects at METU-MEMS Center will be presented.

Since the first introduction in 1970's, MEMS technology is becoming popular in many different application areas, including military, automotive, and consumer electronics, as it provides cheap, small, and smart sensors and actuators. This technology is especially critical for biomedical applications, resulting in a new research area shortly called BioMEMS. BioMEMS can be defined in general as "devices or systems constructed using techniques inspired from microfabrication that are used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities [1]." Application areas of BioMEMS range from diagnostics to micro-fluidics, systems for drug delivery, tissue engineering, and implantable systems.

One of the most interesting application areas for this technology is the micro total analysis systems (Micro-TAS). Biological samples can be analyzed in a very small area with considerably reduced cost and time, by forming micro-fluidic channels on silicon substrate and combining them with onchip electronics. Some examples for such applications include on-chip electrophoresis systems, polymerized-chainreaction (PCR) units, DNA sequencing chips, and complex lab-on-a-chip devices [2-6].

There are currently various BioMEMS related projects going on at METU-MEMS, including DNA electrophoresis systems [3], dielectrophoresis chips for cell seperation [4], gravimetric sensors for cancer cell detection, microvalves and pumps for lab-on-a-chip systems [5], and electrochemical sensors for bacteria and toxin detection.

References

[1] R. Bashir, "BioMEMS: state-of-the-art in detection, opportunities and prospects," Advanced Drug Delivery Rev., Vol: 56, 11, 1565-86, 2004.
[2] V.M. Ugaz et. al. "Microfabricated electrophoresis systems for DNA sequencing and genotyping applications: current technology and future directions," Philos Transact A Math Phys Eng Sci.; 362: 1105-1129, 2004.
[3] S. Sukas et. al., "A parylene based double-channel microelectrophoresis system for rapid mutation detection via heteroduplex analysis," Electrophoresis J., Vol. 29, No. 18, pp. 3752-58, 2008.
[4] G. Yılmaz et. al. "A Dielectrophoretic Cell/Particle Separator Fabricated By Spiral Channels and Concentric Gold Electrodes," TRANSDUCERS'07, pp. 73-76, June 2009.
[5] E. Yıldırım et. al." An Electrostatic Parylene Microvalve for Controlling In-Plane Flow," µTAS 2009.
[6] A.C.R. Grayson et. al., "A BioMEMS review: MEMS technology for physiologically integrated devices," Proceedings of the IEEE, Volume 92, Issue 1, pp. 6-21, Jan. 2004.

FORMATION AND EVALUATION OF MOLECULARLY IMPRINTED POLYPYRROLE

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Molecularly-imprinted polymers (MIPs) are mimicking action of affinity based structures that are applied biosensor (e.g. immunosensor) design. The MIPs based sensors are interesting because of their relatively low costs and good selectivity towards imprinted analyte.

In this presentation chemical and electrochemical polymerization of polypyrrole (Ppy) will be overviewed. Possibilities to apply both methods of Ppy synthesis for the formation of polypyrrole layers, which were molecularly-imprinted by caffeine and theophylline (MIP-Ppy) will be presented. Synthesis of MIP-Ppy is possible on various surfaces. Formed MIP-Ppy layers could be evaluated by electrochemical methods, quartz crystal microbalances (QCM), surface plasmon rseonance and optical ellipsometry. Polymerization process could be estimated by analysis of chronoamperometric and QCM data. Cottrell equation is mostly applied for the integration of total charge, which is passing during electrochemical polymerization based on potential pulses.

In this study a unique home-made flow-through QCM cell was used for the formation of MIP-Ppy and the same cell was applied for QCM measurements. The main advantages of here applied flow-through cell design were (i) reduced volume of the cell and (ii) the possibility to perform continuous association/dissociation processes. The performed QCM measurement data proved that the QCM sensor modified with MIP-Ppy is more sensitive to imprinted molecules.

In addition to QCM affinity and dielectric properties of MIP-Ppy based thin film were evaluated by electrochemical methods and ellipsometry. The most interesting electrochemical information was observed when potentiodinamic methods (cyclic voltammetry, pulsed amperometric detection and electrochemical impedance spectroscopy) were applied.

Evaluation of association/dissociation kinetics between caffeine imprinted MIP-Ppy and both xanthine derivatives (caffeine and theophylline) was evaluated and some parameters of thermodynamics were calculated. The evaluation demonstrated significant differences in interaction between MIP-Ppy and imprinted analyte or not-imprinted homologous materials.

The equilibrium constant K_{eq} of MIP-Ppy/theophylline and MIP-Ppy/caffeine complexes were calculated and from these K_{eq} values standard Gibbs free energy ($\Delta G°$) for formation of MIP-Ppy/theophylline and MIP-Ppy/caffeine complexes were estimated.

OLIGONUCLEOTIDE APTAMERS: EMERGING AFFINITY PROBES FOR BIOANALYTICAL MASS SPECTROMETRY AND BIOMARKER DISCOVERY

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Selective isolation of biological important molecules and their functional characterization is one of the primary goals of bioanalytical chemistry. Several different affinity tools such as antibodies, affimers, nanobodies, DARPins have been explored to achive these goals. In recent years, oligonucleotide based affinity tools called aptamers have become progressively attractive and the research in this area has seen an exponential increase. Aptamer probes have been explored in many different areas of bioanalytical chemistry such as electrical and optical biosensor development, targeted drug delivery, logic gates, DNA nanotechnology, point of care diagnostics. However aptamers are still largely overlooked in mass spectrometry (MS) and biomarker discovery. After the completion of human genome project, the focus has shifted towards functional genomics and to understand the living systems by deciphering the functions of proteins and metabolites. Therefore identification and functional characterization of these molecules is of outmost importance. While identification of isolated biomolecules and analysis of simple biological mixtures using MS has become relatively simple, the power of MS gradually decreases as the complexity of the biological mixtures increases. Therefore development of selective and targeted approaches is at the forefront of mass spectrometry. Aptamers have great potential in affinity mass spectrometry to improve selectivity, specificity and throughput. This talk will discuss the development of new oligonucleotide aptamer-based MS methods. Specifically, aptamer-decorated nanostructures and their applications in analyzing real biological samples and next generation native mass spectrometry methods for biophysical characterization of aptamer-ligand interactions will be discussed.

ELUTION ORDER IN CHIRAL CHROMATOGRAPHY THROUGH COMPUTATIONAL STUDIES

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In the last few decades, there has been a significant increase in the development of chiral compounds. Thus, access to enantiomerically pure compounds is presently a major focus of pharmaceutical research and, in particular, chiral HPLC separations have gained great importance for semi-preparative and industrial isolation of enantiopure compounds. Chemoinformatic procedures as well as molecular modelling and quantum chemistry techniques can be successfully applied to address chirality related problems especially when enantiomerically pure reference standards are missing. In this scenario, we made computational efforts to explain the mechanism of enantioselective recognition and hence to rationalize the enantiomer elution order (EEO) with both low- and high-molecular weight chiral selectors (SOs). The following selected examples are the subject of the present communication.

With the chiral ligand exchange chromatography (CLEC) system based on the *N*,*N*-dimethyl-(*S*)-phenylalanine as the chiral mobile phase additive (CMPA) to the eluent, the EEO of amino acid analytes (SAs) was found to depend on the different water coordination capability on copper ion in the formation of the mixed ternary complexes. The use of a decision tree model enabled to explain the observed EEO of a pool of amino acids in the presence of the O-benzyl-(*S*)-serine-based CMPC-CLEC system. After the calculation of numerous 3D descriptors on quantum mechanically optimized SA/Cu(II)/SO complexes, the delta-E_{sol} was selected as the suitable one for correctly classifying the submitted species according to the relative chromatographic behaviour. A computational protocol consisting in molecular dynamic simulations was applied to gain insight into the overall stereorecognition mechanism of N^{α}-Boc-N^{4}-(hydroorotyl)-4-aminophenylalanine with a quinine-based zwitterionic chiral stationary phase (CSP). The use of two energy descriptors, INTER and SELF, explained the observed stereoisomeric elution order. Finally, electronic circular dichroism studies on the two enantiomers isolated with a cellulose *tris*(4-chloro-3-etylphenylcarbamate-based CSP, combined with time-dependent density functional theory calculations allowed to characterize the configuration of the enantiomers of the species 2-(5-bromo-2-ethoxyphenyl)-6-trifluoromethyl-*H*-1,4-benzothiazin-3(4*H*)-one and determine a (*R*) < (*S*) EEO.

GENETIC POLYMORPHISMS CAN BE PROGNOSTIC AND PREDICTIVE BIOMARKERS FOR CANCER RISK

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Reactive species (RS) including free radicals derived from cellular metabolism in aerobic organisms and from exogenous sources such as ionizing radiations, carcinogenic compounds, and environmental toxins. Defense mechanisms, such as, antioxidant, metabolizing and DNA repair systems; exist in living organisms to encounter the production and effects of RS. If the prooxidant–antioxidant balance is disturbed in favor of the former, a state of oxidative stress can occur, leading to oxidative damage to biomolecules including DNA, proteins and lipids [1]. Oxidative DNA damages that escape repair before replication may lead to mutagenesis and be a risk in the development of cancer. Oxidative damages are repaired by a variety of repair mechanisms. Base Excision repair (BER) is the main pathway for the repair of these damages and Nucleotide Excision Repair (NER) pathway also is included in the repair to some degree. Single Nucleotide Polymorphisms (SNPs) are DNA base variants present in the human population at a frequency >1%. There has been an increasing focus on the role of SNPs in the development and progression of cancer but also on their role in diagnostics and risk prediction. Non-synonymous coding SNPs and regulatory SNPs in DNA repair genes can result in reduced DNA repair capacity, which can underlie a higher mutation rate and increased cancer risk [2]. Most cancers are the result of the interactions of multiple genes and environmental factors. Reliable knowledge on which BER sequence variants are associated with cancer risk would help elucidate the mechanism of cancer [3, 4, 5]. If a polymorphism is in a repair gene that plays a pivotal role in the removal of oxidative DNA damage, for instance, the resulting reduced repair activity will increase the possibility of cancer. Studies on polymorphisms, therefore, provide an opportunity to discover candidate susceptibility alleles. Data from separate association studies are often conflicting. Meta-analysis can be used to integrate conflicting findings across several studies of the same genetic variant [6]. It is to provide develop database which a relatively comprehensive assessment of the relationship gene polymorphisms and the cancer risk.

References

[1] M. Dizdaroğlu, Oxidatively induced DNA damage and its repair in cancer, Mutation Res., 763, 212-245, 2015.
[2] B. Karahalil, V.A. Bohr, D.M. Wilson 3rd., Impact of DNA polymorphisms in key DNA base excision repair proteins on cancer risk, Hum. Exp. Toxicol., 31, 981-1005, 2012.
[3] B.Karahalil, A.B. Engin, E. Coşkun, Could 8-oxoguanine DNA glycosylase 1 Ser326Cys polymorphism be a biomarker of susceptibility in cancer? Toxicol. Ind. Health, 30, 814-825, 2014.
[4] B. Karahalil, V.A. Bohr, N.C. De Souza-Pinto, Base excision repair activities differ in human lung cancer cells and corresponding normal controls, Anticancer Res., 30, 4963-4971,2010.
[5] A.B. Engin, B. Karahalil, A. Engin, A.E. Karakaya, DNA repair enzyme polymorphisms and oxidative stress in a Turkish population with gastric carcinoma. Mol. Biol. Rep., 38,5379-5386, 2011.
[6] P.T. Zhou, B. Li, J. Ji, M.M. Wang, C.F. Gao, A systematic review and meta-analysis of the association between OGG1 Ser326Cys polymorphism and cancers, Med. Oncol., 32,472, 2015.

DRUG-INDUCED LIVER INJURY

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Drug induced liver injury (DILI) is an uncommon but important and challenging form of liver disease. Many people use different kind of drugs, vitamin-minerals, nutritional supplements and herbal preparations. Some of these items have a potential for liver injury. DILI can not be differentiated easily from acute or chronic hepatitis, acute liver failure, biliary obstruction, or fatty liver disease. To detect specific drug that cause liver damage and to find out specific manifestations of DILI is challenging area. DILI can be severe and sometimes fatal, but is usually ameliorated by finishing the use of responsible drug. A lot of drugs were withdrawn from the market due to their potential to cause liver injury. Hepatic damage due to drugs is classifed as nonidiosyncratic (predictable), as in the case of acetaminophen, or idiosyncratic (unpredictable). In the United States, about 2000 acute liver failure are occured every year, and 50% of them is caused by drugs. Big portion of this cases (about 40%) was caused by acetaminophen. It was reported in a recent and well-documented population based study that drug-induced liver injury (DILI) was 19.1 (95% CI, 1.54-23.3) cases per 100,000 persons yearly. DILI is caused by different factors such as dose, lipid solubility of drugs, strength of hepatic biotransformation, race, age, sex, chronic liver disease etc. Genetic predispositon is another important parameter for DILI. In general, severe DILI is caused by predominantly hepatocellular injury. Hepatocellular injury is indicated by rises in blood alanine or aspartate aminotransferase (ALT or AST). The level of these enzymes is the main dertermimant of severity of DILI. However these enzyme level is not always a reliable predictor for hepatotoxicity of drugs. Some of drugs cause a high ALT or AST level transiently but repeated use of this kind of drugs do not cause severe DILI. Functional ability of liver like bilirubin clearence, prothrombin and other coagulation factor sythesis is another indicator of severe DILI. On the other hand many of drugs which cause severe DILI in human does not cause this toxic effect in animal. For this reason it is not easy to predict DILI, and there are not a clear genetic, metabolic or spesific factors which predict DILI in an individual. Among the hepatotoxic drugs, amoxicillin/clavulanate, isoniazid, and nonsteroidal antiinflammatory drugs are at the first place. In this presentation DILI will be discussed briefly with the knowledge that is available in the literature.

BIOMARKERS OF INSULIN-MEDIATED DNA DAMAGE

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Diabetes mellitus type II (DM II) affects almost 350 million people worldwide. Among other health threatening consequences, patients develop an increased rate of malignancies. Hyperinsulinemia, which is present during the early phase of the disease, is considered a risk factor for cancer development. We have shown that an elevated insulin level can induce oxidative stress, resulting in DNA damage in colon cells in vitro and in kidney cells in vitro and in vivo. The signaling pathway for this is started by phosphorylation of the insulin and insulin-like growth factor-1 receptors, followed by activation of PI3K, which then activates Akt. Subsequently, NADPH oxidase isoforms and mitochondria produce increased amounts of reactive oxygen species (ROS), which can attack the DNA. Inhibition of the tumor suppressor Pten, which is involved in the negative regulation of PI3K/Akt and its downstream targets, increased ROS production and genomic damage in cell lines of the liver and the kidney. Knock-down of pten in a mouse model yielded increased oxidative stress and genomic damage in the liver. The antidiabetic drug metformin did not show intrinsic antioxidant activity in the cell free assay, but when combined with insulin, it protected cultured cells from insulin mediated oxidative stress, DNA damage and mutation. Treatment of rats with metformin protected their kidneys from oxidative stress and genomic damage associated with hyperinsulinemia. Since DM II is often associated with obesity, and bariatric surgery has been shown to reduce insulin resistance effectively, we investigated the influence of bariatric surgery as well as caloric restriction on oxidative stress and genomic damage in a rat obesity model (Zucker$^{fa/fa}$ rats). In urine, kidney and colon samples, a reduction of oxidative stress and DNA damage was observed after weight loss by either method.

If the same mechanisms are active in patients, the use of antioxidants and/or ROS production inhibitors might exert protective effects. Weight loss in overweight individuals and certain pharmacological treatments may protect patients from genomic damage and may support efforts to reduce the elevated cancer risk that is associated with hyperinsulinemia.

TOWARDS ACCELERATED LEAD FINDING: NMR-BASED METABOLOMICS

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Per definition metabolomics represents a comprehensive holistic approach, defined as systematic identification and quantification of all metabolites in an organism, at given conditions. The comprehensive analysis of the chemical fingerprints left by metabolic processes started to play a crucial role in the personalized medicine [1].

Since the term 'metabolome' has been introduced several platforms and techniques for high throughput analyses of targeted metabolites have been developed (mainly mass spectrometry and nuclear magnetic resonance spectroscopy). Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, because it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of ^{1}H NMR-spectrometry over the other analytical platforms (MS in particular) is the possibility for (relative) quantification and thus the direct comparison of concentrations of all compounds present in the sample, as the signal intensity is only dependent on the molar concentration of the solutes [2, 3].

Some case studies of the application of NMR-based metabolomics concept in natural products research, biotechnology and lead finding [3-6] will be presented and thoroughly discussed.

References

[1] J. K. Nicholson, Metabolomics, *Nature,* 463, 32, 2010.
[2] H. K. Kim, Y. H. Choi, R. Verpoorte, NMR-based metabolomics analysis of plants, *Nature Protocols* 5, 536, 2010.
[3] M. I. Georgiev, K. Ali, K. Alipieva, R. Verpoorte, Y. H. Choi, Metabolic differentiations and classification of *Verbascum* species by NMR-based metabolomics, *Phytochemistry* 72, 2045, 2011.
[4] G. Zahmanov, K. Alipieva, S. Simova, M. I. Georgiev, Metabolic differentiations of dwarf elder by NMR-based metabolomics, *Phytochemistry Letters,* 11, 404, 2015.
[5] K. Alipieva, L. Korkina, I. Erdogan Orhan, M. I. Georgiev, Verbascoside – A review of its occurrence, (bio)synthesis and pharmacological significance, *Biotechnology Advances,* 32, 1065, 2014.
[6] D. Marcoccia, M. I. Georgiev, K. Alipieva, S. Lorenzetti, Inhibition of the DHT-induced PSA secretion by *Verbascum xanthophoeniceum* and *Serenoa repens* extracts in human LNCaP prostate epithelial cells, *Journal of Ethnopharmacology,* 155, 616, 2014.

IMPROVING THE PHARMACOKINETIC DISPOSITION AND PHARMACOLOGY OF THE BIOACTIVE HERBAL MOLECULES THROUGH PHARMACEUTICAL FORMULATION

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13α(21)-Epoxyeurycomanone (EP) and eurycomanone (EN) are two major bioactive quassinoids in a standardized extract of *Eurycoma longifolia* (TAF273) that have contributed towards the traditional use of the plant to increase spermatogenesis and fertility [1]. The quassinoids possess low oral bioavailabilities, due to high aqueous solubility and poor membrane permeability, resembling the Biopharmaceutical Classification System (BCS) Class 3 drugs. The present study formulated a lipid-based solid dispersion mixture (TAF273-SD) of the quassinoids in TAF273 using Gelucire® 44/14 and Span 60 to improve the gut absorption, oral bioavailability and efficacy of spermatogenesis in rats. The intestinal absorption of EP and EN were assessed using an *in vitro* everted rat gut sac method. The relative bioavailability of the quassinoids was measured following a three-way crossover treatment design in rats, administered TAF273-SD and TAF273 intravenously and orally. The spermatozoa were collected from the rat epididymis after 42-day oral treatment and then counted following the WHO protocol. The formulated TAF273-SD significantly improved the oral pharmacokinetic disposition of EP and EN in rats. The plasma EP and EN versus time curves from TAF273-SD and TAF273 displayed double-peak profiles, prompting the investigation of the contributing factors involved and measurement of the gastro-intestinal transit-time with reference markers, theophylline and sulfasalazine orally co-administered with the formulated TAF273-SD. Both EP and EN took longer time to reach the rat small intestine and were mostly detected in the large intestine at concentrations of 74.36 % and 80.27 %, respectively. The rat oral bioavailabilities of EP and EN in TAF273-SD were 1.69 ± 0.34 and 2.75 ± 0.53 fold, respectively higher than the non-formulated TAF273. The rat spermatozoa count of the formulated TAF273-SD was also significantly higher than the non-formulated TAF273. The non-formulated TAF273 extract at 0.028 ppm and its major quassinoid, EN at 0.005 ppm reduced acetylcholine- and histamine-induced guinea pig ileum contractions, suggesting that a muscle relaxant effect may contribute importantly to the delay in gastric-emptying and intestinal absorption. In conclusion, the formulated quassinoids further improved the rat oral bioavailability and spermatozoa count. The double-peak pharmacokinetic profile of the quassinoids persisted, due to reduction in gut motility that caused delay in gastric emptying and intestinal absorption.

References

[1] K.L. Chan, B.S. Low, C.H. Teh, P.K. Das, The effect of *Eurycoma longifolia* on sperm quality of male rats, Nat Prod Commun, 4: 1331-1336, 2009.

MODIFICATION STUDIES ON CYCLOARTANE-TYPE SAPOGENOLS

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Saponins, high-molecular weight secondary metabolites, are utilized in cosmetic to pharma, beverage to sugar industries. These compounds have received considerable attention in drug discovery studies due to their wide range of bioactivities (cytotoxic, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal, antifungal, antiparasitic, antiviral, anti-HIV). Saponins can be divided into two main groups, the triterpenoid and the steroid saponins. There has been extensive search on steroidal saponins, considered as one of the most marketed pharmaceutical products. Besides being an important starting material for the production of steroidal hormones, there are many steroidal compounds as active pharmaceutical ingredients in the market (anti-inflammatory, antitumor, antiviral, antiallergic). From a bioactivity perspective, the most important triterpenoid structures are recognized as oleanane, ursane, lupane, and dammarane carbon skeletons, which are also commercially available for further semi-synthesis and biotransformation studies [1]. Since drug-discovery programs on the triterpenoids have mainly focused on these skeletons providing extensive bioactivity data, the less common miscellaneous aglycones such as cycloartanes have been disregarded for long time.

The cycloartanes, unique triterpenoids with a characteristic 9,19-cyclopropane ring, occupy a special position among low molecular bioregulators since cycloartenol is a key intermediate in the biosynthesis of different phytosterols. For this reason, cycloartenol and its weakly polar derivatives are widespread in the plant kingdom. The plants of *Astragalus* genera are the richest source of this class of compounds. Cycloastragenol (CA), is the main sapogenol of many cycloartane-type glycosides found in the *Astragalus* genus. Recently CA, 20(R),24(S)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane, has attracted attention due to its unique bioactivity, viz., telomerase activation [2].

Taking into account, the results of our comprehensive studies and preliminary screenings in addition to recent progress in the literature, our research group has decided to focus on *Astragalus* cyloartanes to form a compound library for advance bioactivity studies such as anti-cancer, anti-aging and anti-inflammatory. Therefore the microbial transformation and semi-synthesis studies were conducted on 3 cycloartane-type sapogenols (Cycloastragenol, astragenol and cyclocanthogenol). Details of our modification studies will be presented.

References

[1] P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural Product Reports*, 23: 394-411, 2006.
[2] C. B. Harley, S. P. Khar, M. Ramaseshan, P. Ramiya, Z. Z. Pirot, S. Fauce, T. Lin, Compositions and Methods for Increasing Telomerase Activity, US Patent 20100292197 A1, Nov. 18 2010.

A SYSTEMS MEDICINE APPROACH FOR PERSONALISED CANCER CHRONOTHERAPY DELIVERY

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Chronotherapeutics aim at improving treatment outcomes through the delivery of medicines according to the Circadian Timing System (CTS), a complex hierarchical and dynamic network system involving molecular clocks located in all cells in the body. Thus circadian timing modified up to 10-fold the tolerability of over 30 anticancer medications in mice or rats. This finding translated into clinical benefit for 5-fluorouracil-leucovorin (FL), oxaliplatin (O), carboplatin, cisplatin, doxorubicin, theprubicin, vinorelbine and irinotecan in cancer patients. However, sex and circadian robustness/disruption appeared as independent determinants of the optimal chronotherapeutic schedule, in meta-analyses of international studies involving patients with metastatic colorectal cancer. Stochastic and deterministic mathematical models further identified circadian robustness in healthy cells, cell cycle length around 24 h and large variability in cancer cells, as critical parameters for best achieving improved efficacy on cancer cells and best tolerability in healthy cells. Moreover, optimal chemotherapy timing differed by up to 8 h according to genotype and sex in mice. Such differences in optimal timing were recapitulated, through modeling the reciprocal regulation of clock genes *Rev-erb*α and *Bmal1*. As a result, optimal circadian timing of irinotecan was predicted irrespective of sex and genotype based on a simple CTS molecular model. The relevance of *Bmal1* was further demonstrated for the chronopharmacokinetics-pharmacodynamics of irinotecan in synchronized cell cultures. We are currently investigating whether circadian rest-activity and temperature circadian patterns also carry out any predictive information for optimizing chronotherapy in individual cancer patients.

Supported by the Coordinated Action on Systems Medicine (CaSyM, FP7)

DRUG TRANSPORTERS AND CANCER: CIRCADIAN RHYTHMS AND THERAPEUTIC IMPLICATIONS

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The circadian timing system (CTS) is a complex system that coordinates bodily and cellular functions along 24h. The CTS composed of a central pacemaker the suprachiasmatic nuclei (SCN) located in the hypothalamus and at the top of the system. The SCN coordinates molecular or circadian clocks consisted of core clock genes such as *Clock* and *Bmal1.* The CTS generates daily rhythms in cellular and organism physiology and adjusts them to environmental cycles [1]. Circadian changes modulate drug metabolism-detoxification and disposition processes. Some drugs are transported via carriers, e.g., ATP-binding cassette (ABC) transporters in particular P-glycoprotein (P-gp), as it confers the multidrug resistance (MDR) to the variety of antineoplastics. Recently, it was reported that circadian rhythms may influence the pharmacokinetics of drugs and play a role in the pharmacokinetic processes when affected by ABC carriers. This is particularly relevant for anticancer drugs, with a narrow therapeutic index [2, 3]. Indeed, circadian timing modifies the toxic effects of 40 anticancer medications in rodents and in patients. The mechanisms of drug detoxification involve the cellular efflux of medications and/or their metabolites via transporters. This detoxification rhythm can importantly contribute to host tolerability for some anticancer drugs such as irinotecan, docetaxel which effluxed by ABC's. However, we should consider also that several mechanisms jointly account for the chronopharmacology of antineoplastics. The challenge is to identify the optimal chronotherapeutic schedules that will best spare healthy tissues from toxic insults in an individual patient [2, 3].

References

[1] Lévi F, Schibler U., *Annu Rev Pharmacol Toxicol*, 47, 593-628, 2007.
[2] Lévi F, Okyar A., *Expert Opin Drug Deliv*, 8, 1535-1541, 2011.
[3] Lévi F, Okyar A et al., *Annu Rev Pharmacol Toxicol,* 50, 377-421, 2010.

DRUG DEVELOPMENT IN ONCOLOGY YESTERDAY, TODAY & TOMORROW

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Drug development is stepwise process progressing from preclinical to clinical evaluation. Drug development differs according to target disease and the patient population because of the risk benefit ratio for a drug. In oncology the life threatening nature of advanced cancer allows the acceptance of considerably more risk than would be acceptable in other conditions. There is an apparent need to improve the speed and efficiency of drug development esp. in oncology. Esp. we need to question how to develop new targeted agents; this cannot be done effectively using response criteria of conventional cytotoxic chemotherapy. Classical approach which is to determine dose based on maximum tolerability and efficacy based on objective tumor response may not be suitable for targeted agents. For standard cytotoxic agents usually objective tumor response is considered as the primary end-point. However this response may not be adequate to identify promising activity with cytostatic targeted agents. Instead, exploratory trials of targeted agents may have to focus on other end-points such as pharmacological effects and disease stabilization. Since many of them have a wide therapeutic index and inhibit tumor growth without demonstrable cytotoxicity.

A primary objective of Phase 1 trials of standard cytotoxic chemotherapeutic agents is ordinarily to define maximum tolerated dose. This dose is typically recommended for exploratory Phase 2 efficacy trials, under the assumption that the higher the dose the greater the antitumor activity. However, molecularly targeted agents are generally less toxic than standard cytotoxic drugs and treatment effects may occur at doses much lower than the maximum tolerated dose. Also these toxicities may not be only hematological toxicities and we also need to look for cumulative toxicities as well.

The way we further develop oncology drugs should be question as well. Today if we have shown that once a new agent has been shown to be effective in one cancer, much effort will be devoted to further investigations of the same drug in various combinations for different disorders. While this approach has led to advances in the treatment of many childhood cancers, were not successful in common adult cancers. The other problem is that early clinical trials new drugs can only be tested against advanced and usually heavily pretreated disease; it is unlikely that dramatic responses will occur. Therefore today good phase II data may not mean that successful phase III trial on the way.

There is an increasing interest in making the best possible use of biomarkers and pharmacogenomics in early phases of drug development. Developing reliable, specific biomarkers could enhance diagnosis, prognosis, targeted therapy and therapy monitoring and could also stimulate the discovery and clinical development of more targeted therapies for metastatic cancer. However, there are currently very few biomarkers in clinical use in oncology; for many cancers, there are no biomarkers at all.

PI3K/AKT/mTOR CELL SURVIVAL PATHWAY: TARGET FOR CANCER DRUG DISCOVERY

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The phosphatidylinositol-3-kinase(PI3K)/AKT/mammalian-target-of-rapamycin (mTOR) cell survival pathway is altered to be hyperactivated in many cancer types. The members of this signalling network regulate a broad range of cellular processes including survival, proliferation, growth, metabolism, angiogenesis and metastasis. The key role of AKT kinase in this network is due to its regulation by a wide-range of upstream signalling elements and downstream effectors through crosstalks with various signalling pathways including RAF/MEK/ERK. Limited clinical success of the available targeted therapeutic agents and challenges mediated by tumour heterogeneity across different cancer types indicate the importance of the PI3K/AKT/mTOR pathway in targeted therapeutics approaches. This talk focuses on the importance of PI3K/AKT/mTOR network that represents the intricate crosstalk between with other pathways involved in cell proliferation for targeted cancer drug discovery. Examples from the molecular actions of the PI3K/AKT inhibitors in liver cancer cells for synergistic therapeutic strategies will be discussed in detail.

MEDULLOBLASTOMA EXTRACELLULAR VESICLES CARRY OTX2 THAT PROMOTES TUMOR GROWTH AND SERVES AS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER

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Medulloblastoma (MB) is the most malignant childhood brain tumor. Current therapy includes surgery followed by whole neuroaxis irradiation, and chemotherapy. Such treatment causes long-term morbidity including endocrine and growth disturbances, as well as neurocognitive dysfunction, which is particularly severe in young children. Therefore, there is an immediate need for the development of biomarker(s), which can be identified through a simple noninvasive tool to promote early diagnosis, subgrouping accurately, and monitor treatment response. OTX2, which is normally silenced in the adult brain, but is overexpressed by genomic amplification or other mechanisms in the majority of MBs (60-70%).

In this study, we hypothesized that the OTX2 mRNA can serve as an early diagnostic and/or prognostic tumor-biomarker, which can be detected in EVs of patient in a non-invasive way. To test this hypothesis, we first collected EVs from MB patients and performed qRT-PCR reactions for the OTX2 mRNA from RNAs isolated from EVs.

In our discovery cohort, we determined that OTX2 mRNA was significantly upregulated in patient serums compared to healthy control subjects. The OTX2 mRNA levels were significantly dropped down to control levels and become undetectable, in some samples, 10-14 days after operation. We performed two independent validation studies and found that OTX2 mRNA can also serve as a prognostic marker to monitor the treatment regimes.

Taken together, this study provides the first genetic tumor biomarker, the OTX2, in serum EVs for MBs and can be used as a genetic screen tool in young children, which then might provide better treatment options.

NETWORK MODELING IDENTIFIES PERSONALIZED THERAPEUTIC STRATEGIES IN GLIOBLASTOMA

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ENDOGENOUS-INSPIRED HYDROPHOBIC DRUG DELIVERY TO CANCERS: LDL-LIKE NANO PARTICLES DESIGNED TO *"PUT THE DRUG IN THE CANCER'S FOOD"*

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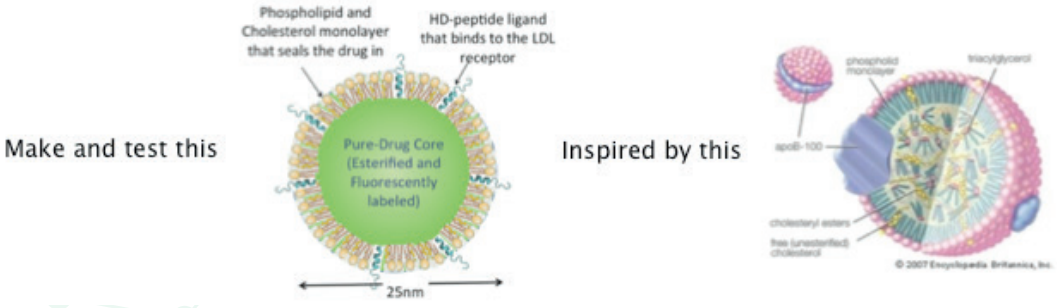
This presentation will discuss our new approaches to nanoparticle therapeutic drug and imaging agent delivery, as especially applied to hydrophobic drugs for metastatic cancer. Reverse-engineering the LDL as inspiration for nano-particle anti-cancer drug delivery we are inspired to create a new pure-drug, ligand-targeted, PET-imageable, nanoparticle, especially for metastatic disease. Motivation for this approach includes the fact that rapidly growing cancer cells have high numbers of LDLRs; numerous malignancies over-express LDLR; and in patients with cancer, their Low Density Lipoprotein (LDL) count is even known to go down. Furthermore, an abundance of LDLR is a prognostic indicator of metastatic potential, and a propensity to store cholesteryl ester is a sign of the aggressiveness of a patient's cancer. Our choice of drugs focuses on pathway-specific growth and metabolic targets in cancer that are themselves quite hydrophobic, such as: Niclosamide (Wnt pathway). Thus, *"Can we put the drug and the imaging agent in the cancer's food?" "Can endogenous uptake mechanism be used to make cancer cells take up a drug or imageable nanoparticle as though it was an LDL of essential materials?"* But instead of being nutrients that feed the cell, Pure-Drug Nanoparticle (PDN) would retard the cells growth, kill it out right, or cause it to kill itself.

All this is building towards an approach to personalized medicine from the Diagnostic and Therapeutic side (Diapeutics), utilizing PET-imagable metabolic indicators, EPR evaluation, and pure-drug nanoparticle delivery.



Acknowledgements

The new Center for Single Particle Science and Engineering (SPSE) established under the auspices of the Danish National Research Foundation's Niels Bohr Professorship award to Needham and SDU.

POLYMERIC NANOPARTICLES FOR TARGETED DRUG DELIVERY

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In our department thermosensitive and biodegradable polymeric micelles based on poly(ethylene glycol)-*b*-poly[*N*-(2-hydroxypropyl) methacrylamide-lactate] (mPEG-*b* pHPMAmLac) have been developed for passive and active drug targeting. Their small size (60-80 nm) and hydrophilic corona result in enhanced blood circulation and tumor accumulation exploiting the enhanced permeation and retention (EPR) effect. Doxorubicin was covalently linked to the core of these micelles through a hydrolytically sensitive hydrazone spacer and an increased *in vitro* and *in vivo* efficacy was observed. In a follow up study, we conjugated an antiepidermal growth factor receptor (anti-EGFR) nanobody as a targeting ligand to the micellar surface. It was demonstrated that the coupling of the nanobody on the surface of the micelles resulted in increased *in vitro* cytostatic activity (14C cells), and also significantly enhanced the antitumor activity and survival of 14C tumor-bearing mice *in vivo*. In another study, polymerizable and hydrolytically cleavable dexamethasone (DEX) derivatives were covalently entrapped in core-crosslinked polymeric micelles in order to achieve highly effective glucocorticoid targeting for rheumatoid arthritis (RA) therapy. By varying the oxidation degree of the thioether in the drug linker, the hydrolysis rate – and therefore the release kinetics of DEX – could be tightly controlled, with half-lives ranging from 10 to 170 days. Upon a single i.v. injection of the most rapidly releasing DEXmicelles, highly efficient disease treatment was achieved in two different animal models of inflammatory arthritis, with clinical signs of arthritis returning to levels observed for healthy controls. In a recent approach we developed polymeric micelles with aromatic benzyl groups in the core which had a high loading for the anticancer drug paclitaxel. Moreover these drug loaded micelles had excellent stability in the circulation and showed very good antitumor activity in different mice models.

References

[1]Talelli M, Rijcken CJF, van Nostrum CF, Storm G, and Hennink WE. Micelles based on HPMA copolymers. *Advanced Drug Delivery Reviews* 62, 231-239, 2010.

[2] Talelli M, Rijcken CJF, Oliveira S, van der Meel R, van Bergen en Henegouwen PMP, Lammers T, van Nostrum CF, Storm G, and Hennink WE. Nanobody-shell functionalized thermosensitive core-crosslinked polymeric micelles for active drug targeting. *Journal of Controlled Release* 151, 183-192, 2011.

[3] Crielaard BJ, Rijcken CJF, Quan L, van der Wal S, Altintas I, van der Pot M, Kruijtzer JAW, Liskamp RMJ, Schiffelers RM, van Nostrum CF, Hennink WE, Wang D, Lammers T, and Storm G. Glucocorticoid-loaded core-crosslinked polymeric micelles with tailorable release kinetics for targeted therapy of rheumatoid arthritis. *Angewandte Chemie* 51, 7254-7258, 2012.

[4] Talelli M, Oliveira S, Rijcken CJF, Pieters EHE, Etrych T, Ulbrich K, van Nostrum CF, Storm G, Hennink WE, and Lammers T. Intrinsically active nanobody-modified polymeric micelles for tumor-targeted combination therapy. *Biomaterials* 34, 1255-1260, 2013.

[5] Shi Y, van Steenbergen MJ, Teunissen EA, Novo L, Gradmann S, Baldus M, van Nostrum CF, and Hennink WE. Stacking increases the stability and loading capacity of thermosensitive polymeric micelles for chemotherapeutic drugs. *Biomacromolecules* 14, 1732-1740. 2013.

ADVANCES IN GENE DELIVERY

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With recent successes in gene therapy clinical trials for hemophilia and retinal diseases, gene therapy is once again gaining attention. For successful gene therapy, it is essential to deliver gene delivery vectors that can meet clinical requirements. Therefore it is needed to make improvement in vectors and generally best gene delivering vector has not yet been developed. Current methods for gene transfer include 2 groups; viral, non-viral [physical methods including]. As viral vectors, retrovirus, adenovirus, adeno-associated virus and herpes simplex virus are used. Systemic administration of these viral vectors may cause serious side effects such as immunogenicity and mutagenicity and had even caused the death of patients. To improve viral vector safety and efficacy, new strategies are required. Non-viral vectors are based on cationic lipids and cationic polymers, which can complex with negatively charged nucleic acids. These are preferred because of safety problems with viral vectors. However they must overcome different obstacles to improve the delivery efficiency. These obstacles are interactions with blood components, enzyme effect, uptaken by the reticulo-endotheliel systems, internalization of cells and cellular trafficking after uptaken. Developments include; improved targeting, enhanced intracellular uptake and reduced toxicity of vectors. For specificity, targeted transcription (specific promoter/enhancer element) was used.

The other concepts for improving vector are properties development of hybrid vectors, chemically modified viral vectors (chemo-viruses), synthetic viruses (virus like systems), modifying the surface particles with polyethyleneglycol, chemically coupling approaches and inorganic nanoparticles.

TARGETING A DISEASE TOLERANCE DEFENSE PATHWAY FOR NOVEL THERAPEUTIC OPPORTUNITIES

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Disease tolerance is defined as the ability of host organisms to fight against pathogens and parasites, limiting the impact of infection on host health, performance and fitness [1]. We have recently reported that tryptophan catabolism and the activation of the aryl hydrocarbon receptor (AhR) play a fundamental role in bacterial lipopolysaccharide (LPS)-induced endotoxin tolerance, contributing to host fitness [2].

In this communication we report the findings of a study aimed at investigating the binding mode of tryptophan metabolites to AhR and its functional correlation with the regulation of transcriptional events of specific target genes [3]. The obtained results open new avenues for the design and development of AhR modulators that, by targeting specific conformations of the receptor associated- gene modulation, may offer novel therapeutic opportunities in infectious diseases.

References

[1] E.L. Simms, Defining tolerance as a norm of reaction. *Evolutionary Ecology*, 563-570, 2000.
[2] A. Bessede, M. Gargaro, M.T. Pallotta, D. Matino, G. Servillo, C. Brunacci, S. Bicciato, E. Mazza, A. Macchiarulo, C. Vacca, R. Iannitti, L. Tissi, C. Volpi, M.L. Belladonna, C. Orabona, R. Bianchi, T, Lanz, M. Platten, M. Della Fazia, D. Piobbico, T. Zelante, H. Funakoshi, T. Nakamura, D. Gilot, M.S. Denison, G.J. Guillemin, J.B. DuHadaway, G.C. Prendergast, R. Metz, M. Geffard, L. Boon, M. Pirro, A. Iorio, B. Veyret, L. Romani, U. Grohmann, F. Fallarino, P. Puccetti. Aryl hydrocarbon receptor control of a disease tolerance defense pathway. *Nature,* 184-190, 2014.
[3] R. Nuti, M. Gargaro, D. Matino, D. Dolciami, U. Grohmann, P. Puccetti, F. Fallarino, A. Macchiarulo, Ligand binding and functional selectivity of L-tryptophan metabolites at the mouse aryl hydrocarbon receptor (mAhR). *J Chem Inf Model.*, 3373-3383, 2014.

EXPLOITING THE SULFUR ASSIMILATION PATHWAY IN SEARCH FOR NOVEL ANTIBACTERIALS. DESIGN AND SYNTHESIS OF CYCLOPROPANE-BASED INHIBITORS OF O-ACETYLSERINE SULFIDRILASE (OASS)

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Cysteine is synthesized in bacteria through two interconnected pathways which converge on O-acetylserine sulfidrilase (OASS), a PLP-dependent enzyme wihich replace acetyl with bisulfide to afford cysteine. Cysteine plays important roles in diverse cellular processes like bacterial swarming motility and oxidative-stress responses. Because of the central role played by cysteine in bacterial physiology, it has been proposed that enzymes involved in its biosynthesis and OASS in particular can be targeted in search of new antibacterials.

Here we report on the rational design, based on the combined use of extended molecular dynamics experiments and NMR data, the synthesis and the preliminary biological evaluation of a series of disubstituted carboxycylopropanes, some of which are endowed with a low nanomolar activity as inhibitors of the two OASS isoforms.

STRUCTURE- AND COMPUTER-BASED DESIGN OF EPIGENETIC INHIBITORS FOR ANTI-PARASITIC THERAPY

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Schistosomiasis, caused by S. mansoni, is a tropical disease that affects over 200 million people worldwide. A novel approach for targeting eukaryotic parasites is to tackle their dynamic epigenetic machinery that is necessary for the extensive phenotypic changes during their life cycle. We identified S. mansoni histone deacetylase 8 (smHDAC8) and Sirtuin2 as potential targets for antiparasitic therapy [1-3]. Here we present results from virtual screening, in vitro testing and crystallization of smHDAC8 inhibitors as well as first in vitro results of smSirt2 inhibitors. A variety of computer-based methods were applied in order to identify novel selective inhibitors. In case of smHDAC8, benzhydroxamates and several sulfonamide-thiazole derivatives were identified by a target-based virtual screening [2]. In vitro testing of 75 compounds identified eight benzhydroxamates as potent and lead-like inhibitors of the parasitic HDAC8. Solving of the crystal structure of smHDAC8 with two of the virtual screening hits confirmed the predicted binding mode. Subsequent chemical optimization resulted in highly potent smHDAC8 inhibitors that are able to kill the parasite in cell cultures. Besides killing S. mansoni, some compounds are also potent inhibitors of different Plasmodium falciparum strains.

References

[1] M. Marek, S. Kannan, et al. Structural Basis for the Inhibition of HDAC8, a Key Epigenetic Player in the Blood Fluke Schistosoma mansoni. *Plos Pathogen* 9(9):e1003645, 2013.
[2] S. Kannan, J. Melesina, et al. Discovery of Inhibitors of Schistosoma Mansoni HDAC8 by Combining Homology Modeling, Virtual Screening and In Vitro Validation. *J Chem Inf Model*, 54, 3005-19, 2014.
[3] M. Schiedel, M. Marek, et al. Fluorescence-based screening assays for the NAD^+-dependent histone deacetylase smSirt2 from Schistosoma mansoni. *J Biomol Screen*. 20, 112-21, 2015.

GIVEN THE NEW SITUATION IN PROFESSIONAL TERMS: CLINICAL PHARMACY

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Traditional pharmacy practice has been continuously progressing for thousands of years and consequently has also been evolving in professional sense. In this context, the concept of clinical pharmacy or pharmaceutical care have been one of the hot debates in the pharmacy world since the latter half of the last century. Traditional pharmacy practice has investigated the specification of the drug product for long centuries. The provision of pharmaceutical active ingredients in pharmaceutical production, packaging, preservation and delivery to patients, is presented as the first application as professional goals. Also in this period, although the patient-focused presentation of drugs has started in professional practice, the importance of this approach has been better understood in the last fifty-sixty years. Clinical Pharmacy can be defined as the rational use of drugs (RUD) and a patient-focused practice on the field of pharmacy. The Pharmaceutical Care is another patient-focused professional concept with the same meaning. Thus, the question on how these modern services differs from one another is important. Pharmaceutical Care is the philosophy of patient-centered practice of pharmacy profession. Clinical pharmacy is the application of this philosophy in professional field. This presentation will describe and discuss the recent developments in Turkey around this main focus.

CLINICAL BASED EDUCATION IN CLINICAL PHARMACY GRADUATE PROGRAMS

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The clinical based education is an essential process in the courses of clinical pharmacy and pharmaceutical care to improve pharmacists' practice on the most of theoretical topics during pharmacy education. The pharmacists could be more competent and self-confident during their professional life after the clinical based education including clinical rotations and interactive case studies. Clinical rotations could provide ideal opportunities for the pharmacists to observe and practice most of the patient oriented services such as patient counselling, drug information, therapeutic drug monitoring, and medication use review. The inter-professional education is aim to improve quality of patient care, lower costs, decrease patients' length of stay, and reduce medical errors [1]. As a result of spending a longer time with patients and medical team, the pharmacists could have better communication skills and also gain empathic feelings towards patients' health conditions and utilization of medications. To get lifelong learning skills, students were encouraged during the hospital activities to take responsibility for their education and practice [2]. In this presentation, it is aim to comprehensively review the Marmara University Clinical Pharmacy Graduate Programs, which has been continued since 1990s with competent academic staffs.

References

[1] Buring SM, Bhushan A, Broeseker A, Conway S, Duncan-Hewitt W, Hansen L, Westberg S. Interprofessional Education: Definitions, Student Competencies, and Guidelines for Implementation. *American Journal of Pharmaceutical Education*, 73(4) Suppl, 2009.

[2] Rita Shah, Improving Undergraduate Communication and Clinical Skills: Personal Reflections of a Real World Experience. *Pharmacy Education*, 4(1), 1-6, 2004.

EMERGING FACE OF CLINICAL PHARMACY, ONCOLOGY PHARMACY

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The value of the oncology pharmacist as a collaborative member of an interdiscipli-nary cancer care team to best support the complex drug therapy needs of the indi-vidual with cancer has been recognized and described in the literature [1]. Oncolo-gy pharmacists are viewed as the cancer drug therapy experts, based on their train-ing, expertise and function. The role of the oncology pharmacist has evolved to ad-dress many aspects of direct patient care and to support overall cancer care. The role continues to evolve in Turkey through the dedication and efforts of oncology pharmacists and Turkish Oncology Pharmacy Association (TOPA). The advance-ment of the role of the oncology pharmacist is key in emerging health systems. Clin-ical pharmacy, as a discipline, may not be known by all physicians but underlines the evolution of the profession of trained pharmacists from drug distribution and chemotherapy preparation to patient-centered services. Clinical pharmacy in on-cology is not very well described; a PubMed search using the terms “clinical phar-macy services and oncology” only retrieves 229 articles since 1976. In oncology, these services include comprehensive medication reviews integrating chemothera-py, supportive care and ambulatory treatment for co-morbidities, therapeutic drug monitoring (anticancer agents, anti-infective agents, immunosuppressive drugs in recipients of allogeneic stem cell transplantation), supportive care counseling (nutri-tional support, pain management, chemotherapy side-effects, prophylaxis and treatment), medication information for the medical staff and patients including pro-motion of adherence to ambulatory treatments, elaboration of therapeutic guide-lines, optimal use of economic resources [2]. Consequently, clinical pharmacists support the multidisciplinary management of patients with cancer.

References

[1] Holle LM, Michaud LB. Oncology pharmacists in health care delivery: vital members 3.Pauwels K, Si-moens S, Casteels M, Huys I. Insights into European drug shortages: a of the cancer care team. J Oncol Pract, 10(3), e142-145, 2014.
[2] Liekweg A, Westfeld M, Jaehde U: From oncology pharmacy to pharmaceutical care: New contribu tions to multidisciplinary cancer care. Support Care Cancer ,12, 73-79, 2004.

CLINICAL PHARMACY IN TURKISH REBUPLIC OF NORTHERN CYPRUS: EDUCATION AND SERVICES

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The curriculum of Near East University (NEU), Faculty of Pharmacy include two theoretical course such as Clinical Pharmacy I and II for 4th year students and Clinical Pharmacy Practice (CPP) course for the 5th year ones. CPP program consists of working in an institutional setting for 8 weeks. The primary objective of this practice experience is to introduce students to a hospital pharmaceutical care setting where they will develop basic technical skills, knowledge, application skills, professional judgment, communication skills, and competency necessary in the profession of pharmacy. The students work under close supervision of the clinical preceptors. These preceptors demonstrate to the students how to apply knowledge they learn in the classroom to the daily practice of pharmacy. Our department manage 5 clinical rounds in the hospital. 5th year students have to take 3 round as compulsory, one of them has to be hospital pharmacy, for the other two round, students can decide which round they want to complete according to their interests (internal medicine, cardiology, respiratory clinic or drug information center (DIC)). The content of the overall course address the NEU Faculty of Pharmacy competencies include patient centered professional approach, works ethically, decision making, team work, communication skills, full knowledge on medicines, consultation services to the patient and patient relatives, therapeutic outcomes monitoring, health promotion, general health advise.

NEU Hospital Clinical Pharmacy Team consists of highly trained drug experts, qualified for providing highly evaluated clinical pharmacy services in tertiary healthcare setting. Degrees of drug specialist include those with a Doctor of Pharmacy Degree, PhD, MD and MSc in clinical pharmacy and M.Pharm with clinical training and experience in specialized clinical areas. Clinical Pharmacy and Drug Information Center was established on the 2nd of June, 2015. This center has the title of being the first center in Turkey and Northern Cyprus for clinical pharmacy practice in the hospitals. The scope of this center is to provide optimal pharmaceutical care and services to all patients at NEU hospital, and to achieve positive patient outcomes. Clinical pharmacists work in close collaborative practice with physicians, residents and other healthcare providers so to achieve a multidisciplinary team healthcare system for NEU hospital patients.This Service is intended to be used for informational purposes that will support all healthcare professionals during their practice at Near East Hospital. Strategy is set to expand and provide drug information site, publishing drugs monographs, clinical guidelines, newsletters and regulatory agencies news and reports regarding new drugs approvals. Also plans are set to cover all health settings and population of Northern Cyprus during first five years of establishment, so to serve as a major national health promoting center in Northern Cyprus.

HERBAL MEDICINAL PRODUCTS – REQUIREMENTS ON MANUFACTURE AND QUALITY ASSURANCE

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As all medicinal products phytotherapeutics (Herbal Medicinal Products, HMPs) come within the same provisions of drug law. In principle same requirements have to be applied for HMPs as for chemical drugs with regard to pharmaceutical quality, safety and efficacy.

Herbal preparations in HMPs obtained from herbal substances consist of multicomponent mixtures of phytochemical constituents. Furthermore, herbal substances are natural in origin and consequently their chemical composition varies. With regard to this complex nature the requirements for the quality of HMPs are defined in specific guidelines, covering the quality of starting material, herbal preparations and finished product. These EMEA/HPMC guidelines on herbal quality are legally binding in the European Community and should be read in conjunction with current EU/ICH guidelines.

As HMPs resp. herbal preparations are essentially defined by the starting material (herbal substance) and the production process, specifications are (only) one part of the control strategy of HMPs to ensure product quality and consistency. Challenges for the implementation of the requirements in practise concern e.g. the search for appropriate markers, especially in combination products, which are intended for quantitative analytical purposes. These markers have to be specific and stable and should be contained in batch to batch consistent quantifiable amounts in the starting material, herbal preparation and finished product respectively. Issues regarding principle approaches will be considered and demonstrated exemplarily.

INTENTIONAL ADULTERATION AND COUNTERFEITING ISSUES IN PHYTOPHARMACEUTICALS: SAFE OR RISKY?

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Phytopharmaceuticals and related products such as nutraceuticals and dietary supplements are currently the popular segments of the pharmaceutical industry, which are in great demand worldwide. The term “phytopharmaceutical” is derived from combination of “phyto” and “pharmaceutical” that afford health benefits, including the prevention and treatment of diseases by herbal extracts. Due to the quick expansion in this area, a splendid number of phytopharmaceuticals offering health assistance is available in world markets. Although phytopharmaceuticals and dietary supplements are often regarded as low risk, a great concern about these products has been arising due to the fact that their health claims are sometimes mistrustful according to various market analyses reported from many countries. The regulations on phytopharmaceuticals or dietary supplements are different in Europe and USA and the most frequent negative issues with these products are intentional adulteration, contamination, counterfeiting, misreporting on the claimed content by the manufacturers, which may cause serious threats to human health. These risky cases are often encountered in sexual enhancers, slimming preparations, antidiabetics of herbal origin.

In this presentation, an updated overview on adulteration, counterfeiting, and contamination cases reported in phytopharmaceuticals and dietary supplements will be presented using reported market analyses.

PHARMACOINFORMATICS IN DRUG R&D PROCESS

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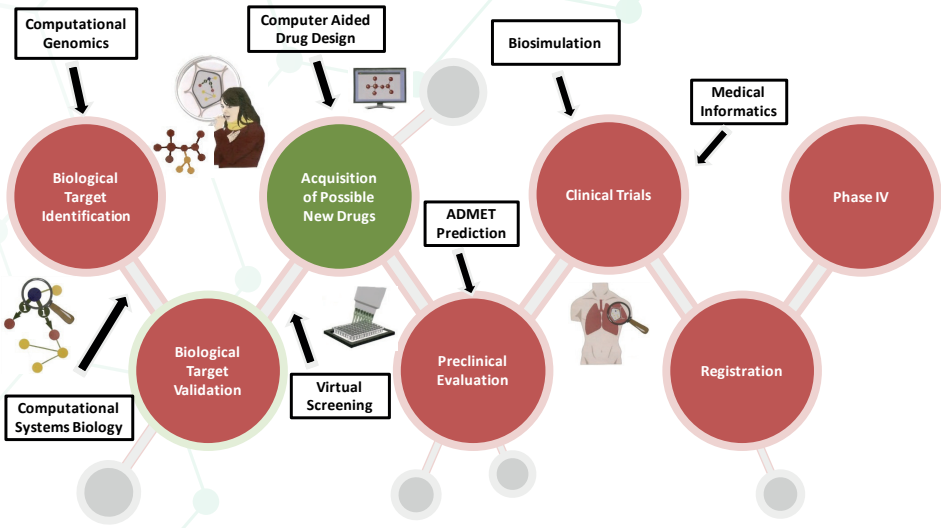
Drug discovery and development requires the integration of multiple scientific and technological disciplines in chemistry and biology; such as biochemistry, molecular and systems biology, pharmaceutical chemistry, analytical chemistry, pharmacology, pharmaceutical toxicology, pharmaceutical technology and extensive use of information technology. The latter is increasingly recognised as Pharmacoinformatics. In this wide area, we are building a modular and scalable computational cloud based web platform to try to help researchers during the drug discovery and development pipeline. The Evias Web Platform [1] is currently designed to efficiently perform Virtual Screening, aimed to identify commercially available lead-like and drug-like compounds to be acquired and tested. The same platform could also be adapted to be included in different stages of the R&D process, for example to help the analytical chemists in the identification of enantiomers and diasteroisomers by their chiroptical properties: by simulating *in silico* the electronic circular dichroism (ECD) [2] or by applying Molecular Dynamics protocols to reproduce a chiral column environment [3].



References

[1] http://www.evias.com.tr
[2] Journal of Chromatography A 10/2014; 1363; 162-168.
[3] Journal of Separation Science 10/2014; 37(19); 2696-2703.

LC/MS BASED SOLUTIONS FOR CHARACTERIZING THERAPEUTIC PROTEINS

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Advancement in the mass spectrometry technology provides new potential for structure analysis of protein drugs. We will present here a new Drift Ion Mobility Q-TOF System that reveals greater details in Bio-similarity studies

ACCREDITATION OF PHARMACY EDUCATION AND BENEFITS OF SMART PHARMACY

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Pharmacy practice and education are facing tremendous changes following new scientific discoveries, technological innovations and evolving patient needs, as well as the advanced competencies required of pharmacists for current and future practice. There is a need to assure the development of an adequate and appropriately trained pharmacy workforce, along with the structures and resources to deliver the required competency-based education and training. Beyond initial preparation, pharmacists must also ensure that their competence is maintained and enhanced throughout their careers.

Many countries are undertaking major transformation of pharmacy education and continuing education. Such developments must be accompanied by robust systems to assure the quality of the educational *context*, *structure*, *process*, *outcomes* and *impact* – the pillars of educational quality [1, 2]. The most visible outcomes of an educational program are the graduates who should be competent and capable of performing safely, effectively and professionally in their practice setting and contributing to the delivery of health care. Educational providers – both pre-service and continuing - must ensure that they are socially accountable and demonstrate how they contribute to addressing national needs and priorities and improved health care outcomes.

Quality assurance systems for education differ from country to country. Traditionally, this role has been performed by governments, but as the level of oversight and public accountability of health professionals has increased, the global trend is moving towards the establishment of independent or semi-independent accreditation agencies, using a peer-review process and experts in the profession. Accreditation systems and standards can be major drivers for a change in a profession, especially when needed change is challenging to implement. Just as accreditation standards can drive changes in education, practice standards and quality indicators can facilitate practice advances. New educational models for self-directed lifelong learning can better support and motivate pharmacists to develop competencies needed to introduce new services, leading to better patient care and other economic benefits.

The presentation will discuss key principles behind accreditation of pharmacy education and describe how such principles and quality indicators – as used in the SMART Pharmacy Project – can drive positive changes in education and practice.

References

[1] International Pharmaceutical Federation. Quality Assurance of Pharmacy Education: the FIP Global Framework. 2nd Ed. 2014.
[2] A. Meštrović, M. Rouse, Pillars and Foundations of Quality for Continuing Education in Pharmacy. American Journal of Pharmaceutical Education, 79(3), 45, 2015.

SMART PHARMACY EXPERIENCE: PROS AND CONS

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Pharmaceutical care is the responsible pharmacists practice, which provide safe and best available therapy for the patient. It is the professional activity in which the pharmacist, using his knowledge and experience, revealing patients' needs, set priorities in the treatment process, and takes responsibility for a positive outcome of drug therapy. That responsibility is shared with the doctor who determined the diagnosis and prescribed therapy, and with patients, encouraging them to the compliance, frequent check and counseling about responsible treatment. Today, many countries are trying to incorporate this new concept in its health care system, and although such attempts are of great interest to national and international pharmacy organizations, many challenges often appear in the implementation of this concept. Some of the difficulties may include: attitudes and opinions of other health professionals, lack of cooperation, and inadequate communication between them, an insufficient number of pharmacists, space or equipment for the provision of pharmaceutical care, including the structure and organization of health care.

Pharmaceutical care derives from the principles and postulates of clinical pharmacy, which pharmacists recognize as the scientific basis for intervention in the treatment of patients. The concept of clinical pharmacy clarifies the role of the pharmacist in the process of providing health care. It involves different ways of cooperation of health professionals in which science and practice can be linked to patient care. But to make this impact had the biggest impact possible, it is necessary to develop clinical knowledge, but also communication skills, judgment and decision-making.

Indicators of quality of pharmaceutical care are equally appropriate for in-patient and community settings, for hospital and community pharmacists, and other healthcare professionals, as applicable, in low-, middle-income and industrialized countries in Europe and other regions of the world. The indicators provide information about the range; quantity and quality of pharmaceutical care interventions/services delivered.

The presentation will discuss details of SMART Pharmacy Project connected with quality indicators and competency development.

ORAL PRESENTATIONS

EXPRESSIONS OF GGT1, GGT5 AND GGT6 GENES IN DIFFERENT TYPES OF CANCERS

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Measurement of gamma-glutamyl transferase (GGT) activity in serum has been found useful in showing of tissue damage. In addition to GGT1, different related genes or sequences in the human genome had been reported by pre-genome studies. Two of them, GGT5 and GGT6, are active genes and have similar nucleotide or amino acid sequence to GGT1. In the current study, we measured GGT1, GGT5, and GGT6 mRNA expressions in normal and tumour tissues of 26 patients with breast, gastric and colorectal cancers using RT-PCR method. Serum GGT levels was measured by a spectrophotometric method. While GGT1 and GGT5 expressed in almost all normal and tumour tissues of the patients, GGT6 observed in half of the both normal and tumour tissues. In total patients, we did not find any significant differences mean mRNA expression levels of GGT1, GGT5 and GGT6 ($p>0.05$), although GGT1 were higher in tumour tissues than those in normal tissues. GGT1 overexpressed in half of the patients (13/26) and overexpressions were positively correlated with gender ($p<0.05$) and age ($p<0.05$). In female patients, serum GGT levels was positively correlated with tumour tissue GGT1 ($p<0.01$) and GGT6 ($p<0.05$). In conclusion, this is the first clinical study examining all three genes simultaneously and showing GGT5 for the first time in both normal and tumour tissues of the breast, gastric and colon. Our results may suggest that tumour GGT1 mainly responsible for the increase in the serum GGT levels in female patients.

CYCLOHEXADIENONES AS MUTANT SPECIFIC INHIBITORS OF K-RAS G12C

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Despite the significant progress in the field of cancer biology and mechanism based drug discovery, the global burden of cancer has continued to rise and is expected to increase 70% by 2030 [1]. In part this has been due to the difficulty in targeting some of the key proteins known to drive tumorigenesis. The Ras subfamily (K-Ras, H-Ras, and N-Ras) are amongst such proteins that are the most powerful drivers of oncogenesis and their aberrant activation is associated with some of the least tractable carcinomas including lung (25%) and pancreatic cancer (90%) [2].

Ras (**Ra**t **S**arcoma) proteins are small GTPases that function as molecular switches that promote cell growth when bound to guanosine triphosphate (GTP) and halt it when the GTP is hydrolyzed to guanosine diphosphate (GDP) in the active site. However, somatic mutations of the Ras protein can make this molecular switch faulty by impairing GTP hydrolysis and thus rendering the protein constitutively active. This can result in aberrant cell proliferation that may lead to tumor development [3].

One of the most frequent point mutations found in K-Ras, occurring close to the binding site, is the substitution of glycine 12 for a cysteine residue and accounts for 10-20% of all Ras-driven cancers [4]. Several efforts have been made towards mutant specific targeting of K-Ras G12C by taking advantage of the reactive cysteine group. However, to date such approaches have suffered from either a lack of target selectivity [4] or insufficient potency [5].

Our research is based on direct targeting of K-Ras G12C by using cyclohexa-2,5-dienones as the reactive war head to trap the mutant cysteine residue. Accordingly, we have prepared several novel covalent-binding inhibitors that incorporate this *bis*-Michael acceptor functionality and tested their activity in a cell viability assay, using SW48 colon cancer cell lines expressing wild-type or mutant K-Ras G12C. From this study, several compounds were found to induce a strong cytotoxic effect at the lowest test concentration of 1 nM in both cell lines. Current work is directed towards further characterization of the cytotoxic activity of the lead compounds and the design of new derivatives using molecular docking approaches.

References

[1] A. Motin, M. Ferrari, Redefining global health priorities: Improving cancer care in developing settings, *J Glob Health*, 1-5, 2014.
[2] H. Ledford, the Ras Renaissance, *Nature*, 278-280, 2015.
[3] D. Cox, J. Der, Drugging the undruggable Ras: Mission impossible? *Nature reviews*, 828-851, 2014.
[4] M. Lim, S. Gray, Therapeutic targeting of oncogenic K-Ras by a covalent catalytic site inhibitor, *Angew. Chem. Int. Ed.*, 199-204, 2014.
[5] M. Ostrem, M. Shokat, K-Ras (G12C) inhibitors allosterically control GTP affinity and effector interactions, *Nature*, 548-551, 2013.

E-CADHERIN AND TGF-β LEVELS IN PATIENTS WITH ENDOMETRIAL AND OVARIAN CANCERS

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Endometrial and ovarian cancers are located in the first place as most common gynecologic malignancy in women. There is doubtless that cancer cell dispersion and metastases are strongly related with the loss of cell-cell adhesion. E-Cadherin is an epithelial adhesion molecule, the intact function of which is crucial for the establishment and maintenance of epithelial tissue polarity, structural integrity, cell proliferation and recognition [1]. TGF-β (Transforming growth factor beta) are widely expressed in all tissues and has a significant role in tumor growth and angiogenesis. Actually, TGF-β enters into a dual role in cancers, acting both as a tumor suppressor and as a promoter of tumor metastasis. Taken into consideration, we determine to examine the serum TGF-β and E-Cadherin levels. Sixty eight total patients (N=68) and forty one healthy controls (N=41) were involved in this study. Forty of them (N=40) were ovarian cancer and the rest of the patients were endometrial cancer (N=28). In the present study, serum TGF-β and E-Cadherin levels were measured by ELISA method. In total patient group, a significant difference was found in TGF-β and E-Cadherin levels compared to controls ($p<0.05$, $p<0.01$, respectively). E-Cadherin were decreased significantly in both endometrial and ovarian cancers ($p<0.01$). Conversely, TGF-β were increased significantly in ovarian cancers as compared to control group ($p<0.01$). In one of them, researchers shows that patients with gastric carcinoma had increased TGF-β compared to normal mucosa [2]. But, there are conflicting results about E-Cadherin in cancer. One study revealed that the serum E-Cadherin in breast cancer patients were significantly higher than controls [3]. Another study suggest that E-Cadherin was lower in patients with prostate cancer compared to controls [4]. As a conclusion, our study revealed that E-Cadherin expression importantly declined but TGF-β expression enhanced in total patients as compared to controls. Significant differences were found in endometrial and ovarian cancer patients compared to controls in terms of E-Cadherin levels. This finding suggest that abnormal TGF-β signaling induces to promote invasion and metastasis of tumor cells through reduced expression of E-Cadherin. So, TGF-β and E-Cadherin appear to be useful biomarkers of prognosis for patients with endometrial and ovarian cancer.

References

[1] E. Mărgineanu, CE. Cotrutz, C. Cotrutz, Correlation between E-cadherin abnormal expressions in different types of cancer and the process of metastasis, *Rev. Med. Chir. Soc. Med. Nat. Iasi,* 112(2):432-6, 2008.
[2] M. Naef, T. Ishiwata, H. Friess, MW. Buchler, LI. Gold, M. Korc, Differential localization of transforming growth factor-beta isoforms in human gastric mucosa and overexpression in gastric carcinoma, *Int. J. Cancer,* 71:131-137, 1997.
[3] Z. Liang, XY. Sun, LC. Xu, RZ. Fu, Abnormal expression of serum soluble E-cadherin is correlated with clinicopathological features and prognosis of breast cancer, *Med Sci Monit.*, 20:2776-82. doi: 10.12659/MSM.892049, 2014.
[4] C. Bonaldi, LA. Azzalis, VB. Junqueira, CG. de Oliveira, VA. Vilas Boas, TM. Gáscon, FS. Gehrke, RK. Kuniyoshi, BC. Alves, FL. Fonseca, Plasma levels of E-cadherin and MMP-13 in prostate cancer patients: correlation with PSA, testosterone and pathological parameters, *Tumori.*, doi: 10.5301/tj.5000237, 2015.

SURFACE ENHANCED RAMAN SCATTERING BASED DETECTION OF GROUP A BETA-HEMOLYTIC STREPTOCOCCUS

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The rapid detection of bacteria has been one of the most important and recent issues for diagnostic, environment and food industry analysis. Many serious and even fatal medical conditions result from bacterial infection or contamination. There are a lot of methods that are developed for the detection of bacteria, however, early diagnosis can not be made with this method. In this work, a sensitive, Surface enhanced Raman scattering (SERS) based method was developed to detect group A beta-hemolytic streptococcus bacteria using modified nanoparticles. Antibody modified Fe_3O_4@Au nanoparticles are employed for capture probe of bacteria and antibody modified gold nanorod particles are used for Raman labeling of *bacteria*. After interacting between the capture probe with solutions of *E. coli* having different initial cell concentrations (15-1x10^8 cfu/mL), SERS measurements are taken. Measured intense Raman peaks are used for quantitative detection of *E. coli* by using plotted calibration curve. Total detection time was less than 2 hours which shows big advantage of this method compared with plate counting classical methods. Our results demonstrate the potantial use of this method to samples obtained by using rod swabs from different cultures to SERS based detection of group A beta-hemolytic streptococcus. In addition, proposed assay was tested on paper-based immunuoassays. The particle modification and introduction of the sample are compatible with the paper-based system.

The authors acknowledge TUBITAK project no: 114Z783 for the financial support.

GENETICALLY MODIFIED ANIMALS, CURRENT PRODUCTS AND REGULATORY APPROACHES

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The history of genetically modified organisms (GMO) is all about conflict and debate; while media and public approach is fearsome. However, 18 million farmers in 28 countries planted more than 181 million hectares of genetically modified (GM) plants in 2014 [1]. Trade in food is sensitive to both cultural values and economic interests and has been severely affected at both national and international levels by the polarization of the GMO debate. USA product-approach to GMOs was strongly supported by the scientific and biotech industry communities and USA became the world's major exporter, while in the EU, opposing interest groups helped shape the tight process-approach regulatory framework. But this was only the first wave. The rapid development of modern biotechnology challenges governments and public; a new subject is here: GM Animals. A genetically modified animal is one whose genetic material has been altered by adding, changing or removing certain DNA sequences in a way that does not occur naturally. GM animals are being developed for research purposes and are confined to laboratories although some GM animals are also being produced for animal breeding, xenotransplantation, molecular pharming or even pet developing. The GM animal future is dependent on the response of the regulatory landscape and its associated range of interest groups at national and international levels. This work focus on the EU and the USA GM animal developments and all ready products while discussing the regulatory approach to them and also discuss the Turkey's current state on the subject.

References

[1] http://isaaa.org/resources/publications/briefs/49/executivesummary/default.asp

DETERMINATION OF THE WOUND HEALING EFFECT OF *Calendula officinalis* L. BY USING *IN VIVO* AND *IN VITRO* MODELS

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Calendula officinalis L. is an annual plant which belongs to the family Asteraceae. It is known as "pot marigold", and has been cultivated as a food and medicinal plant since middle ages. In folk medicine, it has been employed in the treatment of inflammation and skin wounds [1,2]. In the light of this information, wound healing activity of *n*-hexane and aqueous methanol extracts prepared from the flowers of *C. officinalis* was evaluated by using *in vivo* circular excision and linear incision wound models and *in vitro* elastase, hyaluronidase and collagenase enzyme inhibition assays. According to the results, aqueous methanol extract displayed the most potent *in vivo* wound healing activity with the contraction value of 87.82% and with the increase in the tensile strength value of 41.13%. As well as hyaluronidase, elastase and collagenase enzyme inhibitory activities with the values of 49.06%, 52.48%, 33.96%, respectively. On the other hand, *n*-hexane extract showed significant but lower *in vivo* and *in vitro* wound healing activity.Therefore, *n*-hexane and aqueous methanol extracts were subjected to HPLC analysis to determine the major compounds. According to the previous studies, it was reported that *C. officinalis* provided a fast healing by increasing collagen, hydroxyproline and hexosamine contents [3] as well as exerting antimicrobial and antioxidant properties [4]. The results of the present study was found to be in accord with the previous findings. In conclusion, it has been demonstrated that aqueous methanol extract of *C. officinalis* flowers possesses wound healing activity by inhibiting collagenase, elastase and hyaluronidase enzymes. However, we suggest that, a total extract prepared from *C. officinalis* should be used for the treatment of wounds, as *n*-hexane extract also displayed significant wound healing activity, indicating both polar and nonpolar compounds could be responsible from the wound healing potential.

References

[1] E. Efstratiou, A.I. Hussain, P.S. Nigam, J.E. Moore, M.A. Ayub, J.R. Rao, Antimicrobial activity of *Calendula officinalis* petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens, *Complementary Therapies in Clinical Practice*, 173-176. 18, 2012.
[2] L.R. Della, A. Tubaro, S. Sosa, H. Becker, S. Saar, D. Isaac, The role of triperpenoids in the topical antiinflamatory activity of *Calendula officinalis* flowers, *Planta Medica*, 516-520. 60, 1994.
[3] P.K. Chandran, R. Kutton, Effect of *Calendula officinalis* flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns, *Journal of Clinical Biochemistry and Nutrition*, 58-64. 43, 2008.
[4] M. J. Leach, *Calendula officinalis* and wound healing: A systematic review, *Wounds*, 1-7. 20(8), 2008.

INVESTIGATIONS ON TRANSPLANTABLE BIOARTIFICIAL PANCREAS DEVICE

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Islet cell transplantation could become an ideal treatment for those patients with severely damaged pancreas to prevent hypoglycemia shock and irreversible diabetic complications. This may overcome the major and unresolved obstacles such as limited donor supplies for organ transplantation and side effects caused by permanent immunosuppressant use. Approximately 30 years ago, some groups made successfull attemps in improving the blood glucose of diabetic animals by transplanting encapsulated islets with semi-permeable membranes consisting of polymer. A semi-permeable membrane protects both the inner islets from mechanical stress and the recipient's immune system (both cellular and humoral immunities), while allowing bidirectional diffusion of nutrients, oxygen, glucose, hormones and wastes, i.e., immune-isolation. This device, which enables immune-isolation, is called encapsulated islets or bio-artificial pancreas. In this study, islet cells were encapsulated in chitosan-alginate beads (CAB). Trials on rats have demonstrated the feasibility of chitosan-alginate encapsulated islet cells for the treatment of type 1 diabetes. Encapsulated islets can be protected from the host's immune system, remain viable and functional following transplantation. However, the long-term success of these therapies requires that microcapsules maintain their immunoprotective capacity and stability in vivo for sustained periods. Alginate composition (proportion of M- and G- blocks), alginate purity, the cross-linking ions (calcium or barium), and the presence or absence of additional polymer coating (e.g. chitosan) layers influences the success of cell encapsulation. Formulation parameters were investigated with respect to their effect on insulin secretion. Beads were generated using barium alginate and chitosan. These beads were superior to intact islets in terms of survival and function in low-oxygen culture and during transplantation and are likely to provide more efficient utilisation of islet tissue, a finding of importance for the future of cell therapy for insulin dependent diabetes.

References

[1] T. D. Hatziavramidis, M.T. Karatzas, G.P. Chrousos, *Annals of Biomedical Engineering,* 41, 469-476, 2013.
[2] Z.-l Zhi,. A. Kerby, A. J. F. King, P. M. Jones, J. C. Pickup. *Diabetologia,* 55, 1081-1090, 2012.

DIRECT ANALYSIS OF DRUGS IN BIOLOGICAL FLUIDS BY ON-LINE CHROMATOGRAPHY

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In pharmaceutical, bioanalytical and biomedical sciences analysis of drugs and metabolites in biological fluids is essential for bioequivalence/bioavailability, therapeutic drug monitoring and drug abuse studies. An optimal and effective sample preparation method plays the most important role since the depletion of the matrix in biological fluids is the biggest issue for a trouble-free analysis. It is impossible to inject the biofluid directly to the chromatographic system with traditional methods but this challenge was overcome by the on-line methods. Combination of solid phase extraction (SPE) with high performance liquid chromatography allows direct analysis of small molecules (i.e.drugs) in biofluids. The method, in summary, depends on connection of the SPE column, coated with various packing materials, directly to the analytical column (where the analytes are separated) through a switching valve and injection of the sample to the system. Following the injection, i) the matrix components are depleted and ii) the analytes are separated in the column. Thus, sample preparation step from the biological fluid is completely eliminated and the sample can be directly injected to the system resulting with high reproducibility in the analyses.

IN SITU NIOSOME FORMING PRONIOSOMAL TABLETS: IN VITRO AND IN VIVO EVALUATION

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Conversion of the vesicular systems such as liposomes and niosomes into commercial products is quite limited due to the critical physical stability problems. Formulation of vesicular systems as more stable provesicular systems by preserving their superior properties may substantially provide the introduction of these systems to patient usage[1,2].The aim of the present study is to formulate candesartan cilexetil (CC) as proniosomal tablet forms to improve its low oral absorption. Slurry method was used to prepare CC loaded proniosomes. Several critical parameters of the production process were changed to obtain an optimum proniosomal formulation. The proniosomes were characterized in terms of recovery, drug loading, drug release and the influence of the production parameters were discussed. The niosomes derived by the hydration of proniosomes were also evaluated for the changes on their particle size and zeta potential. Upon the hydratation of proniosomes, niosomes were formed with a mean particle size of 204 ± 2 nm and with a negative charge of -43.65 ± 0.54 mV. Drug was successfully loaded in the niosomal carriers with a percentage of 99.09 ± 0.04 %. The flowability, compressibility and consolidation properties were good both alone and after mixing with the tableting agents (microcrystalline cellulose and cross-linked poly vinylpyrrolidone). Prevention of the initial niosomes properties were confirmed after the hydration of proniosomal tablets. The in vitro drug release studies revealed the enhanced drug release via proniosomal formulation. The oral bioavailability of CC was evaluated on Wistar rats and a 1.86-fold ($p<0.01$) increase was observed on the relative bioavailability of CC via proniosomal tablets when compared to the pure drug.The plasma concentration of candesartan in rats was higher than that of pure CC. The estimated Tmax was 1.19±0.0.19 h for proniosomes tablet and this was significantly shorter than the Tmax (3.5±0.63 h) for pure drug ($p<0.01$). The increase in the rate and extent of absorption for the proniosomal tablet could be attributed to the increase in the rate and extent of drug dissolution in gastrointestinal tract. The more rapid absorption of drug from proniosomal tablets would be beneficial in the treatment of hypertension or heart failure, particularly in case of emergency. (This study has been supported by TUBITAK (The Scientific and Technological Research Council of Turkey) under grant 111S283.)

References

[1] Z.S. Bayindir, N. Yuksel, Provesicles as novel drug delivery systems, *Curr Pharm Biotechno*, 16:344-364, 2015.
[2] C. Marianecci, L. Di Marzio, F. Rinaldi, C. Celia, D. Paolino, F. Alhaique, S. Esposito, M. Carafa, Niosomes from 80s to present: the state of the art, *Adv Colloid Interfac*, 205:187-206, 2014.

ABILITY OF *Viburnum opulus* L. TO INHIBIT THE DEVELOPMENT OF ENDOMETRIAL IMPLANTS IN RATS: SURGICALLY-INDUCED ENDOMETRIOSIS MODEL

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Endometriosis is a common gynecologic disorder in which cells of endometrium grow outside the uterine cavity due to the improper functions of endometrial glands and stroma in women. Current therapeutic aprroaches mainly focus on the molecules that possess positive effects on one or more stages [1]. Traditional medicines could also be used for the treatment of endometriosis due to their low cost and high efficacy. Indeed, there are several plants commonly used for this purpose worldwide. In traditional medicine the fruits of *Viburnum opulus* L. (Caprifoliaceae) have been used to treat a wide range of maladies including dysmenorrhea and ovarian cysts [2]. The aim of this study is to explore the activity of a previously unexplored plant, *V. opulus,* in the treatment of endometriosis in order to find scientific evidence for the folkloric use of this plant by comparing the effect with buserelin acetate, a GnRH agonist. Experimental endometriosis was induced in six-week-old female, nonpregnant, Sprague Dawley rats. 15 mm piece of *endometrium* from uterine corn was *sutured* into abdominal wall. After twenty-eight days, a second laparotomy was performed. The endometrial foci areas were calculated and intra-abdominal adhesions were scored. The abdomen was closed. *n*-Hexane, ethyl acetate (EtOAc) and methanol (MeOH) extracts prepared from the fruits of *V. opulus* were administered *per os* once in a day throughout the experiment. At the end of the treatment, all rats were sacrified and endometriotic foci areas and intra-abdominal adhesions were reevaluated and compared with the previous findings. The tissues were histopathologically investigated. Moreover, peritoneal fluid was collected to detect tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) levels. Post-treatment volumes were found to be significantly decreased from 96.8 to 30.1 mm^3 in EtOAc group and from 102.3 to 34.7 mm^3 in MeOH group. No adhesion was detected in EtOAc group. The levels of TNF-α, VEGF and IL-6 reduced to 4.78; 15.85 and 31.69 pg/ml, respectively in EtOAc group; and decreased to 4.41; 16.54 and 33.02 pg/ml, respectively in MeOH group. *V. opulus* appears to have promising effects in the treatment of endometriosis.

References

[1] M.A. Demirel, I. Süntar, M. Ilhan, H. Keles, E. Kupeli Akkol, Experimental endometriosis remission in rats with *Achillea biebersteinii* Afan.: Histopathological evaluation and determination of cytokine levels, European *Journal of Obstetrics & Gynecology and Reproductive Biology*, 175, 172-177, 2014.
[2] C.E. Dennehy, The Use of Herbs and Dietary Supplements in Gynecology: An Evidence-Based Review, *J Midwifery Womens Health,* 51(6), 402-409, 2006.

POSTERS

PREPARATION AND CHARACTERIZATION OF DEXKETOPROFEN TROMETHAMOL LOADED DYNASAN® 114 SOLID LIPID NANOPARTICLES (SLNs)

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Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed medications in the World [1]. NSAIDs are used for the treatment of musculoskeletal disorders such as osteoarthritis and rheumatoid arthritis [2].

Dexketoprofen trometamol is the dextrorotatory enantiomer of ketoprofen formulated as tromethamine salt [3]. Dexketoprofen trometamol's distribution and elimination half-lives are 0.35 and 1.65 hr, respectively [4].

SLNs are colloidal carriers developed especially for the delivery of lipophilic compounds at the beginning of the 1990s as an alternative system to existing traditional carriers like emulsions, liposomes and polymeric nanoparticles [5].

Preparing and characterizing SLNs for oral controlled analgesic delivery was aimed in this study. Dynasan® 114 (Trimyristin/Glyceryl Trimyristate) was selected as the solid lipid. Dexketoprofen tromethamol loaded SLNs were prepared by hot homogenization method followed by probe ultrasonication and characterization was achieved by particle size/PDI and zeta potential measurements, DCS thermograms, SEM imaging FT-IR and NMR spectroscopic analyses.

This study was financed by Anadolu University Scientific Research Project No: 1502S081.

References

[1] C. Sostres, C. J. Gargallo, M. T. Arroyo, A. Lanas, Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract, *Best Pract Res Clin Gastroenterol*, 24; 121-132, 2010.
[2] T. Komatsu, T. Sakurada, Comparison of the efficacy and skin permeability of topical NSAID preparations used in Europe, *Eur.J.Pharm.Sci.*, 47, 890-895, 2012.
[3] D. Burke, J. Bannister, Dexketoprofen trometamol in post-operative pain management, *Acute Pain*, *5*, 57-62, 2003.
[4] https://www.medicines.org.uk/emc/medicine/17099
[5] F. Castell, C. Puglia, M. G. Sarpietro, L. Rizza, F. Bonina, Characterization of indomethacin-loaded lipid nanoparticles by differential scanning calorimetry, *Int.J.Pharm.*, 304, 231-238, 2005.

DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPY METHOD FOR DOMPERIDONE MALEATE IN PHARMACEUTICAL DOSAGE FORMS

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Domperidone maleate (DM) is a lipophilic drug and it is classified in the Biopharmaceutics Classification system as a class II drug [1]. It is an antiemetic drug with low oral bioavailability (about 15%) [2].The aim of this study is to develop and validate an analytical method of DM in pharmaceutical dosage form. The HPLC system consisted of a gradient pump, thermostable column department and a UV detector supplied by Agilent 1100. The column was a C18 column (5µm,150x4.6mm). The quantification was performed at 270 nm. The injection volume was 20µL and the retention time of DM was about 1.96 min. The mobile phase was mixture of acetonitrile: methanol (30:70) (v/v) pumped at 1 mL/min. The HPLC method was validated partially with respect to linearity, limit of detection (LOD) and quantitation (LOQ), precision and accuracy. In addition DM solubility in water, ethanol, various oils, surfactants, gastric and intestinal mediums were determined and also lipid water partition coefficient (Log P) was evaluated with validated HPLC method.

The linearity between peak area and concentration was analyzed using three calibration curves obtained in the different days with standard solutions of DM at ten different concentrations ranging from 1 to 17 ppm for DM. Ten samples prepared at the same concentration (11 ppm) to evaluate method precision, standard deviation and coefficient of variation. The prepared three standard solutions (8, 11, 14 ppm) were injected three times at different levels as a test sample. Data indicate that DM peak area is linear over concentration range of 1-17 ppm. The R^2 for regression line is 0.9997 with slope of 11.1 and y + intercept of -0.8447. LOD was found to be 0.0672 µg/mL and LOQ was found to be 0.2037 µg/mL. The maximum solubility of DM was measured in PEG 400, Tween 20 and Tween 80 was found to be 8.622±0.0089 mg/mL, 5.897 ±0.0087 mg/mL and 5.037±0.0055 mg/mL respectively. In addition, the log P value for DM was detected as 1.23± 0.031.

In this study, the high recovery and low relative standard deviation confirm the suitability of the method for determination of domperidone in pharmaceutical dosage forms. The method was validated for accuracy, precision, specificity, and linearity. In conclusion, an efficient HPLC method was developed and validated for DM in pharmaceutical dosage formulations.

References

[1] S.B. Choudhari, M. Nilesh, A.V. Bhalerao, Self microemulsifiying drug delivery system for treatment of emesis, *AJPTI*, 2, 1-10, 2014.
[2] P. Laddha, V. Suthar, S. Butani, Development and optimization of self emulsifiying drug delivery of domperidone, *Braz J Pharm Sci*, 50, 92-99, 2014.

BENZOCAINE LOADED MICROEMULSION-BASED HYDROGELS: PREPARATION, CHARACTERIZATION AND *IN VITRO* RELEASE

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Benzocaine (BZN) is a local anesthetic used primarily to relieve pain or irritations on the skin and mucosal surfaces [1]. While microemulsions offer various advantages for topical delivery, it is difficult to stabilize the system because of low viscosity [2]. In order to overcome this problem, microemulsion-based hydrogels (MBH) could develop. The purpose of this study was to prepare and evaluate the potential use of MBH for dermal delivery of BZN.

The microemulsions were prepared using isopropyl myristate as oil phase, Span 20, Tween 20, Tween 80, Cremophor EL and Cremophor RH40 as surfactants, ethanol as co-surfactant and distilled water as aqueous phase. MBHs were prepared using 1% or 2% of Carbopol 940 by swelling in the microemulsion under magnetic stirring for 24 h. To find out the suitability of formulations for dermal applications, the characteristic properties of formulations such as pH, viscosity, refractive index, electrical conductivity droplet size, polydispersity index (PDI) and zeta potential were evaluated. *In vitro* release and stability studies were also performed.

After BZN (2% (w/w)) was entirely dissolved in the microemulsion, the clear formulation was obtained with no phase change. The droplet size of microemulisons was found between 61.62±1.423 nm and 167.9±7.807 nm. PDI is below 0.3 could be used as an indication of uniformity of droplets. Zeta potential values of microemulsions were found neutral. The conductivity of microemulsions was found between 36.7±0.043 $mS/cm^{-1}$and 53.8±0.027 mS/cm^{-1}. The pH of the formulations was found in between 4.962±0.043 and 6.460±0.116. The drug content in BZN loaded MBHs was within the range between 95.91±0.26 and 99.21±0.19%. The MBHs had pH values varying from 6.72±0.013 to 7.75±0.032, suitable for topical application. Addition of 1% Carbopol was not significantly affect flux value ($P>0.05$) because of slightly increase viscosity of microemulsions. BZN loaded M3 and M3 based hydrogels ($M3BH1_{BZN}$ and $M3BH2_{BZN}$) were higher flux values than M1 and M1 based hydrogels. According to the results of characterization, stability and *in vitro* release studies, the most desirable formulation for topical delivery of BZN were considered the M3BH1 which contains Span 20 and Cremophor EL as surfactants.

References

[1] M. Köllmer, C. Popescu, et al. Stability of benzocaine formulated in commercial oral disintegrating tablet platforms, *AAPS PharmSciTech*, 14(4), 1333–1340, 2013.
[2] W. Zhu, C.Guo , et a. Microemulsion-based hydrogel formulation of penciclovir for topical delivery, *Int J Pharm,* 378, 152–158, 2009.

IN VITRO EVALUATION OF MUCOADHESIVE PROPERTIES OF CLINDAMYCIN PHOSPHATE LOADED CHITOSAN PERIODONTAL FILMS

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Clindamycin phosphate (CP) is an antibiotic against gram(-) anaerobic pathogens that cause periodontitis [1]. Oral absorption of clindamycin is high and it is an effective agent in the field of dentistry [2]. Chitosan is a cationic polysaccharide and presents electrostatic interactions with potential bioadhesive forces. Additionally, chitosan increases the absorption of hydrophilic drugs in the oral mucosa by neutralizing the anionic sites of mucosal cells [3].

This study was designed to understand the effects of some formulation parameters on mechanical properties of chitosan films. Clindamycin phosphate loaded (1%) chitosan films were prepared by solvent casting technique with low (LC), medium (MC) and high (HC) molecular weight chitosan at 1%, 2% or 3% polymer concentration in 1,5% v/v aqueous acetic acid. Thickness, viscosity (Brookfield Viscometer / RVDV-2, T Spindle No: 93), degree of swelling (DS%) measurements and *in-vitro* drug release studies were performed. Adhesiveness measurements were carried out for each film formulation using texture analyzer (TA.XT plus, Stable Micro Systems).

According to the results given in Table 1; thickness, DS% and adhesive properties of chitosan films changed by the increase in polymer concentration. This change was especially significant when the concentration increased from 1% to 2%. Polymer type was not found as effective as concentration on the physicochemical characteristics of films. However, viscosity was affected by both polymer concentration and type.

Table 1. Effect of different amount and different molecular weight of chitosan on the thickness, viscosity, degree of swelling and adhesiveness of films (± SD)

Code	Thickness (μm)	Viscosity (cP)	DS (%)	AUC (kg.sec)	Peak Force (kg)
HC-1	541.7 ± 20.4	941.70 ± 52.0	268.9 ± 48.5	0.035 ± 0.022	0.176 ± 0.121
MC-1	515.0 ± 24.3	416.70 ± 28.9	205.6 ± 98.6	0.016 ± 0.010	0.105 ± 0.064
MC-2	875.0 ± 37.8	5467.0 ± 57.7	525.5 ± 266	0.020 ± 0.009	0.072 ± 0.061
MC-3	778.3 ± 63.7	18400 ± 100	595.3 ± 472	0.021 ± 0.020	0.099 ± 0.099
LC-1	660.0 ± 44.3	21.500 ± 3.35	290.1 ± 177	0.018 ± 0.004	0.062 ± 0.016
LC-2	741.7 ± 65.2	383.30 ± 116	226.0 ± 122	0.009 ± 0.001	0.045 ± 0.020
LC-3	731.7 ± 27.1	683.30 ± 76.4	191.0 ± 127	0.003 ± 0.001	0.009 ± 0.009

References

[1] A. H. El-Kamel, L. Y. Ashri, I. A. Alsarra, Micromatricial metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases, *AAPS pharmscitech*, E184-E194, 2007.
[2] M. Kilicarslan, S. A. Ozkan, T. Baykara, LC Determination of Clindamycin Phosphate from Chitosan Microspheres, *Chromatographia*, 72, 799-805, 2010.
[3] I. S. M. Costa, R. P. Abranches, M. T. J. Garcia, M. B. R. Pierre, Chitosan-based mucoadhesive films containing 5-aminolevulinic acid for buccal cancer's treatment, *Journal of Photochemistry and Photobiology B: Biology*, 140, 266–275, 2014.

ERLOTINIB LIPOSOMES AND NANOCOCHLEATES

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Liposomes are small vesicles and having phospholipid bilayers. Nanocochleates are also made of phospholipids and they are formed cigarette-like helical structure. Phospholipids and cholesterols make them biocompatible with biological membranes. Nanocochleates and liposomes are therefore accepted to be biocompatible. Nowadays, liposomes or cochleates are frequently used in cancer treatments as smart drug-delivery systems for providing effective and targeted therapies. They can be targeted to the tumors using folic acids and some surface modifiers. Both liposome and nanocochleate have some advantages and disadvantages. Lipophilic and hydrophilic drugs can be loaded into liposomes. On the other hand, oxidation of phospholipids seems to be a problem for liposomes but it is rarely observed in nanocochleates due to lack of aqueous core [1]. In this study, liposomes and nanocochleates are prepared by modified Bangham method and optimum formulations are evaluated considering encapsulation efficiency (EE), zeta potential (ZP), particle size distribution (PSD), polydispersity index (PI). The preparation method for liposome as follows: dipalmitoyl phosphatidylcholine (DPPC), cholesterol, methoxy-poly(ethyleneglycol)2000-di stearoylphosphatidylethanolamine (PEG-PE) (1:1:1 molar ratio), dexketoprofen trometamol (DEX), Erlotinib HCl (ERLO) and Folic acid (FA) were dissolved in methanol: chloroform (1:1 v/v) and the solvent was removed at 44°C under vacuum using rotary evaporator. After a dry homogeneous film was formed, the film was re-hydrated with distilled water and particle size was reduced using extruder. The preparation method for nanocochleate as follows: 1,2-dioleoyl-sn-glycerol 3-phospho-L-serine (DOPS), cholesterol, PEG-PE (1:1:1 molar ratio), DEX, ERLO and FA were dissolved in chloroform. The mixture was dried at 42°C under reduced pressure using rotary evaporator and then the solid film was re-hydrated with distilled water. 2 ml calcium chloride solution (6 mM) was added dropwise to 1 ml of the suspension and then the mixture was kept in a refrigerator at +4°C for overnight period. Particle size was reduced by using extruder. Prepared formulations were centrifuged and drug substances in supernatant were analyzed by UPLC (Ultra Performance Liquid Chromatography) and EEs were determined. ZP, PSD and PI were also determined by Malvern Zetasizer Nanoseries. Formulation characteristics of optimum formulations are given in Table1.

Table-1: Formulation characteristics of prepared formulations.

Formulation	EE			ZP (mV)	PSD (nm)	PI
	DEX	**ERLO**	**FA**			
Liposome	70,7%	78,3%	99,4%	-21,4±1,8	166,8±10,6	0,292±0,049
Nanocochleate	52,9%	86,2%	99,4%	-21,1±0,9	210,6±2,0	0,285±0,074

References

[1] T.T.H. Pham, C. Gueutin, M. Cheron, S. Abreu, P. Chaminade, P.M. Loiseau, G. Barratt, Development of antileishmanial lipid nanocomplexes, *Biochimie*, 1-11, 2014.

TAMOXIFEN LOADED LIPOSOMES/NANOPARTICLES

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Tamoxifen, is the first representative of the SERM (Selective Estrogen Receptor Modulators) group of drugs which has been approved by FDA on prevention and the treatment of estrogen positive breast tumors[1][2].

In this study, chitosan containing liposomes and chitosan nanoparticles of tamoxifen were developed having dimethyl-β-cyclodextrine or sodium taurocholate as absorption enhancers. The absorption properties of these liposomes and nanoparticles through Caco-2 cell monolayers were investigated. MTT tests were performed to confirm usable concentrations of tamoxifen, dimethyl-β-cyclodextrine and sodium taurocholate for Caco-2 cells. The concentrations of tamoxifen, dimethyl-β-cyclodextrine and sodium taurocholate were selected according to cytotoxicity tests results (120 µg/ml, 0.15% and 0.005 mM, respectively). Blank liposome and nanoparticle formulations were found to be non-cytotoxic, the viability values were above 85% for all tested concentrations. Caco-2 cells were grown up for 21 days (60000 cells/ml) on Transwell® polycarbonate membranes and placed between apical and basolateral compartments of vertical diffusion chambers. Transport experiments were performed at 37ºC, from apical to basolateral compartment. Samples were withdrawn at the particular time periods from the basolateral side, amounts of tamoxifen were analyzed and permeability coefficients were calculated. In vivo studies were also evaluated by oral administration of liposomes/nanoparticles to Balb-C type female mice. Blood samples were collected at specific time intervals and tamoxifen analyzed from plasma by HPLC.

According to the results, developed liposome formulations with absorption enhancers has appeared to be promising to achieve enough oral absorption for tamoxifen. High in vitro/in vivo correlation results have confirmed that Caco-2 cell line is a good model for predicting oral absorption of tamoxifen loaded lipomes and nanoparticles.

References

[1] R. M. O'Regan, V. C. Jordan, The evolution of tamoxifen theraphy in breast cancer: selective oestrogen- receptor modulators and downregulators, The Lancet Oncology, 3, 207-14, 2002.
[2] M. Clemons, S. Danson, A. Howell, Tamoxifen ('Nolvadex'):a review, Cancer Treatment Reviews, 28, 165-180, 2002.

PRODUCTION OF FUNGAL CHITOSAN

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Chitosan, copolymer of glucosamine and N-acetyl glucosamine is mainly derived from chitin. Currently, industrial production for chitin and chitosan is from the shell wastes. The cell wall of fungi is an alternative source for chitosan production. Chitosan being polycationic, nontoxic, biodegradable as well as antimicrobial finds numerous applications especially in the agriculture, food, and pharmaceutical industries [1]. Chitosan has been isolated from *Ganoderma lucidum.* To characterize the extracted material, its FTIR spectrum is compared with that of shrimp chitosan (Alfa Easer- standard). Final degree of deacetylation which determined by FTIR spectrum as 43% for chitosan *G. lucidum*. Therefore *G. lucidum* was found to be the producer of the moderate amounts of chitosan. This study was supported ERU BAP TSA-11-3580

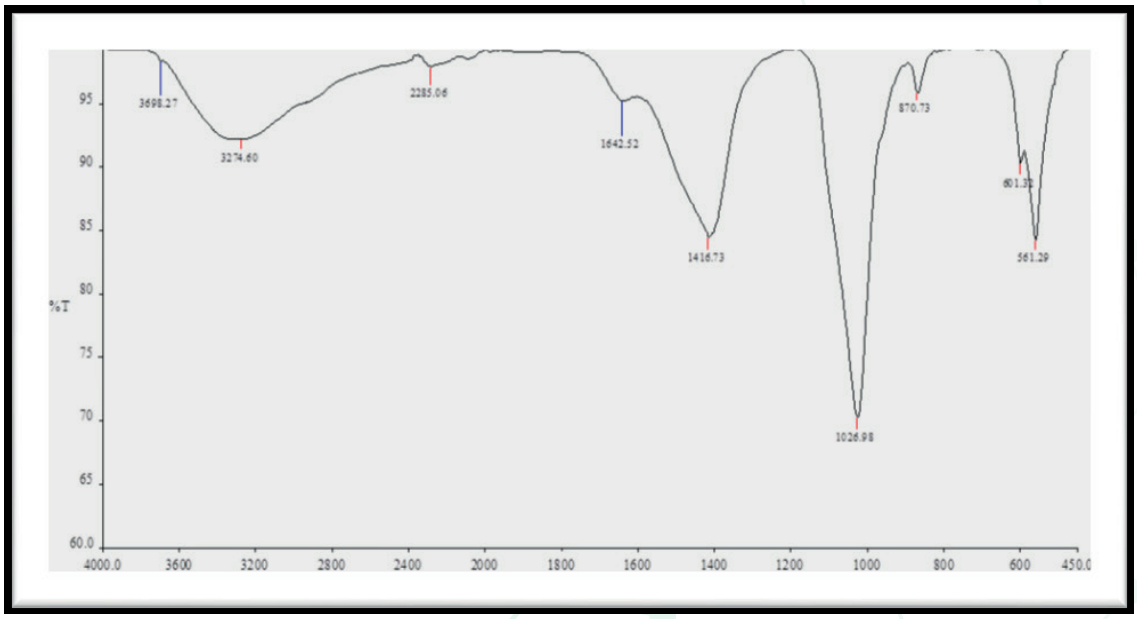

Figure. FTIR Spectrum of Chitosan from *G. lucidum*

References

[1] S. Chatterjee, M. Adhya, AK. Guha, BP. Chatterjee, Chitosan from *Mucor rouxii*: production and physico-chemical characterization, Process Biochemistry, 395-400, 2005.

THE EFFECT OF PREPARATION METHOD ON SIZE OF POLY (2-ETHYL-2-OXAZOLINE) (PEtOx)-b- POLYCAPROLACTONE (PCL) POLYMERIC MICELLES

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Poly (2-ethyl-2-oxazoline) (PEtOx) is a novel hydrophilic polymer [1]. PEtOx has similar biocompatibility and stealth function as PEG and it has also more advantage like chemical versatility, stability and pH sensitivity [2]. Micellar systems are an aggregate composed of amphiphilic block copolymer which formed a core-shell structure and good candidate for drug and gene delivery applications. Also the preparation technique is quite simple by using chemical and physical methods. Among these, dialysis and thin-film hydration methods are often used due to their simplicity and ability to yield low size and uniform particles [3]. In this study, we prepared blank PEtOx-b-PCL polymeric micelles by three methods as dialysis, self-assembly solvent evaporation and thin-film hydration method. We aimed to determine the effect of preparation method on micelle size, zeta potential and polydispersity index (PDI) to obtain optimum micelle formulation. We used $PEtOx_{4500}$-b-PCL_{2250} copolymer with low molecular weight to investigate the optimum micelle formulations. The fresh resulting micelles were characterized for size, zeta potential and PDI. Results showed that optimum micelle formulation was obtained by dialysis method as 53.8±1.4 nm, -2.38 ± 0.28 mV and 0.39 ± 0.04 for size, zeta and PDI, respectively. Also, the results of thin film hydration method were obtained similar to dialysis method (71.2±0.2 nm, -1.27±0.85 mV and 0.19±0.01 for size, zeta and PDI, respectively). According to solvent evaporation method, results significiantly higher than other methods, size 246.1±7.1 nm, zeta -11.6±0.42 mV, PDI, 0.38±0.05. As it was seen from the results, preparation methods considerably affect the micelle formulation. Thus, dialysis method was determined as an ideal method to prepare optimum PEtOx-b-PCL micelle formulation. These PEtOx-b-PCL micelles which have optimum size, are promising vehicles for gene and drug delivery and further research should be performed on these micellar systems. (This study was supported by TUBITAK research grant 213M728).

References

[1] Victor R. de la Rosa,. “Poly(2-oxazoline)s as materials for biomedical applications”, *J Mater Sci: Mater Med.,* (2014) 25:1211-1225

[2] L. Yan and L. Qiu, “Indocyanine green targeted micelles with improved stability for near-infrared image- guided photothermal tumor therapy,” *nanomedicine*, vol. 10, pp. 361–373, 2015.

[3] X. Ai, L. Zhong, H. Niu, and Z. He, “Thin-film hydration preparation method and stability test of DOX-loaded disulfide-linked polyethylene glycol 5000-lysine-di-tocopherol succinate nanomicelles,” *Asian J. Pharm. Sci.*, vol. 9, no. 5, pp. 244–250, 2014.

POLYMERIC MICELLES BASED ON POLY (2-ETHYL-2-OXAZOLINE) AS DRUG CARRIERS

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Biocompatibility and stealth behavior are the reasons of recent popularity of poly(2-oxazoline) s in biomedical applications. Due to advantages such as the easy variation of the monomer composition and the introduction of side-chain functionalities, poly(2-oxazoline)s could be an alternative to polyethylene glycol (PEG) which is the gold standard for stealth behavior. Polymeric micellar systems formed from block copolymers have been studied as drug delivery systems especially for drug targeting of anticancer drugs. Block copolymers consisting poly(2-ethyl-2-oxazoline) (PEtOx) as hydrophilic segment and poly (ε-caprolactone) (PCL) as hydrophobic segment are promising micellar carriers for drug and gene delivery. In this study, effect of the molecular weight of hydrophilic block on micelle size, zeta potential and polydispersity index (PDI) was investigated using solvent evaporation technique. PEtOx-b-PCL copolymers with two different molecular weights ($PEtOx_{7400}$-b-$PCL_{1500,}$ $PEtOx_{6400}$-b-PCL_{1500}) were used for investigation of the properties of micelles.

Micelles were prepared by dissolving diblock copolymer in tetrahydrofuran (THF) and then adding to distilled water via dropping. THF was evaporated by using rotary evaporator. The resulting micelles were characterized for size, zeta potential and PDI. Effect of freeze-drying on micelle size, zeta potential and PDI was also investigated. Results showed that $PEtOx_{7400}$-b-PCL_{1500} copolymer formed micelles with the average diameter 106.3±1.2 nm and zeta potential -14.3± 0.6 mV, PDI 0.5±0.0 and freeze-drying did not affect the average size. Micelles based on $PEtOx_{6400}$-b-PCL_{1500} copolymer had average diameter 89.6±2.1 nm, zeta potential -9,0±0,5 mV, and PDI 0.4±0.0. However, the size of freeze-dried micelles was increased by two-fold compared to freshly prepared ones. Our results indicated that PEtOx-b-PCL micelles have appropriate size and zeta-potential values for delivering of drug and genes. It was also found that molecular weight of hydrophilic block caused only a slight difference on these values. (This study was supported by TUBITAK research grant 213M728).

References

[1] R. Hoogenboom,Poly(2-oxazoline)s: A polymer class with numerous potential applications, *Angew. Chemie - Int. Ed.*, 48, 7978-7994, 2009.

IN VITRO CHARACTERIZATION AND EVALUATION OF PLA NANOPARTICLES LOADED WITH TELMISARTAN

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Telmisartan is an angiotensin II receptor type 1 blocker, widely used for the treatment of hypertension. It has also been characterized as a peroxisome proliferator–activated receptor-γ (PPAR- γ) agonist [1]. PPAR-γ agonists induce cell-cycle arrest and apoptosis in breast cancer cells [2]. In this study telmisartan was encapsulated to poly (d,l-lactide) (PLA) nanoparticles and cytotoxic effects of the nanoparticles were investigated on breast cancer (MCF-7) and mouse fibroblast (L-929) cell lines. Blank and drug loaded nanoparticles were prepared by nanoprecipitation method. PLA and telmisartan was dissolved in 5 mL dimethylsulfoxide (DMSO) and added dropwise to the 10 mL of ultra pure water containing 0.5% Pluronic-F-68 (w/v) under magnetic stirring. The nanoparticle suspension was allowed to stir for 20 min and then concentrated to 10 mL under vacuum using rotavapor. Then nanoparticle suspension was centrifuged at 14,000 rpm for 20 min and washed twice with ultra pure water to remove DMSO. The washed nanoparticles were characterized for particle size, zeta potential, drug encapsulation and in vitro release profile. For examining antitumor effects of formulations, telmisartan solution, blank and drug loaded nanoparticles were applied to cells and their effects on cell viability were evaluated by MTT assay after 72 hours incubation. Average particle size and zeta potential values were 205.9±57.3 and 22.6±1.0 respectively. Drug encapsulation was 89.4 % and 6.8 % of drug was released after 24 hours in phosphate buffered saline at 37°C. Cell viability of MCF-7 cells was 39.0, 37.4, 49.7 % for telmisartan loaded nanoparticles, blank nanoparticles and telmisartan solution respectively. Telmisartan loaded nanoparticles appeared slightly more cytotoxic than telmisartan solution. Drug release from nanoparticles may not enough to produce strong cytotoxic effect at determined timing period. There was no significant cytotoxicity on L-929 cell line for nanoparticles and drug solution. From these results, we may conclude that a controlled release of telmisartan can be obtained by drug loaded PLA nanoparticles and they can be used as therapeutic agent for the treatment of breast cancer. Further studies will be conducted in order to investigate this approach.

References

[1] S. C. Benson et al., Identification of Telmisartan as a Unique Angiotensin II Receptor Antagonist With Selective PPAR -Modulating Activity, *Hypertension*, vol. 43, no. 5, pp. 993–1002, 2004.
[2] M. H. Fenner, E. Elstner, Peroxisome proliferator-activated receptor-γ ligands for the treatment of breast cancer, *Expert Opin. Investig. Drugs*, vol. 14, no. 6, pp. 557–568, 2005.

APPLICATION OF QUALITY BY DESIGN (QbD) TOOLS AND BIOPHARMACEUTICAL CLASSIFICATION SYSTEM (BCS) IN DEVELOPING pH-INDEPENDENT CONTROLLED RELEASE MATRIX TABLET

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The major objective of the present study was to develop a robust, clinical safe and efficient pH-independent controlled release matrix tablet for BCS Class III, weak acidic drug Valsartan, using Quality by Design (QbD) approach. For this purpose Risk Assessment, Design Space and Absorption Simulations within BCS concept have been performed [1-3]. Matrix tablets were prepared by using valsartan (Sanovel Pharmaceuticals), sodium citrate (Yılmaz Chem.,Turkey) and Lutrol F127 (BASF, Germany). Diovan ® 80 mg tablet (Novartis) has been used as innovator pH-dependent immediate release tablet. Risk assessment studies has been performed with screening and quantitative tools. After optimization studies, FMEA has been revised with identified control strategy. “Dissolution” has been determined as critical quality attribute (CQA); and “Solubility enhancer level (Lutrol F127)” and “pH modifier level (Sodium Citrate)” have been identified as critical material attributes (CMA) based on risk assessment. Matrix tablet formulation has been optimized with central composite design. Thirteen batches were prepared and evaluated for percent of drug released in 2 h (Y1), percent of drug released in 8h (Y2). Optimum formulation with optimal pH-independent controlled release has been identified based on Design Space approach. Dissolution profiles of valsartan matrix tablets were determined using the USP 38 <711> Dissolution, Delayed Release Dosage Forms, Method A monograph. For a successful high quality, clinical safe and efficient drug development, it is very important to link biopharmaceutical properties of active pharmaceutical ingredient (API) with CQAs of the drug product with appropriate risk assessment tools. In this manner BCS concept can be considered as regulatory risk management tool and a main element for QbD. For this purpose absorption behavior of the optimum formulation has been simulated with using ACAT model, GastroPlus ® (Simulations Plus) software. According to the risk assessment tables and graphics, Valsartan found to be a high risky API with low permeability property based on BCS framework. Following, the high risky attributes of matrix tablet have been determined as “Solubility enhancer level (Lutrol F127)” and “pH modifier level (Sodium Citrate)” on dissolution. It has been concluded that the optimum formulation, provided an optimal release and enhanced bioavailability properties with higher regional absorption.

References

[1] G. L. Amidon, et al., A Theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability, *Pharmaceutical Research*, 12, 413-420, 1995.
[2] ICH Q8: Pharmaceutical Development, 2009.
[3] ICH Q9: Quality Risk Management and local implementation in the regulatory systems, 2005.

DEVELOPMENT AND *IN VITRO* EVALUATION OF GABA-LOADED POLYMERIC NANOPARTICLES PREPARED BY INVERSE EMULSION POLYMERIZATION FOR BRAIN DELIVERY

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Epilepsy is characterized by abnormal electrical activity within the brain which can result in either generalized or partial seizures [1]. Gamma-aminobutyric acid (GABA) plays a pivotal role in suppressing the origin and spread of seizure activity. Inhibition of GABA synthesis and blockage in release or post synaptic reaction were determined to provoke epileptic convulsions [2]. It is hypothesized that poor seizure control is associated with low brain GABA levels [3].

Targeting antiepileptic drugs to the brain is one of the challenging issues for epilepsy treatment since many therapeutic agents are unable to cross the blood-brain-barrier. In an attempt to overcome the limitations, nanocarriers were investigated as drug delivery vehicles for antiepileptics [4].

Depending on the most recent theory regarding reduced GABA levels in epileptic brains, incorporation of GABA into polymeric nanoparticles was aimed in this study for developing brain-targeted, nanosized, nontoxic, biocompatible and highly specific formulations. Polymeric nanoparticles (PNPs) were prepared by inverse emulsion polymerization method to investigate the effects of stirring type and rate on the characteristics of the GABA-loaded PNPs. During the characterization studies particle size, polydispersity index, morphology and zeta potential analyses were performed. DSC, XRD and FTIR analyses were also achieved in order to evaluate the changes in polymeric structures prepared. PNPs were furthermore evaluated for drug loading using HPLC and for *in vitro* GABA release using dialysis membrane.

As a result of the study GABA-loaded PNPs could be prepared using different amounts of crosslinking agent. Particle sizes of all PNPs were found to be in the nanoscale range. Morphological studies demonstrated spherical shape of all PNPs. Zeta potential values measured displayed neutral or positively charged characteristic. Possible GABA-matrix interaction was shown with DSC, XRD and FTIR analyses. GABA loading of 14-17 % was obtained for PNPs. GABA release from all PNPs prepared was much slower than the pure GABA. In conclusion, incorporation of GABA into PNPs was performed successfully and prolonged release behavior was obtained.

References

[1] M.F. Bennewitz, Saltzman, W.M., Nanotechnology for the delivery of drugs to the brain for epilepsy, *Neurother.*, 6 (2), 232-336, 2009.
[2] T. Şahiner, Epilepside Temel Mekanizmalar. In: Epilepsi, Bora, İ., Yeni, SN.., Gürses, C. (Eds.), Nobel Tıp Kitabevi, İstanbul, s. 29-36, 2008.
[3] O.A.C. Petroff, D.L. Rothman, K.L. Behar, R.H. Mattson, Low brain GABA level is associated with poor seizure control, *Ann. Neurol.*, 40, 908-911, 1996.
[4] T. Patel, J. Zhou, J.M. Piepmeier, W.M. Saltzman, Polymeric nanoparticles for drug delivery to the central nervous system, *Adv. Drug Deliver.Rev.*, 64 (7), 701-705, 2012.

BIOEQUIVALENCE STUDY OF MEMANTINE HYDROCHLORIDE XR 28 MG MICROPELLET CAPSULES IN COMPARISON WITH NAMENDA XR 28 MG EXTENDED RELEASE CAPSULES IN HEALTHY MALE SUBJECTS UNDER FASTING CONDITIONS

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Bioequivalence studies are required by regulatory authorities in order to ascertain that pharmaceutical products containing the same drug entity at the same dose in equivalent pharmaceutical formulations can be safely and effectively interchanged in human therapy. Nobel İlaç Sanayii ve Ticaret A.Ş.-Turkey developed a generic form of memantine, **Memantine Hydrochloride XR 28 mg Micropellet Capsules**. Presently, one of the marketed formulations is **Namenda XR 28 mg Extended Release Capsules** from Forest Pharmaceuticals Inc.-U.S.A. A comparative bioavailability study under fasting conditions is performed to determine whether **Memantine Hydrochloride XR 28 mg Micropellet Capsules** is bioequivalent to **Namenda XR 28 mg Extended Release Capsules** with respect to the rate and extent of absorption as characterised by C_{max} and $AUC_{0\text{-tlast}}$, respectively or not [1].

48 subjects were randomly assigned into two groups according to a computer-generated randomization scheme. The administration of the respective trial medication was done in the morning around 08:00 on an empty stomach after an overnight fast (minimum 10 hours). After 30 days washout period; in Period II, the subjects have been administered by the other drug that they have not been administered in the Period I. 44 subjects completed the clinical phase of the study as planned. Totally 25 blood sample points were selected including predose. The quantitative determination of memantine in (K_2EDTA) human plasma using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLCMS/ MS) has been developed and validated [2].

Since the 90% confidence interval values for the test/reference mean ratios for C_{max} and $AUC_{0\text{-tlast}}$ of memantine are contained within the acceptance limits preset in the Clinical Study Protocol, 0.80-1.25; according to the applied bioequivalence study, **it is concluded that test and reference memantine hydrochloride products are bioequivalent.**

References

[1] Guideline on the investigation of bioequivalence: CPMP/QWP/EWP/1401/98 Rev. 1/Corr., London, EMEA, 20 January 2010
[2] EMA- Guideline on bioanalytical method validation. 21 July 2011 EMEA/CHMP/EWP/192217/2009 Committee for Medicinal Products for Human Use (CHMP)

PENETRATION PROPERTIES OF DEXKETOPROFEN FROM SYNTHETIC CARBON NANOTUBE MEMBRANE

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In the present work, we aimed to evaluate the penetration properties of Dexketoprofen (DKP) through carbon nanotube (CNT) composite membranes. It was also aimed to compare of CNT membrane with other commercial membranes such as dialysis, cellophane, tuffryn, strat-m membrane. Multlwalll carbon nanotubes (MWCNTs) were used to prepare carbon nanotube membrane. To mimic the lipids and protein barrier properties of the skin, a variety of lipids as cholestrol, L-Alpha-Dipalmitoyl phosphatidylcholine (DPPC) and different protein bovine serum albumin (BSA) were added to the CNT membranes.The penetration properties of DKP were determined using Franz type diffusion cells. Ultra high performance liquid chromatography (UPLC) system was used for analysis of DKP. The steady-state permeation and flux (Js) were determined from the slope of the cumulative permeated DKP versus time graph [1].

The permeability coefficient (K_p) indicated the relation between the flux and the concentration DKP at the donor compartment (K_p = Js/Cd). Statistical analysis was performed using an ANOVA/ SPSS tests. A p-value less than 0.05 was considered significant. The physical appearance of the resulting membrane was determined by scanning electron microscopy (SEM) (Fig. 1).

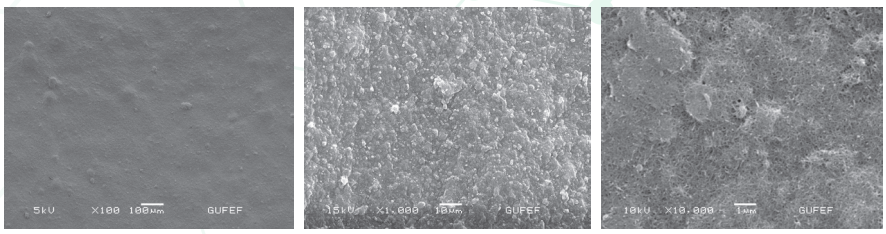

Fig. 1. SEM images of MWCNT membrane

We examined flux through CNT membrane and results were compared with dialysis, cellophane, tuffryn membrane and the impregnated DPPC-CNT membrane. CNT membrane gave significantly different flux values. However the CNT membrane, strat-m membrane, cholesterol-CNT membrane, BSA-CNT membrane, BSA-cholesterol-CNT membrane, BSA-DPPC-CNT membrane flux values were similar to each other and there was no statistically significant difference between them. However the permeability coefficient of DKP from CNT membrane is statistically different as compared to dialysis and cellophane membranes.

This work was supported by the Scientific and Technological Research Council (TUBITAK) grant number 114S133. We acknowledge to Prof. Dr. Zekiye Suludere and Assoc. Prof. Dr. Demet Çetin for SEM measurements.

References

[1] T. Caon, A.C.O. Costa, M.A.L. Oliveira, G.A. Micke, C.M.O. Simões, Evaluation of the transdermal permeation of different paraben combinations through a pig ear skin model, *Int J Pharm*, 391, 1-6, 2010.

ROLE OF SURFACTANTS AND pH ON DISSOLUTION PROPERTY OF CARVEDILOL

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Careful choice of surfactant media is required to perform dissolution studies of poorly soluble drugs for appropriate quality control or surrogate tests, as well as in vitro-in vivo correlations [1,2]. The purpose of the present study was to investigate the effects of different types of surfactants on the solubility of poorly soluble weak base, carvedilol and evaluate the dissolution of its innovator and five generic products to identify the most suitable surfactant medium reflecting the in vivo dissolution of this nonselective β-blocker. Sodium lauryl sulfate (SLS), hexadecyltrimethylammonium bromide (CTAB) and polysorbate 80 were used as anionic, cationic and nonionic surfactants, respectively. The solubility of carvedilol in buffers and surfactant media (0.5, 1 and 2%) within the physiological *pH* range was measured. Dissolution behaviors of the commercial tablets were studied using USP apparatus II in pH 1.2, 4.5 and 6.8 buffers and pH 6.8 dissolution media with 0.5% SLS, CTAB and polysorbate 80. Solubility of carvedilol increased significantly as pH increased from 1.2 to 4.5, and it decreased as pH further increased, giving low solubility value at pH 7.4 (7.0 µg/mL). Drug solubility increased gradually with the increased concentrations of all surfactants at pH 1.2 and 6.8. (r^2 = 0.928-0.999). Polysorbate 80 enhanced the solubility of carvedilol irrespective of pH. SLS and CTAB exhibited larger solubilization effect than polysorbate 80 depending on pH and ionic nature of the surfactant (2.5-192 and 7.3-235 vs. 3.1-27 fold, respectively). 85% of the labeled amount was dissolved within 30 min for the reference and all test products at pH 1.2 and 4.5. One test formulation showed a difference in dissolution profile at pH 4.5. The others showed dissolution profiles similarity to the reference at pH 1.2 and 4.5. In pH 6.8 phosphate buffer, drug dissolved from all products were in the range of 52-68% in 2 h. Dissolution rates and extents of the products increased most in the presence of CTAB (0.5%) at pH 6.8. However, the dissolution profiles of two test products were different from that of reference in CTAB medium. SLS was found to be inappropriate due to the potential interaction between SLS and excipients of the formulations and formation of a less soluble complex with the cationic drug. In 0.5% polysorbate 80 medium, the dissolution rates and extents of carvedilol from the reference and test products were less than those in CTAB medium. However, the number of in vitro dissolution profiles similarity of test products to the reference increased when 0.5% polysorbate 80 was added to pH 6.8 dissolution medium (f_2 > 50 for four out of five). In conclusion, the study demonstrated that polysorbate 80 containing dissolution medium (pH 6.8) seems to be the most biorelevant medium which successfully reflects the bioequivalence of test products to the reference product of carvedilol.

References

[1] G.L. Amidon, H. Lennernäs, V.P. Shah, J.R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, *Pharm Res* 12: 413-420, 1995.
[2] S.-H. Park, H.-K. Choi, The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs, *Int J Pharm* 321: 35-41, 2006.

ACCURACY AND PRECISION OF TABLET SPLITTING BY HAND AND A TABLET CUTTER

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Tablet splitting is a common practice in clinical settings to lower dose, facilitate swallowing or save costs. Splitting devices can be used when hand splitting is difficult or painful [1, 2]. However, data on the comparison of accuracy of tablet splitting devices and hand splitting are limited. The first purpose of this study was to evaluate the accuracy and precision of tablet splitting by a healthy volunteer and compare two splitting techniques. The second purpose was to evaluate if the split tablets of different brands were likely to comply with the current regulatory requirements. 600 mg scored gabapentin tablets from three different manufacturers were tested for weight variation, loss of mass, disintegration, friability and hardness. 15 tablets for each product were split by hand and a commercially available tablet cutter (Minion tablet cutter, Gozde Medical Equipment Company, Turkey). The accuracy of weight of split tablets were in the range of 95.7-104 vs. 91.4-107%, 92.5-107 vs. 83.6-116% and 91.2-108 vs. 84.3-113% when split by hand and the tablet cutter for product A, B and C, respectively. No significant difference in accuracy was determined between left and right sides of product B split by hand ($p = 0.535$). However, in all other cases, significant differences in accuracy were determined between left and right sides ($p < 0.05$). The precision of weight of split tablets were 1.95 vs. 3.96%, 3.12 vs. 8.57% and 4.21 vs. 7.02% (CV%) when split by hand and the tablet cutter for product A, B and C, respectively. Of the nonmechanically split tablet portions, none of the products deviated by more than 10% from the ideal weight. 4, 8 and 14 out of 30 bisected tablets had a loss of mass greater than 3% for product A, B and C, respectively. The number of bisected tablets showing loss of mass greater than 3% increased 2-3 times for the mechanically split tablets. 26.7 and 16.7% deviated by more than 10% from the ideal weight for product B and C, respectively. The split tablet portions of all products *met USP standards* for *friability (< 1%)*. No significant differences existed between the disintegration times of intact and half tablets for product A and B (21 vs. 21 min and 18 vs. 17 min, respectively). However, splitting shortened the disintegration time of product C (1 vs. 6 min). There was no correlation between the disintegration times and hardness values (81±11, 181±8 and 120±18 N for product A, B and C, respectively.) Overall, the accuracy and precision of hand splitting was more favourable than the tablet cutter. Only mechanically split product B failed to comply with the subdivision test of European Pharmacopoeia. However, none of the products complied with the FDA requirement for loss of mass. The study demonstrated that splitting of gabapentin tablets may result in inaccurate dosing. Therefore, appropriate measures regarding tablet splitting devices should be taken to ensure good patient care by the health authorities.

References

[1] D.A. van Riet-Nales, M.E. Doeve, A.E. Nicia, S. Teerenstra, K. Notenboom, Y.A. Hekster, B.J.F. van den Bemt. The accuracy, precision and sustainability of different techniques for tablet subdivision: Breaking by hand and the use of tablet splitters or a kitchen knife, *Int J Pharm* 466: 44-51, 2014.

[2] S.A. Helmy. Tablet splitting: Is it worthwhile? Analysis of drug content and weight uniformity for half tablets of 16 commonly used medications in the outpatient setting. *J Manag Care Spec Pharm* 21: 76-86, 2015.

DERMAL BEHAVIOUR OF SEMISOLID SLN DISPERSIONS OF ETOFENAMATE

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Solid lipid nanoparticles (SLN) are prepared with lipids which are in solid form at both body and room temperature. SLNs attract considerable interest for topical applications because they are prepared using non-irritant and nontoxic lipids and show excellent tolerability, good physical stability, and high drug-loading capacity. A novel strategy of the topical administration is preparing the semisolid form of the SLNs instead of incorporating the nanoparticles into conventional dosage forms [1]. An anti-nflammatory drug etofenamate (ETF) loaded semisolid SLNs were succesfully prepared by a novel one step production method using the lipids, Compritol 888 ATO and Precirol ATO 5, previously. Occlusive and mechanical characterization of the formulations and commercial gel product were also evaluated [2]. The main objective of this study is to investigate the ex vivo dermal behavior and skin localisation of these new semisolid SLNs and conventional gel product. For this purpose, the ex vivo penetration/permeation of ETF loaded semisolid SLNs and commercial gel were investigated using rat skin. A fluorescent dye cumarin-6 was also used to visualize the dermal distribution of the semisolid SLN formulations. For monitorizing the penetration of fluorescent dye into the skin samples Confocal Laser Scanning Microscopy (CLSM) was employed. No permeation was observed at the end of the 24h period for all semisolid SLNs and also commercial product of ETF. These findings showed that no systemic absorption was obtained for all of the formulations. The cumulative ETF uptake was found to be 7.4 ± 0.2% for Compritol 888 ATO SLNs; 5.8 ± 0.5% for Precirol ATO 5 SLNs and 9.5 ± 1.7% for gel product. The reason of the increased penetration of ETF with the gel product was attributed to the extremely high percentage of the various penetration enhancers used in the commercial product. According to CLSM images, it is possible to see that greater fluorescence intensities and deeper penetrations were obtained with both of the semisolid SLNs in comparison to gel product of pure dye. When the pure dye which mimics ETF was used solely, although a poor emission was monitorized through the hair shafts, no florescence intensity was observed in the deep epidermis. These findings indicated that the superiorities of the novel semisolid SLNs as a topical drug delivey systems compared to the conventional dosage form.

References

[1] U. Badilli, C. T. Sengel-Turk, A. Onay-Besikci, N. Tarimci, Development of Etofenamate-Loaded Semisolid SLN Dispersions and Evaluation of Anti-Inflammatory Activity for Topical Application, *Current Drug Delivery*, 12, 200-209, 2015.
[2] U. Badilli, C. T. Sengel-Turk, G. Amasya, N. Tarimci, Evaluation of Occlusive ond Mechanical Characteristics of Etofenamate Encapsulated Semisolid SLN Formulations, 11th International Symposium on Pharmaceutical Sciences, Poster No: P-110, June 9-12 2015, Ankara Turkey.

DESIGN AND CHARACTERIZATION OF HIBRID NANOPARTICULATE SYSTEMS FOR TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

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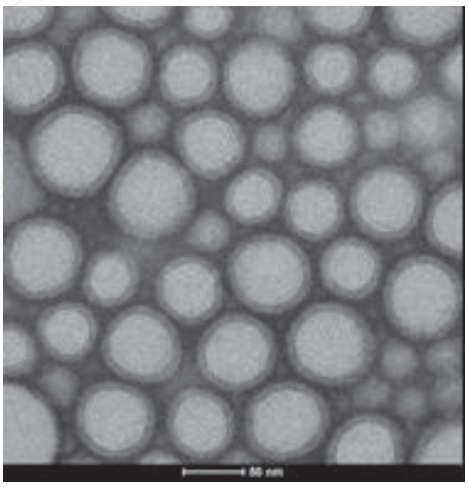
Benign prostatic hyperplasia (BPH) is recognized as a chronic progressive disease associated with considerablemorbidity such as hematuria, urinary infections, and the risk of acute urinary retention. Due to increased life expectancy, the proportion of men at risk for BPH and its morbidity is increasing. Effective treatment of this disease is essential to improve patient well-being [1]. Lonidamine, a derivate of indazole-3-carboxylic acid, is disrupted energy metabolism by interfering with glycolysis and caused cell apoptosis. Based on its mechanism of action, lonidamin is a promising new agent for the treatment of BPH [2]. The aim of this research is to prepare poly(D,L-lactic-co-glycolic acid)-lecithin-poly(ethylene glycol) hibrit nanoparticulate systems of lonidamine and evaluate the physicochemical characteristics of the particles. For this purpose, lonidamine loaded various formulations were produced successfully by a modified technique. Lonidamin was encapsulated in the range of 48.36-98.93% into the particles. The mean particle size of the hybrid formulations ranged from 110.3 to 173.0 nm with a unimodal size distribution (PDI ≤0.250). The hybrid structure and the spherical shape of the particles were proved based on the TEM micrographs. DSC analysis indicated that there was no interaction between lonidamin and the lipid-polymeric materials. In-vitro release experiments demonstrated that poly(D,L-lactic-co-glycolic acid)-lecithin-poly(ethylene glycol) hibrit nanoparticulate systems had exhibited a slow and continuous release of lonidamin.



References

[1] J.Lin, J. Zhou, Qianlienning capsule treats benign prostatic hyperplasia via suppression of the EGF/STAT3 signaling pathway. *Experimental and Therapeutic Medicine,* 1293-1300, 2013.

[2] P. Ditonno, M. Battaglia, Clinical evidence supporting the role of lonidamine for the treatment of BPH, *Reviews in Urology* S27-S33, 2005.

THE EFFECT OF TYPE OF ORGANIC PHASE SOLVENTS ON THE CHARACTERISTICS OF GEMCITABINE HCl LOADED POLY(LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES

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Gemcitabine HCl is a highly hydrophilic molecule and the formulation of hydrophilic molecules in nanoparticles suffer from low entrapment efficiency because of the drug rapid partitioning to the external aqueous phase [1]. The objective of this study was to screen different solvents for optimizing nanoparticle preparation in terms of particle size, entrapment efficiency, polydispersity index and zeta potential. Particle size, polydispersity index and zeta potential were determined by using a Zetasizer Nano ZS (Malvern Instruments, UK). The entrapment efficiency of gemcitabine HCl were determined via high performance liquid chromatography. The nanoparticles containing Gemsitabine HCl were prepared using double emulsion solvent evaporation, double emulsion solvent diffusion and modified double emulsion solvent diffusion/evaporation method [2]. Briefly poly (lactic-co-glycolic acid) (PLGA) was dissolved in organic solvent/solvent system. Ethyl acetate (EA, partially water-soluble organic solvent), acetone (ACE, fully water-soluble organic solvent) and dichloromethane (DCM, water-immiscible organic solvent) were used as organic solvents either individually or in combinations. Gemcitabine HCl was dissolved in aqueous solution of polyvinyl alcohol (PVA). The aqueous and oil phase were then mixed by sonication with a probe sonicator. The primary emulsion (w/o) was transferred into external aqueous phase of PVA and the mixture was sonicated. After evaporation of organic phase, nanoparticles were recovered by centrifugation at 18.000 rpm for 45 min. Nanoparticles were then washed with distilled water and lyophilized for 40 h at -55°C. The present investigation suggests that organic solvents play a significant role in nanoparticle formulations. The solubility of organic phase solvents in water was an important parameter affecting the mean size of PLGA nanoparticles. When EA and ACE were used small particles of 230-255 nm in size were obtained. Nanoparticles prepared with only DCM showed the largest particle size (485 nm) and widest size distribution. The low entrapment efficiency was obtained when the polymer was dissolved in DCM, EA or ACE individually. Nanoparticles with highest entrapment efficiency and low particle size were obtained when the mixture of DCM/ACE (1:2, v/v) was used as the organic phase. The values of the zeta potential for all formulations were found similar so there was no surface charge difference for gemcitabine HCl loaded nanoparticles prepared by different organic solvents. (This study was supported by TUBITAK research grant 113S841).

References

[1] E. Cohen-Sela, M. Chorny, N. Koroukhov, H.D. Danenberg, G. Golomb, A new double emulsion solvent diffusion technique for encapsulating hydrophilic molecules in PLGA nanoparticles, *Journal of Controlled Release*, 90-95, 2009.
[2] J.Liu, Z. Qiu, S. Wang, L. Zhou, S. Zhang, A modified double-emulsion method for the preparation of daunorubicin-loaded polymeric nanoparticle with enhanced in vitro anti-tumor activity, *Biomedical Materials*, 1-10, 2010.

DEVELOPMENT AND EVALUATION OF ORALLY DISINTEGRATING TABLETS OF FOSINOPRIL SODIUM BY DIRECT COMPRESSION METHOD

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Fosinopril sodium is an orally angiotensin converting enzyme inhibitor. It is used in the treatment of various cardiovascular disorders such as heart failure, stroke, myocardial infarction and cardiac death in high-risk patients and hypertension [1]. Hypertension and related heart disease are usually seen in geriatric patients and have a high incidence. In this group of patients, problems arise regarding treatment compliance due to difficulty in swallowing conventional oral solid-dosage forms. The aim of this study is to develop orally disintegrating tablets of fosinopril sodium and thus to increase treatment compliance by eliminating the swallowing problems. Orally disintegrating tablet formulations were prepared using diffferent superdisintegrants. These are croscarmellose sodium, crospovidone, sodium starch glycolate and low-substituted hydroxypropyl cellulose. Developed formulations were characterized in vitro. Friability was observed between 0.53-0.72% which were below 1% indicating that sufficient mechanical integrity and strength of the prepared tablets. According to EP, orally disintegrating tablets disintegrate within 3 min using conventional disintegration apparatus. Disintegration time for all formulations were below this value. The tablets containing Low-Substituted Hydroxypropyl Cellulose showed longest disintegration and wetting time, whereas the tablets containing crospovidon showed shortest times. In conclusion, fosinopril sodium orally disintegrating tablets with sufficient crushing tolerance and short disintegration time can be prepared using different superdisintegrants (This study was supported by TUBITAK research grant 114S931).

References

[1] R.N.G Rao, M. Kumar, M.S. Reddy, B.M. Reddy, C. Kistayya, Development and Evaluation of Fast Dissolving Tablets of Fosinopril by Direct Compression Method, *International Research Journal of Pharmacy*, 3(10), 123-129, 2012.

EFFECTS OF DIFFERENT FILLERS AND DISINTEGRANTS ON MIRTAZAPINE ORALLY DISINTEGRATING TABLET CHARACTERISTICS

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Orally disintegrating tablets (ODTs) are attractive drug delivery systems for pediatric, geriatric, and psychiatric patients with dysphagia. Aim of this study was to evaluate the effects of different fillers on physical characteristics, disintegration and dissolution properties of mirtazapine ODT formulations in comparison with reference product (Remereon SolTab). Coacervation method was used for taste masking of mirtazapine and 6% (w/w) Eudragit® E-100 was employed as coating material since it presented more similar results to the reference product in pre-formulation studies [1].

Different fillers and disintegrants were tested in Group B and Group C formulations, respectively. In Group B formulations, effects of Ludiflash®, Phamaburst™ C1, Galen IQ®, F-Melt® and Mannitol Parteck® M100 were tested in terms of the effects disintegration and dissolution profiles. Optimum results were obtained using Ludiflash®. In group C formulations, Kollidon® CL, Ac-Di-Sol®, Explotab® and Kollidon® CL / Amberlit® IRP-88 mixture were tested as disintegrants. Other excipients and their amounts were kept unchanged. Formulations, which were prepared with Galen IQ® and F-Melt®, presented poor dissolution profiles when compared to the other group B formulation contains Ludiflash®. Hence, using Galen IQ® or F-Melt® as diluents has no favorable effect on dissolution profile. The most convenient dissolution profile at 0,1N HCl medium were achieved with formulation C3 which contains only Kollidon® CL as disintegrant from among group C formulations. Disintegration studies also indicated that shortest disintegration time was achieved with Kollidon® CL.

Based on the results of this study, an ODT formulation for mirtazapine will be developed using Ludiflash® and Kollidon® CL as filler and disintegrant respectively. Effects of other parameters such as slug tablet granules, citric acid amount and sodium bicarbonate amounts will also be evaluated in order to achieve the optimum properties.

References

[1] Yıldız S., Aytekin E, Bozdag Pehlivan S, Ünlü N. Characterization of Orally Disintegrating Tablets Containing Taste-Masked Mirtazapine Granules with Different Coating Ratio. 2015 CRS Annual Meeting, 26-29 July 2015, Edinburgh-Scotland.

POTENTIAL NOSE-TO-BRAIN DELIVERY OF HYBRID NANOPARTICLES IN THE TREATMENT OF GLIOBLASTOMA INDUCED FEMALE WISTAR RATS

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Following the administration to the nasal cavity, pharmacological agents, are transmitted to the brain via the olfactory and trigeminal nerves. Intranasal administration is used as an alternative route for non-invasive delivery of drugs to the brain. This path bypasses the blood-brain-barrier (BBB) and eliminates systemic side effects. The goal of this study was to evaluate the antitumor efficacy of farnesylthiosalicylic acid (FTA) loaded (lipid/cationic) lipid-PEG-PLGA hybrid nanoparticles (HNPs) after intranasal application in brain tumor induced rats.

Once daily, 1 mL of 1 mg/mL cyclosporine in 0.9% NaCl solution was applied intraperitoneally to female Wistar rats (250-300 g) for 5 days before tumor cell implantation. Then, at day 0 the rats were anesthetized and placed under a stereotaxic instrument. After creating a hole in the skull of the rat, a volume of 5 µL RG2 cell suspension with $50x10^4$ cells was implanted unilaterally into the right striatum of the rat brain with the use of a Hamilton glass syringe [1, 2]. At day 10, the first MRI scans were conducted to analyze the tumor size. Brain tumor bearing rats (range 8-25 mm^2) were divided into 2 groups of 7 rats. Empty **(±106 nm)** and FTA loaded 30% PEG HNPs (±163 nm) were prepared with the emulsion sonication method [3]. A volume of 20 µL of drug dispersion prepared in TPBS (PBS containing 0.1% w/v Tween-20) was administered at the same day (day 10) to the brain tumor bearing rats by intranasal application. Intranasal application was performed by pipetting the formulation into the right nostril over a period of 2 minutes at supine position. At day 15, second MRI scans were conducted to analyze tumor size of post-treatment [1-3].

The in vivo study showed that intranasally applied FTA loaded 30% PEG HNPs were able to reach the glioblastoma in the brain and effectively reduced the tumor area with 31.0% respectively. From this study it can be concluded that **(**lipid/cationic) lipid-PEG-PLGA HNPs for intranasal delivery of FTA is a potential non-invasive approach in the treatment against glioblastoma.

References

[1] I. K. Geletneky, A. Ayache, R. Koch, M. Herrero, Y. Calle, L. Deleu, C. Sommer, N. Thomas, J. Rommelaere, J. R. Schlehofer, Regression of advanced rat and human gliomas by local or systemic treatment with oncolytic parvovirus H-1 in rat models, *Neuro Oncol*, 12(8): p. 804-14, 2010.
[2] M. Yemisci, S. Bozdag, M. Cetin, F. Soylemezoglu, Y. Capan, T. Dalkara, Treatment of malignant gliomas with mitoxantrone-loaded poly(lactide-co-glycolide) microspheres, *Neurosurgery*, 59(6), p. 1296-1302, 2006.
[3] R. H. Fang, C. M. Hu, L. Zhang, Quick synthesis of lipid-polymer hybrid nanoparticles with low polydispersity using a single-step sonication method, *Langmuir*, 26, p. 16958-62, 2010.

EMBRYONIC STEM CELL AND INSULIN-LOADED PLGA NANOPARTICLES AND EVALUATION OF EFFICIENY ON PANCREATIC BETA TC CELLS

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Diabetes is one of the most common diseases that occur globally. In diabetes, insulin is the most effective drug for the control of blood glucose levels [1]. Embryonic stem cells (ESCs) derive from the inner cell mass of the blastocyst, an early-stage embryo. The ESCs subsequently have the potential to differentiate into various types of cells [2]. ESCs have been proposed as a potential source of pancreatic beta cells as well as a powerful tool for cell replacement therapy in the treatment of diabetes [3]. Nanoparticles (NPs) are spherical structures ranging around nanometer in size and prepared from natural and synthetic polymers [4]. In this study, we determined insulin and ESC-loaded NPs efficiencies on diabetic pancreatic beta TC cell line. NP formulations were prepared using poly lactid-co-glycolide acid (PLGA) as a polymer. For amounts of captured insulin and ESC, lowry protein determination method was used. In additon, for ESC encapsulation efficiency, cell counting and Western blot analysis were performed. The type of NPs were determined using a transmission electron microscope (TEM). For in vitro characterisation of insulin and ESC NPs, particle size, zeta potential, polydispersity index and encapsulation efficiencies were determined. In vitro insulin release experiment from NPs was performed with a dialysis membrane using Franz-type diffusion cells in pH 7.4 phosphate buffer at 37 ºC. In cell culture studies, cytotoxicity test was carried out to determine the toxic effects of insulin and empty nanoparticle. Insulin and empty nanoparticles were also not found to be toxic to cells at any concentrations. Transport of insulin from the NP formulation through pancreatic beta TC cell from the apical side to the basolateral compartment were evaluated. Cumulative amounts of insulin at the end of the 48 h time period were calculated. We investigated the relationship between glucose and insulin concentration in diabetic cell incubated with glucose and streptozocin (STZ). The reduction of insulin concentration was synchronous with increasing glucose level. After applying insulin and ESC loaded NPs to diabetic pancreatic beta TC cells for 48 h, insulin levels were increased for both glucose and STZ-induced diabetic cell groups. It was concluded that insulin and ESC loaded NPs improved the decreased insulin levels inducing glucose and STZ. These NPs 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.

References

[1] P. He, H. Liu, Z. Tang, M. Deng, Y. Yang, X. Pang, X. Chen, Poly(ester amide) blend microspheres for oral insulin delivery, *International Journal of Pharmaceutics*, 455, 259-266, 2013.
[2] S.Y. Goh, R.B. Abdullah, W.E. Wan Khadijah, Characterization of caprine embryonic stem cell-like outgrowths derived from the inner cell mass isolation, *Small Ruminant Research*,107, 38– 44, 2012.
[3] T. Fujikawa, S. Oh, L. Pi, HM. Hatch, T. Shupe, BE. Petersen, Teratoma formation leads to failure to treatment for Type I diabetes using embryonic stem cell-dervied insulin-producing cells, *American Journal of Pathology,* 166, 1781-1791, 2005.
[4] F. Danhier, E. Ansorena, JM. Silva, R. Coco, A. Breton, V. Preat, PLGA-based nanoparticles: An overview of biomedical applications, *Journal of Controlled Release*, 161, 505-522, 2012.

PREPARATION OF CHITOSAN NANOPARTICLES BY NANO SPRAY DRYING TECHNOLOGY

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Nano spray drying technology is a breakthrough innovation in order to obtain submicron particles from a solution. Up to date the Buchi Nano Spray Dryer B-90 is the newest available device for spray drying process to obtain nano size particles. The device enables to produce very small particles due to its special spray head system. It is also possible to decrease sample loss via laminar airflow in this closed-system and the electrostatic particle collector enables to get particles with high yield [1,2]. In this study, it was aimed to reveal some of critic parameters of this technique and to evaluate their influences on manufacturing process, particle characteristics and operation capacity. Chitosan was chosen as a model polymer and a series of chitosan solutions at two different concentrations were prepared. Prepared solutions were sprayed using nano spray dryer system. The studies were performed in duplicate to understand variabilities. In terms of determining the critical steps in manufacturing process; the effects of orifice (sieze) on the spray head, the spray capacity and the chitosan polymer concentrations were investigated. Particle sizes, polydispersity indexes and zeta potentials were measured as particle characteristics. Product yield and sprayed sample volume/process time were also evaluated.

Finally, it was concluded that the smaler orifices on spray head provided smaller particle sizes, but it requires more processing time. Also, the spraying volume had no effective on particle sizes but effects the processing time. On the other hand, polymer concentration directly affected both production yield and sprayed sample volume/processing time.

References

[1] X. Li, N. Anton, C. Arpagaus, F. Belleteix, T.F. Vandamme, Nanoparticles by spray drying using innovative new technology: The Büchi Nano Spray Dryer B-90, *Journal of Controlled Release*, 304-310, 2010.
[2] R. P. Aquino, M. Stigliani, P. Del Gaudio, T. Mencherini, F. Sansone, P. Russo, Nanospray Drying as a Novel Technique for the Manufacturing of Inhalable NSAID Powders, *The Scientific World Journal*, Article ID 838410, 2014.

EVALUATION OF CHARACTERIZATION PARAMETERS OF METHOTREXATE LOADED CHITOSAN NANOPARTICLES

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The aim of this study was to evaluate characterization parameters of chitosan nanoparticles (CSNPs) in absence and presence of methotrexate (MTX) for development of intravenous drug delivery system. CSNPs were prepared by using ionic gelation process. CS was dissolved in 1% acetic acid solution. TPP solution was prepared with deionized water and MTX was dissolved in it. Then, MTX-TPP solution was added dropwise under constant stirring to equal volume CS solution. For characterization and stability studies, the particle size and zeta potential of CSNPs were measured at initial time and 6 months by using Malvern Zeta Sizer. The composition of CSNPs and characterization parameters were shown in Table 1 and 2, respectively.

Table 1. Composition of CSNPs

Formulation	Composition of CSNPs		
	CS (%)	TPP (%)	MTX (mg)
F1	0.25	0.125	-
F2	0.50	0.125	-
F3	0.75	0.125	-
F1-MTX	0.25	0.125	1
F2-MTX	0.50	0.125	1
F3-MTX	0.75	0.125	1

Table 2. Characterization parameters of CSNPs

Formulation	$T_{initial}$		$T_{6\ month}$	
	Particle size (nm) ± ss	Zeta potential (mV) ± ss	Particle size (nm) ± ss	Zeta potential (mV) ± ss
F1	144,767 ± 2,754	19,000 ± 0,648	210,000 ± 7,000	21,240 ± 1,665
F2	377,033 ± 9,445	36,333 ± 1,322	378,000 ± 8,000	29,458 ± 3,288
F3	750,567 ± 61,186	33,633 ± 1,613	-	-
F1-MTX	169,000 ± 10,316	20,133 ± 1,144	188,857 ± 7,721	24,703 ± 1,347
F2-MTX	427,633 ± 61,312	29,067 ± 2,207	377,363 ± 9,728	35,240 ± 1,589
F3-MTX	1768,000 ± 409,821	34,233 ± 0,737	-	-

According to characterization results, F1 formulation with and without MTX was chosen for ideal formulation. The particle size of F1 was available for intravenous drug delivery. These formulations could provide a promising strategy for drug delivery system. (This study was supported by Ege University Scientific Research Project Comission (project number: 14/ECZ/037) and Aliye Uster Foundation.)

DEVELOPMENT OF THERMOSENSITIVE GEL SYSTEM COMBINED WITH ENZYME, ANTIBIOTIC AND MICROSPHERES FOR THE ERADICATION OF BACTERIAL BIOFILM

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Medical device-related osteomyelitis (MDRO) is a common occurring problem due to tissue damage and inflammation during implantation procedure, device dysfunction and systemic infections. The treatment of MDRO is difficult, time-consuming and expensive procedure owing to slow healing of infected tissue depend on bone necrosis and poor vascular perfusion. Furthermore, medical device is a suitable substrate for bacteria in order to form biofilm which causes a high resistance to used antibiotics and incomplete penetration of antibiotic to infected area [1].

The aim of this study was to develop and characterize thermosensitive gel formulations combined with serratiopeptidase (SP) enzyme, vancomycin HCl (VA) and VA loaded poly(ε-caprolactone) (PCL) microspheres in order to simultaneously obtain the eradication of the biofilms, immediate and sustained drug release in the target site.

Empty thermosensitive pluronic hydrogels were prepared by the “cold method” using Pluronic® F 127 [2]. Viscosity measurements, drug and enzyme release, in vitro enzyme activity and in vitro anti-biofilm activity studies were performed to characterize the formulations. 67.8% of total VA was released at the end of the first day, 69.9% at the end of the second day, and 74.5% at the end of the twenty-fifth day. The enzyme activity was measurable for 10 days starting with a burst release of 41.8 % in the end of the first day. According to in vitro antibiofilm activity assays and release studies, the gel formulation containing 0,8% free VA, 5% SP and 40% of VA loaded high molecular weight PCL microspheres was chosen as the most promising candidate formulation for in vivo studies.

This study was supported by The Scientific and Technological Research Council of Turkey, TÜBİTAK (SBAG,111S275).

References

[1] Francolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. *FEMS Immunol. Med. Microbiol*., 59(3), 227-38, (2010).
[2] Choi, HG, Jung JH, Ryub JM, Yoob SJ, Oha YK, Kima CK. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. *Int. J. Pharm*, 1998. 165(1), 33-44.

EVALUATION OF RHEOLOGICAL AND TEXTURAL PROPERTIES OF TOOTHPASTES

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The viscosity, flow properties and texture of a toothpaste are very important parameters that influence production and customer satisfaction. Whether a toothpaste has suitable rheological properties (viscosity, pseudoplasticity, thixotropic property, very low yield value) and texture structure depends on the properties and ratios of the substances used in its formulation. These properties and ratios are naturally different in every commercial toothpaste. A toothpaste must form a homogenous ribbon upon extrusion from the tube; must not flow out of an uncapped tube in the absence of an extrusion pressure; must require minimal effort to be extruded from the tube; must form a sharp and clean ribbon of desired amount on the toothbrush following extrusion; must be able to stand up on the toothbrush, and not sink between the bristles; and must disperse quickly in the mouth after brushing is initiated. Whether a toothpaste satisfies these conditions can be determined by using suitable devices that evaluate its rheological and textural properties. Texture analysis measurements provide important information about a toothpaste's extrusion and firmness, as well as the cohesiveness and consistency of the paste mass.

The aim of this study was to determine whether the rheological properties and texture analysis parameters of toothpastes can be evaluated together. To this end, commercially marketed children's toothpastes were evaluated using a rotational viscometer (Brookfield RVDVIII, Rheocalc V2.4, cone spindle no: XXX) and texture analyzer (TA.XT.Plus, Stable Micro System).

References

[1] D. Laba, 1993, Rheological properties of cosmetics and toiletries, Marcel Dekker Inc., 246-336, 1993.
[2] L. Gilbert, C. Picard, G. Savary, M. Grisel, Rheological and textural characterization of cosmetic emulsions containing natural and synthetic polymers: relationships between both data, 150-163.

NEW PERSPECTIVE FOR MEMANTINE ORALLY DISINTEGRATING TABLET: SEDEM EXPERT SYSTEM

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SEDEM Diagram Expert System is a prospective methodology to obtain an optimum formulation for direct compression by predicting whether a disintegrant or another excipient is suitable for API or not.[1] Orally disintegrating tablets (ODTs) are solid dosage forms containing special excipients to disintegrate in few minutes when placed on the tongue. ODTs have some advantages especially for geriatric patients who has some difficulties in swallowing tablets and capsules. Memantine is a BCS class I drug which used in Alzheimer's disease medication. Therefore, memantine was chosen as suitable drug for ODT formulation.

The aim of this study is to select the most appropriate superdisintegrating agent (Ludiflash®, Ludipress®, Parteck®) for ODT formulation of memantine by using SEDEM Diagram Expert System. Sedem Diagram Expert System enables us to evaluate dimension, compressibility, flowability/powder flow, lubricity/stability and lubricity/dosage parameters of superdisintegrating excipient and Memantine. Hardness, friability, disintigration time properties of compressed tablets were examined. SEDEM Diagram is shown in Fig 1.

'Stat Graphs Programme' was used to attain SEDEM diagram. According to the obtained diagrams, Parteck® ishowed optimum physical properties (Fig 2a-2b). Consequently, SEDEM Diagram Expert System is a promising method for direct compression ODT formulation studies.

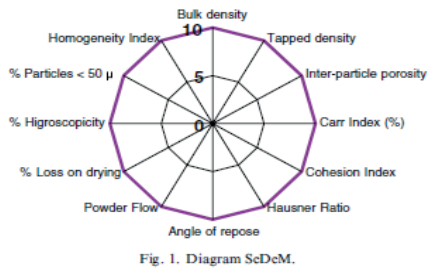

Fig. 1. Diagram ScDeM.

Fig 1

Memantine

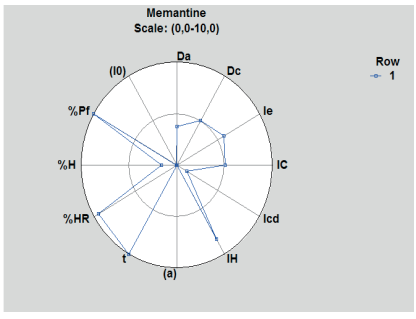

Fig 2a

Memantin-Parteck

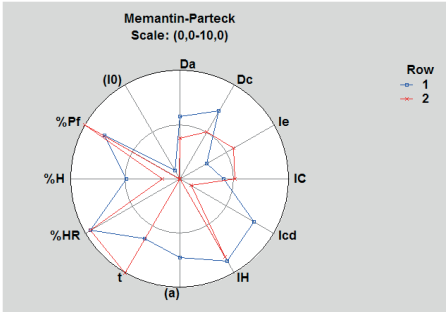

Fig 2b

References

[1] J. M. S. Negre, SeDeM Diagram: A New Expert System for the Formulation of Drugs in Solid Form, Expert Systems for Human, *Materials and Automation*, pages 17-34, 2011.

INVESTIGATION OF MUCOADHESIVE GEL AND LIPOSOME FORMULATIONS FOR NASAL DELİVERY OF OVALBUMIN: AN IN VITRO EVALUATION USING TEXTURE ANALYZER

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The emergence of new diseases and the lack of efficient vaccines against numerous non-treatable pathogens require the development of novel vaccination strategies. To date, only a few mucosal vaccines have been approved for humans. Recent advances make feasible the development of efficacious mucosal vaccines with adequate safety profile. Thus, currently intranasal vaccines represent an attractive and valid alternative to conventional vaccines [1]. The objectives of this study were to prepare gel and liposome formulations for nasal vaccination, to investigate and evaluate mucoadhesive and textural properties on sheep nasal mucosa. For this purpose we have developed ovalbumin (OVA) containing gel formulations using chitosan (2 % w/v) and Carbopol® 974P NF (0,25% w/v) which are composed of polymers that show promising potential as mucosal vaccine delivery systems. We have also developed OVA containing liposome and gel contains liposome formulations. The mucoadhesive and mechanical properties of these formulations were determined. The mucoadhesion testing method was based on the measurement of the force and the work needed to detach a sample of sheep nasal mucosa from a gel formulation. The forces involved in the detachment process were measured by a TA-XT Plus Texture Analyzer in adhesive mode. Maximal detachment force (F_{max}) and the work of adhesion (W_{ad}) values were the mean of six experiments performed at 37°C. Texture profile analyses of the prepared gels were evaluated using TA.XTPlus Texture analyzer equipped with 5 kg load cell in texture profile analysis (TPA) mode. Each formulation was transferred into a universal bottle (25 mL) to a fixed height of 8 cm. An analytical probe of diameter 10 mm was depressed twice into each sample to a defined depth (15 mm), at a defined rate (2 mm/s), with a defined recovery period (15 s), between the end of the first compression and the beginning of the second. At least six replicate analyses of each sample were performed at 37±0.5°C. The higher work of mucoadhesion was determined in the formulations including chitosan. Chitosan gel formulation was more mucoadhesive in the case of sheep nasal mucosa than carbopol. Ideally, formulations designed for nasal drug delivery should have high adhesiveness, cohesiveness and elasticity values. The carbopol gel formulation exhibited the greatest adhesiveness and cohesiveness values. The adhesive and mucoadhesive properties of the gel containing liposomes were low. The results suggest that chitosan and carbopol gels appear to be the most suitable mucoadhesive polymer for nasal drug delivery systems.

References

[1] P. Riese, P. Sakthivel, S. Trittel, C. Guzmán, Intranasal formulations: promising strategy to deliver vaccines, *Expert Opin Drug Deliv.* 11(10), 1619-34, 2014.

STUDIES ON IMPROVEMENT OF WATER-SOLUBILITY WITH ELECTROSPUN NANOFIBERS I: CURCUMIN

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Electrospinning has been utilized for preparing polymeric nanofibers which is simple and efficient technique. Electrospinning method provides high surface area to volume ratio and ensures increased solubility of poorly water soluble drugs [1]. Curcumin (Cur) has antitumor, antioxidant, anti-inflammatory properties. But curcumin has low solubility and permeability thus classified as BCS Class 4 [2]. The aim of this study was to improve the solubility of curcumin by preparing its electrospun nanofibers.

Curcumin was purchased from Sigma Chemicals, Germany. Hydroxypropyl methyl cellulose (HPMC K100M LV) and polyethylene oxide (PEO, Polyox WSR205, Mw 600.000) was gift from Colorcon, India. Electrospun nanofibers were produced using electrospinning unit, Inovenso NE-100, Turkey. Before electrospinning procedure, conductivity, viscosity and surface tension values of each polymer solution and the final mixture were measured. Curcumin nanofibers were prepared from a mixture, which contained HPMC and PEO polymers. Electrospinning process was performed at 14 kV voltage using a flow rate of 0,6mL/h for 3 hours. The distance of needle tip to the collector was 21 cm. The morphologies and the mean diameter of the fibers were determined through scanning electron microscopy (SEM). DSC and FT-IR studies of nanofibers were also carried out. Drug loading, solubility and drug release studies were carried out at distilled water and pH 1.2 solution.

The viscosity, conductivity and surface tension values of PEO, HPMC and final mixture solutions were shown in Table 1. Final mixture had the lowest surface tension value thus provided better electrospinning process. Drug loading was found to be 79,53 µg per cm^2. Curcumin was found to be insoluble in distilled water and pH 1.2 solution. The solubility of curcumin was increased from practically zero to 7,66mg/L and 1,57mg/L by the producing of the curcumin nanofibers, in water and pH 1.2 solution respectively.

It was concluded that electrospinning was a useful technique for he improvement of the water-solubility of poorly-soluble drugs.

Table 1. Mean values of the physicochemical properties of solutions

	Viscosity (cPs)	Conductivity (µs/cm)	Surface Tension (dyn/cm)
PEO 3%	911	90	57,39
HPMC K100M LV 3%	777	244	58,63
Final Mixture (PEO 3%, HPMC 3%, Cur 1 mg/mL)	8114	124	33,27

References

[1] D. Yu, LM. Zhu, K. White, C. Branford-White, Electrospun nanofiber-based drug delivery systems, *Health*, 1, 67-75, 2009.
[2] B. Wahlang, YB. Pawar, AK. Bansal, Identification of permeability-related hurdles in oral delivery of curcumin using the Caco-2 cell model *European Journal of Pharmaceutics and Biopharmaceutics*, 77, 275–282, 2011.

SODIUM ALGINATE NANOBEADS FOR DELIVERING ANTICANCER DRUGS

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The aim of this work was to determine optimum formulation parameters of alginate beads which are going to be uptaken by macrophages and go through Peyer's Patches and lymphatic system. Particle size is one of the most important factors for the uptake of nanoparticles by macrophages in Peyer's Patches.[1] The uptake of 100 nm biodegradable particles in the rat intestine was reported to be significantly higher in comparison with larger particles of 1 and 10 μm [2]. In this study, the alginate nanobeads were developed using different concentrations of sodium alginate, PEG, Tween 80. Nanobeads were prepared using dripping method with Ultra-Turrax mixing. The formulations were prepared by 2^3 factorial design. Mean particle sizes, polydispersity index (PDI) and zeta potantials were determined. Here we demonstrate the effect of sodium alginate concentration, polyethylene glycol (PEG), Tween 80 on particle size were optimized. The most important factor was found to be the amount of sodium alginate concentration and PEG. Optimised formulation has the particle size around 200nm.

References

[1] A. Awaad, M. Nakamura, K. Ishimura, Imaging of size-dependent uptake and identification of novel pathways in mouse Peyer's patches using fluorescent organosilica particles, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 8, 627-636, 2012.
[2] P. D. Manisha, L. Vinod, L. A. Gordon, J. L. Robert, Gastrointestinal uptake of biodegradable microparticles: effect of particle size, *Pharm Res,* 13, 1838-45, 1996.

NANOEMULSION FORMULATIONS: OPTIMIZATION AND DESIGN FOR ENHANCED ORAL ABSORPTION

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Lipophilic drugs which are poorly water soluble need specific applications like lipid based delivery systems to increase their oral absorption across gastrointestinal tract [1]. Lipid suspensions, emulsions and solutions are becoming increasingly popular to overcome of this problem. Also self-nanoemulsifying systems are considered one of this lipid based delivery technics. Making decision to select of suitable self-emulsifying formulation is important and self-emulsification region is obtained in the pseudoternary phase diagrams. For each combination of self-nanoemulsifying systems, ternary diagrams of surfactants (Labrasol, Labrafil), co-surfactants (Plurol Oleique, Transcutol, Lauroglycol FCC) and oils (Labrafac Lipophile WL1349, Labrafac PG) were constructed to recognize the zone of nanoemulsion formation. Thirty-two samples were prepared and for each sample oil and surfactant: co-surfactant ratio were mixed in ratios ranging from %10:90 - %90:10 like 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 1:9 and pseudoternary phase diagrams were drawn (Figure 1). Convenient formulations were choosen and budesonid active pharmaceutical ingredient was loaded. Each formulation was evaluated for their physicochemical characteristic such as droplet size, drug content, etc. (This study supported by Istanbul University BAP no: 47691)

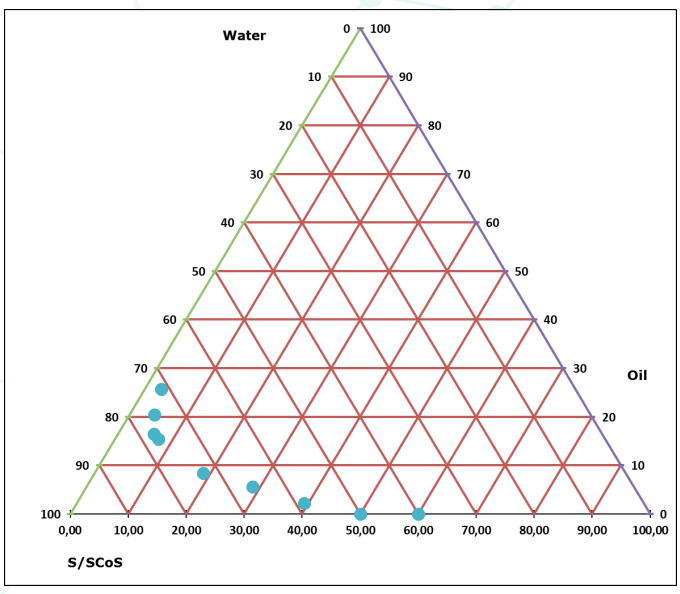

Fig. 1: Pseudoternary phase diagram of formulation E

References

[1] C.J.H. Porter, C.W. Pouton, J.F. Cuine, W.N. Charman, Enhancing intestinal drug solubilisation using lipid-based delivery systems, *Adv drug del rev*, (60) 673-691, 2008.

INFLUENCE OF VERAPAMIL ON METFORMIN PERMEABILITY ACROSS Caco-2 CELLS

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Metformin hydrochloride (MH) is a biguanide derivative oral antidiabetic agent mainly prescribed for the treatment of non-insulin dependent diabetes. MH is a known substrate of various uptake transporters (OCT1, OCT2, OCT3, and PMAT) expressed in the gut and liver [1]. The main purpose of this study was to determine permeability of MH across Caco-2 cells in the absence and presence of PMAT inhibitor verapamil [2]. Caco-2 cells were seeded (30.000 cells/well) on polycarbonate membrane inserts (1µm). Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, penicillin (50 unit/mL) and streptomycin (50 µg/mL) was used as the growth medium, and HBSS containing 25 mM D-glucose and 10 mM HEPES was used as the transport buffer (TB). Monolayer integrity of the cells was assessed 21 days after seeding by measuring the TEER values, and the cells with TEER values > 600 $\Omega.cm^{-2}$ were used for transport studies. MH in the absence (80 µM) or presence of verapamil (50 µM) was dissolved in TB, and then added to the apical side (250 µL). Basolateral side was added only drug free TB (750 µL). At the end of 2-hour incubation, samples were removed from both sides, and MH concentrations in samples were analyzed by means of a validated HPLC method using a Waters Spherisorb ODS2 C18 (250x4.6 mm 5 um; USA) column. The HPLC system was operated (25 °C, 232 nm) using a mobile phase consisted of phosphate buffer (pH 3; 100 mM)–acetonitrile (30:70, v/v), and delivered at a flow rate of 1 mL/min. In the absence and presence of verapamil, permeability values (P_{app}) of MH were calculated as P_{app}= Rate of transport/(surface area × initial concentration). The results revealed that transport of MH across Caco-2 cells was decreased significantly by verapamil ($p<0.05$, Figure 1), indicating that PMAT plays an important role in the absorption, and hence bioavailability of MH.

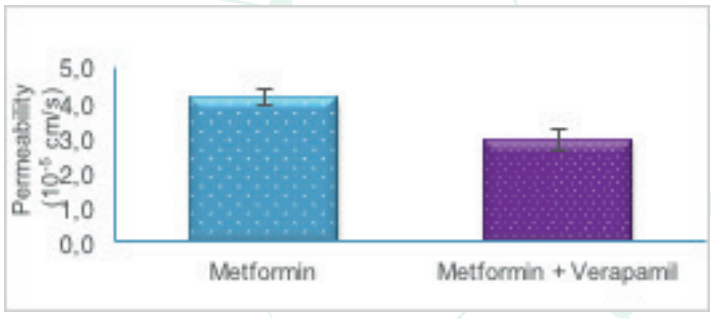

Figure 1. Permeability values of metformin (mean ± SE, n=6).

References

[1] KL. Garrison, S. Sahin, LZ. Benet, Few drugs display flip-flop pharmacokinetics and these are primarily associated with classes 3 and 4 of the BDDCS. *Journal of Pharmaceutical Sciences*, 3229-3235, 2015.
[2] K. Engel, J. Wang, Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Molecular Pharmacology*, 1397-1407, 2005.

CONCOMITANT MONTELUKAST AND RUPATADINE AS A TREATMENT FOR SEASONAL ALLERGIC RHINITIS: A FORMULATION DEVELOPMENT PERSPECTIVE

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Seasonal allergic rhinitis (SAR) is prevalent in world and more common in children among age 1-12 year. Montelucast sodium is leukotriene receptor antagonist and used effectively in treatment of SAR, Exercise-Induced Bronchoconstriction and asthama [1]. Rupatidine is non sedating, H_1 receptor antagonist and platelet activating factor (PAF) inhibitor. Rupatidine used for treatment of symptomatic treatment of seasonal & perennial allergic rhinitis and idiopathic urticaria [2]. New studies have demonstrated that rupatadine inhibits PAF effects in nasal airways and produces a greater reduction in nasal symptoms than levocetirizine [3]. It was reported that leukotrine inhibitor and antihistamine agents in combination has profound effect than used as single. Montelucast sodium and Loratidine combination are approved in market and proved to be beneficial. All the drugs are available in individual dosage form in US and EU market. The novel combination of Montelucast sodium with Rupatidine was designed for investigation. This combination especificaly Rupatidine has more advantages over Desloratidine/Loratidine in already approved combination Montelucast and Loratidine. Pharmacokinetic studies shows that Rupatidine is rapidly absorbed in 45-60 minutes while Loratidine requires 2-4 hrs [4]. The newly developed pharmaceutical formulation includes inhibiting chemical interaction of rupatadine and montelukast. The design of formulation involves manufacturing of two components individually and compressed as bilayer tablet. The two components are prepared in such a way that both drugs have minimal interaction in bilayer tablet. The stability of formulation evaluated using validated stability indicating methods. The combination involves Montelukast sodium 10 mg and Rupatidine 10 mg strengths which are same as individual dosage form strength. The fixed dose combination (FDC) product was evaluated against Individual marketed product for dissolution profiling. The bioequivalence study of novel combination formulation was performed against reference products and found that all BE parameters are complying limits. This robust, stable formulation will fulfill the need of patient compliance with synergistic effect.

References

[1] Singular®(Montelukast sodium) Tablets, Oral granules, Patient information leaflet (PIL).
[2] M Sánchez-Borges, Treatment of Recalcitrant Chronic Urticaria With Nonsedating Antihistamines: Is There Evidence for Updosing?, *Journal of Investigational Allergology & Clinical Immunology*, 23(3), 141-144, 2013.
[3] J. Mullol, Update on rupatadine in the management of allergic disorders, *Allergy*, volume- 70, Supplement S100, 1-24.
[4] K F Lukat, A direct comparison of efficacy between Desloratadine and Rupatadine in seasonal allergic rhinoconjunctivitis: a randomized, double-blind, placebo-controlled study, 6, 31-39, 2013.

DELAYED RELEASE FIXED DOSE COMBINATION OF NON-STEROIDAL ANTI INFLAMMATORY DRUGS AND PROTON PUMP INHIBITORS

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most frequently prescribed medications worldwide in pain management.It can be complications of ulcers, hemorrhages and perforation,cause gastrointestinal (GI) bleed. Co-administration of proton pump inhibitors (PPIs) to prevent these GI complications [1]. American College of Rheumatology use of PPIs along with NSAIDs for osteoarthritis (OA) patient for long-term treatment [2]. The combination of PPIs along with NSAIDs prevent GI toxicity and the drugs in this fixed dose combination (FDC) has been shown bioequivalent with respect to rate and extent of drug absorption [3]. Diclofenac sodium is effective in treating the signs and symptoms of arthritic conditions. It is an inhibitor of prostaglandin synthetase (cyclooxygenase). Delayed release dosage form give peak plasma concentrations after approximately 2.5 hours and plasma half-life for the terminal elimination phase is 1-2 hours [4]. Rabeprazole sodium is a anti-secretory compounds, the substituted benzimidazoles,20mg dose the onset of the anti-secretory effect occurs within one hour, maximum effect occurring within 2-4 hours [4]. Present study is about a formulation of delayed release bi-layer tablet of Diclofenac sodium (NSAIDs) and Rabeprazole sodium (PPIs) combination which has advantage of patient compliance over immediate release dosage forms with respect to reduced dosage regimen. Formulation of delayed release bi-layer tablet involved combining Diclofenac sodium (NSAIDs) and Rabeprazole sodium (PPIs) in a delayed release form to achieve better efficacy and prolong duration of action. Formulations were optimized to have desired adhesion characteristics for each layer and a mechanical strength so as to withstand vibration during until packaging line in future commercial production. Formulations were evaluated at each stage of processing with necessary analytical method development and subsequent stability evaluation too done. In vitro dissolution study in media simulation with pH environment of Gastrointistinal tract was done with distinct release profile and Diclofenac sodium (NSAIDs) and Rabeprazole sodium (PPIs) was found to be comparable with referance product Voltarol® 50mg Enterik Tablet, similarly Rabeprazole sodium Gastro-resistance tablet release was found to be comparable with referance product Pariet® 20mg Tablet. Invitro comparable formulation was evaluated for in-vivo Bioequivalence study in fasting and fed state which was found bioequivalent with respective reference product.

References

[1] L. Laine, The gastrointestinal effects of nonselective NSAIDs and COX-2-selective inhibitors, *Semin Arthritis Rheum.*, 32(3 Suppl 1):25–32, 2002.
[2] M. C. Hochberg, R. D. Altman, K. T. April, M. Benkhalti, G. Guyatt, J. McGowan, et al. American College of Rheumatology. American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee, *Arthritis Care Res*, 64, 465-74, 2012.
[3] J. Mukherjee, A. Das, U. S. Chakrabarty, B. Sahoo, P. Sengupta, B. Chatterjee, et al. Bioequivalence study of fixed dose combination tablet containing rabeprazole and diclofenac sodium in healthy Indian subjects, 60, 506-9, 2012.
[4] https://www.medicines.org.uk/emc/

IN VIVO PHARMACODYNAMIC STUDY OF ACECLOFENAC LOADED SNEDDS

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In this study, we aimed to determine the anti-inflammatory activity of two aceclofenac (AC) loaded oil-in-water self nanoemulsifying drug delivery systems (SNEDDSs) using carrageenan induced rat paw edema method and to investigate if there was any gastric ulcerogenic activity of aceclofenac loaded systems with the controling of petechia in the pylorus. The studies were carried out in male Wistar albino rats weighing 200-300 g. The rats were obtained from GUDAM (Gazi University Experimental Research Center). The used aceclofenac dose was 10 mg/kg [1]. The study groups were divided into 17 groups (n=6), including control and acelofenac sample groups. Each one of the sample groups was administered by oral gavage to the rats (0.5 mL sample to 100 g rat). The paw volumes were measured with a plethysmometer at 0th, 90th, 180th, 270th and 360th minutes of administration. Afterwards, acute toxic effects of the groups were investigated and for this purpose mortality presence for the rats were recorded after 48 of the study. For investigating gastric ulcerogenic effect of AC, sample groups were administered to the rats (0.5 mL sample to 100 g rat), the stomach of the rats were removed and examined for any petechia with magnifying lens. As we look at the results, it was seen that the sample control groups had no difference when compared with control-saline group, ($p>0.05$). All of the groups that include AC, showed maximum edema inhibition at 180th min. The value of inhibition in the form of SNEDDS including AC (coded as ND1+AC), was higher at all the time points compared to other groups of AC ($p<0.0001$). ND1+AC group had no ulcerogenic effect according to our findings. None of the SNEDDSs and their nanoemulsion formulations caused petechia dots. There was no acute toxic effect of AC groups and the used control groups, according to the results of mortality studies. This study indicated that the developed SNEDDS including AC, was effective and an alternative drug delivery system in medical treatment.

References

[1] C. Nagda, N. P. Chotai, U. Patel, et al. Preparation and characterization of spray-dried mucoadhesive microspheres of aceclofenac. *Drug Dev Ind Pharm*, 1155-1166, 2009.

DAIDZEIN-LOADED PLGA-GELUCIRE® 44/14 NANOPARTICLES: THE PREPARATION AND *IN-VITRO* CHARACTERIZATION

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Daidzein, structurally or functionally mimic mammalian estrogens, is a water-insoluble natural isoflavone and has low oral bioavailability. It is found in Leguminosae and certain Traditional Chinese Medicinal herbs (e.g. Kudzu). Daidzein is used in the treatment of different disease (hypertension, cerebral thrombosis, menopause syndrome and coronary heart disease). Recent studies showed that daidzein could prevent the onset of diabetes and inhibit the growth of cancer cells. Thus, daidzein is a promising agent for multipurpose treatment [1, 2]. Gelucire® 44/14 (a mixture of *glycerides* and *PEG* esters) as inert semi-solid waxy amphiphilic excipient has been studied extensively for increasing solubility and enhancing oral bioavailability of poorly water-soluble drugs [3]. Besides, Gelucire® 44/14 as a co-surfactant can be useful to *reduce* the particle *size of nanoparticles (NPs)*. The aim of this study was to prepare and characterize daidzein-loaded PLGA 75:25-Gelucire® 44/14 NPs. The NPs were prepared using a mixture of DCM and EA (1.5:1) containing daidzein, PLGA 75:25 and Gelucire® 44/14 as *organic phase* and 1% (w/v) of PVA solution as aqueous phase. The results of characterization studies were given in Table 1. In phosphate buffer pH 7.4, about 50% and 90% of daidzein were released from NPs within 7 days and 24 days, respectively. PLGA-Gelucire® 44/14 NPs might be useful systems for the sustained release of daidzein, enhancing its bioavailability and also providing the use in the treatment of different diseases (This study was supported by TUBITAK 115S085)

Table 1. The results of characterization studies.

	EE% (n=3)	DL% (n=3)	Zeta Potential (mV) (n=3)	Particle Size (nm) (n=6)
Mean	84.85	4.20	-14.7	409.12
SD	2.20	0.12	0.36	51.69

EE: Encapsulation efficiency; DL: drug loading; SD: standard deviation.

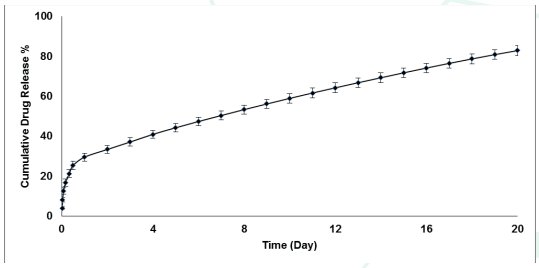

Fig. 1. In vitro release profile of daidzein-loaded nanoparticles (Mean±SD, n=3).

References

[1] Y. Gao, W. Gu, L. Chen, Z. Xu, Y. Li, The role of daidzein-loaded sterically stabilized solid lipid nanoparticles in therapy for cardio-cerebrovascular diseases, *Biomaterials*, 4129-36, 2008.
[2] Y.Ma, X.Zhao, J. Li, Q. Shen, The comparison of different daidzein-PLGA nanoparticles in increasing its oral bioavailability. *Int J Nanomedicine*, *7*, 559–70, 2012.
[3] A. Karataş , N. Yüksel , T. Baykara, Improved solubility and dissolution rate of piroxicam using gelucire 44/14 and labrasol, *Farmaco*. 60(9):777-82, 2005.

DESIGN AND EVALUATION OF ORODISPERSIBLE TABLETS (ODTs) CONTAINING METFORMIN HYDROCHLORIDE USING LYOPHILIZATION TECHNIQUE

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Metformin HCl (MHCl) is widely used for the treatment of type 2 diabetes, and has a relatively low oral bioavailability (BA;~50-60%) [1]. ODTs are becoming popular due to their advantages (e.g. pleasant taste, rapid drug dissolution, increasing bioavailability of active substances due to pre-gastric absorption and ease of administration), and have significant impact on the patient compliance. The EP defines ODTs as having a disintegration time of less than 3 min, however, the disintegration time recommended by FDA is approximately 30 sec or less (when placed upon the tongue) [2]. The aim of this study is to prepare MHCl-ODTs by *lyophilization technology* using gelatin as a matrix former (Table 1). The results of control tests and digital photograph of ODTs were given in Table 2 and Fig.1. The percent weight variation of the formulation was within the pharmacopoeial limits (EP-7). The preferable strength of the ODTs is ranged of 30-80 N [3]. Mannitol can affect the hardness of ODT (ODTs with higher hardness value can be prepared using the higher the amount of mannitol in the formulation) [4]. Friability study showed that the formulation prepared with gelatin:mannitol (1:3) was non-friable and the friability value (FV) was less than 1% (USP 30-NF 25). Shoukri et al. [5] reported that ODTs formulated with the high amounts of gelatin (2% and 3%) were non-fragile (FV=<1%). Generally, the time for disintegration of ODTs is less than 1 min. 81.82±4.93% of the MHCl is dissolved within 4 minutes in 900 mL of PB at pH 6.8 (37±2 ºC) (Fig. 2). Lyophilized MHCl-ODTs were successfully prepared using gelatin. ODTs might be useful in increasing the BA of MHCl (This study was supported by Ataturk University Research Foundation, 2014/39).

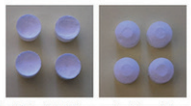

Fig.1. The digital photograph of formulation

Table. 1. Compositions of ODT formulations

Formulation	Amount (mg)					
	Metformin HCl	Gelatin	Mannitol	Pluronic F68	PEG 4000	Distilled Water
	125	15	45	7.5	7.5	400

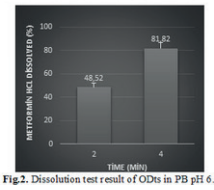

Fig.2. Dissolution test result of ODts in PB pH 6.8

Table 2. The results of ODTs control tests (Mean±SD)

Evaluation of ODTs	Formulation
Weight variation (g)	0.204±0.001
Hardness (N)	58.09±1.21
Thickness (mm)	6.98±0.12
Friability (%)	0.50±0.26
Drug Content (%)	104.73±6.40
Disintegration Time (sec)	23.28±3.06
Water Absorption Ratio (%)	36.29±1.12
Dispersion Time (sec)	88.56±4.49

References

[1] B. Viollet, B. Guigas, et al. Cellular and molecular mechanisms of Metformin: an overview, *Clin Sci,* 253-70, 2012.
[2] P. Arora, V.A. Sethi, Orodispersible tablets: a comprehensive review, *IJRDPL,* 270-84, 2013.
[3] L. Hu, D. Gu, Q. et al. *Anal Acta* 4: 216. doi:10.4172/2153-2435.1000216.
[4] K.J. Tanuwijaya, D. Fatma, Formulation of ibuprofen orally disintegrating tablets (ODTs) by lyophilization method using gelatin and mannitol, *Int.J. PharmTech Res,* 996-1002, 2014.
[5] R.A. Shoukri, I.S. Ahmed IS, et al, In vitro and in vivo evaluation of nimesulide lyophilized orally disintegrating tablets, *Eur J Pharm Biopharm,* 162-71, 2009.

PROLIFERATION PROPERTIES OF RESVERATROL ORGANOGELS PREPARED BY MICROWAVE IRRADIATION

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Resveratrol is a polyphenolic compound useful for the proliferation of fibroblasts, and improvement of the skin structure. However it has low water solubility and to provide its stability against environmental factors is very difficult in various formulations [1,2]. In this study, polyethylene glycol (PEG) organogels of 50 mM resveratrol were prepared with Carbopol 974 at concentrations of 1%, 2%, 3% and 4%. Carbopol 974 was dispersed in 25 ml of PEG (200, 400, 600) separately, and the dispersion was homogenized (Ultra-Turrax Ika T25) for 5 min at 24000 rpm. Then it was poured into glass Petri dishes and exposed to micro-irradiation (Arcelik MD574; 1,200 W/1 h) for 2 min [3]. Differential Scanning Calorimetry (DSC) and rheological analyses was performed. After the optimization studies the most proliferative formulation was chosen by using normal human dermal fibroblast cells for wound models (CytoSelectTM). The proliferation effect was detected with the DAPI and Giemsa stain models and microscopic (Cell Biolabs San Diego, USA) observations.

DSC data revealed that resveratrol was dissolved in organogels. The formulations were shear thinning systems and gelation occurred over 2%. All of the organogels including resveratrol has more migration activity than control groups. The percentages of covering the open areas of the organogels prepared with 3% Carbopol in PEG-400, PEG-200 and PEG-600 are %314, %150.3 and %186.1, respectively. The maximum wound closure rate was determined with PEG 400. This study demonstrated that organogels of resveratrol prepared with 3% Carbopol 974 in PEG 400 showed high rates of migration activity. The developed formulation can be available for different uses such as pharmaceuticals and cosmetics.

(This study was supported by TUBITAK 2209-A research grant 1919B011501311).

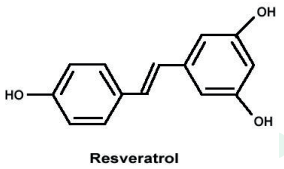

Figure 1. The chemical structure of trans-resveratrol

References

[1] M. Abril, A. I. Negueruela, C. Perez, T. Juan, G. Estopanan, Preliminary Study Of Resveratrol Content İn Aregon Red And Rose Wines, *Food Chemistry*, 92; 729-736, 2005.
[2] İ. Eroğlu, E. H. Gökçe, N. Tsapis, S. Tuncay Tanrıverdi, G. Gökçe, E. Fattal, Ö. Özer, Evaluation of characteristics and in vitro antioxidant properties of RSV loaded hyaluronic acid-DPPC microparticles as a wound healing system, *Coll & Surf B.* 126: 50-57, 2015.
[3] Gökçe E.H., Yurdasiper A., Korkmaz E., Özer Ö. A novel preparation method for organogels: high speed homogenization and microirradiation, *AAPS Pharm. Science and Tech.,* 63:517-529, 2013.

DESING AND EVALUATION OF TELMISARTAN PRESS-COATED TABLETS FOR PULSATILE DRUG DELIVERY

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Controlled release drug delivery systems are designed to provide constant blood drug levels regardless of diurnal physiological and biological variations of the human body, which called circadian rhythm. But this constant blood drug levels created by controlled release drug delivery systems can cause problems such as resistance, tolerability, and drug side effects. On the other hand, some diseases, which show circadian variation, needs to synchronize effective drug levels to body's circadian rhythm so that it can improve drug efficacy, and reduce the toxicity [1].

Hypertension is a chronic disorder affecting up to 35% of human adults. Blood pressure displays appreciable predictable-in-time circadian variation. The chronotherapy of hypertension takes into account the clinically relevant features of the 24-h pattern of blood pressure, e.g. the accelerated morning rise and night time decline during sleep, plus potential administration circadian time determinants of the pharmacokinetics and dynamic of antihypertensive medications. The administration of the antihypertensive drug at appropriate timing is more important in treating patients with resistant hypertension than changing the drug combination to control blood pressure and revert to normal blood pressure pattern. Pulsatile drug delivery system are gaining importance as these systems deliver the drug at specific time as per the pathophysiological need of the disease, resulting in improved patient therapeutic efficacy and compliance [2].

The purpose of this study was to enhance aqueous solubility of telmisartan by preparing solid dispersions with Pluronic® F68 as carrier and to develop press-coated tablets for pulsatile drug delivery of telmisartan. The oral press-coated tablet was developed to achieve the time-controlled disintegrating or rupturing function with a distinct predetermined lag time. Press-coated tablet containing telmisartan and other excipients in the inner core was formulated with an outer shell by different weight ratio of hydrophobic and hydrophilic polymers. In this study, the influence of the type and amount of polymers in outer shell on the time-lag and rupturing function of press-coated tablet was investigated. The core tablet, prepared by a direct compression method, was designed to dissolve quickly. The lag time and time-controlled release behavior of telmisartan from press-coated tablets could be modulated by changing the type of polymers in outer coating and the type of excipients in core tablets.

References

[1] S. Y. Lin, Y. Kawashima, Current status and approaches to developing press-coated chronodelivery drug systems, *Journal of Controlled Release*, 331–353, 157, 2012.
[2] R. C. Hermida, D.E. Ayala, F. Portaluppi, Circadian variation of blood pressure: The basis for the chronotherapy of hypertension, *Adv. Drug Del. Rev.*, 904-922, 2007.

INVESTIGATION ON THE COMPLEXATION OF QUERCETIN WITH METHYL-*β*-CYCLODEXTRIN

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Quercetin (Qu) has many biological activities such as antitumor activity, cardiovascular protection, anti-inflammatory, estrogenic and antiallergy activity and Qu act as an effective radical-scavenger against oxidative cell damage [1]. In spite of being so effective in a wide range, its use in pharmaceutical field is limited by its low aqueous solubility. The purpose of this study was to enhance aqueous solubility, dissolution rate and antioxidant activity of Qu by complexation with methyl-β-cyclodextrin (M-β-CD). The inclusion compounds were prepared by modified co-lyophilisation (L1), lyophilisation (L2) and evaporation (L4) methods, using a molar ratio Qu/M-β-CD, 1:1. The quantity of Qu was determined by HPLC method. The characteristics of the complexes were evaluated by DSC, XRD, ^{1}H-NMR, FT-IR, SEM, encapsulation efficacy, *in-vitro* dissolution rate analyses. Dissolution experiments studied in 50 ml pH 1.2 by adding pure Qu (1 mg), physical mixture or the complexes containing an equivalent amount of Qu. Antioxidant activity of the pure Qu and complexes were evaluated *in vitro* by DPPH assay [2]. The drug-entrapment efficiency were found 52.33 %, 96.22 % and 90.99 % for L1, L2 and L4 formulation respectively. Analyses results demonstrated that formation of complexation strongly increases the aqueous solubility of Qu at 25°C. The results of characterization studies suggest that the Qu molecule was located inside the M-β-CD cavity and, consequently a Qu/ M-β-CD inclusion complexes was formed. The pure Qu and physical mixture showed a low dissolution rate with 36 % and 30 % drug dissolved within 120 min respectively. On the other hand, 72 % and 70 % of drug was released within 5 min for L1 and L4 complexes respectively. According to results of DPPH studies, scavenging effect of Qu increased with an increased Qu concentration. It was noted that the antioxidant activity of Qu was influenced by M-β-CD. The highest antioxidant activity were observed in L2 complex (62.11 % for 1 mg Qu concentration and 80.74 % for 5 mg Qu concentration).

References

[1] Z. Zhang, S. Gu, Y. Ding, M. Shen, L. Jiang, Mild and novel electrochemical preparation of β- cyclodextrin/graphene nanocomposite film for super-sensitive sensing of quercetin, *Biosens. Bioelectron.,* 57, 239-244, 2014.
[2] A. Kumari, S. Kumar Yadav, Y.B. Pakade, B. Singh, S. Chandra Yadav, Development of biodegradable nanoparticles for delivery of quercetin, *Colloids Surf., B,* 80, 184-192, 2010.

Acknowledgements

This study was financed by Anadolu University Scientific Research Project Foundation (No: 1502S059). We thank, Anadolu University, Plant, Drug and Scientific Research Center (AUBIBAM) for ^{1}H-NMR analyses.

EVALUATION OF IN VITRO DISSOLUTION CHARACTERISTICS OF NON-STEROIDAL ANTIINFLAMMATORY DRUGS IN THE TURKISH DRUG MARKET-1 : FLURBIPROFEN, A BCS CLASS IIa DRUG

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Some BCS Class IIa weak acidic compounds, particularly the small molecule NSAIDs, can dissolve quickly and behave like BCS Class I drugs at intestinal pH (6.5-7.0) in the GI tract, even though they exhibit low solubility at acidic pH. These drugs are potential canditates for biowaiwers. Flurbibuprofen is a BCS Class IIa drug which has a low solubility below its pK_a value (4.22) and has an increasing solubility with higher pH values [1].The aim of this study was to compare in vitro dissolution behaviors of innovator (IN), Majezik®, and six generic (GE) products, containing 100 mg flurbibuprofen, available on the Turkish Drug Market. The dissolution profiles comparisons were done at compendial dissolution media (pH 4.5 and pH 6.8) and biorelevant media. Dissolution tests were carried out at 37 ± 0.5ºC in 900 mL dissolution media at 50 rpm by USP 2 Paddle Apparatus. Samples were taken at 5, 10, 15, 20, 30, 45 and 60 min time intervals, filtered through 0.45 µm filters. The samples in compendial dissolution media pH 4.5 and pH 6.8 were analyzed by UV-spectrophotometry at 246 nm. The samples in biorelevant media were analyzed by a HPLC system with a UV detector at 247 nm to avoid the peak integration of the dissolution components and the active substance. Data were assessed by model-independent and model dependent methods. The similarity factor (f_2) was determined. DDsolver® kinetic program [2] was used for model-dependent evaluations. It was observed that the dissolution profiles of all GE products were found to be similar to the IN product in pH 6.8. The IN *vs* the GE3 gave similar dissolution curves with f_2>50 for FaSSIF and FeSSIF (Table 1). Cumulative dissolved values reached ~100% in FaSSIF and FeSSIF for all products. It was determined that, flurbiprofen solubility was affected by pH value and also concentrations of sodium taurocholate and lecithin. Dissolution kinetics were evaluated with DDsolver® which were mainly fit to Gombertz Model for FaSSIF and Logistic Model for FeSSIF.

Table 1. Comparison of f_2 values for IN *vs* GEs products

	pH 4.5	pH 6.8	FaSSIF	FeSSIF
IN *vs* GE1	39	57	48	48
IN *vs* GE2	46	57	48	56
IN *vs* GE3	32	58	62	52
IN *vs* GE4	43	69	49	42
IN *vs* GE5	55	64	49	57
IN *vs* GE6	51	58	64	47

References

[1] Y. Tsume, D. Mudie, P. Langguth, GE. Amidon, GL. Amidon, Biopharmaceutics Classification System: Subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC, *E.J.Pharm.Sci,* 57, 152-163, 2014.
[2] Y. Zhang, M. Huo, J. Zhou, A. Zou, W. Li, C. Yao, S. Xie, DDSolver: An add-in program model modeling and comparison of drug dissolution profiles, *AAPS J.*;12, 263-271, 2010.

EVALUATION OF IN VITRO DISSOLUTION CHARACTERISTICS OF NON-STEROIDAL ANTIINFLAMMATORY DRUGS IN THE TURKISH DRUG MARKET-2 : IBUPROFEN, A BCS CLASS IIa DRUG

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BCS Class II drug product dissolution *in vivo* and *in vitro* is highly dependent on the acidic or basic nature of the drug, the drug solubility and formulation factors, in addition to the *in vivo* luminal environment. Recently, it is suggested a BCS Class II sub-classification with a, b, and c subclasses dependent on the acidic (a), basic (b), or neutral (c) characteristics of the drug in the physiological pH range (~pH < 7.5). Some BCS Class IIa weak acidic compounds, particularly the small molecule NSAIDs, can dissolve quickly and behave like BCS Class I drugs at intestinal pH (6.5-7.0) in the GI tract. Ibuprofen is a BCS Class IIa drug which has a low solubility below its pK_a value (4.5) and has an increasing solubility with higher pH values [1]. The aim of this study was to compare in vitro dissolution behaviors of innovator (IN), Brufen®, and two generic (GE) products, containing 600 mg ibuprofen, available on the Turkish Drug Market. The dissolution profiles comparisons were done at compendial dissolution media (pH 4.5 and pH 6.8) and biorelevant media. Dissolution tests were carried out at 37 ± 0.5ºC in 900 mL dissolution media at 50 rpm by USP 2 Paddle Apparatus. The samples in compendial dissolution media pH 4.5 and pH 6.8 were analyzed by UV-spectrophotometry at 222 nm and 264 nm, respectively. The samples in biorelevant media were analyzed by a HPLC system with a UV detector at 235 nm to avoid the peak integration of the dissolution components and the active substance. Data were assessed by model-independent and model dependent methods. The similarity factor (f_2) was determined. DDsolver® kinetic program [2] was used for model-dependent evaluations. It was observed that the dissolution profiles of IN and GE1 products were similar in all dissolution media. However, IN and GE2 did not give similar dissolution curves with f_2=48 for FaSSIF (Table 1). Cumulative dissolved values were > 90% in FaSSIF and FeSSIF for all products. It was determined that, ibuprofen solubility was affected by pH value and also concentrations of sodium taurocholate and lecithin. Dissolution kinetics were mainly fit to Hopfenberg Model for pH 6.8 and Gompertz Model for FaSSIF and FeSSIF.

Table 1. Comparison of f_2 values for IN *vs* GEs products

	pH 4.5	pH 6.8	FaSSIF	FeSSIF
IN *vs* GE1	72	76*	69	55
IN *vs* GE2	91	54*	48	56

**Since both IN and GE products dissolved > 85 % of drug in 15 minutes the profile comparison with an f_2 test is unnecessary.*

References

[1] Y. Tsume, D. Mudie, P. Langguth, GE. Amidon, GL. Amidon, Biopharmaceutics Classification System: Subclasses for in vivo predictive dissolution (IPD) methodology and IVIVC, *E.J.Pharm.Sci,* 57, 152-163, 2014.
[2] Y. Zhang, M. Huo, J. Zhou, A. Zou, W. Li, C. Yao, S. Xie, DDSolver: An add-in program model modeling and comparison of drug dissolution profiles, *AAPS J.*;12, 263-271, 2010.

IN VITRO & IN VIVO EVALUATION OF PECTIN BEADS CONTAINING DIFLUNISAL

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Pectin gel beads have been developed in recent years as a unique vehicle for oral drug delivery due to their excellent biocompatibility, biodegradability, simple method of preparation, low cost and minimal processing requirements. The objectives of this study were to evaluate the drug-polymer interaction in pectin-alginate beads containing diflunisal and then, to determine anti-inflammatory effect of beads as well as its influence on gastric mucosa. Diflunisal loaded pectin beads were successfully prepared by ionotropic gelation. The pectin beads were characterized by size, Scanning Electron Microscopy (SEM), weight uniformity and drug entrapment efficiency. The existence of a possible interaction between diflunisal and the grafted co-polymers used was investigated by Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD) and Fourier Transform Infra-Red (FTIR) analysis. Drug loaded beads were spherical to oval in shape with relatively low drug entrapment efficiency. The drug was found to be present inside the beads as crystalline to semicrystalline form with no significant physical or chemical interaction between drug and excipients. Anti-inflammatory activity of encapsulated beads was determined on Wistar albino rats (n=6/group) employing paw aedema test in comparison to plain drug. Encapsulated beads showed similar therapeutic activity. In order to determine whether encapsulation reduces gastric side effects of diflunisal, beads were administered to rats orally. Tryphan blue solution was injected into tail vein. 24 hours after injection, animals were sacrificed and stomach lesions were examined microscopically. Less gastric lesion was determined following the administration of encapsulated diflunisal. The results implied that pectin-alginate beads can be used as a suitable enteric dosage form for diflunisal.

References

[1] F. Castelli, P. Giunchedi, O. La Camera, U. Conte, *Drug Delivery,* 7, 45-53, 2008.

PREPARATION, CHARACTERIZATION, AND IN VIVO EVALUATION OF SOLID LIPID NANOPARTICLES CONTAINING DICLOFENAC AFTER INTRAVITREAL ADMINISTRATION

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For improvement of drug availability after intravitreal administration, Solid Lipid Nanoparticles (SLNs) containing diclofenac as a novel drug delivery system was prepared in the present study. These nanoparticles were compared with conventional formulation in animal model after intravitreal injection. In this experimental study, 18 albino rabbits were included. In right eyes of all rabbits, SLNs of diclofenac (0.3 mg drug) were injected intravitreally. On the left eyes of the rabbits, commercial form of diclofenac (0.3 mg drug) was injected. Rabbits were categorized into five main groups based on the time of sampling. One, four, twelve, twenty four and forty eight hours after injection, vitreous and aqueous humor samples were obtained in all cases. Concentration of diclofenac sodium was evaluated in all samples using HPLC method. Size of nanoparticles was around 170 nm after preparation. Drug concentration in eyes injected by SLNs was significantly higher than left eyes injected by commercial product up to 4 hours after intravitreal injection ($P<0.05$). Diclofenac was quantified in samples up to 48 hours after intraocular injection. Four hours after intravitreal injection, concentration of diclofenac in vitreous and aqueous humor of eyes received SLNs was respectively 2.5 and 6.5 times higher than eyes injected by commercial form of drug. Also, we found that although the drug was injected intravitreally, it has been found in the aqueous humor in a significant concentration. Here we demonstrate the potential of SLNs as a carrier of diclofenac for intraocular injection in order to prevent the systemic effects of the drug, increase the injection intervals and improvement of patient compliance (This study was supported by Mashhad University of Medical Sciences research grant 911039).

LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF ESCITALOPRAM IN HUMAN URINE

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Escitalopram oxalate (ESC) is an orally administered highly selective serotonin-reuptake inhibitor, developed for the treatment of depression and anxiety disorders [1]. ESC is metabolized to S-demethylcitalopram (S-DCT) and S-didemethylcitalopram (S-DDCT). In humans, unchanged ESC is the predominant compound in plasma. Following oral administration of ESC, the fraction of drug recovered in the urine as ESC and S-demethylcitalopram (S-DCT) is about 8% and 10%, respectively [2]. The main objectives of this work were to determine the drug in pharmaceutical dosage form and spiked ESC in human urine consequently, a new method of a liquid chromatography/tandem mass spectrometry (LC-MS/MS) was developed. The method involved liquid–liquid extraction of the analyte from human urine after alkalization with ammonium hydroxide then extraction with hexane. The absolute recovery of spiked ESC from urine was more than 70%.The chromatographic separation was achieved within 3.36 min by using mobile phase consisted of a binary gradient elution profile comprising 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Zorbax Eclipse C18, 50 mm x 2.1mm analytical column; the flow-rate was 0.35 ml/min. The mass transitions were selected according to their stabilities and intensities. As precursor ion, the protonated molecular ion [M+H] was observed at 325.0 m/z. The transition of 325.0 109.0 m/z was selected as quantifier and 325.0 262.0 m/z as qualifier. The developed method was validated according to the ICH Q2(R) 1 guideline. The method was linear over the range of 0.1-500 ng/ml with 1/x weighted regression coefficient of 0.99878.The LLOQ by using CV% was found to be 0.1ng/mL. The proposed procedure was successfully applied for determination of ESC in urine and different pharmaceutical dosage forms.

References

[1] W. J. Burke, “Escitalopram.,” *Expert Opin. Investig. Drugs*, vol. 11, no. 10, pp. 1477–1486, 2002.
[2] Forest Laboratories Inc. Lexapro® (escitalopram oxalate) tablets and oral solution: US prescribing information [online]. Available from URL: http://www.frx.com/pi/ lexapro_pi.pdf [Accessed 2015 Aug15].

SPECTROFLUORIMETRIC DETERMINATION OF ATENOLOL FROM HUMAN URINE USING MOLECULARLY IMPRINTED SOLID-PHASE EXTRACTION

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In this study, a molecularly imprinted solid-phase extraction (MISPE)-spectrofluorimetric approach, which shows desirable sensitivity and selectivity for ATE determination in human urine, is reported. ATE is effective in the treatment of hypertension and in the prophylactic management of angia [1]. Molecularly imprinted polymers (MIPs) for the recognition of ATE were synthesized using a non-covalent molecular imprinting technique. During the synthesis of MIPs, acrylic acid, ethylene glycol dimethacrylate, dibenzoyl peroxide and dichloroethane were used as a functional monomer, crosslinker, initiator and porogen, respectively. Non-imprinted polymers were also prepared using the same procedure but in the absence of ATE. Calibration curve was in the range of 0.1-2.0 µg/mL. LOD and LOQ values were found to be 0.032 and 0.099 µg/mL, respectively. Moreover, relatively low within-day (0.38-1.03%) and between-day (0.47-2.05%) precision values were obtained. The developed MISPE-spectrofluorimetric method was successfully applied to the determination of ATE in human urine.

References

[1] R.C. Heel, R.N. Brogden, T.M. Speight,. Atenolol: A Review of its Pharmacological Properties and Therapeutic Efficacy in Angina Pectoris and Hypertension, *Drugs*, 425-460, 1979

DETERMINATION OF MONOSODIUM GLUTAMATE LEVELS BY HPLC METHOD IN INSTANT SOUPS MARKETED IN ANKARA

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The aim of this study is to determine monosodium glutamate (MSG) amounts in instant soups marketed in Ankara and to evaluate those levels' convenience with Turkish Food Codex. In this research, total number of 100 instant soup samples regarding 5 different firms (A, B, C, D, E) with particular series numbers and each of them collected from markets in various districts of Ankara, were analyzed. In order to determine the levels of MSG in instant soup samples, high pressure liquid chromatography (HPLC) method was used. The samples were analyzed with fluorescence detector and derivatized with 5-dimethylamino-naphthalene-1-sulfonyl chloride (dansyl chloride). The average MSG amounts of the samples regarding A, B, C, D and E firms were detected as 22,72±4,51 g/kg, 4,17±1,23 g/kg, 1,14±0,49 g/kg, 6,63±2,03 g/kg and 35,97±2,29 g/kg respectively. According to the results of the study, while the determined MSG amounts of B, C and D firms were under the limit of the codex value which is 10 g/kg, the detected MSG levels of A and E firms exceeded the limit value of the codex. Especially, in the case of going over the limit values, MSG could create health risks. Thus, it should not be used and added to foods in an insensible way. Therefore, it is important to investigate commonly consumed foods in terms of MSG levels. (This study was supported by Turkish Higher Education Council and Gazi University OYP program, Ankara, Turkey)

L-CYSTEINE CAPPED MN-DOPED ZnS QUANTUM DOTS *AS A* ROOM TEMPERATURE PHOSPHORESCENCE SENSOR FOR *IN-VITRO* BINDING ASSAY OF IDARUBICIN AND DNA

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L-cysteine capped Mn doped ZnS quantum dots (QDs)/Idarubicin (IDA) nanohybrids were used as novel room temperature phosphorescence (RTP) sensor to detect double stranded deoxyribonucleic acid (ds-DNA)/drug interaction. IDA, anthracycline derivative anticancer drug, was adsorbed on the surface of the QDs as an electron acceptor to quench the RTP emission. The RTP intensity of QDs was quenched quickly upon addition of quencher and the reaction reached equilibrium within 2 min. The quenching mechanism of phosphorescence of Mn-doped ZnS QDs by IDA is combined dynamic and static quenching. The static and dynamic quenching constants were found as 1.1×10^{5} M^{-1} and 8.7×10^{4} M^{-1}, respectively. The addition of ds-DNA caused formation of ds-DNA/IDA complex and recovered the RTP signal of Mn-doped ZnS QDs, which allowed qualitative analysis. Under optimal conditions, RTP intensity of QDs/IDA nanohybrids increased linearly with the concentration of ds-DNA from 1.2 to 6.0 µM. This method is simple, low cost and avoids from some interferences such as autofluorescence, some cation. The illustrated results on binding mode of IDA and ds-DNA would provide further information on the mechanism of anticancer drugs' binding with DNA, and they will benefit the designing of new drugs.

DETERMINATION OF TOTAL ARSENIC IN MEAT SAMPLES BY ATOMIC FLUORESCENCE SPECTROMETRY

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Inorganic arsenic is an important natural environmental contaminant to which humans are routinely exposed and causes cancer and possibly other adverse health outcomes such cardiovascular and neurological diseases and diabetes. The adverse health effects of arsenic depend strongly on the dose, species and duration of exposure [1]. Although natural contamination of drinking water with inorganic arsenic is the primary source of environmental exposure, organoarsenical compounds used in food-animal production are of a growing concern [2,3]. Until recently chicken producers in several countries in Latin America, USA and China were routinely supplement poultry feed with arsenical drugs such as roxarsone, (3-nitro-4-hydroxyphenylarsonic acid) which also treats intestinal parasites in birds [3,4]. Reports have indicated that the use of arsenic based-drugs contributes to dietary inorganic arsenic exposure in consumers [4]. Even though roxarsone is banned in the European Union and Turkey, to get an idea on the exposure from other potential sources, such as drinking water, there is a need for a simple and fast analytical method for routine control of arsenic in meat samples. A few studies have determined total arsenic concentrations in the tissues of chickens. In this study, total arsenic was determined in lamb, beef, chicken thigh, chicken wings and turkey meat using hydride generation atomic fluorescence spectrometry. The samples (n= 31) were obtained from local markets in Ankara and digested using HNO_3, $HClO_4$, and H_2SO_4 acid mixture. Arsenic concentrations were between 1.06-16.06 ng/g As and all the samples were well below the FDA tolerances for arsenic residues (500 ng/g) in poultry meat [3]. Accuracy of the sample preparation and analysis were checked using a certified reference material (CRM) namely, DOLT 3 (Dog fish liver, NRC, Canada). The arsenic concentration in the CRM was found as 10.3 ± 0.4 µg/g, while certified arsenic concentration in CRM was reported as 10.2 ± 0.5 µg/g. The proposed method required simple and inexpensive instrumentation with a detection limit of 0.3 ng/g As.

References

[1] International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans. Arsenic, Metals, Fibres, and Dusts. Volume 100 C: A Review of Human Carcinogens. Lyon, France: International Agency for Research on Cancer, 2012.
[2] A.R. Sapkota, L.Y. Lefferts, S. McKenzie, P. Walker, What do we feed to food-production animals? A review of animal feed ingredients and their potential impacts on human health, *Environ. Health Perspect.*, 115, 663-670, 2007.
[3] E. K. Silbergeld, K. Nachman, The environmental and public health risks associated with arsenical use in animal feeds, *Ann. NY Acad. Sci.*, 1140, 346-357, 2008.
[4] K. E Nachman, P.A. Baron, G. Raber, K. A. Francesconi, A. Navas-Acien and D. C. Love, Roxarsone, inorganic arsenic, and other arsenic species in chicken: A U S based market basket sample, *Environ. Health Perspect.,* 121(7), 818-824, 2013.

A RAPID AND SIMPLE METHOD FOR THE DETERMINATION OF TRIPHENYLMETHANE DYE RESIDUES IN CULTURE FISH BY COMBINING A SIMPLE LIQUID-LIQUID EXTRACTION (LLE) WITH PSEUDO ISOTACHOPHORESIS STACKING TECHNIQUE

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Because of low price and easy to use, malachite green (MG) and crystal violet (CV), the triphenylmethane dyes are commonly used in the prevention and control of fungus diseases in fish. They have also been used as a disinfectant to extend the survival time of fish in the transport process [1]. In this study a simple and rapid method was developed for extraction, preconcentration and quantification of ultratrace residues of triphenylmethane dyes in culture fish samples using a new developed simple extraction procedures followed by capillary electrophoresis (CE) determination. Pseudo Isotachophoresis (p-ITP) stacking technique was used for the separation and online concentration of MG, CV and their leuco metabolites. The separation was performed using a leading electrolyte of 1.80M acetate, 30mM phosphate and 25% acetonitrile at the pH of 3.5. Electrokinetic sample injection (12kV, 50s) followed by pressure injection from BGE (50mbar, 5s) was used. Separation voltage of 17kV and cassette temperature of 17 ^{o}C was chosen as optimum values. The detection wavelengths were 590nm for MG and CV and 214nm for the leuco metabolites. The intra- and inter-day variability (RSD) of peak areas were in the range of 0.81–4.61% and 3.81-8.72%, respectively. The limit of detection (LOD) (S/N = 3) of MG, CV, LMG and LCV were 2.3, 1.0, 1.6 and 3.2 ng/g, respectively. The recoveries of MG, CV, LMG and LCV in the extracts of culture fish were 80.2-102.9% with RSD values less than 3.9. The MG, CV, LMG and LCV in spiked (10 ng.g^{-1}) culture fish were successfully determined with satisfactory repeatability and recovery. This method required simple and inexpensive devices and relatively small volumes of organic solvents, so it is a handy, efficient, and convenient method for extraction and determination of ultratrace amounts of the triphenylmethane dyes in culture fish samples.

References

[1] D. J. Alderman, Malachite green: A review. *J. Fish Dis*. 8: 289-298, 1985.

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION PRIOR TO CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF PARABENS IN PERSONAL CARE PRODUCTS, HUMAN URINE AND SALIVA

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Parabens are extensively employed as preservatives in personal care products due to their low toxicity, neutral pH, lack of perceptible odor and thermal stability [1]. However, a controversy surrounding their use has been mounting since 2004 when five of them were found in human breast cancer tissues [2]. DLLME (Dispersive liquid-liquid microextraction) has drawn much attention as an outstanding technique that offers simple and cheap extraction of analytes within a very short time [3]. In this study, DLLME was combined with CE (capillary electrophoresis) for the determination of four parabens [i.e., methyl- (MP), ethyl- (EP), propyl- (PP) and butylparaben (BP)]. The analytes were separated at 12 °C and 25 kV with a background electrolyte of 25 mM borate buffer at pH 9.2 containing 5.0% (v/v) acetonitrile. Optimum extraction conditions were as follows: 200 µL chloroform (extraction solvent), 1.0 mL acetonitrile (disperser solvent) and 1 min extraction time. Back-extraction of parabens from chloroform into a 50 mM sodium hydroxide solution within 10 s extraction time facilitated their direct injection into CE. Enrichment factors were in the range of 4.3-13.1 and limits of detection ranged from 0.03 to 0.32 µg mL^{-1}. Calibration graphs showed good linearity with coefficients of determination (R^2) higher than 0.9911 and %RSD (relative standard deviations) lower than 5.1%. DLLME-CE was demonstrated to be a simple and rapid method for preconcentration, separation and determination of parabens in personal care products, human urine and saliva with relative recoveries in the ranges of 83.0-108.8%.

References

[1] M. G. Soni, I. G. Carabin, G. A. Burdock, Safety assessment of esters of p-hydroxybenzoic acid (parabens), *Food and Chemical Toxicology,* 43(7), 985-1015, 2005.
[2] P. D. Darbre, A. Aljarrah, W. R. Miller, N. G. Coldham, M. J. Sauer, G. S. Pope, Concentrations of parabens in human breast tumours, *Journal of Applied Toxicology,* 24(1), 5-13, 2004.
[3] U. Alshana, N. Ertaş, N. G., Göğer, Determination of parabens in human milk and other food samples by capillary electrophoresis after dispersive liquid–liquid microextraction with back-extraction, *Food Chemistry,* 181, 1-8, 2015.

A NEW SORBENT FOR SOLID-PHASE EXTRACTION OF SOME TRACE ELEMENTS AND ITS APPLICATION

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Solid phase extraction is the very popular separation and preconcentration technique currently available for analytes in different matrices. The selection of an appropriate SPE extraction sorbent depends on interaction between the sorbent and analytes of interest. Thus, selection of sorbents used in solid phase extraction is very important. There are many commercially sorbents for solid phase extraction. Besides, it is used in solid phase extraction of sorbents obtained in various chemical process [1,2].

In this study, composite of Graphene-Poly (Methacrylamide-co-divinylbenzene) as sorbent for solid phase extraction procedure was synthesized. Characterization of synthesized sorbent was performed by FT-IR, thermo gravimetric analysis and SEM.

Synthesized sorbent is investigated using for proconcentration and separation of Cu(II), Cd(II), Cr(III), Pb(II), and Bi(III). Analytical parameters such as pH (2-10), sample flow rate (1-10 ml/min), eluent type (HNO_3/Acetone, HNO_3/dH_2O, HCl/Acetone, HCl/dH_2O) and sample volume (50-1000 ml) were investigated.

Optimum parameters were determined as pH 8.0, 5 ml/min, 1 M HNO_3 in acetone, 100 ml respectively. Additionally, the proposed solid phase extraction method was used for determination of trace metal ions in tap water, mineral water, sea water and pharmaceutical samples.

References

[1] F. Sabermahani, M. A. Taher. Application of a new water-soluble polyethylenimine polymer sorbent for simultaneous separation and preconcentration of trace amounts of copper and manganese and their determination by atomic absorption spectrophotometry, *Analytica Chimica Acta,* 152–156, 2006.
[2] K. Kocot, R. Sitko. Trace and ultratrace determination of heavy metal ions by energy-dispersive X-ray fluorescence spectrometry using graphene as solid sorbent in dispersive micro solid-phase extraction, *Spectrochimica Acta Part B: Atomic Spectroscopy,* 7–13, 2014.

NON-CHROMATOGRAPHIC INORGANIC ARSENIC SPECIATION ANALYSIS IN WATER SAMPLES BY HYDRIDE GENERATION ATOMIC FLUORESCENCE SPECTROMETRY

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The toxicity of arsenic depends strongly on its chemical forms. Inorganic arsenic species are more toxic than the organic ones and also inorganic trivalent (As^{3+}) forms are more toxic than the pentavalent (As^{5+}) forms [1]. Therefore, it is necessary to determine the individual species in order to estimate its environmental impact and health risks. Although a number of methods have been developed, using hydride generation method followed by analysis with an element selective detector has been the preferred technique for determination of arsenic [2]. The use of hydride generation (HG) can separate analytes from sample matrices, thereby reducing or eliminating potential chemical and spectral interferences. Atomic fluorescence spectrometry (AFS) has been used for determination of hydride-forming elements because of its high sensitivity, wide linear range, ease of use and low cost [3]. The coupling of HG with AFS has been widely reported for arsenic determination and/or speciation [1,4,5]. Arsenic speciation with HG-AFS is generally carried out by coupling a chromatographic technique such as high performance liquid chromatography (HPLC) but these hypenated techniques are tedious and time consuming. Thus, there is a need for alternative methods for arsenic speciation analysis.

A simple non-chromatographic procedure is proposed for determination of inorganic arsenic species (As^{3+} and As^{5+}) in water samples. Determination of arsenic species are carried out without using a chromatographic method and achieved by using HG-AFS with hydrogen diffusion flame. Non-chromatographic speciation of arsenic species are performed by oxidation state-specific hydride generation. In this procedure arsines from As^{3+} were generated without using a pre-reduction reagent such as thioglycolic acid or *L*-cysteine. After As^{3+} is determined, total arsenic would be determined by pumping the pre-reduction reagent to the flow system and online reduction of As^{5+} species to As^{3+} would occur. The concentration of As^{5+} is calculated by the difference between total arsenic and As^{5+} concentration, and inorganic arsenic speciation would thus achieved. The proposed method would be an alternative, fast and simple method for inorganic arsenic speciation analysis.

This study was supported by Gazi University Research Fund, Project No: 02/2015-09.

References

[1] X. Yan, X. Yin, X. He, Y. Jiang, *Anal. Chem*, 74, 2162, 2012.
[2] M. Burguera, J.L. Burguera, *Talanta*, 44, 1587, 1997.
[3] H. Sun, F, Qiao, R, Suo, L. Li, S. Liang, *Anal. Chim. Acta*, 505, 255, 2004.
[4] A.M. Featherstone, P.R. Boult, B.V. O'Grady, E.C.V. Butler, *Anal. Chim. Acta*, 409, 215, 2000.
[5] P. Cava-Montesinos, M.L. Cervera, A. Pastor, M. de la Guardia, *Talanta*, 60, 787, 2003.

DETERMINATION OF LEAD IN SOME COSMETIC PRODUCTS BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

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Lead was determined in 25 lipstick samples using continuous flow hydride generation atomic absorption spectrometry (CF-HG-AAS). The lipstick samples were digested with HNO_3: HF (7:2) at 100 °C in teflon beaker. The parameters that affect the lead absorbance signal such as the acidity, the concentrations of oxidizing and reducing agent, the flow rate of the solutions and the carrier nitrogen gas were optimized. Linear calibration range for the lead concentrations in the aqueous media at 283.3 nm is 2.5-100 µg l^{-1}. The equation of calibration curve obtained by using linear regression was found as A= 0.0059 C + 0.0049 (R^2= 0.9987) (A: absorbance, C: µg l^{-1}).The concentrations of lead in lipstick samples were found in the range of 1.68 ± 0.72 mg kg^{-1} within the confidence limits of 95%. The detection limit, and the characteristic sensitivity were found as 0.51 µg l^{-1} and 0.74 µg l^{-1}, respectively. Also, limit of quantitation (LOQ) was calculated as 1.70 µg l^{-1}. Precision of the measurements (n= 11) at 20 µg l^{-1} Pb level was 4.2 % RSD (relative standard deviation). The recovery was found as 98.14 ± 1.88 within the confidence limit of 95%.

ANALYSIS OF SOME FATTY ACIDS BY USING HEAD-SPACE GAS CHROMATOGRAPHY

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Head-space gas chromatography coupled to a flame ionization detector (HS-GC–FID) was used to determine the fatty acids such as conjugated linoleic acid, linoleic acid and linolenic acid in the lipids. It is diffucult to determine the long chain fatty acids with gas chromatography because of their polar carboxylated groups. To analyse the fatty acids by gas chromatography methylation was carried out in order to convert them to less polar methyl ester derivatives. This process was accomplished chemically with excess methanol and catalysed with sulphuric acid. Some parameters such as temperature, amount of acid, and alcohol that affect the ester yield were optimized. 60 °C, 2 % H_2SO_4, (6:1) methanol:lipid ratio were chosen as an optimum condition. Na_2SO_4 was used to remove the water from the medium. 180 °C and 30 min were used as an optimum condition in the head space sample preparation. Rt-2560 (100 m × 0.25 mm i.d., 0.20 µm thickness) column was used in gas chromatography. Sample was injected in splitless mode, inlet temperature and detector temperature were 200 °C. Oven temperature program was as follow; 50 °C (2.0 min), 20 °C/min ramp to 120 °C (0 min), 5 °C/min ramp to 160 °C (2.0 min), 5 °C/min ramp to 220 °C (20.0 min). Helium was used as a carrier gas (1 mL/min flow rate).

ADSORPTION OF ISONIAZID BY ACTIVATED CARBON

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The presence of pharmaceuticals (and their metabolites) in the environment, notably the aquatic compartment, has been a growth area in environmental chemistry [1]. Recent studies have showed that the elimination of many pharmaceutically active compounds in sewage treatment plants is often incomplete and these are directly discharged to surface waters [2,3]. Removal of pharmaceutically active compounds from secondary effluents prior to water reuse is of paramount importance, because augmentation of water supply by wastewater reclamation has recently gathered substantial momentum [4].

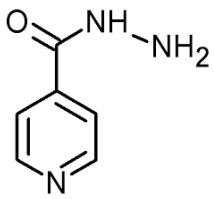

Figure 1. Chemical structure of isoniazid

In this context, the removal of isoniazid by activated carbon was studied spectrophotometrically in aqueous solutions. The adsorption experiments were carried out as a function of time, initial concentration and temperature. Adsorption kinetic data were modeled using the Lagergren first order and the pseudo-second order kinetic equations. Intraparticle diffusion graphics were also plotted. The data obtained from kinetic studies are fitted to the pseudo-second order kinetic equation better than the Lagergren first order kinetic equation. The Giles isotherms were used to understand the adsorption mechanism of isoniazid. Isotherms plotted appear to fit L-type according to Giles isotherm classification. The Langmuir and Freundlich isotherms were used to model the equilibrium data. According to experimental results both isotherm equations can be used to model the equilibrium data, but Langmuir model was better to fit the isotherm data at 298 K.

References

[1] O.A. Jones, J.N. Lester, N. Voulvoulis, Pharmaceuticals: a threat to drinking water?, *Trends in Biotechnology*, 23 (4), 163-167, 2005.
[2] R. Andreozzi, M. Raffaele, P. Nicklas, Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment, *Chemosphere*, 50, 1319-1330, 2003.
[3] M.D. Hernando, M. Mezcua, A.R. Fernandez-Alba, D. Barceló, Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments, *Talanta*, 69 (2), 334-342, 2006.
[4] L.D. Nghiem, A.I. Schafer, M. Elimelech, Pharmaceutical Retention Mechanisms by Nanofiltration Membranes, *Environ. Sci. Technol.,* 39, 7698-7705, 2005.

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION OF CAFFEINE FROM COLA SEEDS AND ITS QUANTITATION USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Caffeine is an important natural compound and recently caffeine and caffeine-containing natural sources (e.g., green tea, guarana, mate, etc.) have been added to weight loss herbal products. Seeds of cola species also contain caffeine [1]. In this study, three cola seed samples (i.e., white, red and bitter cola) from Nigeria found in markets were investigated for their caffeine content by HPLC after its extraction from the seeds using dispersive liquid–liquid microextraction (DLLME).

Since the introduction of DLLME in 2006 [2], it has gained widespread acceptance as a simple, fast and miniaturized sample cleanup and analyte preconcentration technique. Owing to its simplicity of operation, rapidity, low cost, high recovery and low consumption of organic solvents and reagents, it has been applied for the determination of a vast variety of organic and inorganic compounds in different matrices [3].

Optimum chromatographic conditions were achieved with an Agilent Eclipse XDB-C18, 4.6 mm ID x 250 mm (5 µm) column, a mobile phase of 70% (v/v) methanol in water at a flow rate of 1.0 mL min^{-1}, a temperature of 25 °C and sample injection volume of 20 µL. CF was monitored using a diode-array detector at 273 nm. Optimum DLLME conditions were as follows: 250 µL chloroform (extraction solvent), 0.5 mL methanol (disperser solvent) and a 60-s extraction time. Back-extraction of CF from chloroform into a 40% (v/v) methanol in water solution within 90 s of vortexing time enabled its direct injection into HPLC. The method's limit of detection (LOD) and limit of quantitation (LOQ) were found as 0.56 and 1.86 µg mL^{-1}, respectively. The calibration graph was linear over the range of 1.86 to 30.0 µg mL^{-1} with a coefficient of determination (R^2) of 0.9951 and %RSD lower than 3.3%. DLLME was demonstrated to be an efficient, cheap and effective for the extraction of CF from kola nuts prior to its quantitation by HPLC.

References

[1] E. Lopez-Garcia, R.M. van Dam, S. Rajpathak, W.C. Willett, J.E. Manson, F.B.Hu, Changes in caffeine intake and long-term weight change in men and women, *American Journal of Clinical Nutrition*, 83, 674-80, 2006.
[2] M. Rezaee, Y. Assadi, M. R. M. Hosseinia, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction, *Journal of Chromatography A*, 1116, 1-9, 2006.
[3] M. Saraji, M. K. Boroujeni, Recent developments in dispersive liquid–liquid microextraction, *Analytical and Bioanalytical Chemistry*, 406, 2027-2066, 2014.

EVALUATION OF THE TOXIC EFFECTS OF CYPERMETHRIN ON THE MALE REPRODUCTIVE SYSTEM

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As components which complement modern agriculture, pesticides make up an important group of weapons which have begun to be used for beneficial purposes such as combating pests and increasing production. The interest in pesticides has grown steadily, in particular because of the toxicity they produce over time both in the human being and in wildlife. In our day efforts are concentrated on developing pesticides with a higher safety index and on reducing the number of cases of pesticide related poisoning as well as of deaths caused by it. Synthetic pyrethroid insecticides are among the most commonly available to consumers today due to increased use in recent years. A consequence of the increased availability, use, and broad-spectrum applicability of pyrethroid insecticides is widespread exposure among the general population. Diet is a primary route of exposure to pyrethroids among non-occupationally exposed individuals, but they have also been measured in a high proportion of household dust samples suggesting that the home environment may also comprise a major exposure source. Synthetic pyrethroids possess hormonal activities and have been classified as endocrine-disrupting compounds (EDCs) and linked to reproductive and development impairment. In humans, several recent non-occupational studies have reported significant or suggestive associations of urinary pyrethroid insecticide metabolite concentrations with reduced sperm concentration, motility and morphology, and increased DNA damage. Experimental studies have also implicated pyrethroid insecticides in altered thyroid function although these associations remain untested in human studies. Among pyrethroids, cypermethrin is a fourth-generation synthetic and a type II pyrethroid insecticide and has been widely used to control noxious insects in agriculture, forestry, households, horticulture, and the public health. Like other insecticides, the widespread use of cypermethrin has been associated with adverse effects on non target species. Human data on altered reproductive or endocrine function ensuing from cypermethrin exposure are limited, but animal and in vitro studies indicated that some cypermethrin may have endocrine disrupting properties. Therefore, the present study was planned and executed to evaluate the reproductive toxicity of cypermethrin in human.

INVESTIGATION OF RESPIRATORY SENSITIZATION POTENCY OF DIACETYL BY DETERMINING CYTOKINE RELEASE PROFILES IN MURINE MODEL

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Exposure to chemical sensitizers in workplace settings leads to significant occupational diseases related to respiratory tract. In recent years, diacetyl, which is used as a food additive, is considered to be associated with various diseases such as bronchiolitis obliterans and asthma. Therefore diacetyl is thought to be as an important hazard for worker's health. In this study we aimed to characterize the sensitization potency of diacetyl for the purpose of its hazard evaluation. Animal welfare caring and non-radioactive *ex vivo* Local Lymph Node Assay (LLNA):BrdU-ELISA method with short term and long term dermal exposure protocols were conducted for determining the sensitization potency of diacetyl with negative control (2,4-dinitrochlorobenzene), positive control (trimellitic anhyride) and vehicle control (acetone : olive oil) groups. Due to significant role of cytokines and antibodies in immune reactions and with limited knowledge about the whole cytokine profile of diacetyl up to now, cytokine profiling and Mouse IgE test were incorporated into research. As endpoints lymphocyte proliferation, cytokine levels (interleukin 2 (IL-2), IL-4, IL-5, IL-10 and IL-13) and serum total immunoglobulin E (IgE) concentrations were measured. Lymphocyte proliferation which was induced with a concentration of 10% (w/v) of diacetyl was not statistically significant relatively to the stimulation of vehicle control neither after short term nor long term exposure. After short term dermal exposure to diacetyl, primary T helper 1 cytokine IL-2 and T helper 2 cytokines IL-4 and IL-13 levels were significantly increased relatively to vehicle control, whereas such significant increases were not observed in long term exposure. According to our measurements of IgE levels after both exposure models, diacetly led to significant increase in serum IgE levels relatively to vehicle control. In conclusion we suppose that the use of cytokines and serum total IgE concentration as end points is not sufficient to determine respiratory sensitization potency of diacetyl. However, significant increases in IL-13 and IgE levels induced with diacetyl might be related to immunologic effects of this chemical. This research might support the studies in the field of hazard assessment of diacetyl for occupational safety and might lead up to adopt new end points to determine sensitization potency of chemicals.

para-PHENYLENEDIAMINE AND HEAVY METAL LEVELS IN COMMERCIAL BLACK HENNA TATTOO MIXTURES

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The incidence of allergic reactions to black henna tattoos definitely appears to be rising. Henna has a very low allergic potential, however, severe allergic contact dermatitis reactions are mainly caused by *para*-phenylenediamine (PPD) which is added a coloring agent in temporary henna tattoos and also by heavy metals. Although PPD is limited in hair dye to 6%, it has still been found in unregulated temporary tattoos and black henna products at alarming levels-ranging up to 64%. Few studies are available in which the components of commercial henna preparations have been analyzed [1-5]. Thus, it is important to increase awareness of the hazards of these products, and the potentially serious long-term implications. In this study, the presence and concentrations of PPD and heavy metal contaminants (cobalt-Co, nickel-Ni, lead-Pb and chromium-Cr) in commercial black henna tattoo mixtures (n=25) from the Ankara, İstanbul, İzmir, Adana and Mersin cities were analyzed using high pressure liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) techniques. PPD levels were detected in the black henna tattoo mixtures at concentrations between 3.37% and 51.59%. 88 % of commercial black henna samples have much higher PPD values than regulation value which were allowed to only in hair dyes (6%) were found. The total Co, Ni, Pb and Cr contaminant levels in black henna tattoo mixtures were determined as 0.115-4.583 ppm, 0.596-3.657 ppm, 0.930-29.300 ppm and 6.440-116.14 ppm, respectively. Co, Ni, Pb and Cr contaminant levels were found in black henna tattoo mixtures dissolved by water as 0.123-0.205 ppb, 0.141-0.755 ppb, 0.479-1.00 ppb and 0.101-0.773 ppb, respectively. Our results suggest that commercial black henna mixtures containing PPD levels up to 52% as well as heavy metal contaminants may increase the risk of allergic contact dermatitis among users. Practitioners and consumers should be aware of allergic sensitization of PPD and heavy metals and of possible subsequent allergic reactions while using black henna tattoos. Furthermore legal arrangements must be enforcement for the public health.

This study was supported by Mersin University Department of Scientific Research Projects (Grant number: BAP-SBE EMBB (AA) 2013-3YL).

References

[1] Goldenberg A, Jacob SE. Paraphenylenediamine in black henna temporary tattoos: 12-year Food and Drug Administration data on incidence, symptoms, and outcomes. *J Am Acad Dermatol*., 2015;72(4):724-6.
[2] Scientific Committee on Consumer Safety, SCCS, Opinion on p-Phenylenediamine, COLIPA n° A7 SCCS/1443/11, Revision of 18 September 2012.
[3] Almeida PJ, Borrego L, Pulido-Melian E, Gonzalez-Diaz O. Quantification of p-phenylenediamine and 2-hydroxy-1,4-naphthoquinone in henna tattoos. *Contact Dermatitis*, 2011; 66, 33–37.
[4] Kazandjieva J, Grozdev I, Tsankov N. Temporary henna tattoos. *Clinics in Dermatology*, 2007; 25: 383–387.
[5] Kang IJ, Lee MH. Quantification of para-phenylenediamine and heavy metals in henna dye. *Contact Dermatitis* 2006; 55: 26–29.

THE FREQUENCY OF MICRONUCLEUS AND OTHER NUCLEAR ANOMALIES IN EXFOLIATED BUCCAL CELLS OF INDIVIDUALS WHO CHRONICALLY EXPOSED TO LOW TO MODERATE LEVELS OF ARSENIC VIA DRINKING WATER

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Inorganic Arsenic (iAs) is one of the most hazardous contaminants in drinking water through natural (geologic) sources or anthropogenic activities. Chronic iAs exposure leads to various diseases such as internal organ cancers (skin, bladder, lung, kidney, etc.), dermal lesions, cardiovascular diseases, peripheral neuropathy and hepatic injury. The maximum acceptable concentration (MAC) for As in drinking water has been established by World Health Organisation as 10 µg/l (WHO, 1993). Geological studies have recently shown that As levels in drinking water ranged from 11 to 500 µg As/l in Nevsehir Province, Turkey. However, no data is available on As exposure and its toxic effects in this area.

In this study, we aimed to determine the level of iAs exposure and possible genotoxic effects in buccal epithelial cells (BEC) of individuals living in this area. 99 subjects living in the region with more than 50 µg As/l in drinking water (52.1 to 256.5 µg As/l) as an exposed group and 95 subjects living with less than 10 µg As/l as a control group were included.Total As concentrations in hair samples and time-weighted average drinking water-iAs (TWA-As) values were used as an As exposure indicators. The frequency of micronucleus (MN), and other nuclear anomalies [nuclear bud (NB), binuclear, condensed chromatin, karyorrhexis, pyknosis and karyolysis] were scored in BEC. Mean values of TWA-iAs were significantly different between exposed and control groups *($p<0,001$).* Hair As levels in exposed group indicated that these individuals had been exposed to As chronically. Median (min-max) value of MN frequency in exposed group [1(0-8)] was similar with those of control subjects [1(0-5)]. However, among the other nuclear anomalies, only nuclear bud and pyknotic cell frequencies were significantly higher in exposed group *($p<0,001$).* In conclusion, As levels in drinking water in our study did not induce genotoxic damage in BEC of individuals. However, significant increase of NB frequency might be associated with iAs-induced DNA damage related with gene amplification process. It could be suggested to consider other tissues (e.g. peripheral blood lymphocytes, exfoliated bladder cells) in larger population size.

This study was supported by The Scientific and Technological Research Council of Turkey (TUBİTAK), Project No. 109S419.

DETERMINATION OF BISPHENOL A (BPA) EXPOSURE LEVELS IN PRESCHOOL CHILDREN IN TURKEY

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There is general concern regarding environmental chemical exposures and the impact these may have on human health, but this is particularly important for vulnerable populations such as infants and children during critical periods of development. One of the biggest concerns of the WHO for children is exposure to chemicals during the intrauterine and childhood period and health problems that arise from in the later stages of the life.

Bisphenol A (BPA) is one of the endocrine disrupters chemicals and due to features and widespread usage (toys, baby bottles, plastic storages, heating containers for food and beverages, the lining of metal cans, medical equipment, consumer electronics and dental sealants etc.) of BPA in many consumer products in all over the world, BPA has played an important role during the last 30 years in our daily life.

BPA has weak estrogenic activity and binds both nuclear estrogen receptor (ER)α- and β- and plasma membrane-bound ERs. Moreover, BPA has been shown to interact with other endocrine receptors, e.g., thyroid hormone receptors. Because of these reasons, BPA exposure especially during the childhood is important in terms of health effects that may occur in the future. Also, determination of BPA levels in children, in order to clarify the role of the BPA in health problems such as obesity will lay the groundwork for future studies.

The aim of this study was to conduct a preliminary investigation of BPA in urine collected from 3-6 years old children living in Ankara. For this purpose, after provided spot urine samples from children (n=100), BPA levels were determined by the high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Free BPA, BPA β-D-glucuronide (BPA-gluc) and total BPA concentrations in the urine samples of children were determined in this study. Total BPA was detected in 79% of spot urine samples of preschool children from Ankara city, with concentrations ranging from 0.0 to 1.84 µg/g creatinine.

This study is funded by The Scientific and Technological Research Council of Turkey TUBİTAK. Project No:115S654)

DETERMINATION OF POSSIBLE GENOTOXIC DAMAGE AND NICOTINE EXPOSURE OF ELECTRONIC CIGARETTE (E-CIGARETTE) USERS

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An e-cigarette is a battery-powered device that simulates tobacco smoking. The use of electronic cigarettes (e-cigarettes) has increased dramatically over the past few years because e-cigarettes have been widely advertised in many countries in the past few years, mostly through the internet. Distributors of e-cigarettes promote the product as completely free of harmful substances. Nonetheless, some distributors present their products as an alternative to tobacco smoking, suggesting that e-cigarettes can be used to aid smoking cessation. The benefits and risks of e-cigarette use are uncertain as the technology is relatively new. Limited number of analyses showed that e-cigarettes and vapor from the e-cigarette contains some toxic chemicals such as nitrosamines, diethylene glycol, acetaldehyde, and some metals etc. Moreover, no sufficient scientific evidence is available confirming the safety and efficacy of e-cigarette's which is needed for helping the decisions of consumers and regulators. In this study we aimed to investigate the possible genotoxic damage and to determine comparatively nicotine exposure of e-cigarette users, classic cigarette smokers and passive smokers.

E-cigarette users (n=25), classic cigarette smokers (n=30), passive smokers (n=25) and healthy non-smoker individuals as control subjects (n=30) were included in this study. DNA damage in peripheral blood samples of our study subjects was comparatively tested by the Comet assay. Urinary cotinine (main metabolite of nicotine) concentrations were determined by the gas chromatography-mass spectrometry (GC-MS) and expressed as ng/g creatinine.

Cotinine levels in e-cigarette users (1580.4±1703.9) were significantly higher ($p<0.001$) than those of passive smokers (86.0±109.6) ($p<0.001$).

The tail moment (mean±SD) in peripheral lymphocytes of e-cigarette users, cigarette smokers, passive smokers and controls were found to be 1.46 ±0.42; 1.16±0.50; 1.13±0.52 and 0.93±0.39 respectively. Tail moment scores of e-cigarette users are significantly higher than those of control subjects ($p<0.0001$), passive smokers ($p<0.05$) and cigarette smoker ($p<0.05$) subjects. These results prove that e-cigarette users are exposed to nicotine as much as cigarette smokers and may speculate that e-cigarette might lead to the DNA damage as much as smoking cigarette.

(This study is funded by The Scientific and Technological Research Council of Turkey TÜBİTAK. Project No:113S837)

CYTOTOXIC EFFECT OF CAPE ON BREAST CANCER MCF-7 AND MDA-MB-231 CELLS

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Natural products play a relevant role in cancer therapy today with substantial numbers of anticancer agents used in the clinic being either natural or derived from natural products from various sources such as plants, animals and microorganisms. Large-scale anticancer drug discovery and screening programs such as those promoted by the National Cancer Institute (NCI) have played an important role in the development of anticancer natural compounds. Because of their safety and low toxicity, natural products as Caffeic Acid Phenethyl Ester (CAPE) are being investigated for the prevention of cancer [1, 2].

CAPE, which is specific inhibitor of Nuclear Factor kappa B (NF-κB), is a natural phenolic bioactive compound obtained from propolis. It has anticancer activity suppressing the growth and inducing the apoptosis of various type of tumor cells as well as antiviral, anti-inflammatory, antibiotic, antifungal, antioxidant and immunomodulatory properties [3-5]. The aim of this study was to investigate the cytotoxic effects on breast cancer cells (MCF-7 and MDA-MB-231).

Cell proliferation was assessed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay. The cells were seeded (4 x 10^3) onto treated various concentrations of CAPE (5-80 μM) for 48h. After 48 hrs, optical density was measured at 570-nm wavelength using a microplate reader. For determining the responses of the cell lines (MCF-7 and MDA-MB-231) colony formation to exposure with CAPE, the cells were seeded (500 cells/well) onto 6-well plates. After 24 hrs, the cells were treated with 20 μM CAPE in every 4 days, and 10 days later. The cells were washed with PBS and stained with crystal violet (0.05%).

After 48 hrs, induced antiproliferative effects in association with a dose-dependent decrease of MCF-7 and MDA-MB-231 cells proliferation were determined. Colony formation after exposure to CAPEanda considerable decrease in colony numbers at 20 μM concentration in both cells were observed.

References

[1] S. Nobili, D. Lippi, E. Witort, M. Donnini, L. Bausi, E. Mini, S. Capaccioli, Natural compounds for cancer treatment and prevention, *Pharmacological Research,* 59, 365-378, 2009.
[2] A. R. M. Ruhul Amin, O. Kucuk, F. R. Khuri and D. M. Shin, Perspectives for Cancer Prevention with Natural Compounds, *Journal of Clinical Oncology,* 27(16), 2712-2725, 2009.
[3] D. Wang, D. B. Xiang, Y. J. He, Z. P. Li, X. H. Wu, J. H. Mou, et al. Effect of caffeic acid phenethyl ester on proliferation and apoptosis of colorectal cancer cells in vitro, *World J Gastroenterol*, 11, 4008-12, 2005.
[4] K. Natarajan, S. Singh, T. R. Burke Jr, D. Grunberger, B. B. Aggarwal, Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa, *B.Proc Natl Acad Sci,* 93, 9090-5, 1996.
[5] R. M. Johnson, Honey bee toxicology, *Annu Rev Entomol,* 7, 415-34, 2015.

GENE EXPRESSION LEVELS IN INDIVIDUALS EXPOSED TO INORGANIC ARSENIC VIA DRINKING WATER

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International Agency for Cancer (IARC) classified arsenic as a human carcinogen. Drinking water is the main source of environmental exposure to arsenic. Chronic exposure to low levels of As is a serious health problem. Therefore, determination of exposure and effect biomarkers in human is very important in terms of classifications of health risks. The aim of this study was to determine gene expression variations in individuals living in Nevşehir district where drinking water arsenic levels lower than 50µg/l and higher than 50µg/l by microarray and quantitative Real-Time PCR application. The individuals in our study are selected from the project (SBAG-109S419, 2010) which was carried out in villages of Nevsehir district. Of these individuals 88 were from Kucukayhan and Emmiler village (drinking water As is >50 µg/l;Group I); 50 were from Kızılkaya village (drinking water As is 10-50 µg/l; Group II); 57 were from Eskiyaylacik, Civelek and Alkan villages (drinking water As is <10 µg/l; Group III). RNAs were isolated and cDNAs were obtained from peripheral blood samples of these residents. Affymetrix microarray-based genome wide analyses of expression patterns was performed in 12 samples. According to the microarray analysis, statistical and pathway comparisons between 3 groups (I, II and III), 4 genes (MSH6, PTK2, MAP3K7 and IL4) were selected from differentially expressed ~140.000 genes. These 4 genes were confirmed by RT-PCR.

This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) project no:109S419 and G.U Scientific Research Projects Department, project no: 02/2012-12 and 02/2012-14.

QUANTITATION OF ORGANIC IMPURITIES IN DRUG PRODUCTS CONTAINING MONTELUKAST AND EVALUATION OF THEIR PROBABLE GENOTOXIC EFFECTS

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Pharmaceutical regulatory agencies have concentrated on monitoring and evaluation of impurities -particularly genotoxic impurities- in drug substances and products. This study aims the analysis of organic impurities in montelukast drug products which is used in asthma therapy and their in vitro toxicological assessments.

Quantitation of impurities namely sulfoxide, cis-isomer, Michael adducts I and II, methylketone, methylstyrene, already indicated in USP was conducted using RP-HPLC analysis on pediatric and adult drug products on market. Validation of analytical method was carried out according to ICH guideline. For genotoxicological assessment of the impurites, miniaturized bacterial gene mutation test (Ames MPF Penta I), mitotic index determination and in vitro chromosomal aberration test with/without metabolic activation system were conducted. In addition, for sulfoxide impurity, found to be above the qualification limit, in silico mutagenicity prediction analysis was done.

Impurities except sulfoxide were under qualification limits in the analysis of different batches of 20 products at 4 mg/10 mg doses for 11 companies. The sulfoxide exceeded qualification limits in 2 companies' pediatric chewable tablets and in 7 companies' adult tablets. Leadscope and ToxTree programs predicted sulfoxide impurity as nonmutagenic. It was also found that none of the impurities have mutagenic effects in Salmonella typhimurium TA98, TA100, TA1535, TA1537, E. coli wp2[pKM101] and wp2uvrA with/without metabolic activation system. There was dose and concentration dependent reduction in mitotic index in all impurities except methylstyrene. It was determined that sulfoxide, cis isomer, and methylstyrene impurities did not induce chromosomal damages in human peripheral lymphocytes whereas michael adducts I&II and methylketone impurities induced chromosomal damages under certain test conditions. Results have shown the evaluated impurities as nonmutagenic which may be classified as ordinary impurities according to current guidelines.

This study is funded by The Scientific and Technological Research Council of Turkey. Project No:111T506

EVALUATION OF ADVERSE EVENTS OBSERVED IN BIOEQUIVALENCE STUDIES CONDUCTED IN TURKEY

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Although Bioequivalence (BE) studies are being performed routinely by Contract Research Organisations (CROs) in clinical research centers in Turkey, an overall evaluation about the attended volunteers, examined Active Pharmaceutical Ingredients (APIs) and their subclasses and the observed adverse events were not studied. Herewith our aim was to revisit the registered BE studies of a CRO (N-CRO) in Turkey between 2000-2013. We have concentrated mainly on an overall statistical evaluation with regards to the frequency, type, drug subgroups, cut off (split points using GINI INDEX) values and probable causes of adverse events in those BE studies. Accordingly, 1642 adverse events in 7828 volunteers attended to 261 BE studies were found. The frequency of adverse events were calculated as 6,29 per study and 0,21 per volunteer. The most frequently observed adverse events were; headache, somnolence, nausea, dizziness and vomiting, respectively. Adverse events were seen mostly in “genitourinary system and sex hormones” subgroup drugs. It has been found that 109 different API’s were used in 261 studies. The frequency of adverse events between test and reference drugs were not different according to the evaluation of available BE studies. In only 63 studies involving 1992 volunteers; the effect of Maximum Plasma/Serum Concentration (Cmax) and Area under the Plasma/Serum Concentration-Time Curve from Zero up to the Last Quantifiable Concentration (AUC) values to adverse event frequency has been investigated by determining split points by Gini Index Method. Observed adverse event frequency above the identified split points for Cmax and AUC values were higher than the values those were observed below the split points. As a conclusion, the review of 13 years period of BE studies of N-CRO mainly revealed that the design of the studies and the demographic properties of the volunteers were consistent with both national and international guidelines as expected. It is suggested that some of these adverse events can be related with psychological stress. The highlighting outcome of our overall evaluation could be the increase of the adverse event frequencies above the estimated split points which has been used for first time by our group. This method is promising for the future studies and showing the importance of the likely individual pharmacokinetic differences in the adverse event occurrence.

DETERMINATION OF 5-HYDROXYMETHYL-2-FURALDEHYDE AND 2-FURALDEHYDE COMPOUNDS LEVELS IN SOME FRUIT JUICES

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Processed fruit juices may contain 5-Hydroxymethyl-2-Furaldehyde (HMF) and 2-Furaldehyde (F) compounds that are known as an indicator of product quality. Whether consumption of food borne HMF pose a potential health risk for humans or not is arguable. HMF is present at high levels in several foods and can be metabolized to 5-sulfooxymethylfurfural, which is mutagenic and carcinogenic. Our aim was to determine the levels of 5-Hydroxymethyl-2-Furaldehyde (HMF) and 2-Furaldehyde (F) compounds in 100 commercial fruit juice samples (apple juice, apricot nectar, cherry and peach nectar) of five different brands (A, B, C, D and E) sold in Ankara, Turkey. HMF and F compounds were determined by high-performance liquid chromatography (HPLC) with diode array detector (DAD). HMF and F were determined in all of the samples. Also, HMF levels were higher than F levels of samples. The mean HMF values (±SE) in samples of A, B, C, D and E brands were determined to be 85.82±3.51, 97.60±3.85, 101.08±4.50, 105.84±4.35 and 104.27±4.51 mg/L, respectively. Mean F values (±SE) in samples of A, B, C, D and E brands were also determined to be 1.11±0.16, 1.34±0.12, 1.24±0.09, 1.15±0.11 and 1.46±0.24 mg/L, respectively. Minimum and maximum HMF and F levels were determined as 63.89-162.27 mg/L and 0.19-4.85 mg/L, respectively. At the present time, no values have been established in the Turkish Food Codex for HMF and F compounds levels in fruit juices or nectars.

ANTIMICROBIAL AND ANTIBIOFILM EFFECTS OF SELECTED FOOD PRESERVATIVES AGAINST *SALMONELLA* SPP. ISOLATED FROM CHICKEN SAMPLES

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Salmonella spp. are widespread foodborne pathogens that contaminate egg and poultry meats. Attachment, colonization, as well as biofilm formation capacity of *Salmonella* spp. on food and contact surfaces of food may cause continuous contamination. Biofilm may play a crucial role in the survival of salmonella under unfavorable environmental conditions, such as in animal slaughterhouses and processing plants. This could serve as a reservoir compromising food safety and human health. Addition of antimicrobial preservatives extends shelf lives of food products, but even when products are supplemented with adequate amounts of preservatives, it is not always possible to inhibit the microorganisms in a biofilm community. In this study, our aims were i) to determine the minimum inhibitory concentrations (MIC) and minimum biofilm inhibitory concentrations (MBIC) of selected preservatives against planktonic and biofilm forms of *Salmonella* spp. isolated from chicken samples and *Salmonella* Typhimurium SL1344 standard strain, ii) to show the differences in the susceptibility patterns of same strains versus the planktonic and biofilm forms to the same preservative agent, and iii) to determine and compare antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. For this purpose, *Salmonella* Typhimurium SL1344 standard strain and 4 *Salmonella* spp. strains isolated from chicken samples were used. Investigation of antimicrobial and antibiofilm effects of selected food preservatives against *Salmonella* spp. was done according to M100-S18 guidelines of Clinical and Laboratory Standards Institute and BioTimer assay, respectively.

As preservative agents, pure ciprofloxacin, sodium nitrite, potassium sorbate, sodium benzoate, methyl paraben, and propyl paraben were selected. As a result, it was determined that MBIC values are greater than the MIC values of the preservatives.

As a result, resistance may be seen in a biofilm community to food preservatives. And, this should not be ignored in food applications.

DETERMINATION OF ASPARTAME LEVELS IN SOFT DRINKS CONSUMED IN ANKARA, TURKEY

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Aspartame is commonly used as artificial sweeteners in several food products. Excess levels of aspartame can be harmful to human health. Therefore, the investigation of aspartame levels in foods is important. The aim of this study was to determine levels of aspartame in soft drinks and to evaluate whether these levels were within the Turkish Food Codex values or not. For this purpose, total number of 90 soft drink samples (A, B, C, D, E and F brands) including 15 from each brand were collected from supermarkets in Ankara province, Turkey. In this study, spectrophotometric method was used for the quantitative determination of aspartame in the samples. Mean levels (± S.E) of aspartame in samples of A, B, C, D, E and F brands were found as 156.81±7.29 mg/L, 208.67±8.97 mg/L, 236.58±17.91 mg/L, 299.54±26.19 mg/L, 202.39±8.08 mg/L and 223.28±14.08 mg/L, respectively.

In the Turkish Food Codex, aspartame is regulated with maximum levels as 600 mg/kg or mg/L in different foodstuffs. Our data revealed that mean levels of aspartame were found within Turkish Food Codex in all samples. However, some samples were not found appropriate according to the label information.

INVESTIGATION OF AFLATOXIN M1 LEVELS IN INFANT FOLLOW-ON MILKS AND INFANT FORMULAS SOLD IN THE MARKETS OF ANKARA, TURKEY

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Aflatoxins are fungal toxins known to be carcinogenic and are classified as food contaminants. This study was performed to investigate aflatoxin (AF) M1 levels in baby foods sold in Ankara (Turkey) and to evaluate the obtained results according to the Turkish Food Codex (TFC). For this purpose, a total of 84 baby food samples (50 follow-on milks and 34 infant formulas) were obtained from different markets in Ankara and the presence of AFM1 in the samples was analyzed by Enzyme Linked Immunosorbent Assay (ELISA). In 32 (38.1%) of 84 infant food samples, the presence of AFM1 was detected in concentrations ranging between 0.0055 and 0.0201 µg/kg. The mean level (± standard error) of AFM1 was found to be 0.0089 ± 0.0006 µg/kg in positive infant follow-on milks. Aflatoxin M1 was detected in only 1 infant formula sample (2.94%) at a concentration of 0.0061 µg/kg. The extrapolated levels of AFB1 contamination in feedstuffs were calculated based on levels of AFM1 in baby food samples. The data estimating AFB1 contamination in dairy cattle feedstuff indicate that contamination may range from 0.3410 to 1.2580 µg/kg, with the mean level ± standard error being 0.5499 ± 0.0385 µg/kg, which is lower than the level set by the TFC and European Union regulations (5 µg/kg). According to the obtained results, the levels of AFM1 in analyzed samples were within the allowed limit (0.025 µg/kg) set in the TFC. Low levels of AFM1 in infant follow-on milks and infant formula samples obtained during the study do not pose a health risk to infants.

This study was supported by Gazi University Scientific Research Department (Ankara, Turkey; Project No. 02/2012-37).

EVALUATION OF THE GENOTOXICITY OF DICHLOROPHENOXYACETIC ACID (2,4-D) IN TISSUE CULTURED *ALLIUM* ROOTS BY COMET ASSAY

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2,4-Dichlorophenoxyacetic acid (2,4-D) is used as a synthetic plant growth regulator in plant tissue cultures and as a herbicide in agriculture [1, 2]. The aim of this study was to investigate DNA damaging effects of different doses of 2,4-D (0.67, 1.34, 2.01, 2.68, 3.35 and 4.02 mg/L) in *Allium cepa* bulblets' root tips treated for 24 and 48 h in tissue culture. The comet tail intensity (%) and tail moment of 25 cells for each treatment were examined, using specialized Image Analysis System. The results of this finding emphasise that 2,4-D did not significantly increase the comet tail intensity and tail moment at all concentrations. Whereas, 4.02 mg/L concentration of 2,4-D induced toxic effects at 48 h treatment. The results of this study showed that 2,4-D may not have genotoxic potential at low concentrations in cultured *A. cepa* root tips.

References

[1] E. Truta, M. M. Zamfirache, C. Rosu, Z. Olteanu, C. Mihai, D. Gherghe, Cytogenetic effects induced by 2,4-D and Kinetin in radish and common bean root meristems, *Rom. Agric. Res.* 28, 207-215, 2011.
[2] L. Kaynak, M. Memiş, Bitki büyüme engelleyici ve geciktiricilerinin etki mekanizmaları, *Akd.* Üniv. *Zir. Fak. Dergisi*, 10, 237-248, 1997.

TRAGACANTH GUM OBTAINED FROM *ASTRAGALUS* AS REPLACEMENT OF AGAR IN PLANT TISSUE CULTURE STUDIES

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Agar is an important solidifying ingredient in all scientific research. Agar is obtained from red algae. With increasing population and increased scientific research, use of agar is also growing. There is danger that, if an alternative to agar is not searched, we may not be able to actively participate in scientific studies in future, due to lack of this important solidifying agent. Tragacanth gum is obtained from *Astragalus* that widely grows on barren lands in Anatolia. It is popularly used as a stabiliser, thickener, emulsifier and suspending agent in the food, pharmaceuticals, cosmetics, textile and leather industries as well as in technical applications based on its high viscosity at low concentrations, good suspending action, unusually high stability to heat and acidity and effective emulsifying properties. The study evaluated effects of different concentrations of tragacanth gum used as solidifying agent replacing agar for germination of the seeds of Samsun cultivar of tobacco. A control using agar was also planted for comparison purpose. The outcomes were very encouraging and showed that out of 6 tragacanth gum concentrations used, 11 g/l concentration had longest shoots (17 cm) roots (8.54 cm), maximum number of leaves (10.50) and maximum average leaf length (3.14 cm) and width (1.47 cm). Morphological analysis of the plants showed no abnormality among seed grown plants on agar and tragacanth gum. Sharp improvements in results were noted over the developments observed on seeds germinated on agar. It is concluded that tragacanth gum could be safely used for *in vitro* cultures during tissue culture studies. It can be safely suggested that tragacanth gum has high economic potential as gelling agent and can replace widely used, though not indispensable, gelling agent agar.

SERUM ALLANTOIN LEVELS IN PRIMARY LUNG CANCER PATIENTS

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Allantoin which is the first and major oxidation product of uric acid is presented as a sensitive marker of oxidative stress in vivo. In this study, the levels of serum allantoin and uric acid, and serum total antioxidant capacity for patients diagnosed with lung cancer (n=28) and healthy controls (n=25) were measured and the allantoin/uric acid ratios were calculated. The serum allantoin levels were measured by a Gas-Chromatography-Mass Spectrometry (GC-MS) method. Serum uric acid levels were measured by High Pressure Liquid Performance (HPLC) method, and total antioxidant activity was measured by a kit method. Allantoin levels and allantoin/uric acid ratios were significantly higher ($p<0.02$ and $p<0.002$, respectively) and uric acid levels and total antioxidant capacity were significantly lower ($p<0.0001$ and $p<0.02$, respectively) in patients than in controls. In conclusion, increased allantoin levels and allantoin/uric acid ratios and decreased antioxidant capacity support the studies suggesting possible participation of oxidative stress in lung cancer. The increase in the conversion of uric acid to allantoin, only as a result of the oxidation reaction in human, supports the thought of uric acid as an antioxidant. As far as we know, this is the first study measuring serum allantoin levels in lung cancer.

THE EFFECTS OF INFLIXIMAB AND 3-AMINOBENZAMIDE ON INDOLAMINE 2,3-DIOXYGENASE (IDO) ENZYME IN TNBS-INDUCED COLITIS MODEL

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Inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) is chronic or relapsing activation of the immune system and also inflammation of the gastrointestinal (GI) tract. Indolamine 2,3-dioxygenase (IDO1) initiates the kynurenine pathway of tryptophan metabolism. Promotion of T-cell mediated tolerance and antimicrobial effects are among the variety of functions attributed to IDO1 activity. We aimed to evaluate the effects of TNF-α inhibitor, (infliximab) and Poly(ADP-ribose) polymerase (PARP) inhibitor (3-aminobenzamide) on the activity and tissue levels of IDO enzyme in TNBS-induced colitis model.

Rats were randomly divided into five groups (n=9). Group 1(vehicle group) received intraperitoneal normal saline. Induction of colitis was performed by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in colitis groups. Group 2 (colitis control) received normal saline, Group 3 received 3-aminobenzamide (10mg/kg/12h), Group 4 received infliximab (10mg/kg/day), Group 5 received infliximab(10mg/kg/day) and 3-aminobenzamide(10mg/kg/12h). After 7 day, the rats were sacrificed and the colon was removed and assessed for macroscopic and microscopic changes. Indolamine 2,3-dioxygenase enzyme protein levels were determined in colonic tissue homogenates. Serum tryptophan and kynurenine levels were measured with HPLC UV detection. Kynurenine/tryptophan ratio was evaluated for IDO enzyme activity. Tumor necrosis factor-alpha (TNF-α) levels were measured by ELISA method in rat plasma.

When compared with control group we observed increased TNF-α levels in TNBS-induced colitis groups, except for group 4 received infliximab. Tissue IDO levels and Serum IDO activities did not show statistically significant differences among groups. Results show that infliximab and 3-aminobenzamide did not affect the IDO enzyme activity and levels in TNBS-induced rat colitis model.

PREDICTIVE AND METABOLIC BIOMARKERS IN PREDIABETICS: POSSIBLE ASSOCIATION WITH VASCULAR DAMAGE

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Prediabetes is characterized by intermediate hyperglycemia with glycemic parameters above normal but below the diabetes threshold. Prediabetes is considered to be a risk state in the development of diabetes. The development of complications of diabetes include numerous different factors such as hyperglycemia, oxidative stress, insulin resistance, dyslipidemia, carbonyl stress, inflammation, reactive oxygen species (ROS), reactive nitrogen species (RNS) and formation of advanced glycation end products (AGEs). Overproduction of ROS/RNS, lowered antioxidant defense and alterations of enzymatic pathways in humans with poorly controlled diabetes mellitus can contribute to endothelial, vascular and neurovascular dysfunction. This study was designed to determine AOPP (advanced oxidation protein products) and AGEs levels as an oxidative stress biomarkers and adiponectin levels as an anti-inflammatory and anti-atherogenic factor in prediabetics and healthy controls and to evaluate the relationship among them. Furthermore, influences of the factors of age, gender, HOMA-IR (homeostasis model assesment-insulin resistance), CIMT (carotid intima-media thickness), and biochemical parameters upon measured parameters. The subjects of this study consisted of 152 individuals, with 74 control (34 male, 40 female) with no metabolic disease and 78 prediabetic subjects (13 male, 65 female) with no receive a treatment. Prediabetics were divided into three groups: the groups of patients of impaired fasting glucose (IFG), of impaired glucose tolerance (IGT), and of complex prediabetes (IFG-IGT). Plasma adiponectin, AGEs and AOPP levels were determined using kits. Determination of biochemical parameters was based on spectrophotometric method. CIMT as an early marker of atherosclerosis was measured by ultrasonography. Significant differences were observed among all prediabetic groups and healthy individuals according to AOPP, AGEs and adiponectin levels. AOPP and AGEs levels of total prediabetics were found significantly higher ($p<0.05$, $p<0.05$), while adiponectin levels were found significantly lower than the control group ($p<0.05$). In conclusion, the observed significantly higher AGEs and AOPP levels and also lower adiponectin levels support that these parameters may be diagnostic biomarkers in diabetes.

EFFECT OF ALDOSE REDUCTASE INHIBITION ON NEUROINFLAMMATION IN MICROGLIAL CELLS

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In the central nervous system, microglial over-activation is considered to be a central event in neuroinflammation [1]. Aldose reductase (AR) has a key role in several inflammatory diseases. Therefore, AR inhibition seems to be a useful strategy for anti-inflammation therapy [2]. In the present study, we found that Quercetin and monochloropivaloylquercetin showed potent inhibition on aldose reductase expression and anti-neuroinflammatory effects in β-amyloid peptide (Aβ, 5μM, 24 hrs) induced inflammatory process by inhibiting expression of inflammatory mediators in microglial cells. Furthermore, mechanistic studies showed that ablation of aldose reductase (siRNA transfection) caused a significant reduction on Cox-2 expression in Aβ-induced neuroinflammation. Quercetin and monochloropivaloylquercetin suppressed Cox-2 mRNA expression, which further resulted in downstream inhibition prostoglandin E2 (PGE2) release in Aβ induced neuroinflammatory process. Also treatment with Sorbinil (AR inhibitor) caused to decrease PGE2 release in Aβ induced neuroinflammatory. Additionally, Aβ treatment resulted with activation of MAPK (phosphorylation of c-Jun N-Terminal kinase(JNK), extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase p38).

Quercetin and Sorbinil significantly reduced the activation of MAPK in Aβ induced neuroinflammation. Furthermore, inhibition of MAPK pathway with Quercetin and monopivaloylquercetin treatment caused a dimunition in proinflammatory cytokine (TNF-α) release in Aβ inducued neuroinflammation.

These findings suggested that AR is a potential target for treatment of neuroinflammation and that quercetin and monochloropivaloylquercetin could be an effective agent for treating or preventing neuroinflammatory diseases.

References

[1] ST. Dheen, C. Kaur, EA. Ling, Microglial activation and its implications in the brain diseases. *Curr. Med. Chem,* 1189-1197, 2007.

[2] KW. Zeng, J. Li, X. Dong, YH. Wang, ZZ. Ma, Y. Jiang, HW. Jin, PF. Tu, Anti-neuroinflammatory efficacy of the aldose reductase inhibitor FMHM via phospholipase C/protein kinase C-dependent NF-κB and MAPK pathways. *Toxicol Appl Pharmacol,* 159-171, 2013.

PRODUCTION AND OVEREXPRESSION OF Na^+/H^+ ANTIPORTER

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The family of Na^+/H^+ exchangers (NHEs) have a role in control of cell cycle and cell proliferation, transepithelial Na^+ movement, salt tolerance, vesicle trafficking, and biogenesis in mammals and also pathophysiological conditions such as hypertension, epilepsy, post-ischemic myocardial arrhythmia, gastric and kidney disease, diarrhea, and glaucoma were found to be associated with NHE dysfunction [1]. Na^+/H^+ antiporters were first described by [2] and are found in the cytoplasmic membranes of almost all cells and in many organellar membranes. Relationship of NHE with disease makes the mechanism and structure of NHE important for drug formulations. For example, overactivation of the NHE1 antiporter in heart muscle cells during open-heart surgery in humans has harmful consequences, which are reduced by the use of drugs inhibiting the antiporter [3]. In our study, Na^+/H^+ antiporter *NHA1* is heterologously overexpressed in different *Saccharomyces cerevisiae* yeast strains (FGY217 (*MATα ura3-52 lys2Δ201 pep4*Δ), AB11c (ena1-4Δ nha1Δ nhx1Δ), KTA40-2 (*ena1Δ::HIS3::ena4Δnha1Δ::LEU2 kha1Δ::KanMX*)) and tolerance of *S. cerevisiae* mutant strains overexpressing Nha1p to Na^+ and different pH were investigated to understand the effect of Na^+/H^+ antiporter.

References

[1] C. L. Brett , D.N. Tukaye, S. Mukherjee, R. Rao, The yeast endosomal Na^+K^+/H^+ exchanger Nhx1 regulates cellular pH to control vesicle trafficking. *Mol Biol Cell,* 16(3), 1396-405, 2005.
[2] I. C. West, P. Mitchell, Proton/sodium ion antiport in *Escherichia coli*. *Biochem J.,* 144(1): 87-90, 1974.
[3] C. Hunte, E. Screpanti, M. Venturi, A. Raman, E. Padan, H. Michel, Structure of a Na^+/H^+ antiporter and insights into mechanism of action and regulation by pH. *Nature* , 435(7046):1197-202, 2005.

HOW THE ORGANIC DERIVATIVES OF VANADIUM AFFECT THE LEVEL OF GLUTATHIONE IN THE NZO MOUSE LIVER

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Glutathione (GSH) remains in the state of metabolic change balance. It is continually actively synthetized and degraded. Intracellular concentration of GSH is peculiar for a given type of cells and ranges from 5 to 10 mM. Taking into consideration various disorders induced by reactive oxygen species (ROS), it seems crucial to understand the ways of controlling the availability and amount of GSH with the use of diet and pharmacological complexes. Organic derivatives of vanadium, which may play the role of anti-diabetic treatment, were tested. They showed influence on the level of glutathione in the liver homogenates (Fig.1). In NZO mice, the administration of the [VO(L^4)(EtO)] complex containing vanadium(IV) in the dose of 20 mg/kg body mass by gavage once a day for 5 weeks caused a statistically significant increase of the glutathione level.After the administration of the [VO(L^3)phen] 0.5 H_2O and [VO(L^5)phen] complexes, a significant decrease of the GSH amount was observed. Glutathione reacts with ROS and in this way it protects the thiol groups of proteins against an irreversible inactivation caused by ROS [1].

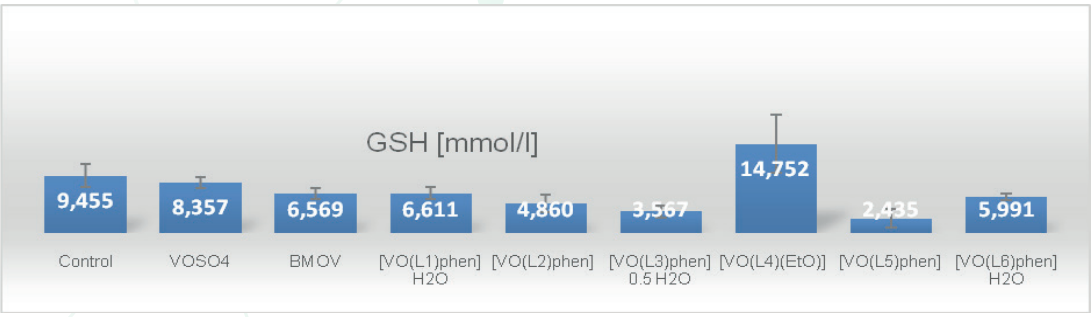

Fig.1 Level of gluthation in the liver NZO mouse fed vanadium compounds

This work was partly financed by the European Regional Development Fund under the Innovative Economy Programme 2007-2013 (WND POIG.01.03.01-174/09).

References

[1] E. Yologlu, M. Ozmen, Low concentrations of metal mixture exposures have adverse effects on selected biomarkers of Xenopus laevis tadpoles, *AquatToxicol.* 18, 168, 19-27, 2015.

BROMOPHENOL BLUE (BPB) OXIDATION BY HYDROXYL RADICAL. A DFT STUDY

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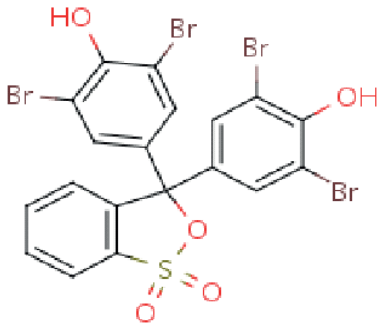
Bormophenol blue (BPB) is a prominent acid dye, accomplished of making direct links with basic groups in tissue-constituents, used as a color mark to monitor the process of agarose gel electrophoresis and polyacrylamide gel electrophoresis, coloring proteins in paper electrophoresis. Bromophenol blue is also a pH indicator at neutral pH [1] and it is an anionic dye and a member of triphenylmethane dyes family. It usually used in many industrial areas for various purposes, e.g. textile, biological stain, dermatological agent, veterinary medicine [2]. Due to various harmful effects of these dyes, the degradation of them was subjected of various works [3]. In this study DFT(Density functional theory) was used to propose a possible mechanism for the radical oxidation of the bromophenol.



Bromophenol Blue

References

[1] Rafia Azmat, Zahida Khalid, Marium Haroon, Khalida Perveen Mehar, Spectral Analysis of Catalytic Oxidation and Degradation of Bromophenol Blue at Low pH With Potassium Dichromate, *Advances in Natural Science*, 6(3), 38-43, 2013.
[2] Babak Samiey, Kamal Alizadeh, Mohammad Ali Moghaddasi, Mir Fazlolah Mousavi, and Nader Alizadeh, Study of Kinetics of Bromophenol Blue Fading in the Presence of SDS, DTAB and Triton X-100 by Classical Model, *Korean Chem. Soc.* 25, 726-736, 2004.
[3] Alireza Nezamzadeh-Ejhieh, Hamidreza Zabihi-Mobarakeh, Heterogeneous photodecolorization of mixture of methylene blue and bromophenol blue using CuO-nano-clinoptilolite, *Journal of Industrial and Engineering Chemistry,* 20, 1421-1431, 2014.

FROM METABOLOMICS STUDY OF AGE RELATED MACULAR DEGENERATION (AMD) TO THE DEVELOPMENT OF NEW PDK INHIBITORS

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Metabolomics is one of the most recent technologies in the Omics sciences defined as “the comprehensive characterization of small molecules (called metabolites) in different biological samples.” This methodology can be applied in many areas, such as biomarker discovery, clinical studies, drug efficacy and toxicity evaluation, diagnostic tools, quality control or drug discovery. Its capability to extract biochemical information associated with a cellular or biological system makes this technique a powerful tool for Medicinal Chemistry. In this work, we present a ^{1}H NMR metabolomics study applied to therapeutic target discovery.

Age-related macular degeneration (AMD) is a leading cause of blindness in the elderly population of industrialized countries. This blindness results from the deterioration of the macula, a small part of the retina specialized for the high-acuity vision. Exudative AMD, called “wet”, is characterized by the formation of new blood vessels growing under the retina according to a process named choroidal neovascularization (CNV). Currently, the aetiology and pathogenesis of AMD remain unclear. Nevertheless, a recent metabolomics study performed on the serum of “wet” AMD patients and on a CNV murine model, that mimics the effect of “wet” AMD, have demonstrated that lactate level is clearly involved in the severity of the pathology as well as the relationship between lactate, CNV and AMD [1].

According to this result, we suggest a new therapeutic approach of AMD based on the normalization of blood lactate level. The modulation of the lactate plasma concentration by treatment of the animals with synthetic compounds and more specifically Pyruvate Dehydrogenase Kinase (PDK) inhibitors significantly decrease the CNV. Starting from these results, development of new PDK inhibitors could open the way to innovative treatment opportunities in AMD disease.

References

[1] Lambert, V.; Lecomte, J.; Hansen, S.; Blacher, S.; Gonzalez, M. L.; Struman, I.; Sounni, N. E.; Rozet, E.; de Tullio, P.; Foidart, J. M.; Rakic, J. M.; Noel, A., Laser-induced choroidal neovascularization model to study age-related macular degeneration in mice. *Nature protocols,* 8 (11), 2197-211, 2013.

EFFECT OF TOPICAL KAEMPFEROL USAGE ON THE SKIN OF EXPERIMENTAL DIABETIC RATS' EXICION-INCISION WOUND HEALING

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In this study, we aimed to investigate the therapeutic effect of topical application of ointments containing kaempferol on the excision and incision wounds of skin in rats conceived diabetes experimentally.

In this study 60 male Wistar rats weighing 200-250 g were used. The rats were randomly divided into 4 groups with 15 animals in each group. Diabetes was formed by injecting a single dose of streptozotocin [45mg/kg] intraperitoneally. After 7 days of streptozotocin administration, if fasting blood sugar was 350 mg/dL or above, we considered the rats as diabetic. Blood glucose levels were measured at regular intervals for follow-up of diabetic rats. We created 1.5cm diameter exicion and 4cm long incision wounds under anesthesia in the rats which were diabetic after 7 days of streptozotocin adminisration. All wounds were cleaned with sterile saline every day and after cleaning, betadine cream base [glycolstearat to, propyleneglycoland liquid paraffin [3:6:1] and 0.5 -1% of the dose was administered as a topical ointment kaempferol. After the treatment process, macroscopic, histological, biomechanical and biochemical investigations were made in tissue samples taken.

In macroscopic observation of all treatment and control groups, there were significant differences in excision and incision wounds, dependent to dosage and treatment time. Incision wounds were examined by biomechanical investigations. In the skin stretching resistance (7 days) statistically significant differences were detected in group applied 0.5% kaempferol compared to the control group.In excision wounds, hydroxyproline content was found to be significantly increased in 0.5% kaempferol group compared to the control group. According to the pathology results, after kaempfreol cream usage, significant results were obtained in the evaluation of reepitelization, the thickness of granulation tissue and angiogenesis.There were no differences in dermal inflammation, inflammation, mast cells, collagen deposition and accumulation of fibrosis.

As a result, kaempferol ointment has healing effect on excision and incision wounds of diabetic rats compared to the control group. Healing of wounds depends on application dosage.

Acknowledgements

This study was carried out with the permission of the Dumlupınar University Animal Ethics Committee. Decision No: 2012 / 8.1.

EFFECT OF TRIMEBUTINE MALEATE ON ACETYLCHOLINE, KCl AND ATP-INDUCED CONTRACTIONS OF DETRUSOR SMOOTH MUSCLE

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Trimebutine maleate (TMB) has been widely prescribed to treat of both hypermotility and hypomotility disorder of gastrointestinal tract [1,2]. The mechanism of its action remains still unclear. It has been reported that TMB has local anesthetic, antimuscarinic and weak mu opioid agonist effects. Morever, TMB has also inhibitor activity on some ion channels such as L-type Ca^{++} channels, Ca^{++}-activated K^{+} channels that play role in smooth muscle contractility [3]. The effects of TMB on colonic motility were well clarified even at mechanistic level. However, the effects of TMB on detrusor smooth muscle (DSM) contractility have not been definitely established. The aim of this study was to investigate the action of TMB on acetylcholine (AcH), K^{+} and ATP-induced DSM contractions. To test the effects of three different doses of TMB (10,50,100 µM), we performed in vitro isolated organ bath studies on rat DMS strips (5-6 mm long and 2-3 mm wide) placed in Tyrode's solution. Three different doses of TMB (10, 50 and 100µM) were added to the organ baths. After the incubation period with different TMB doses; the responses were obtained. TMB (10, 50 and 100µM) caused a concentration-dependent decrease in AcH-induced contractions of the strips (15%, 22% and 48% respectively). The KCl (60 mM)-induced contractions of DSM strips incubated with TMB (10, 50 and 100µM) were significantly decreased (33%, 77% and 93% respectively), especially 100 µM TMB almost completely abolished the contractile response. ATP (10^{-4} M) caused neither increase nor decrease in the strips incubated with 10 µM TMB. However pre-treatment with 50µM and 100µM doses of TMB reversed ATP- induced contractions. In conclusion, our findings suggest that TMB inhibits ATP, KCl and AcH-induced contractions dose-dependently in DSM. TMB may be effective drug for the treatment of detrusor overactivity due to its multiple targets. Further studies are needed to confirm this hypothesis and to exhibit certain effect of TMB on DSM contractility.

References

[1] H.Lee, B.J. Kim, Trimebutine as a modulator of gastrointestinal motility, *Arch Pharm Res,* 34(6), 861-864, 2011
[2] W.Tan, H. Zhang, H.Luo, H. Xia, Effects of Trimebutine maleate on colonic motility through Ca^{+2}- activated K^{+} channels and L-type Ca^{+2} channels, *Arch Pharm Res,* 34(6), 86, 979-985, 2011.
[3] W.Tan, H. Zhang, H.Luo, H. Xia, Effects of Trimebutine maleate on colonic motility through Ca^{+2}- activated K^{+} channels and L-type Ca^{+2} channels, *Arch Pharm Res,* 34(6), 86, 979-985, 2011.

DETERMINATION OF CYTOTOXICITY OF DEXAMETHASONE SODIUM PHOSPHATE ON MCF-7 AND L929 CELL LINES

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Glucocorticoids have potent anti-inflammatory activities and have been widely used to treat chronic and severe inflammatory diseases [1]. Among them Dexamethasone sodium phosphate (DEX-NP) is a synthetic glucocorticoid, has the properties of glucocorticoid hormones of the adrenal glands and has an anti-inflammatory and immunosuppressive effects. Lymphocytes, fibroblasts, macrophages and other immune cells' functions are inhibited by DEX. Leukemia, lymphoma, multiple myeloma and lung cancer are also treated by this drug [2]. In this study, we aimed to determine the cytotoxicity of DEX-NP on MCF-7(human breast adenocarcinoma cell line) and L929 (Mouse fibroblast cell line). Cells were treated with the specified concentration of DEX-NP. Eight different concentrations of DEX-NP were applied to the MCF-7 and L929 cells. Cell viability was evaluated by MTT assay after 24 h incubation. Cell viability of MCF-7 cells were 74, 75.3, 70.3, 85.9, 86.9, 108, 115 and 144 % for 2000, 1000, 500, 250, 125, 62, 31, 15 µg/mL of DEX-NP concentrations, respectively. Cell viability values for L929 cells were determined as 67.3, 67.6, 68,6, 77.3, 78.3, 89.7, 98.8 and 98.8% at the same concentrations. It was found that DEX-NP had proliferative effect on the MCF-7 cells at low concentrations. Whereas, L-929 cells were not affected at these concentrations. At increasing concentrations of DEX-NP have caused cell death for both cell lines. As a result of this study, we may suggest to use high concentrations of DEX-NP during cancer treatment.

References

[1] J. Cao, M. Naeem, J.-K. Noh, E. H. Lee, and J.-W. Yoo, Dexamethasone phosphate-loaded folate-conjugated polymeric nanoparticles for selective delivery to activated macrophages and suppression of inflammatory responses, *Macromol. Res.*, vol. 23, no. 5, pp. 485-492, 2015.
[2] D. Georgieva, B. Kostova, and D. Rachev, Review Article Nanosized polymeric carriers for dexamethasone controlled delivery, vol. 7, no. 3, pp. 1767-1772, 2015.

DRUG-DRUG INTERACTION BETWEEN PPI AND CLOPIDOGREL

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Proton pump inhibitors (PPIs) are the most prescribed medications in Turkey. The PPI is one of the strongest agents for acid suppression in diseases such as gastro-esophageal reflex and in primary and secondary prevention of NSAIDS and antiplateles associated bleeding disorders specially upper gastrointestinal hemorrhage [1]. Clopidogrel needs metabolic activation predominantly by the hepatic cytochrome P450 isoenzyme Cytochrome 2C19 (CYP2C19) and proton pump inhibitors (PPIs) are extensively metabolized by the CYP2C19 isoenzyme as well. The use of proton pump inhibitors is one of the risk factors associated with treatment failure or unresponsiveness for clopidogrel therapy [2]. Our objective is to describe the prescribing patterns of proton pump inhibitors among the cardiovascular patient in Turkish population.

A retrospective study was conducted in 100 patient's prescriptions that contain clopidogrel were collected in a period of 3 months from three community pharmacies. Demographic variables were collected from the pharmacy online system and indications for both proton pump inhibitor and clopidrogel were identified. The use of PPI like Lansoprazol, Esomeprazol, Pantoprazole, Omeprazole, Rabeprazole, and Clopidogrel were evaluated by using pharmacy medication record system within 6 months period. Prescriptions that include PPI and clopidogrel simultaneously were highlighted. Drug-drug interaction was checked using Medscape drug interaction checker.

In the current study, findings showed that, 74% of patients receiving clopidogrel and PPI simultaneously where 26% of patients had received clopidogrel with PPI at the same prescription. The prescription patterns of PPI were found as 25%, 13%, 28%, 8% for lansoprazole, esomeprazole, pantoprazole and rabeprazole respectively. Significant drug-drug interaction between PPI and clopidogrel was detected in 38% of patients, 31.7% of these patients received both drugs at the same prescription. 22.9% of the patients had different PPI in the medication histories, 16% of these changes were incorrectly and 6.9% were correctly done.

Although most of the prescriptions contain pantoprazole or rabeprazole, there are still some prescriptions that contain lansoprazole in combination with clopidogrel, may reduce the effectiveness of clopidogrel in preventing heart attack or stroke. Community pharmacist must be aware of these types of interaction and must play a role in preventing and monitoring them by calling the prescribers if possible. Increasing the community pharmacist's knowledge towards these types of interactions will lead to decrease the negative economic and health impacts of drug interactions.

References

[1] Lin K., Hernandez-Diaz S., Garcia Rodriguez L. Acid suppressants reduce risk of gastrointestinal bleeding in patients on antithrombotic or anti-inflammatory therapy. *Gastroenterology* 141: 71-79, 2011.
[2] AU Goodman SG, Clare R, Pieper KS, Nicolau JC, Storey RF, Cantor WJ, Mahaffey KW, Angiolillo DJ, Husted S, Cannon CP, James SK, Kilhamn J, Steg PG, Harrington RA, Wallentin L, Association of proton pump inhibitor use on cardiovascular outcomes with clopidogrel and ticagrelor: insights from the platelet inhibition and patient outcomes trial. *Circulation* 28;125(8):978-86, 2012.

THE ROLE OF SOCIAL NETWORKING IN THE IMPROVEMENT OF PHARMACIST'S KNOWLEDGE

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Social network have been around for several decades in many different forms. Since 2006 when Facebook website moved to a public site, the way by which social network used were completely changed. 88% of Americans using social media to search for medical information and 20% particularly search on, such as Facebook, Twitter, BlogSpot, and YouTube [1]. In Turkey, over the last few years we have seen several health professionals including pharmacist using Facebook to create groups. These groups have a number of different purposes. A group may be created to share specific health information and discuss interesting issues. Other group used to share social activities or public problems and solutions. Aim of the study to evaluate the impact of social network information sharing in the knowledge and attitude of pharmacists.

The Facebook social network has been used to create clinical pharmacy group since 6 months ago. Only Pharmacists were accepted as a group member. The group was fed by information which includes new drugs, FDA alerts, adverse event report and also drug related problems. Pharmacists were assigned in two major groups, group A active pharmacist who becomes a member of our clinical pharmacy group, share and discuss information through the network and group B who is not a member. A knowledge measurement survey (AMS) was given to both of them. The data analyzed using SPSS `Pearson chi-square test` ($p < 0.05$).

Acknowledge measurement survey was developed and the difference in the score was used to evaluate the difference between the two study groups.

142 pharmacists participated in the study, 34.50% of the participants were a member of our Facebook group and 65.49% of participants were not. 78.87% of participants have only pharmacy bachelor degree and 21.12% of participants have complete or incomplete postgraduate education. 83.67% of the pharmacists who were a group member have a bachelor degree, 76.34% of the pharmacist who do not group member have a bachelor degree. The education level distribution between the two groups was not statistically significant. While 63.64% of the AMS questions were answered correctly in the member group only 44.09% were answered correctly in the non-member group.

The study emphasizes the importance of social network in providing the accurate and fastest information for the daily use of the pharmacists, there is a significant difference in knowledge between the pharmacist who join, share and discuss information on the social network and the one who do not join.

References

[1] Jessica A. Savas, Karen E. Huang, Sara Moradi Tuchayi, Steven R. Feldman, Understanding the influence of social media in medicine: lesson learned from Facebook, *Dermatology Online Journal* 20 (9): 16, 2014.

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *Crocus sativus* L. LEAF

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Saffron (*Crocus sativus* L.) is a plant belonging to the subfamily Crocoideae, in Iridaceae family. *Crocus* genus consists of 88 species distributed in Central and Southern Europe, North Africa and 5 species in Azerbaijan. Among these species *C. sativus* is medicinal and cultivated in Bilgah village of Absheron Peninsula. The species have certain pharmacological activities namely; antispasmodic, sedative [1], tumoricidal [2], free radical scavenging [3] and others. All these properties have been attributed to the stigmas, whereas other parts of the plant have been much less studied. Especially bioactive compounds of leaf have been unstudied. The present study deals with the macroscopical, microscopical and preliminary phytochemical investigation on the leaf of *Crocus sativus* L. The diagnostic features of the leaf are the presence of six major and among them two minor collateral vascular bundles and stomas in cavities on lower epidermis. The total lipid contents and fatty acid profiles were evaluated. In results the major saturated fatty acids were myristic acid (44,45%) and palmitic acid (15,07%). Preliminary phytochemical work of the alcoholic extract showed the presence of flavonoids, carotenoids and lipids. In this study the flavanoid content of the leaves identificated by chromatographic methods. Although quantification of total flavonoid content in leaves was determined by using spectrophotometric method. On the other hand, total flavanoid content of the leaves of *Crocus sativus* L. was evaluated at the various vegetation periods: during the flowering, leaf formation and at the end of vegetation period. Maximum total flavanoid content was seen at the leaf formation period of vegetation.

References

[1] S. R. Sampathu, S. Shivashankar, Y. S. Lewis, A. B. Wood, Saffron (*Crocus sativus* Linn.) Cultivation, processing, chemistry and standardization. *Critical Reviews in Food Science & Nutrition*, 123-157, 1984.
[2] J. A. Fernández, Anticancer properties of saffron, *Crocus sativus* Linn, *Advances in Phytomedicine*, 313-330, 2006.
[3] M. Ajami, S. Eghtesadi, H. Pazoki-Toroudi, R. Habibey, S. A. Ebrahimi, Effect of *Crocus sativus* on gentamicin induced nephrotoxicity, *Biological research*, 43(1), 83-90, 2010.
[4] R. Sánchez-Vioque, M. F. Rodríguez-Conde, J. V. Reina-Ureña, M. A. Escolano-Tercero, D. Herraiz-Peñalver, O. Santana-Méridas, In vitro antioxidant and metal chelating properties of corm, tepal and leaf from saffron (*Crocus sativus* L.). *Industrial Crops and Products*, 39, 149-153, 2012.

CYTOTOXICITY OF METHANOL EXTRACTS OF *Cousinia davisiana* Hub.-Mor. AND *C. ramosissima* DC. ON A-549 AND COLO-205 CELL LINES

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Asteraceae is an exceedingly large and widespread family of flowering plants. The family has more than 1100 genus and 2500 species worldwide [1]. *Cousinia* is one of the largest and most diverse genera in central and south west Asia, with 600- 700 species, [2]. There are 38 species and 6 sections of *Cousinia* genus in Turkey. One of them is Stenocephalae Bunge. section. The species of this section are *C. davisiana* Hub.-Mor., *C. foliosa* Boiss. & Bal., *C. ramosissima* DC. and *C. stenocephala* Boiss. These plants are primarily located in central and south-east Anatolia.

It was reported that *Arctium*, which is a related genus to *Cousinia* showed considarable cytotoxic activity against various cancer cell lines [3]. Indeed, in a previous study, the cytotoxic activity against Fibrosarcoma WEHI 164 cancer cell lines and Matrix metalloproteinase (MMP) inhibitory effects of a total ethanol extract of some *Cousinia* species were revealed [4].

To the best of our knowledge, there have been no phytochemical and cytotoxic activity studies on species of this section reported so far. Therefore, in this study the cytotoxic effects of *C. davisiana* and *C. ramosissima* against A-549 and COLO-209 cancer cell lines were evaluated by MTT method. The phytochemical and activity studies on these species of this section arein progress.

References

[1] P. H. Davis, Flora of Turkey and the East Aegean Islands, Vol 5, Edinburgh University Press, Edinburgh, 1975.
[2] F. Attar, S. B. Djavadi, A taxonomic revision of Cousinia, sect. Cynaroides (Asteraceae, Cardueae) in the flora of Iran, *Iranian Journal of Botany*, 16(1), 130-84, 2012.
[3] S. Susanti, et al., Tumor specific cytotoxicity of arctigenin isolated from herbal plant Arctium lappa L., *J Nat Med,* 66, 614-21, 2012.
[4] A. R. Shahverdi, et al., Concomitant chemopreventive and antibacterial effects of some Iranian plants from the genus Cousinia (Asteraceae), *Brazilian Journal of Pharmacognosy*, 17, 325-30, 2007.

MORPHOLOGICAL CHARACTERISTICS OF *CLINOPODIUM ACINOS* AND *Clinopodium suaveolens* (LAMIACEAE) GROWING IN TURKEY

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A group of Lamiaceae that has caused confusion over its generic boundaries are those species belonging to the complex surrounding the genera *Satureja*, *Calamintha*, *Micromeria*, *Clinopodium* and *Acinos*. In the current study, morphology characteristics of *Clinopodium acinos* (L.) Kuntze and *C. suaveolens* (Sm.) Kuntze previously treated as *Acinos* in Flora of Turkey was determined. *C. suaveolens* grows in North-West and the West Anatolia and it is strongly odorous while *C. acinos* is recorded from North Anatolia and has a slight odor. They are studied for the first time. Detailed descriptions and illustrations of general appearance of plants and their leaf, bract, flower, calyx, corolla and nutlet shapes are described and illustrated.

References

[1] P. H. Davis and A. Doroszenko, *Ballota.* In: Davis, P.H. et al. (eds.), *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Edinburgh, 1982.

COMPOSITIONS OF THE ESSENTIAL OILS OF *BALLOTA NIGRA* L. SUBSP. *UNCINATA* (FIORI & BEG.) PATZAK AND SUBSP. *ANATOLICA* P.H. DAVIS FROM TURKEY

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Ballota nigra L. is commonly distributed in western Turkey and inner Anatolia where flowering aerial parts are used in medicine for treating cough, digestive, diuretic, antiseptic, antiinflammatory and more especially for neurosedative activities. It is a member of Lamiaceae family and is represented in the Flora of Turkey by five subspecies [1]. The medicinal properties attributed to the essential oil of the genus *Ballota*, prompted us to investigate the chemical constituents of the oil of two subspecies of *B. nigra*.

The essential oils from aerial parts of subsp. *uncinata* and subsp. *anatolica* were isolated by steam distillation. The analysis was performed by using a gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously.

Twenty-two compounds were identified from the oil of subsp. *uncinata* representing 96.9% of the total oil and fourteen compounds were identified from the oil of subsp. *anatolica* representing 88% of the total oil. The major components were characterized as caryophyllene oxide (21.2 %), hexadecanoic acid (19.9 %), b-caryophyllene (18.9 %) for subsp. *uncinata* and hexadecanoic acid (40.9 %) and b-bisabolene (13.4 %) for subsp. *anatolica,* respectively.

This study has demonstrated that the sesquiterpene and the other contents, like hexadecanoic acid, possessed the highest value in the oils of subspecies.

References

[1] E. Leblebici, *Clinopodium* In: Davis, P.H. et al. (eds.), *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Edinburgh, 1982.

EXPLORING *IN VITRO* ANTIOXIDANT ACTIVITY OF SOME NATURAL AND SEMI-SYNTHETIC FLAVONOID DERIVATIVES AND THE EXTRACTS FROM *MACLURA POMIFERA* (RAFIN.) SCHNEIDER (OSAGE ORANGE) AND ITS ESSENTIAL OIL COMPOSITION

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Maclura pomifera (Rafin.) Schneider (Moraceae), known by local names such as “osage orange, hedge apple, box wood, and horse apple”, is a tree species natively grown in the United States. However, it is widely cultivated throughout the world for ornamental purposes and, therefore, it is almost naturalized in Turkey. In the current study, we investigated antioxidant potential of the ethyl acetate, ethanol, aqueous, and *n*-butanol extracts prepared from the fruits of *Maclura pomifera* (MP) along with the leaf ethanol extract of the female tree using six experimental models including 2,2’-diphenyl-1-picrylhydrazyl (DPPH), *N*,*N*-dimethyl-*p*-phenylendiamine ($DMPD^{+}$), and nitric oxide (NO) radical scavenging activity, metal-chelating capacity, ferric- (FRAP) and phosphomolibdenum-reducing antioxidant power (PRAP) assays adapted to ELISA microtiter methods. Besides, the major isoflavonoids; osajin and pomiferin, their semi-synthetic derivatives; *iso*-osajin and *iso*-pomiferin, and macluraxanthone isolated from MP were also assayed in the same assays. The fruit aqueous extract exerted higher scavenging activity against and NO radicals, while the fruit ethyl acetate extract was the most active against DPPH radical (68.61 ± 2.53%). Considering the tested compounds, the highest DPPH (91.74 ± 0.26%) and DMPD (30.63 ± 1.31 %) radical scavenging effect was observed with macluraxanthone, while pomiferin and *iso*-pomiferin exhibited better activity than osajin and *iso*-osajin except metal-chelation capacity assay. The essential oil composition of the leaves from the male and female trees and fruits was analyzed by capillary gas chromatography-mass spectrometry (GC-MS). Phytol was the major compound in both the leaf oils, while the fruit essential oil contained β-caryophyllene as the main component (69.3%).

INVESTIGATION OF SOME BIOACTIVITY PROPERTIES AND CHARACTERIZATION OF POLYSACCHARIDES FROM WILD MUSHROOMS

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Today, natural product researches are of great importance for discovering new bioactive sources. Turkey has a rich mushroom flora due to its geographical position and in recent years, phytochemical and biological activity analysis of mushrooms have been increased day by day [1,2]. In this study, it was aimed to investigate some biological activities and characterization of polysaccharides isolated from four wild mushroom species (*Rhizopogan roseolus, Terfezia claveryi, Tricholoma terreum, Leucoagaricus leucothites*) collected from Turkey. Monosaccharide units of fungal polysaccharides were identified by High Pressure Liquid Chromatography (HPLC). The results of monosaccharide analysis in HPLC showed that polysaccharides of four mushrooms composed of mainly glucose and fructose. The polysaccharide extract of *T.claveryi* were found as rich in phenolic compounds when compared the other species and showed the highest antioxidant activity, according to the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) radical cation decolorisation method. It was seen that according to 2,2-diphenyl- 2-picrylhydrazil hydrate ($DPPH^{\bullet}$) radical scavenging activity method, polysaccharide extracts do not have activity. Antimicrobial activities of the fungal polysaccharides were investigated by disc diffusion method and microdilution method against on five menengitidis pathogens. All the polysaccharide extracts were found did not have any antimicrobial activity against on pathogens.

References

[1] G, Güzeldağ. Polyporaceae Türlerinde (*Ganoderma spp.*,ve *Trametes spp.*) Üretim ve Biyoteknolojik Optimizasyon Olanaklarının Araştırılması, Doktora Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana 2007: 1
[2] M, Apaydın. *Tricholoma fracticum* (Britzelm.) Kreisel Mantarının Aktivitesi Ve Kimyasal Bilesimlerinin Araştırılması, Yüksek Lisans Tezi, Muğla Üniversitesi, Fen Bilimleri Enstitüsü,Kimya Anabilim Dalı, Muğla 2011:1

ANTIBACTERIAL EFFECTS OF FOUR DIFFERENT *Rhododendron* L. SPECIES

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Rhododendron which belons to Ericaceae family is the common name of floriferous plants that contains 850 species. Rhododendrons that have various flower and leave structures are decidious as well as being green during all seasons. They are used for the treatment of inflammation and rheumaticdiseases. Rhododendrons used in this work were collectedfrom the cities –Ordu, Artvin and Rize-. Flowers and leaves of *Rhododendron ponticum* L., *Rhododendron luteum* Sweet, *Rhododendron smirnovii* Trautv. *Rhododendron ungernii* Trautv. were extracted with dimethyl sulfoxide (DMSO).The antibacterial activity of the obtained extracts at different concentrations (100x, 50x, 25x and 12,5x dilutions) were investigated. against *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus epidermidis* ATCC 12228, *Kocuria rhizophile epidermidis* ATCC 9341 using diffusion method. For positive control taylosin tartarat and for negative control DMSO were used and the antibacterial effectiveness of the flowers and the leaves of the plant was observed. At the end of this research, it was observed that there was a very clear zone diameter and no bacterial growth in the taylosin tartarat given areas. Leaves of *R. smirnovii* and *R. ponticum* and flowers of *R. ponticum* and *R. luteum* were useful and effective against *P. aeruginosa* strain. No efffectivenes was recorded for the other plant extracts againts other bacteria tested.

DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN THE AERIAL PARTS OF *Onosma sericeum, O. rascheyanum* AND *O. caerulescens* BY GC-MS

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The genus *Onosma* L. (Boraginaceae) includes about 150 species worldwide [1], approximately 70% of the species is found in Anatolia. This genus is represented by 101 species (107 taxa) in Turkey and 50 of which are endemic [2,3]. *Onosma* species are known to contain aliphatic ketones, shikonin, lipids, naphtazarins, phenolic compounds, naphtoquinones and pyrrolizidine alkaloids (PAs) [4]. PAs are generally considered as toxic for human and livestock. Especially, PAs possessing a 1,2-double bond in their base moiety (i.e., necine) exhibit hepatotoxic, carcinogenic, genotoxic, teratogenic and sometimes pneumotoxic effects. PAs pose a serious health risk to human that may be exposed to them through contaminated foodstuffs or when plants containing them are consumed as medicinal plants [5]. In the current study, alkaloid extracts obtained from the aerial parts of three *Onosma* species were analysed by GC-MS to assess their PA content. The total pyrrolizidine alkaloid and tertiary base content of *Onosma* species are shown in the Table 1. Higher percentage of alkaloids was present as their N-oxide derivatives. This is the first report on the pyrrolizidine alkaloid content of *Onosma sericeum, O. rascheyanum* and *O. caerulescens*.

Table 1. Alkaloid contents of *Onosma* species

Plants	Tertiary base %	Total alkaloid %
O. sericeum	0.01	0.134
O. rascheyanum	0.03	0.11
O. caerulescens	0.046	0.15

References

[1] A. El-Shazly, A. Abdel-Ghani, M. Wink, Pyrrolizidine alkaloids from *Onosma arenaria* (Boraginaceae), *Biochem Syst and Ecol*, 31: 477-485, 2003.
[2] R. Binzet, H.I. Teke, The anatomical properties of *Onosma mollis DC.* and *Onosma halophila Boiss. & Helder*. (Boraginaceae) from Turkey, *Pak J Bot*, 46 (5): 1663-1668, 2014.
[3] H. Riedl, 1978. Onosma L. in: Davis, P.H (Ed.), Flora of Turkey and the East Aegean Islands, vol.6. Edinburgh University Press, Edinburgh
[4] I. Ahmad, S. A. Nawaz, N. Afza, A. Malik, I. Fatima, S.B. Khan, M. Ahmad, M. I. Choudhary, Isolation of Onosmins A and B, lipoxygenase inhibitors from *Onosma hispida*, *Chem Pharm Bull*, 53 (8): 907-910, 2005.
[5] H. Wiedenfeld, Plants containing pyrrolizidine alkaloids: toxicity and problems, *Food Additives and Contaminants*, 28 (3): 282-292, 2011.

UREASE INHIBITORY AND ANTIOXIDANT ACTIVITY OF *Ferulago blancheana Post ex Boiss.*

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In this study, it was aimed to determine the urease inhibitory and antioxidant activities, total phenol and flavonoid content of *Ferulago blancheana* Post ex Boiss. For this purpose, aqueous and methanol extracts of the aerial parts and roots of *F. blancheana* were prepared and subjected to liquid-liquid fractionation with dichloromethane, ethyl acetate and *n*-butanol. Obtained extracts and subfractions were used in the experiments mentioned below.

Antioxidant activities were determined with 1,1-diphenyl-2-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC) and *2,2'*-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid *(ABT9)* radical cation decolourization assay [1-4]. According to tree antioxidant activity test results, dichloromethane, ethyl acetate and *n*-butanol subfractions of aerial parts and ethyl acetate subfraction of the roots attract attention with high activity.

Total flavonoid contents were determined by using "aluminium chloride colorimetric method" [5]. The highest flavonoid content was determined on ethyl acetate subfraction of the aerial parts and dichloromethane subfraction of the roots (124 and 64 mg rutin equivalent/g extract, respectively). Total phenolic contents were determined with "Folin Ciocalteu Method" [6-7], and ethyl acetate subfractions of roots and the aerial parts were determined to have the highest phenol contents (168 and 168 mg gallic acid equivalent/g extract).

Urease inhibitory activity was determined by measuring ammonia production using indophenol method [8-9]. *n*-Butanol subfraction of the aerial parts presented the highest urease inhibitory activity (IC_{50}: 459,2 µg/ml).

References

[1] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir. A novel total antioxidant capacity index for dietary polyphenols, vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, *Journal of Agricultural and Food Chemistry,* 52, 7970-7981, 2004.
[2] İ. Gülçin, Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids, 32, 431-438, 2007.
[3] B. Matthaus, Antioxidant activity of extracts obtained from residues of different oilseeds, *Journal of Agricultural and Food Chemistry,* 50, 3444-3452, 2002.
[4] R, Re, N. Pellegrini, A. Protegente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free radical biology & medicine*, 26, 1231-1237, 1999.
[5] R.G. Woisky, A. Salatino, Analysis of propolis: some parameters and procedures for chemical quality control, J*ournal of Apicultural Research*, 37, 99-105, 1998.
[6] V.L. Singleton, J, A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, 16, 144-158, 1965.
[7] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventos, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent, *Methods in Enzymology,* 299, 152-178, 1999.
[8] K.M. Khan, S. Iqbal, M.A. Lodhr, G.M. Maharvf, S. Perveen, M. L Choudhary, A. Z. H. Chohan, C. T. Supuran, Synthesis and Urease Enzyme Inhibitory Effects of Some Dicoumarols, *Journal of Enzyme Inhibition and Medicinal Chemistry,* 19, 367-371, 2004.
[9] M.W. Weatherbum, Phenol-Hypochlorite Reaction for Determination of Ammonia, *Analytical Chemistry,* 39, 971- 974,1967.

TWO ANTI-ULCEROGENIC FLAVONOID GLYCOSIDES FROM *Sideritis caesarea* Duman, Aytaç & Başer

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In our previous studies, the aqueous and 80% ethanol extracts of *Sideritis caesarea* were shown to have potent anti-ulcerogenic effect against ethanol-induced gastric ulcer model in rats. The ethanol extract was fractionated by solvent-solvent extraction and column chromatographic techniques according to bioassay guided fractionation process, but the active conmpound(s) could not be determined before [1, 2].

In this study, two known flavonoid glycosides were obtained from subfractions of ethyl acetate extract by bioassay guided fractionation techniques using the same experimental gastric ulcer model. Their structures were determined as isoscutellarein-7-O-[6"'-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6"-*O*-acetyl-β-D-glucopyranoside and 4'-*O*-methylhypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6"-*O*-acetyl-β-D-glucopyranoside according to NMR and LC-MS data. These compounds were orally administered to rats and their gastroprotective effects were tested using the ethanol-induced gastric ulcer model. According to the results of the bioactivity experiment, both flavonoid glycosides have almost equally and significant gastroprotective activities [70% and 69%, respectively (*: $p<0.05$)] against ethanol–induced experimental ulcer model in rats. In addition, our bioassay guided fractionation studies are in progress to obtain other active compound(s).

This study was financially supported by The Scientific and Technological Research Council of Turkey - TUBİTAK (Project no: SBAG-112S581).

References

[1] İ. Gürbüz, A.M. Gençler Özkan, E. Yeşilada, O. Kutsal, Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbası, *Journal of Ethnopharmacology,* 101: 313-318, 2005.
[2] İ. Gürbüz, T. Günbatan, A. M. Gençler *Özkan*, In vivo anti-ulcerogenic effect evaluation of *Sideritis caesarea* Duman, Aytac & Baser (Lamiaceae), Phytopharm 2015, 22-24 July 2015, Bonn, Germany.

ASSESSMENT OF ANTICHOLINESTERASE, METAL-CHELATING AND FRAP ACTIVITY OF SOME ASTERACEAE, FABACEAE AND RUTACEAE HERBS FROM MALAYSIA

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Cholinesterase (ChE) family consists of two sister enzymes; acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), which are associated with the pathology of Alzheimer's disease (AD) according to the "cholinergic hypothesis". Hence, ChE inhibitors are accepted as the most prescribed drug class for the treatment of AD. Since neurodegeneration is strongly linked to oxidative stress and metal accumulation, antioxidant and metal-chelating agents might be also helpful toward AD. In our continuing studies to explore new ChE inhibitors from herbal sources, we have now aimed to screen the ethanol extracts of some members from Asteraceae, Fabaceae, and Rutaceae families growing in Malaysia. The plant samples screened were *Elephantopus scaber*, *Emilia sonchifolia*, *Blumea balsamifera*, *Chromolaena odorata*, *Cosmos caudatus*, and *Gynura procumbens* from Asteraceae, *Mimosa pudica*, *Senna alata*, *Senna obtusifolia*, *Albizia saman*, and *Tamarindus indica* from Fabaceae) as well as *Citrus hystrix* and *Murraya koenigii* from Rutaceae for their ChE inhibitory effect at 200 µg/mL and metal-chelaitng capacity at 125 µg/mL. Our results pointed out that the extracts were more active inhibitors of BChE, which was most effectively inhibited by the Rutaceae species, *e.g. M. koenigii* (90.56 ± 0.15%, IC_{50}= 8.43 µg/mL) and *C. hystrix* (76.12 ± 1.49%, IC_{50}= 25.64 µg/mL). Except *E. scaber* (50.48 ± 1.29%, IC_{50}= 196.20 µg/mL), all Asteraceae and Fabaceae plant samples displayed lower inhibition than 50%. Among the tested species, only *C. hystrix* extract exerted metal-chelating capacity over 50% (57.60 ± 0.70%). All of the extracts were screened for their ferric-reducing antioxidant power (FRAP) at 100 µg/mL of final concentration and the highest FRAP belonged to the extract of *C. caudatus* (1.427 ± 0.079), followed by *M. koenigii* (1.038 ± 0.072).

Our findings indicated that *M. koenigii* and *C. hystrix* contain potential selective BChE inhibitory substances and our work is in progress to identify their active components.

This study has been attributed to dear Prof.Dr. Farida Habib Shah from Malaysia, who passed away in November, 2014. She, as a great natural product chemist, was the collector and identifier of the plants tested herein. May her soul rest in peace.

ANTIFEEDANT ACTIVITY OF AERIAL PART EXTRACTS OF SOME *Vincetoxicum* TAXA AGAINST DESTRUCTIVE PESTS *Spodoptera littoralis* AND *Leptinotarsa decemlineata*

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The genus *Vincetoxicum* N.M. Wolf, (Apocynaceae; subfamily Asclepiadoideae) [1] which is represented with approximately 100 species, is distributed throughout Asia, Japan and Europe [2]. *Vincetoxicum* is one of the largest genera of the subfamily in Anatolia. The genus is represented by 10 taxa three of which are endemic to Turkey [3]. Some species of the genus have been used in Chinese traditional medicine for the treatment of neurosis, malaria, rupture, scrofula, scabies and wounds [4]. In previous studies antibacterial, antifungal [5], antifeedant and growth inhibitory [6] effects were reported as biological activities of *Vincetoxicum* species. Phytochemical investigation showed presence of steroids, triterpenoids, alkanols, glycosides, alkaloids, flavonoids, saponins and phenolic compounds [6]. In the present study four different polarity extracts obtained from aerial parts of five *Vincetoxicum* taxa (*V. canescens* (Willd.) Decne. subsp. *canescens*, *V. canescens* subsp. *pedunculata* Browicz, *V. fuscatum* subsp. *fuscatum* (Hornem) Reichb., *V. fuscatum* subsp. *boissieri* (Kusn) Browicz and *V. parviflorum* Decne.) were investigated for their insect antifeedant activity, chronic toxicity and growth inhibitory effects against polyphagous pest *Spodoptera littoralis* Boisduval (Lepidoptera:Noctuidae) and oliphagous pest *Leptinotarsa decemlineata* Say. (Coleoptera: Chrysomelidae). The tested extracts displayed significant differences between their antifeedant activities in the highest tested dose of 500 µg/cm^2. Among 20 tested extracts, 7 extracts and 4 extracts were selected as the most effective (100.0±0.0) against *L. decemlineata* larvae and *S. littoralis* larvae, respectively. The antifeedant activity of 10 extracts against larvae of *S. littoralis* and 8 extracts against larvae of *L. decemlineata* were in the range of 99.9-50 %. Furthermore the growth inhibition and chronic toxicity on *S. littoralis* larvae were determined. (This work was supported by the Ministry of Agriculture of the Czech Republic Project No: RO0415)

References

[1] V.H. Heywood, R.K. Brummitt, A. Culham, O. Seberg, Flowering plant families of the world, Firefly Books, pp 38-40, 2007.
[2] S. Liede, *Cynanchum-Rhodostegiella-Vincetoxicum-Tylophora* (Asclepiadaceae): new considerations on an old problem, *Taxon*, 45:193-211, 1996.
[3] K. Browicz, *Vincetoxicum* N.M. Wolf. In: Davis P.H., ed., Flora of Turkey and the East Aegean Islands, University Pres, Edinburgh, 6; 163-173, 1978.
[4] I. Sliumpaite, M. Murkovic, A. Zeb, P.R. Venskutonis, Antioxidant properties and phenolic composition of swallow-wort (*Vincetoxicum lutea* L.) leaves, *Ind Crop Prod*, 45:74-82, 2013.
[5] C. Mogg, P. Petit, N. Cappuccino, T. Durst, C. McKague, M. Foster, J.E. Yack, J.T.Arnason, M.L. Smith, Test of the antibiotic properties of the invasive vine *Vincetoxicum rossicum* against bacteria, fungi and insects, *Biochem Syst and Ecol*, 36:383-391, 2008.
[6] R. Pavela, Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. and *Spodoptera littoralis* Bois. larvae, *Ind Crop Prod*, 32:213-219, 2010.

COSMETIC PLANTS OF TURKEY

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Unconscious use of uncertain production methods and non-standardized natural products affect the skin health adversely in our country. Therefore, knowledge on 62 medicinal and aromatic plants growing naturally in Turkey which are used in cosmetics, their botanical properties, active ingredients, production methods and potential dermatoxic effects was updated in this study evaluating 569 literature [1].

As a result of the study, it was found that plants were used in cosmetics owing to their antiinflammatory, antioxidant, photo-stabilizer or tyrosinase inhibitor properties (*eg* apigenin, luteolin, kaemferol, isoliquiritigenin, isosalipurposide, esculetin, isovitexin and malvidin) activities [2-4]. According to the literature, monosaccharides were used as viscosity enhancer, film former and emollient; oligosaccharides were used as stabilizer and bulking agent; and some polysaccharides were used as emollient [5-8]. Some plants containing gum, resin, organic acid or terpenes which mostly belong to Asteraceae, Lamiaceae, Rutaceae and Sapindaceae families irritate the skin causing allergic contact dermatitis and/or creating sensibility or photo-sensibility [9-10]. No record could be found in the literature on toxic effects of some phytocosmetics [11].

References

[1] F.Z. Saltan (Ed.), Kozmetik Amaçla Kullanılan Tıbbi ve Aromatik Bitkiler ve İçerikleri, Nobel Tıp Kitabevleri, ISBN: 978-605-335-141-2, İstanbul, 2015.
[2] F. Patri, V. Silano, *Plants in Cosmetics*, Vol.1, Council of Europe Publishing, Strasbourg, 2002.
[3] A. Benaiges, P. Guillen, *Botanical Extracts.* In: Analysis of Cosmetic Products, Elsevier,The Netherlands, 2007.
[4] Y. Yazan (Ed.), Kozmetik Bilimi, 2. Baskı, Nobel Tıp Kitabevleri, ISBN: 978-975-420-762-0, İstanbul, 2010.
[5] F.S.Sr. D'Amelio, Botanicals. Phytocosmetic Desk Reference. CRC Press, London, 1999.
[6] M.T. Luu, M. Mercier, P. Thau, Sucrose ester multilamellar emulsifiers for skin moisturization, *Cosmetics & Toiletries*, 125 (10), 48-50, 2010.
[7] S. Patel, A. Goyal, Functional oligosaccharides: production, properties and applications, *World Journal of Microbiology and Biotechnology*, 27, 1119-1128, 2011.
[8] W. Johnson, Safety Assessment of Polysaccharide Gums as Used in Cosmetics. CIR Draft Report for Panel Review, November 14, 2014.
[9] I.R. White, Opinion on Fragrance Allergens in Cosmetic Products, Scientific Committee on Consumer Safety, European Union, 2011.
[10] E. Antignac, G.J. Nohynek, T. Re, J. Clouzeau, H. Toutain, Safety of botanical ingredients in personal care products/cosmetics, *Food and Chemical Toxicology*, 49 (2), 324-341, 2011.
[11] F.A. Andersen, Annual review of cosmetic ingredient safety assessments- 2004/2005. *Astragalus gummifer* gum*, International Journal of Toxicology*, 25 (2), 1-89, 2006.

CARBOHYDRATE DIGESTIVE ENZYME INHIBITORY EFFECT AND ANTIOXIDANT POTENTIAL OF *Inula viscosa* (L.) Aiton

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The present study was designed to examine *in vitro* antidiabetic activities of different extracts of flowers, leaves and roots of *Inula viscosa* (L.) Aiton. Methanol extracts of flower and leaf exhibited remarkable α-glucosidase inhibitory activity. Additionally, α-amylase inhibitory activities of the extracts were moderate at only 3000 µg/ml. Based on the results of *in vitro* antidiabetic activity tests; antioxidant activities, total phenol and flavonoid contents of the most promising extracts were evaluated. To identify compounds responsible for the antidiabetic activity, major compounds of *L. viscosa* were analyzed for their *in vitro* enzyme inhibitory activity. Quercetin, luteolin and rutin exhibited a significant inhibition on α-glucosidase at 10 mM concentration. Consequently, *L. viscosa* is considered as an antidiabetic and antioxidant agent that could potentially be used in pharmaceutical industry for preparation of functional food.

COMPARATIVE ANALYSIS OF CHEMICAL PROFILE, ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF *Juniperus foetidissima* Willd. AND *Juniperus sabina* L.

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Fruit and leaves of junipers are commonly used internally as tea and pounded fruits are eaten to lower blood glucose levels in Anatolia. *In vitro* antidiabetic activities of leaf and fruit extracts of *Juniperus foetidissima* and *J. sabina* were examined fortheir inhibitory activity on α-glucosidase and α-amylase enzymes. Owing to promising enzyme inhibitory activities of the extracts, their *in vivo* antidiabetic effects were investigated on streptozotocin-induced diabetic rats. Extracts were administered orally at two different doses (500 and 1000 mg/kg) and glipizide was used as reference. Additionally, antioxidant activities of the extracts were determined by phosphomolybdenum, ferric-reducing antioxidant power and 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity assays. Phytochemical screening tests indicated that *Juniperus* extracts contained mainly flavonoids, tannins, terpenoids and carbohydrates. Content of the major compound amentoflavone in the extracts was determined by high performance liquid chromatography. *In vitro* enzyme inhibitory effects of the extracts were supported by the results of *in vivo* antidiabetic activity studies. As a result, *Juniperus* extracts and its active constituents might be beneficial for diabetes and its complications.

IN-VITRO ANTIDIABETIC AND ANTIOXIDANT EFFECTS AND PHYTOCHEMICAL STUDIES ON *Helianthus tuberosus*, *Cydonia oblonga* AND *Allium porrum*

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Cydonia oblonga Mill. (Rosaceae) leaves, *Helianthus tuberosus* L. (Asteraceae) tubers, and *Allium porrum* L. (Liliaceae) bulb & leaves have been used traditionally to treat diabetes mellitus in Anatolia. In our previous study, the antidiabetic activity of these plants has been observed [1]. In this sudy, inhibitory activities of these plants on α-glucosidase and α-amylase enzymes which is critically effecting carbohydrate digestion and glucose absorption were evaluated. On the other hand, antioxidant activities, total phenol and flavonoid contents of *C. oblonga, H. tuberosus* and *A. porrum* were investigated. The highest ABTS radical scavenging, total antioxidant activities and reducing power were found in *C. oblonga* leaf ethanolic extract. But, tested plant extracts did not show any significant α-amylase and α-glucosidase inhibitory activity. The total phenol and flavonoid contents in the extracts showed different values in the range of 7.91-163.33 mg gallic acid equivalent/g and 27.26-29.60 mg quercetin/g, respectively. Consequently, the mechanism of action of these plants with potential antidiabetic properties was not related to the inhibition of α-amylase and α-glucosidase enzymes.

References

[1] M. Aslan, N. Orhan, D. Deliorman Orhan, F. Ergun, Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes, *J. Ethnopharmacol.*, 384-389, 2010.

ANTICANCER POTENTIAL OF DIFFERENT *ORIGANUM* SPECIES

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Origanum is from family Lamiaceae. The main components of the essential oil were thymol and carvacrol, while their biogenetic precursors, *p*-cymene and γ-terpinene. Also it has other chemical constituents such as flavonoid glycosides, phenolic compounds, and polysaccharides. Due to these components; it has exhibit a lot of pharmacological effect such as anticancer, antioxidant, antimicrobial, also it has been benefit a lot of clinical trial. In this review we aimed to report the anticancer activity of different types of Origanum.

In a study *Origanum acutidens (*OA) inhibited in MCF-7, MDAMB- 468 and MDA-MB-231 cancer breast cell lines proliferation [1]. Other a study the cytotoxicity effect of petroleum ether, ethyl acetate, ethanol, and water of aerials parts of *Origanum compactum* were investigated on breast carcinoma cell (MCF-7). In conclude, determined that ethyl acetate extract (30 mg/L) and ethanol extract (56 mg/L) showed the highest activity against (MCF-7) [2]. Reported that the aqueous extracts of *O. dayi* induce apoptosis in HepG2 cells through mitigation of mitochondrial pathway [3]. Dhaheri and colleagues identified that *Origanum majorana* as a promising chemo preventive and therapeutic candidate that modulate breast cancer growth and metastasis [4]. Sivas and Tomsuk determined that essential oil *Origanum onites* L. and its phenolic constituent carvacrol significantly exhibited cytotoxic and apoptotic effect against HepG2 cell line [5]. Reported that prepared from *Origanum syriacum* L. ethanolic extracts were exhibited anticancer activity against to THP-1 leukemia cell line [6]. Gorbovic and co-workers determined that methanolic extract from *O. vulgare* has cytotoxic and anti-proliferative activity against to HCT-116 and MDA-MB-231 cell line [7]. In another work investigated the effect of *Origanum vulgare* ethanolic extracts on redox balance, cell proliferation, and cell death in colon adenocarcinoma Caco2 cells. Consequently determined that this extract potential agent selective for this cancer cell [8].

This plants use can be useful in many techniques for therapeutic situation especially cancer due to including very rich. This review will increase quality of information and will be the basis for future perspective.

References

[1] E. Tuncer, S. Unver-Saraydin, B. Tepe, S. Karadayı, H. Özer, M. Sen, K. Karadayı, D. Inan, S. Elagöz, Z. Polat, M. Duman, M. Turan, *J BUON, 1*8(1), 77-85. 2013.
[2] F.E. Babili, J. Bouajila, J.P. Souchard, C. Bertrand, F. Belvert, F. Fouraste, C. Moulis, *A Journal of Food Science,* 76(3), 512-518. 2011
[3] D. Bhatia, A. Mandal, E. Nevo, B. Anupam, *Asian Pac J Trop Biomed*, 5(2), 87-92. 2015.
[4] D.A. Dhaheri, S. Attoup, K.Arafat, S. Abuqamar, J. Viallet, A. Saleh, H.Alhagha. R. Iratni, *Plosone.* 8(7), 2013.
[5] H. Sivas, Ö. Tomsuk, *Anadolu Unıversıty Journal of Scıence and Technology*, 1(2), 171-180. 2011.
[6] B.M. Ayesh, A.A. Abed., D.M. Faris, *BMC Research Notes,* 7,612. 2014.
[7] F. Gorbović, M.S. Satnkovic, M. Curcic, N. Dordevic D. Seklic, M.Topuzovic, S.Marcovic, *Plants, 2*, 371-378. 2013.
[8] I. Savini, R. Arnore, M.V. Ctani, L. Avigliano, *Nutrition and Cancer* 61(3), 381-389. 2009.

ANTIOXIDANT ACTIVITIES OF FRESH AND DRIED MINT SAMPLES

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In this study, antioxidant activity of peppermint (*Mentha piperita* L.) was examined. To this end, ethanol, methanol and water extracts were prepared from lyophilized and air-dried mint samples. For determination of phenolic substances Folin-Ciocalteu reagent was used and total antioxidant activity was evaluated via cupper (II) reduction (CUPRAC) method. Additionally, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), *N,N*-dimethyl-*p*-phenylendiamine (DMPD) and superoxide free radical scavenging activities were assigned. Gallic acid, trolox, ascorbic acid, butylhydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as reference substances. The highest total phenolic substance content was determined in water extracts. For air-dried plant 59.91±24.97 mg/g GAE, for lyophilized plant 58.20±2.34 mg/g gallic acid equivalent (GAE) phenolic substance is determined. According to the results of the CUPRAC study, it was found that each state of the plant yields similar level of total antioxidant capacity to standard substances. DPPH radical scavenging activity was found to be high in air-dried plant. ABTS radical scavenging activity was also high in air-dried plant. ABTS radical scavenging effect in ethanol and methanol extracts was higher than ascorbic acid and in water extract was higher than BHT for each sample of the plant. DMPD radical scavenging activity was found to be more effective in air-dried plant sample. For all the extracts of all samples, DMPD radical scavenging effect was higher than BHA. Superoxide radical scavenging activity was found to be more effective in air-dried plant rather than lyophilized plant. Both plant samples showed higher superoxide radical scavenging activity than BHA.

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF *Trigonella mesopotamica* Hub.-Mor and *Trigonella filipes* Boiss. FROM MARAŞ REGION IN TURKEY

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The genus *Trigonella* L. (Fabaceae) is widely distributed in dry regions around the Mediterranean, Southeastern Europe, Western Asia, North and South Africa. It contains approximately 135 species [1,2]. In Turkey, there are 54 *Trigonella* species and 21 of them are endemic [3]. *T. foenum-graecum* L. is the most widely used species in the genus. It is cultivated as a spice in Mediterranean countries, the Middle East, Russia, the Balkans, West Asia and China [1]. This plant has been extensively studied and it was reported that seeds have antidiabetic, antioxidant, anti-inflammatory, antipyretic, immunomodulatory, anticancer, gastroprotective and chemopreventive effects [4-7]. *T. foenum-graecum* is rich in flavonoids and also contains alkaloids, saponins, fixed oils, polysaccharides, proteins and minerals [4].

In the present study, the total phenolic and total flavonoid contents of the seeds and the aerial parts of *T. mesopotamica* Hub.-Mor. and *T. filipes* Boiss. from Maraş region in Turkey were investigated. Total phenolic content in plant extracts was determined using Folin-Ciocalteu assay and total flavonoid content was measured spectrophotometrically with $AlCl_3$ assay. The highest phenolic and flavonoid contents were determined in the aerial parts of *T. filipes.* The antioxidant activities of the extracts were examined using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay. The extract of the aerial parts of *T. filipes* showed the highest DPPH radical scavenging activity (60.16±0.06 % inhibition).

References

[1] D. Bown, Encyclopedia of Herbs & Their Uses, 1st ed. London: Darling Kindersley, 393, 2002.
[2] WC. Evans, Trease and Evans Pharmacognosy, 15th ed. UK: University of Nottingham, 26, 2002.
[3] E. Martin, H. Akan, M. Ekici, Z. Aytaç, Karyology of ten Turkish *Trigonella* L. (Leguminosae) species from section *Cylindricae* Boiss. *Turk J Bot*, 34, 485-494, 2010.
[4] K. Srinivasan, Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects, *Food Rev Int*, 22, 203-224, 2006.
[5] RS. Pandian, CV. Anuradha, P. Viswanathan, Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats, *J Ethnopharmacol,* 81, 393-397, 2002.
[6] FA. Toppo, R. Akhand, AK. Pathak, Pharmacological actions and potential uses of *Trigonella foenum-graecum*: A Review, *Asian J Pharm Clin Res*, 2, 28-38, 2009.
[7] R. Yaday, R. Kaushik, D. Gupta, The health benefits of *Trigonella foenum-graecum*: A Review, *Int J Eng Res Appl*, 1, 32-35, 2011.

FATTY ACID PROFILES AND MINERAL COMPOSITIONS OF THREE *Trigonella* L. SPECIES FROM SILIFKE-GÜLNAR REGION IN TURKEY

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The genus *Trigonella* L. (Fabaceae) is represented worlwide with about 135 species. Most of the species are widely distributed from the Mediterranean regions, Southeastern Europe, Western Asia, North and South Africa [1,2]. *Trigonella foenum-graecum* L., commonly called fenugreek, is the main cultivated species for culinary and medicinal purposes [1]. The seeds have been used in many traditional systems as aromatic, carminative, galactogogue, antibacterial, antidiabetic, hypocholesterolemic, diuretic and analgesic agent [3-6]. *Trigonella* species contain fixed oils, carbonhydrate, mucillagine, proteins, inorganic compounds, choline, flavonoids, saponins, alkaloids and sterols [3,7]. According to the literature, *T. foenum-graecum* is extensively studied but there are some previous studies focusing on the other *Trigonella* species.

In Turkey, the genus *Trigonella* is represented by 54 taxa. Among them *T. spruneriana* Boiss., *T. velutina* Boiss. and *T. strangulata* Boiss. from Silifke-Gülnar region have not been studied phytochemically and pharmacologically. The aim of the present study was to determine the fatty acid composition of the seeds. Additionally, mineral composition of the seeds and the aerial parts of the plants were investigated. The fatty acid composition of the seed oils was analyzed by gas chromatography-mass spectrometry (GC-MS). The mineral contents of the plants were determined by using an inductively coupled plasma mass spectrometry (ICP-MS). α-Linolenic and linoleic acids were the major fatty acids in the oils. The mineral analysis showed that the plants contain various macro and essential trace elements, especially magnesium, phosphorus, potassium, calcium, magnesium, iron, zinc, copper and selenium.

References

[1] D. Bown, Encyclopedia of Herbs & Their Uses, 1st ed. London: Darling Kindersley, 393, 2002.
[2] WC. Evans, Trease and Evans Pharmacognosy, 15th ed. UK: University of Nottingham, 26, 2002.
[3] K. Srinivasan, Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects, *Food Rev Int*, 22, 203-224, 2006.
[4] RS. Pandian, CV. Anuradha, P. Viswanathan, Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats, *J Ethnopharmacol*, 81, 393-397, 2002.
[5] FA. Toppo, R. Akhand, AK. Pathak, Pharmacological actions and potential uses of *Trigonella foenum-graecum*: A Review, *Asian J Pharm Clin Res,* 2, 28-38, 2009.
[6] R. Yaday, R. Kaushik, D. Gupta, The health benefits of *Trigonella foenum-graecum*: A Review, *Int J Eng Res Appl*, 1, 32-35, 2011.
[7] S. Shailajan, S. Menon, A. Singh, M. Mhatre, N. Sayed, A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds, *Pharmaceutical Methods*, 2, 157-158, 2011.

ANALYSIS OF THE FATTY ACIDS FROM THE SEEDS OF THE GENUS *Grammosciadium* DC. GROWING IN TURKEY

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The genus *Grammosciadium* DC. (Apiaceae) is represented by 8 taxa in Turkey; *G. daucoides* DC., *G. macrodon* Boiss., G*. cornutum* (Nábělek) C.C.Towns. *G. confertum* Hub.-Mor. & Lamond, *G. platycarpum* Boiss. & Hausskn. ex Boiss., *G. pterocarpum* Boiss., *G. schischkinii* (V.M.Vinogr. & Tamamsch.) V.M.Vinogr. and *G. haussknechtii* Boiss [1,2]. All species of the genus, except for *G. confertum,* belong to Irano-Turanian element [1]. Moreover, among them, *G. schischkinii, G. haussknechtii* and *G. confertum* are endemic taxa to Turkey [2]. There are several studies on the fatty acid contents of the family Apiaceae seed oils except for the genus *Grammosciadium* [3,4]. According to a literature survey of the genus *Grammosciadium*, there are a few studies on their phytochemical compositions and pharmacological activities. In the present study, the fatty acid compositions of the seed oils of above-mentioned *Grammosciadium* species were analyzed for derived methyl esters of their fatty acids by GC-FID and GC-MS. As a result of the analysis, 13 fatty acids were identified in the samples and the range of total fat in the taxa varied between 73.1 and 98.7%. GC-MS data showed that the main fatty acids were a mixture of petroselinic (18:1, n6) and oleic (18:1, n9) acids (15.1-64.7%). Moreover, we mainly detected linoleic (18:2, n6, 23.6-40.9 %) and palmitic acids (16:0, 4.5-20.1%) as well. This is the first report on the fatty acid compositions of the seeds of the genus *Grammosciadium* growing in Turkey. (This study was supported by Gazi University Research Foundation GUEF 02/2012-24).

References

[1] I.C. Hedge and J.M. Lamond, *Grammosciadium* DC. In: Flora of Turkey and East Aegean Islands. Ed. P.H. Davis, Vol. 4, p. 318-321, 1972.
[2] M.G. Pimenov and M.V. Leonov, The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. *Turk. J. Bot.*, 139-45, 2004.
[3] E. Bagci, Fatty acids and tocochromanol patterns of some Turkish Apiaecae (Umbelliferae) plants; a chemotaxonomic approach, *Acta Bot. Gallica*, 143-51, 2007.
[4] R. Kleiman, G.F. Spencer, Search for New Industrial Oils: XVI. Umbelliflorae-Seed Oils Rich in Petroselinic Acid, *J. Am. Oil Chem. Soc.*, 29-38, 1982.

INVESTIGATION OF ANTICANCER POTENTIAL OF *Pisolithus arrhizus* MUSHROOM GROWN IN TURKEY

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P. arrhizus intended to be used in this study which have high medicinal value and widely distributed in Turkey. In the literature it is reported that anticancer agents are found in some mushrooms while *P. arrhizus*, has not been studied as a source of bioactive agents yet. Colon cancer is one of the most endemic cancers causing significant health problems which decreases life quality and causes job loss. The purpose of this study was to evaluate the cytotoxic and antigenotoxic effects of *P. arrhizus*. The WST-1 cell proliferation assay kit and HT29 cell line was used in cytotoxicity study. The antigenotocity of the mushroom was established by Comet test. As a result of this study, the ethanol and methanol extracts of the mushroom sample have been found to have a high cytotoxicity on colon cancer cells and this effect increased proportionally with increasing concentration. On the other hand, both extracts decreased the effect of H_2O_2 on human lymphocytes. Our results support the use of *P. arrhizus* as a source of anticancer compounds.

CYTOTOXIC COUMARINS FROM THE FRUITS OF *Neocryptodiscus papillaris* (BOISS.) HERRNST. & HEYN

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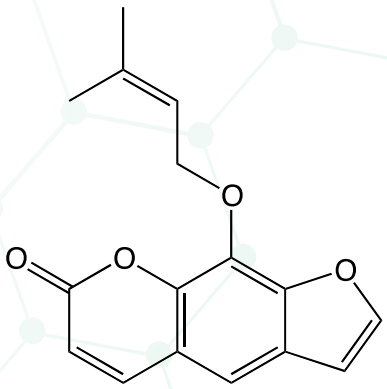
Neocryptodiscus papillaris (Boiss.) Herrnst. & Heyn (Apiaceae) is the only species of *Neocyrptodiscus* genus growing in Turkey [1]. The petroleum ether and dichloromethane extracts of the roots and fruits of this species show cytotoxic activity against MCF-7 breast cancer cell line. Previously, we have reported isolation and structure elucidation of three prenylated coumarins; oxypeucedanin, isoimperatorin and osthol, as the major cytotoxic principles of petroleum ether extract of the roots of *N. papillaris* [2]. In order to identify their cytotoxic compounds, petroleum ether and dichloromethane extracts of the fruits of *N. papillaris* were subjected to a series of chromatographic separations and then structures of the purified compounds were elucidated by spectroscopic methods. In addition to the three major cytotoxic coumarins isolated from the roots, imperatorin (**1**) and suberosin (**2**) were determined as the major cytotoxic compounds of the fruits of *N. papillaris* (This study was supported by the Research Foundation of Gazi University, research grant no. BAP 02/2014-01. We thank Assoc. Prof. Dr. Serdar Öztuzcu, University of Gaziantep, Faculty of Medicine, Department of Medical Biology, for the cytotoxic activity testing).



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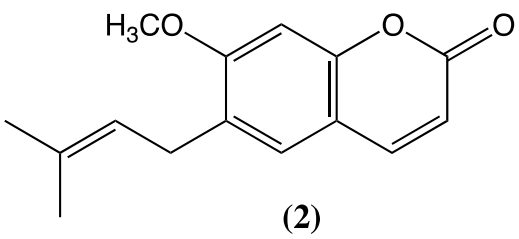

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References

[1] I. Herrnstadt, C.C. Heyn, *Neocryptodiscus papillaris* (Boiss.) Herrnst. & Heyn - a new combination based on *Cachrys papillaris* Boiss. *Candollea* 52(1): 181-184, 1997.
[2] F. Tosun, F. Mıhoğlugil, A. Duran, M. Miski, Cytotoxic Coumarins from the Roots of *Neocryptodiscus papillaris*, 11th International Symposium on Pharmaceutical Sciences, Ankara, Turkey, P-297, 9-12 June 2015.

ANTIPROLIFERATIVE POTENTIAL OF *Pistacia terebinthus* L. ON BREAST CANCER CELL LINES

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Most cancer treatment plans may include surgery, radiation and chemotherapy. Developing and applying of new chemotherapeutic agents and molecular-targeted drugs contributed to the cancer treatment, but are still insufficient because of their toxicity and the failure of chemotherapy due to drug resistance. For that reason, investigators attempt to discover and develop effective and less toxic biocompounds for the treatment. Thus, medicinal plants have become a focus to improve new anticancer agents. *Pistacia* L. species (Anacardiaceae family), are perennial shrubs or small trees with aromatic leaves. Most of the plant parts are used as food and traditional medicine in the region. Different parts of *P. terebinthus* have been used in the treatment of burns, wounds, and stomache, and also have used as antiseptic for respiratory and urinary system diseases, antipyretic and diuretic [1-5].

In the present study, to determine antiproliferative effect of the methanol and aqueous extracts of the fruits and leaves of *P. terebinthus* on two breast cancer cells (MCF-7 and MDA-MB-231), (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) colorimetric assay was carried out. The cells were seeded (4 x 10^3) onto 96-well plates and 24 h later treated with various concentrations methanol and aqueous extracts of *Pistacia terebinthus* (250 µg/ml-500 µg/ml -750 µg/ml and 1000 µg/ml) for 48 h.

P. terebinthus induced a dose-dependent decrease on the proliferation of both cells. But we observed no significant cytotoxic effect at ≤250 µg/ml concentrations of *P. terebinthus* treatment. However, greater than 36% and 20% decrease in cell viability was demonstrated at the concentrations of 500 µg/ml ($p \leq 0.001$) in MCF-7 and MDA-MB-231 cells, respectively.

References

[1] S. Mondal, S. Bandyopadhyay, M. K. Ghosh, S. Mukhopadhyay, S. Roy, C. Mandal, Natural products: promising resources for cancer drug discovery, *Anticancer Agents Med Chem*. 12(1):49-75, 2012.
[2] V. Kuete, T. Efferth, Biodiversity, Natural Products and Cancer Treatment. Publisher: *World Scientific* p: 412, 2014.
[3] R. S. Tagne, B. P. Telefo, J. N. Nyemb, D. M. Yemele, S. N. Njina, S. M. Goka, L. L. Lienou, A. H. Nwabo Kamdje, P. F. Moundipa, A. D. Farooq, Anticancer and antioxidant activities of methanol extracts and fractions of some Cameroonian medicinal plants. *Asian Pac J Trop Med*, 7(Suppl 1): S442-S447, 2014.
[4] I. Hacıbekiroğlu, P. K. Yılmaz, N. Haşimi, E. Kılınç, V. Tolan, U. Kolak, In vitro biological activities and fatty acid profiles of Pistacia terebinthus fruits and Pistacia khinjuk seeds. *Nat Prod Res*. 29(5):444-446, 2015.
[5] I. Erdogan Orhan, et al. Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of Pistacia terebinthus L. and their fatty and essential oil analyses.*Food Chemistry* 130: 882–888, 2012.

IN VITRO SCREENING OF NATURAL DRUG POTENTIALS FOR MASS PRODUCTION

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Plants, plant extracts and compounds synthesized by plants are utilized for treatment of diseases for ages [1]. Nowadays, the demand for natural remedies is remarkable, due to low side effect and easy accessibility, people prefer to use medicinal plants worldwide [2]. Turkey as a mediterranean county and as being a home of the cross road of 3 climate regions has rich natural vegetation. A number of medicinal plants have been grown and exported throughout the world, which need to be worked on. In order to explore new sources and research known sources for their rich content, we have collected some natural grown medicinal and aromatic plants, which have economic value in Bolu district (Gölcük and Seben areas). 17 different plants comprising *Tanacetum parthenium* L., *Sambucus nigra* L., *Equisetum arvense* L., *Achillea millefolium* L. were studied in terms of their *in vitro* activities, phytochemical constituents and mineral content. Polar (methanol) extracts were prepared and analyzed for their total phenolics by Folin-Ciocalteu, total flavonoids, and *in vitro* antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Besides, their metal content analysis of such as iron (Fe), selenium (Se), copper (Cu) were performed by using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) on plant samples and their methanol extracts [3,4,5]. Plants will be evaluated for their content in order to grow as medicinal plant for their economic value. The results will be discussed.

References

[1] S. Sekar, D. Kandavel, Interaction of Plant Growth Promoting Rhizobacteria (PGPR) and Endophytes with Medicinal Plants - New Avenues for Phytochemicals. *J. Phytology*, 2:91-100, 2010.

[2] T. Hussain, M. Arshad, S. Khan, H. Satar, M. S. Qureshi, *In Vitro* Screening of Methanol Plant Extracts for Their Antibacterial Activity. *Pak. J. Bot.*, 43:531-538, 2011.

[3] R. G. Woisky, A. Salatino, Analysis Of Propolis: Some Parameters And Procedures For Chemical Quality control. *Journal of apicultural research*, 37(2), 99-105, 1998.

[4] V. L. Singleton, J. A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158, 1965.

[5] S. Burda, W. Oleszek, Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry*, 49(6), 2774-2779, 2001.

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS FROM *Thymbra spicata* L. AND *Sideritis rubriflora* Hub.-Mor. WITH COMMERCIAL IMPORTANCE IN TURKEY

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Sideritis species are highly endemic, the genus Sideritis has an important place among the other Lamiaceae genera and these species named “Dağçayı or Adaçayı” are used as herbal tea and folk medicine in Turkey. *T. spicata* (Lamiaceae)grows wild in some eastern mediterranean contries and the dried leaves are used as a spice and as a herbal tea. The essential oil of *T. spicata* wasfound to inhibit mycelial growth of the fungi.

The aim of this study was to compare the antifungal effects of essential oils of two plants obtained by different extraction methods.. All essential oils inhibited all fungi at concentrations of 0.1-1/100 (v/v). The essential oil of *S. rubriflora* was more effective than that of *T. spicata*. It can be said that the essential oil of *S. rubriflora* could be used as natural antifungal agents in food preservation and human health.

References

[1] O. Sagdıç, A. Aksoy, G. Özkan, L. Ekici, S. Albayrak, Biological activity of the extracts of two endemic Sideritis species in Turkey, *Innov. Food Sci. Emer. Tech.*, 9, 80-84, 2008.
[2] H. Baydar, O. Sagdıç, G. Özkan, T. Karadoğan, Antibacterial activity and composition of essential oils from Origanum, Thymbra and Satureja species with commercial importance in Turkey, *Food Control*, 15, 169-172, 2004.
[3] J-C. Chalchat, M. Ozcan, Constituents of the essential oil of *Sideritis erythrantha* var. *erythrantha*, *Gen. Appl. Plant Phys.*, 31(1-2), 65-70, 2005.

CHOLINESTERASE AND TYROSINASE INHIBITORY ACTIVITIES OF TWO *ASTRAGALUS* L. SPECIES FROM TURKEY

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Astragalus L. genus that belongs to Fabaceae family is known by the name of “geven” and has 424 species including 224 endemic species in Turkey [1-2]. The roots of some *Astragalus* species have been used in throat and stomach-ache, diabetes, leucaemia, wound-healing and also for their hepatoprotective, diuretic, cardioprotective, antioxidant, immunstimulant and antiviral properties for many years [3]. *Astragalus* species contain polysaccharides, triterpenoid saponins, flavonoids, alkaloids, phenolic compounds and organic acids [4]. In the present research, we aimed to investigate cholinesterase and tyrosinase inhibitory potential of two *Astragalus* species (*A. compactus and A. psoraloides*). The methanol extracts of the roots of these dried and powdered endemic species were tested against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the enzymes linked to Alzheimer’s disease, as well as tyrosinase (TYRO), the target enzyme connected to Parkinson’s disease. Cholinesterase inhibition activity of the extracts were measured using the Ellman’s Colorimetric Method with galantamine hydrobromide as the reference and tyrosinase inhibitory activity was analysed using the Modified Dopachrome Method with 3,4-dihydroxy-L-phenylalanine (L-DOPA) as the substrate and alpha kojic acid as the reference. AChE, BChE and TYRO inhibitory activities of these extracts were measured at the doses of 250 µg/mL, 500 µg/mL and 1000 µg/mL. According to the results, it was demonstrated that *A.compactus* was more active than *A. psoraloides* against these three enzymes at all the doses and also concentration dependent activities were observed for all samples and exhibited as percentage inhibition. The root extract of *A. compactus* exerted the highest inhibition at 1000 µg/mL against AChE (36,71 ± 0,66%), BChE (42,50 ± 0,32%) and TYRO (50,69 ± 0,48%). Similarly the root extract of *A. psoraloides* showed the highest inhibition against AChE (34,29 ± 0,96%), BChE (37,01 ± 2,31%) and TYRO (41,55 ± 0,24%) at the same dose.

References

[1] P. H. Davis, R. R. Mill, K. Tan, Flora of Turkey and the East Aegean Islands, vol. 10, University Press, Edinburgh., 114-124, 1998.
[2] Z. Aytaç, Astragalus L. In: Güner, A., Özhatay, N., Ekim, T., Baser, K.H.C. (Eds.), Flora of Turkey and the East Aegean Islands, vol. 11. University Press, Edinburgh., 79-88, 2000.
[3] L. Ö. Demirezer, T. Ersöz, İ. Saraçoğlu, B. Şener, Tedavide kullanılan Bitkiler ‘’FFD Monografları’’, İstanbul, Nobel Tıp Kitabevi. 219-228, 2007.
[4] E. Bedir, İ. Çalış, R. Aquino, S. Piacenta, C. Pizza, *J. Nat. Prod.*, 62, 563-8, 1999.

ANTIOXIDANT PARAMETERS AND ORGANIC ACID CONCENTRATION IN DIFFERENT GRAPE CULTIVARS GROWN IN SOUTH POLAND

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Over the last few years, vineyards were established in Poland. These vineyards are just beginning to bear fruit and grape is harvested to produce wine. However the climate in Poland is less favorable to viticulture than in countries with warm climates, these conditions may give a specific chemical composition to the grapes [1]. In this study, anthocyanin, total polyphenol, sugar, vitamin C, tartaric acid, malic acid and citric acid contents of 13 grape varieties (white and red) were determined.

Concentration for antocyanins in juice only from red grapes was from 10.46 to 140.94 mg/L, total polyphenols in juice of white grape was from 32.12 to 100.00 mg/L and for red from 67.99 to 140.99 mg/L, sugar in juice for white from 13.17% to 16.35% and for red from 12.56% to 20.40%, vitamin C in juice for white 15.58 to 46.72 mg/L and for red 49.84 to 202.49 mg/L, tartaric acid in juice for white from 3426 to 9067 mg/L and for red 3913-11904 mg/L, malic acid in juice of white from 1835 to 7695 mg/L and for red from 1275 to 4374 mg/L, citric acid in juice for white from 147.8 to 1093.9 mg/L and for red from 442.8 to 1162.9 mg/L.

Obtained results are not significantly differ from the results for the vines from other countries.

References

[1] J.G. Gustafsson, A.Martensson,Potential for extending Scandinavian wine cultivation, *Acta Agriculturae Scandinavica, Section B-Soil and Plant Science,* 55, 82-97, 2005.

ON-LINE SCREENING AND IDENTIFICATION OF ANTIOXIDANT PHENOLIC COMPOUNDS OF *Saccocalyx satureioides* Coss. et Dur.

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Saccocalyx satureioides Coss. et Dur. is an endemic folk medicinal plant of septentrional Sahara. In this study, the chloroform, ethyl acetate and *n*-butanol soluble parts of the hydroethanolic extract (8:2) of the air-dried aerial parts of the species were evaluated for the antioxidant activity based on the results of 2,2'-azino-di(3-ethyl-benzothiazoline-6-sulfonic acid ($ABTS^+$). On-line HPLC-$ABTS^+$ was applied to screen the extracts and subsequent fractionation followed by spectroscopic analysis (HRMS, UV, NMR: ^1H, ^{13}C, COSY, NOESY, HSQC and HMBC) were applied to identify free radical scavengers in *S. satureioides*. Seventeen compounds were identified: piceol (**1**), vanillin (**2**), ferulic aldehyde (**3**), 3,3'-bis (3,4-dihydro-4-hydroxy-6,8-dimethoxy-2H-1-benzopyran) (**4**), 3,3'- bis (3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran) (**5**), dimethylcaffeic acid (**6**), balanophonin (**7**), 7-methyl-sudachitin (**8**), caffeic acid (**9**, *p*-coumaric acid (**10**), isoscutellarein-7-*O*-[β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside (**11**), isoscutellarein-7-*O*-[β-D-allopyranosyl-(1→2)]- 6"-*O*-acetyl-β-D-glucopyranoside (**12**), isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside (**13**),, quercetin (**14**), isoscutellarein-7-*O*-[6'''-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6''-*O*-acetyl-β-D-glucopyranoside (**15**), apigenin-7-*O*-[6''-*trans*-*p*-coumaroyl]-β-D-glucopyranoside (**16**), and sideritiflavone (**17**). These compounds were the dominant free radical scavengers in the species and their trolox equivalent antioxidant capacity (TEAC) were also determined. Quercetin and caffeic acid were the most active components (TEAC: 34. 41 and 31.93 µg/mL respectively), these results were validated by offline antioxidant DPPH and ORAC tests. All these compounds are described for the first time in the genus *Saccocalyx* whereas compounds **1, 3, 16** are described for the first time in the Lamiaceae family.

SECONDARY METABOLITES AND ANTIOXIDANT ACTIVITY OF *Limonium duriusculum* (DE GIRARD) KUNTZE EXTRACTS

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Many species of the genus *Limonium* are used in folk and modern medicine and are rich sources of flavonoids [1]. We report in this study, the chemical composition of the chloroform, ethyl acetate and *n*-butanol soluble parts of the H_2O-MeOH extract of the aerial parts of *Limonium duriusculum* (de Girard) Kuntze [2], and the antioxidant properties of *n*-BuOH extract using different assay systems. Air-dried aerial parts of *L. duriusculum* were used to prepare aqueous-methanolic extract. Then this extract was used to prepare $CHCl_3$, EtOAc and *n*-BuOH extracts. These three extracts were fractionated by silica gel column chromatography. The obtained fractions were purified on TLC silica gel plates giving 10 pure compounds. The antioxidant activity of the *n*-BuOH extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH°) free radical scavenging assay, ferrous chelating effect and inhibition of lipid peroxide (LPO) formation induced by Fe^{2+}/ascorbic acid system.

The structures of the isolated compounds which were identified as: β-sitosterol (**1**), methyl gallate 4-methyl ether (**2**), gallic acid 4-methyl ether (**3**), methyl gallate (**4**), vanillic acid (**5**), gallic acid (**6**), apigenin (**7**), apigenin 7-*O*-β-*D*- (6''-methylglucuronide) (**8**), pinoresinol (**9**) and 3β,5,6,7,8,3',4'-heptahydroxyflavanone (**10**), were established by spectral analysis, mainly ESIMS, UV and 2D-NMR experiments (COSY, HSQC, HMBC and ROESY). Compound **8** was new, and named as duriusculine while apigenin was the major constituent of the three extracts. The *n*-BuOH extract exhibited significant antioxidant activity.

References

[1] Asen, S., Roberts, C. M., Astill, J. D. 4,6,4′-Trihydroxyaurone and other flavonoids from *Limonium*. *Phytochemistry*, 11, 2601-2603, 1972.
[2] Quezel, P. and Santa, S.. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Editions du C.N.R.S, Paris, Tome II, pp. 736, 1963.

ANTIOXIDANT ACTIVITY AND PHENOL CONTENT OF SOME AROMATIC MEDICINAL PLANTS FROM ALGERIA

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Methanolic extract of ten medicinal plants (*Carduncellus pinnatus* (Desf.) DC; *Cédrus atlantica* Manetti; *Cotula cinerea* Delile; *Crateagus oxyacantha* Poir. *Lavandula multifida* L.*; Quercus suber; Osyris quadripartita* Salzm; *Rosmarinus officinalis* L.*; Salvia officinalis* L.; *Zygophyllum album* L.) from Algeria were examined for antioxidant activity, phenol and flavonoid content. Total phenol and flavonoid content and DPPH radical scavenging activity of the extracts were spectrophotometrically determined. Butylhydroxyanisole (BHA), quercetin, and ascorbic acid (Vit C) were taken as standard in case of phenol, flavonoid content and antioxidant activity respectively. The total phenol and total flavonoid contents were observed highest in *Quercus suber*. The DPPH radical scavenging activity was highest in: *Rosmarinus officinalis*; *Salvia officinalis* and *Quercus suber*. There observed a relationship between phenol and flavonoid content but failed to show relationship between phenolics content and antioxidant activity of the methanol extracts of the plants.

ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF PROBIOTIC IN AN EXCISION WOUND MODEL

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Successful wound healing requires resolution of the inflammatory response. But, if this phase continues, the wound may enter to chronic phase and fail to heal. Recent studies have shown that probiotic strains are capable to limit excessive amounts of reactive radicals. This study was conducted to evaluate the anti-inflammatory and antioxidant properties of *Lactobacillus reutery* in an excision wound model.

After creating full-thickness skin wounds on the back of 54 male Sprague–Dawley rats weighing 200–250 g, rats were divided into three groups consisting of 18 rats in each group. *Treatment group animals were topically treated with cream of L. reutery (10^6 cfu/ml) in eucerin base, eucerin group received topical eucerin daily and negative control group did not receive any treatment.* Wound contraction rates were calculated and the wound tissues were harvested at 5, 10, and 15 days for biochemical analysis. Malondialdehyde (MDA) level as an index of lipid peroxidation and myeloperoxidase (MPO) activity as an index of neutrophil infiltration during of inflammatory phase of wound healing wereassessed.

The percentage of the wound contraction was significantly higher at 8, 10 ($p<0.001$), 12 and 15 ($p<0.05$) days in the treated group than those of the control group. A significant decrease was observed in MDA content in the treated group than those of the control group on days 5 and 15 post wounding ($p<0.05$). The MPO activity was significantly decreased in the treated group compared to the control group at 5 and 10 ($p<0.05$) days after injury.

These findings reveal that the anti-inflammatory and antioxidant potential of the probiotic cream provided faster and better wound healing in rats and probiotic cream could be an additional novel therapeutic agent in the management of wound healing.

CHEMICAL COMPOSITON AND CHOLINESTERASE INHIBITORY ACTIVITY OF *Daucus aristidis* Coss. ESSENTIAL OIL FROM TWO STATIONS OF ALGERIA

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The chemical composition of the essential oil obtained by hydrodistillation from the aerial parts of *Daucus aristidis* (Apiaceae) cultivated from two stations of Algeria (Ghofi and Djelfa) at the flowering stage, was investigated for the first time, by GC and GC-MS and evaluated for *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities, the enzymes linked to Alzheimer's disease, by a spectrophotometric method of Ellman using ELISA microplate-reader at 100 µg/ml concentration. The main components of *D. aristidis* oil from Ghofi and Djelfa were α-pinene (74.1%-49%) and β-pinene (11.9%-19.2%) respectively. The oils exhibited a moderate inhibitory activity (over 50%) against both enzymes.

SYNTHESIS AND BIOLOGICAL EVALUATION OF MANNICH BASES OF BENZIMIDAZOLE-2-PHENOL DERIVATIVES AS ANTICHOLINESTERASE AGENTS

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Alzheimer's disease is an age-related neurodegenative disorder which affects more than 35 million people worldwide and involves in 60-80% of the dementia cases [1], [2]. Neurotransmitter acetylcholin's deficiency in central nervous system causes cognitive impairement in Alzheimer patients. The cholinergic approach for the therapy of this disease is using either an agonist or AChE (acetylcholinesterase) inhibitor to increase the cholinergic function [3]. In our previous study, a group of *N*-{2-[4-(1*H*-benzimidazole-2-yl)phenoxy]ethyl}substituted amine derivatives were synthesized and evaluated for their inhibitor activity against AChE [4]. Most of the compounds were exhibited good inhibition. Also we known that, an important number of studies report the activity of Mannich bases as AChE inhibitors [5]. In this study, a series of novel 'mannich bases of 2-phenolsubstitutedbenzimidazole' derivatives (Fig 1) were synthesized and characterized by ^{1}H NMR, mass spectral studies and elemental analysis. The compounds evaluated for the inhibition activity against AChE by using Ellman's method [6]. The final compounds exhibited from moderate to good activities to the AChE enzyme compared to the standart drug.

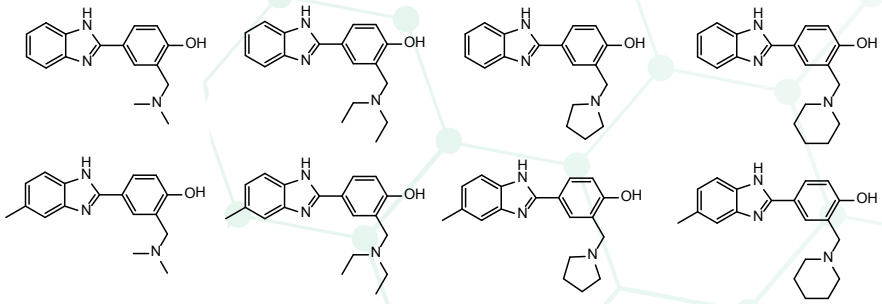

Fig. 1. The structures of synthesized compounds

References

[1] Alzheimer's Association; Alzheimer's disease facts and figures. *Alzheimer's & Demantia*, 5, 234-270, 2009
[2] Deutsche Alzheimer Gesellschaft, Factsheet ''Die Epidemiologie der Demenz". 2010. Quelle:http://www.deutschealzheimer.de/fileadmin/alz/pdf/factsheet/FactSheet01_10.pdf (aufgerufen am 10.02.2012)
[3] U. Holzgrabe, P. Kapkova, V. Alptüzün, J. Scheiber, E. Kugelmann, Targeting Acetylcholinesterase to Treat Neurodegeneration, *Expert Opin. Ther. Targets*, 11(2), 161-179, 2007.
[4] A. S. Alpan, S. Parlar, L. Carlino, A. H. Tarikogullari, V. Alptüzün, H. S. Güneş, Synthesis, Biological Activity and Molecular Modeling Studies on 1*H*-Benzimidazole Derivatives as Acetylcholinesterase Inhibitors, *Bioorg. Med. Chem.*, 21(17), 4928-4937, 2013.
[5] G. Roman, Mannich bases in medicinal chemistry and drug design, *Eur. J Med. Chem.*, 89, 743-816, 2015.
[6] G. L. Ellman, K. D. Courtney, B. J. Andres, R. M. Featherstone, A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity, *Biochem. Pharmacol.*, 7, 88-95, 1961.

ANTI-ALZHEIMER ACTIVITY OF NOVEL 6-SUBSTITUTED-3-(2*H*)-PYRIDAZINONE-2-ACETYL-2-(2,4-DISUBSTITUTED BENZAL) HYDRAZONE DERIVATIVES

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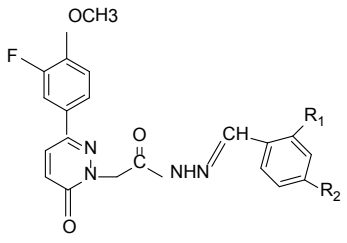
Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system, characterized by loss of cognitive ability and severe behavior abnormalities, which ultimately results in degradation of intellectual and mental activities. To date, the majority of current drug therapeutic approaches to AD follow the cholinergic hypothesis. Therefore, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors have gained a great popularity for the treatment of AD. Current treatment approaches in this disease continue being primarily symptomatic, with the major therapeutic strategy based on the cholinergic hypothesis and specifically on AChE and BChE inhibition [1]. Furthermore, pyridazinone derivatives have been reported to exhibited wide range of pharmacological activities such as antidepressant, antihypertensive, antithrombotic, anticonvulsant, cardiotonic, antibacterial, diuretic, anti-HIV, aldose reductase inhibitors, anti-inflammatory, anticancer. Considering the 6-aryl-3(2*H*)-pyridazinone residue as pharmacophoric group for the activity, in this work; we aimed to obtain some new derivatives of this class. Therefore, we focused on the formation of a ring system on the pharmacophoric pyridazinone residue and its 2,4-disubstitutedbenzalhydrazone analogue on the second position. In the present paper, fifteen 6-Substituted-3-(2*H*)-pyridazinone-2-acetyl-2-(2,4-disubstitutedbenzal) hydrazone derivatives which were synthesized by us [2]. The in vitro inhibition of AChE and BChE for these compounds were determined by the method of Ellman et al. [3] using galantamine as reference.



VI

References

[1] P. Jia, R. Sheng, J. Zhang, L. Fang, Q. He, B. Yang, Y. Hu, Design, synthesis and evaluation of galanthamine derivatives as acetylcholinesterase inhibitors, *Eur. J. Med. Chem.,* 772-84, 2009.
[2] A. B. Özçelik, S. Utku, Ö. Güler, M. Uysal, Novel 6-Substituted-3(2H)-pyridazinone-2-acetyl-(2,4-disubstituted benzal) hydrazone Derivatives: Synthesis and Structure Elucidation" 11th International Symposium on Pharmaceutical Sciences, P177 242-43, Ankara, Turkey, 09-12 June 2015.
[3] G. L. Elman, K. D. Courtney, V. Andres, R. M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharm.*, 88-95, 1961.

INVESTIGATION OF 3-METHYL-5-(4-CARBOXYCYCLOHEXYL METHYL)-TETRAHYDRO-2*H*-1,3,5-THIADIAZINE-2-THIONE GENOTOXICITY IN HUMAN PERIPHERAL LYMPHOCYTES USING SISTER CHROMATID EXCHANGE ASSAY

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Tranexamic acid is a drug, commonly used for curing abnormal bleeding in a variety of diseases. In a previous study, twelve tetrahydro-1,3,5- thiadiazine derivatives were synthesized by Özçelik et al [1] from the amine group of tranexamic acid as a prodrug in order to use tetrahydrothiadiazine derivatives topically as bleeding blocking and antimicrobial agents. These effects of these twelve compounds were compared with tranexamic acid. Among them, 3-methyl-5-(4 carboxycyclohexylmethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione is the most remarkable one and this compound may be used as a drug. In this study, genotoxicity of test compound was investigated using *in vitro* sister chromatid exchange (SCE) assay in cultured human peripheral lymphocytes. In addition, mitotic and replication indices were also calculated. Lymphocytes obtained from two healthy young donors were treated with six different concentrations (078; 1,56; 3,13; 6,25; 12,50 and 25,00 µg/mL) of test compound in culture conditions for 24 and 48 h. A negative, a solvent (PBS+%10 NaOH) and a positive control (mitomycin-C) were maintained for each treatment. According to test results, 3-methyl-5-(4-carboxycyclohexylmethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione significantly increased the SCE/cell ratio at four highest concentrations both 24 h and 48 h periods compared with the negative control (except 6,25 µg/mL for 48 h). In addition, compared with the solvent control, this increase was significant at the four highest concentrations for 24 h treatment, at the two highest concentrations for 48 h treatment. This compound decreased the MI in all concentrations compared with the negative and solvent controls at 24 h treatment period (except 0.78 and 1.56 µg/mL compared with the solvent control). In addition, MI decreased in three highest concentrations of test compound at 48 h period compared with the negative control and only 25 µg/mL was significant compared to the solvent control. On the other hand, no statistically significant deviation was observed in the replication index. In a previous study this compound did not increase the frequency of chromosomal aberrations compared with negative and solvent controls. The SCE assay has been widely used to assess the genotoxic potential of mutagenic and carcinogenic agents although it has to be taken into account that SCEs do not predict cancer as good as other biomarkers like chromosomal aberrations. For this reason, there is need to be done other genotoxicity tests for this compound.

References

[1] A.B. Özçelik, S. Ersan, A.U. Ural, S. Özkan, M. Ertan, Synthesis of 3-Substituted-5- (4- carboxycyclohexylmethyl)- tetrahydro-2H-1,3,5-thiadiazine-2-thione derivates as antifibrinolyticandantimicrobialagents, Arzneimittel-Forschung (DrugResearch), 57(8), 554-559, 2007.

ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY POTENTIAL OF PYRIDAZINONE ANALOGS AS NEW INHIBITORS FOR ALZHEIMER DISEASE

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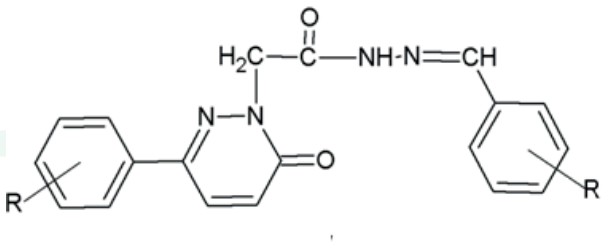
Alzheimer's disease (AD) is a complex neurodegenerative disorder of the central nervous system. It is estimated that nearly 36 million people worldwide are now suffering from AD, and would be increased to about 66 million until 2030. Acetylcholinesterase (AChE), a serine protease, is responsible for acetylcholine hydrolysis and plays a fundamental role in impulse transmission by terminating the action of the neurotransmitter acetylcholine at the cholinergic synapses and neuromuscular junction. Among the various approaches for treating AD, inhibition of AChE is still prevailing intreating or alleviating the symptoms of AD. Tacrine a nonselective AChE/butyrylcholinesterase (BuChE) inhibitor, was the first drug approved by FDA in 1993. Other selective inhibitors, such as donepezil, also reached the market sequentially [1]. However, this type of molecules displayed low AChE/BuChE selectivity, which might lead to undesirable peripheral side effects. We realized that the SAR of donezepil parallel with that our synthesized compound 6-substituted-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstituted benzal) hydrazone derivatives [2]. These pyridazinone molecules to be worth of optimization for finding diverse AChE inhibitors with high potency and AChE/BuChE selectivity The in vitro inhibition of AChE and BChE for the new synthesized title compounds was determined by the method of Ellman et al. [3] using galantamine as reference.



References

[1] W. Xing, Y. Fub, Z. Shi, D. Lu, H. Zhang, Y. Hua, Discovery of novel 2,6-disubstituted pyridazinone derivative as acetylcholinesterase inhibitors, *Eur. J. Med Chem*, 95-103, 2013.
[2] A.G. Akdemir, A.B. Özçelik, S. Utku, Ö. Güler, M. Uysal, H. Temel, Synthesis of 6-Substituted-3(2*H*)-pyridazinone-2-acetyl-2-(substituted/nonsubstituted benzal)hydrazone Derivatives" 11th International Symposium on Pharmaceutical Sciences, P222 (284-285), Ankara, Turkey, 09-12 June 2015.
[3] GL. Elman, KD. Courtney, V. Andres, RM. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem Pharm*, 88-95,1961.

DESIGN AND SYNTHESIS OF NOVEL ACETYLCHOLINESTERASE ENZYME INHIBITORS BASED ON STRUCTURAL SIMILARITY TO DONEPEZIL AND CARBOXYLIC ACID INDUCED ENZYME IMMOBILIZATION

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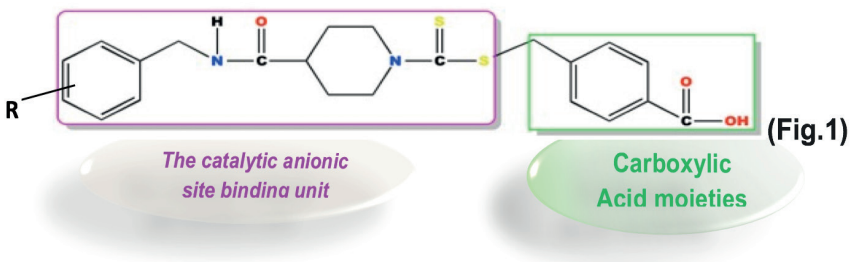
The treatment of Alzheimer's (AD) disease took a major step forward today as scientists unveiled a drug that appears to slow the progression of the disease. Existing drugs simply address the symptoms - and failure to deal with the underlying causes means they quickly wear off, and the disease soon takes its devastating course [1].Thus, over the past decade various cholinergic drugs, primarily inhibitors of the enzyme acetylcholinesterase (AChE) such as tacrine, donepezil, and more recently galantamine have been launched on the market for the symptomatic treatment of AD [2]. It is well known that an enzyme can immobilize by binding covalently to carboxyl, sulfhydryl, hydroxyl group, etc. along with a covalent binding may alter the conformational structure and active center of the enzyme, resulting in a major loss of enzyme activity [3]. Accordingly, in this paper we report the design and synthesis of a new series of compounds containing in their structures a benzylcarbamoylpiperidine as the catalytic anionic site binding unit structurally related to Donepezil and some carboxylic acid moieties as enzyme immobilizer, connected to each other by a linker of suitable length (Fig.1), in order to obtain new and influential derivatives as potent AChE inhibitors, and to cause a twofold increase in the inhibition effect toward the AChE enzyme. The entire synthesized compounds were characterized by the elemental analysis, FTIR, and ^{1}H-NMR methods and were subjected to anticholinesterase activity.



(Fig.1)

References

[1]http://www.dailymail.co.uk/health/article-3170737/Major-Alzheimer-s-breakthrough-scientists-unveil-new-drug-slows-progression-disease.htmlcitalopram.," *Expert Opin. Investig. Drugs*, vol. 11, no. 10, pp. 1477–1486, 2002.
[2] Pilar M, Oz-R, Laura R, Esther Garcı´a-P, Isabel D, Marı´a D, Rita V, Paola U, Celia D, Manuela B, Vincenza A, Axel Bidon-C, Modesto O, F. Javier L, Miguel M, and Ana M, Design, Synthesis, and Biological Evaluation of Dual Binding Site Acetylcholinesterase Inhibitors: New Disease-Modifying Agents for Alzheimer's Disease. *J. Med. Chem*. 2005, 48, 7223-7233.
[3] http://www.slideshare.net/jahir143/enzyme-immobilization-20940809 [Accessed 2015 Aug 27].

SYNTHESIS OF SOME NEW 1(2*H*)-PHTHALAZINONE DERIVATIVES AND EVALUATION OF THEIR ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES

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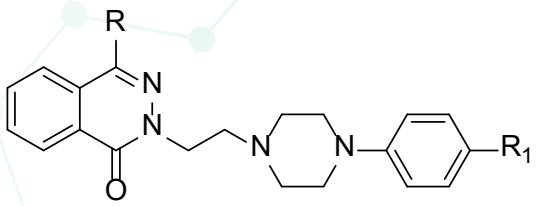
Alzheimer's disease (AD), a most common type of dementia, is manifested by progressive deterioration of intellectual and cognitive functions, memory loss, personality changes and difficulty in carrying out daily activities [1]. Current treatment options for AD are limited, there have been four drugs approved by Food and Drug Administration (FDA), including three cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and one *N*-methyl-D-aspartate (NMDA) receptor antagonist (memantine). However cholinesterase inhibitors are only effective in mild and moderate stages of the disease, their effectiveness varies individually and also they can cause peripheral side effects [2]. That's why new cholinesterase inhibitor development studies continue all over the world.

Consequently, we designed and synthesized twelve new 1(2*H*)-phthalazinone derivatives in order to investigate their cholinesterase inhibitory activities. Cholinesterase inhibitory activities of the synthesized compounds were determined by modified Ellman Assay [3]. (This study was supported financially with grant from Research Foundation of Gazi University Project Number: 02/2012-39).



$R = -H, -CH_3, -C_6H_5$
$R_1 = -H, -OCH_3, F, Cl$

References

[1] Y. Biran, C.L. Masters, K.J. Barnham, A.I. Bush, P.A. Adlard, Pharmacotherapeutic targets in Alzheimer's disease, *Journal of Cellular and Molecular Medicine,* 13, 61-86, 2009.
[2] D.A. Casey, D. Antimisiaris, J. O'Brien, Drugs for Alzheimer's Disease: Are They Effective?, *Pharmacy and Therapeutics*, 35, 208-211, 2010.
[3] C. Yamali, H.O. Gulcan, B. Kahya, S. Cobanoglu, M.K. Sukuroglu, D.S. Dogruer, Synthesis of some 3(2H)-pyridazinone and 1(2H)-phthalazinone derivatives incorporating aminothiazole moiety and investigation of their antioxidant, acetylcholinesterase, and butyrylcholinesterase inhibitory activities, *Medicinal Chemistry Research*, 24, 1210-1217, 2015.

2-PYRIDYL-4-SUBSTITUTED PHENOL THIAZOLE DERIVATIVES; SYNTHESIS, MOLECULAR MODELING AND AROMATASE INHIBITORY ACTIVIES

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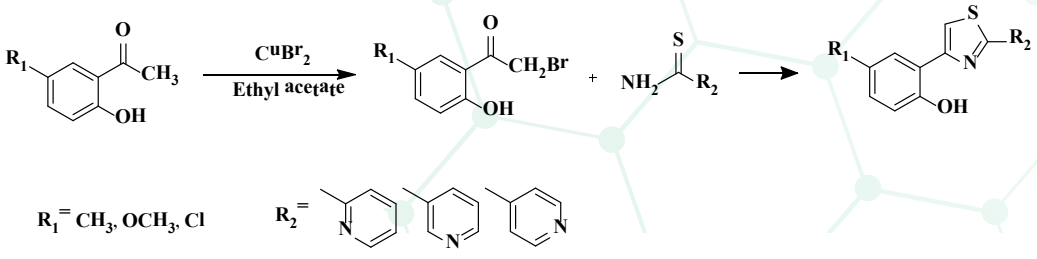
According to WHO (World Health Organisation), breast cancer is the top cancer in women in the developing world. Prolonged exposure to endogenous estrogens, such as early menarche, late menopause, late age at first childbirth, exogenous hormones, oral contraceptive and hormone replacement therapy are among the most important risk factors for breast cancer [1, 2].

Breast cancer treatment involve non-steroidal inhibiton of aromatase enzyme instead of steroidal therapy. Resumption of oestrogen production depends on the synthesis of new aromatase molecules [3]. After the release of "Crystal structure of human placental aromatase cytochrome P450 in complex with androstenedione" (3EQM), it became a possibility to develop selective drugs towards the cytochrome P450 in aromatase. Also, supporting data presented that adding an extra central 1,3-thiazole ring structure to 3,5,4'-trihydroxy-trans-stilbene structure enhances 6000 times aromatase inhibitory activity [4].

In this study, non-steroidal aromatase inhibitors 2-pyridyl-4-(5'-substituted phenol)thiazoles were synthesized and their purity, physical and structural specifications were checked by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, MS techniques and molecular modelling techniques (Scheme 1). Furthermore the compounds aromatase inhibition levels and IC_{50} values were determined using fluorescence based techniques and XTT (2,3-bis-(2-methoxy-4- nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) experiments on different cancer lines. Results of this study have shown promising anticancer activity.



Scheme 1; General synthesis of the proposed compounds.

References

[1] World cancer report, Lyon, International Agency for Research on Cancer, 2008.
[2] Jr. J.V. Lacey et al., Breast cancer epidemiology according to recognized breast cancer risk factors in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial Cohort, *BMC Cancer,* 9, 84, 2012.
[3] B.J.A. Furr, Aromatase inhibitors, Basel, Boston, Birkhäuser, ix, 182, 2006.
[4] T. Ogas, T.P. Kondratyuk, J.M. Pezzuto, Resveratrol analogs: promising chemopreventive agents, *Annals of the New York Academy of Sciences,* 1290:21-9, 2013.

SYNTHESIS OF SOME NEW 4,5-DIPHENYL-3(*2H*)-PYRIDAZINONE DERIVATIVES AND EVALUATION OF THEIR INHIBITORY ACTIVITIES ON ACETHYLCHOLINESTERASE/ BUTYRLCHOLINESTERASE ENZYMES

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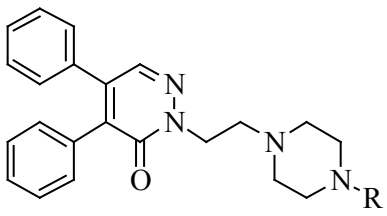
Alzheimer's disease (AD), the most common type of dementia in the elderly population, is a progressive neurodegenerative brain disorder that gradually destroys a patient's memory and ability to learn, make judgements, communicate with the social environment and carry out daily activities. Nowadays, drugs used in the symptomatic treatment of AD are cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and N-Methyl-D-Aspartate antagonist, memantine [1]. Because cholinesterase inhibitor drugs are only effective in mild and moderate stages of the disease and also the number of drugs was limited, new cholinesterase inhibitor development studies continue all over the world. The current approach in the treatment of AD is the cholinergic hypothesis. According to the hypothesis, cognitive decline observed in AD were associated with reduction of the neurotransmitter acetylcholine in the brain. Therefore, the primary goal of treatment is to increase the amount of acetylcholine [2].

In the present study we designed and synthesized eight new 4,5-diphenyl-3(*2H*)-pyridazinone derivatives in order to investigate their acetylcholinesterase/butyrylcholinesterase inhibitory activities. Modified Ellman assay was used for cholinesterase inhibitor activity measurement. [3]. (This study was supported by TUBITAK research grant 114S129)



R; p-substituted phenyl, benzyl or 2-pyridyl

References

[1] S. Salloway, J. Mintzer, M. F. Weiner, J. L. Cummings. *Alzheimers Dement*, 4, 65-79, 2008.
[2] M. Singh, M. Kaur, H. Kukreja, R. Chugh, O. Silakari, D. Singh, *Eur. J. Med. Chem.* , 70, 165-188, 2013.
[3] C. Yamalı, H. O. Gülcan, B. Kahya, S. Çobanoglu, M. K. Şüküroglu, D. S. Doğruer, *Med. Chem. Res.* 24(3), 1210-1217, 2015.

SYNTHESIS, CRYSTAL STRUCTURE, SPECTROSCOPIC AND DENSITY FUNCTIONAL MODELLING STUDIES OF 2-ISOPROPYLBENZIMIDAZOLIUM TETRACHLOROPLATIANTE (II) MONOHYDRATE

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Since the discovery of cisplatin [*cis*-diamminedichloroplatinum(II)] in 1965 and Food and Drug Administration (FDA) approval of cisplatin in 1978 [1] for metastatic ovarian tumors [2], thounds of cisplatin analogues have been synthesized [3]. In this studey, synthesis, crystallographic characterization, spectroscopic and density functional modelling studies of the 2-isopropylbenzimidazolium tetrachlroplatinate(II) monohydrate $(C_{10}H_{13}N_2)_2.[PtCl_4].H_2O$ have been reported. The molecular structure of the compound was determined by single-crystal X-ray diffraction analysis. In the compound, the Pt atoms reside at a center of inversion. The compound is comprised of 2-isopropylbenzimidazolium: $(C_{10}H_{13}N_2)^+$ and $[PtCl_4]^{2-}$ ions, respectively, linked by intermolecular hydrogen bonds N···Cl [3.249(4) from 3.660(7) Å], C···Cl [range from 3.553(7) to 3.895(7) Å] and O atom of a non-coordinating water molecule in the crystal structure N···O [2.728(8) Å], O···Cl [range from 3.234(6) to 3.451(7) Å], C···O [range from 3.350(7) to 3.545(1) Å] for the investigated compound. The molecular structure obtained from X-ray single-crystal analysis of the investigated compound in the ground state has been compared using Hartree-Fock (HF) and density functional theory (DFT), B3LYP and PBE1PBE functional with LANL2DZ basis set. The experimental (spectroscopic) and calculated vibrational frequencies (using DFT) of the title compound have been compared. There exists a good correlation between experimental and theoretical data for the complex.

References

[1] B. Rosenberg, L. Vancamp, J. E. Trosko, V. H. Mansour, *Nature*, 386-386, 1969.
[2] S. Sherman, S. J. Lippard, *Chem. Rev.*, 1153-1181, 1987.
[3] I. Ali, W. A. Wani, K. Saleem, A. Haque, *Anti-cancer Agents in Med. Chem.*, 296-306, 2013.

SYNTHESIS OF PLATINUM (II) COMPLEXES WITH SOME 2-SUBSTITUTED BENZIMIDAZOLE AS CARRIER LIGANDS

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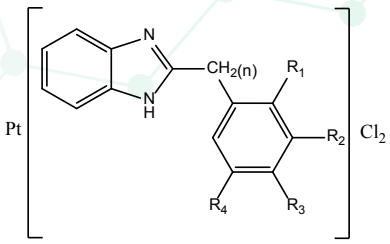
Cisplatin and its analogues carboplatin and oxaliplatin are routinely used worldwide in clinical practise [1]. The clinical efficacy of these anticancer drugs is diminished by intrinsic and acquired tumor resistance and side effects [2, 3]. In the search of new platinum compounds avoiding toxicity and resistance, special attention has been paid to the replacement of one or both NH_3 ligands of cisplatin by other N-donor ligands [4]. It is well-known that benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds and benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allow them to interact easily with the biopolymers of the living system which is responsible for their numerous biological activities and functions. Furthermore, benzimidazoles are known to exhibit a wide variety of pharmacological properties including antitumour activity [5] and inhibition of nucleic acid synthesis [6].

In this study, 5 new Pt(II) complexes with 2-substituted benzimidazole as carrier ligands were synthesized and structurally elucidated by using elemental analysis, ^{1}H-NMR and Mass spectroscopy methods. Our next studies will be focused on investigate the in vitro cytotoxic activities of these compounds.



Compound	n	R^1	R^2	R^3	R^4
1	0	H	H	CH_3	H
2	0	OCH_3	H	H	H
3	0	H	OCH_3	OCH_3	H
4	1	H	H	OCH_3	H
5	1	H	OCH_3	OCH_3	OCH_3

References

[1] L. Kelland, The resurgence of platinum-based cancer chemotherapy, *Nature Reviews Cancer*, 573-584, 2007.
[2] M. A. Fuertes, C. Alonso, J. M. Perez, Biochemical modulation of cisplatin mechanisms of action: Enhancement of antitumor activity and circumvention of drug resistance, *Chemical Reviews*, 103, 645-662, 2003.
[3] S. R. McWhinney, R. M. Goldberg, H. L. McLeod, Platinum neurotoxicity pharmacogenetics, *Molecular Cancer Therapeutics*, 8, 10-16, 2009.
[4] E. Wang, M. Giandomenico, Current Status of Platinum-Based Antitumor Drugs, *Chemical Reviews*, 99(9), 2451-2466, 1999.
[5] A. A. Saposov, I. N. Yozhitsa, L. I. Bugaeva, V. A. Anisimova, Benzimidazole derivatives: Spectrum of pharmacological activity and toxicological properties (a review), *Pharmaceutical Chemistry Journal*, 33(5), 232-243, 1999.
[6] R. A. Bucknall, S. B. Carter, A Reversible inhibitor of nucleic acid synthesis, *Nature*, 213, 1099-1101, 1967.

DESIGN, SYNTHESES AND ANTICANCER ACTIVITY OF 5-(ALKYLSULFONYL)-2-(5-SUBS-1H-INDOL-3-YL)-1-SUBS-1H-BENZO[d]IMIDAZOLES

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Novel indole-benzimidazole derivatives are designed and synthesized, in order to address the therapeutic need for treating cancer types-more specifically breast cancer. Syntheses of the compounds were carried out starting from commercially available aryl sulfonyl chlorides. Alkylation of the sulfonyl chlorides with iodoethane in the presence of tellurium, rongalite, and 1 M aqueous sodium hydroxide [1,2] gave ethylsulfonyl derivatives. This was followed by reaction with conc. H_2SO_4 and potassium nitrate to give nitro intermediates [2] Nucleophilic displacement of the chloro group with several amines in N,N-dimethylformamide, and their reduction with hydrogen gas by using palladium carbon and condensation of these derivatives with appropriate indole carboxaldehydes gave the targeted indole-benzimidazoles (3). Purity control and structural elucidation were controled by using elemental analyser and ^{1}H, ^{13}C-NMR, Mass spectrometers, respectively. Cytotoxic and anti-proliferative effects were measured against three mammalian cancer cell lines – MCF7, MDA-MB-231 and HEPG2. Cytotoxic and anti-proliferative effects were assessed via MTT assays and the half maximal inhibitory concentration [IC_{50}] measurements, to pinpoint candidates for further assays. Within the screening, a range of drug concentrations between 0.25 µM and 40 µM were applied, and a well-known chemotherapeutic agent Camptothecin was used as a positive control meanwhile. Using the MTT assays, we identified several drug candidates with promising IC_{50} measures and inhibitory curves based on the drug concentrations applied. Nonetheless, further evaluations will include assessing additional derivatives, proliferative/apoptotic potentials, changes in the transcriptome, and *in vivo* consequences. This work was supported by TÜBİTAK-SBAG Research Project: 213S037.

References

[1] H. Suzuki, Y. Nishioka, S. I. Padmanabhan, T. Ogawa, A novel synthesis of alkyl aryl sulfones via the telluride ion assisted coupling of arenesulfonyl chlorides with alkyl halides. *Chem Lett*, 72 (7), 728, 1988.
[2] K. Kon-I, M. Matsumizu, A. Shima, Preparation of sulfonyl benzimidazole derivatives useful in treatment of diseases-mediated by CB2 receptor. 2006, U.S. Pat. Appl. Publ., 63 pp. CODEN: USXXCO US 2006094750 A1 20060504.
[3] Z. Ates-Alagoz, Z., C. Kus, T. Coban, Synthesis and antioxidant properties of some novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments, *Journal of Enzyme İnhibition and Medicinal Chemistry*, 20(4), 325-331, 2005.

ACETHYLCHOLINESTERASE INHIBITORY POTENCY OF NOVEL TETRASUBSTITUTED IMIDAZOLE DERIVATIVES

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A deficiency of acetylcholine (ACh) containing neurons in some part of the brain such as cortex and hippocampus is related to the deficits in memory and cognitive function. For this reason, inhibition of acetylcholinesterase (AChE) enzyme, which reduces the level of ACh in central nervous system is a crucial target in the sense of medicinal chemistry. Acetylcholinesterase inhibitors (AChEI) prolong the duration of action of acetylcholine (ACh) and render symptomatic relief in AD. The usage of AChEI foranti-Alzheimer purpose has beneficial effects on cognitive, functional and behavioral symptoms of the disease [1,2]. Due to anticholinesterase potency of imidazole compounds a new series of imidazoles were synthesized to investigate their potency against AChE [3].

Prepration of 4-substituedbenzaldehyde derivatives

4-Fluorobenzaldehyde (10 mmol, 1,075 mL), K_2CO_3 (10 mmol, 1,38 g), appropriate secondary amine (20 mmol), and DMF (5 mL) were added into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 200 °C and 10 bars for 15 min. After the control of reaction by TLC, the mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

Microwave assisted synthesis of 4,5-Bis(4-substituted phenyl)-2-(4-susbtitutedphenyl)-1H-imidazoles (1)

Corresponding benzaldehyde derivative prepared in the first step (10,5 mmol) appropriate 4,4'-disubstitutedsubstituted benzil (10 mmol) and ammonium acetate (80 mmol, 6 gr) were dissolved in glacial acetic acid (10 mL) into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 220 °C and 10 bars for 15 min. The reaction mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

The chemical structures of the compounds were confirmed by spectral data. The anticholinesterase effects of the compounds were determined by Elman's method [3]. Enzymatic studies showed that some modifications on chemical structure of the compounds are required to enhance biological activity.

References

[1] Araújo JQ, Araújo de Brito M, BôasHoelz LV, de Alencastro RB, Castro HC, Rodrigues CR. Receptor-dependent (RD) 3D-QSAR approach of a series of benzylpiperidine inhibitors of human acetylcholinesterase (HuAChE) *Eur J Med Chem.*2011; 46: 39-51.
[2] Dvir H, Silman I, Harel M, Rosenberry TL, Sussman JL. Acetylcholinesterase: From 3D structure to function.*ChemBiol Interact*. 2010; 187: 10-22.
[3] Koolpe GA, Lovejoy SM, Goff DA ,Lin KY, Leung DS, Bedford CD, Musallam HA, Koplovitz I, Harris RN, Quaternary salts of 2-[(hydroxyimino)methyl]imidazole. 5. Structure-activity relationships for side-chain nitro-, sulfone-, amino-, and aminosulfonyl-substituted analogues for therapy against anticholinesterase intoxication. *Journal of Medicinal Chemistry,* 34:4, 1368-76,1991.
[4] Ellman, G.L.; Courtney, K.D.; Andres Jr, V.; Featherstone, R.M., A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology,* 7(2), 88-95, 1961.

SYNTHESIS AND EVALUATION OF SOME NEW PYRIDINE BEARING HYDRAZONE DERIVATIVES AS POTENTIAL ANTIBACTERIAL AGENTS

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In recent years, in terms of spreading worldwide rapidly and developing resistance to antimicrobial agents, bacterial infections become a major health problem [1]. That's why the discovery of new antimicrobial agents which suppress only the evolution of pathogens without harming host cells and the challenge with multidrug-resistance has became a huge interest in medicinal chemistry [2]. Hydrazone derivatives have been found to exhibit antimicrobial activity [3, 4]. One of the most common antibacterial drugs nifuroxazide, possesses a hydrazone moiety in its molecular structure and was used as an intestinal antiseptic [2]. There are many studies confirmed that pyridines have also been found to show antibacterial activity in many studies [5, 6]. Izoniazid, that was used in the treatment of tuberculosis, have both hydrazide and pyridine moieties, which proves that these structures play a very significant role in antibacterial activity [7]. Combination of two active moieties leads to a rise in biological activity.

Based on the advantages of hydrazone and pyridine moieties, we have synthesized a new series of hydrazone derivatives and tested their antibacterial activity. The structures of the obtained compounds have been evaluated using FT-IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral data and elemental analyses results. Variable and modest activity was determined against the investigated species of bacteria and fungi.

References

[1] S. Rollas, S. G. Küçükgüzel, Review biological activities of hydrazone derivatives, *Molecules* 12, 1910-1939, 2007.
[2] Z. A. Kaplancikli, M. D. Altintop, A. Ozdemir, G. Turan-Zitouni, G. Goger, F. Demirci, Synthesis and *in vitro* evaluation of some hydrazone derivatives as potential antibacterial agents, *Lett Drug Des Discov,* 11, 355-362, 2013.
[3] R. Narang, B. Narasimhan, S. Sharma, A Review on biological activities and chemical synthesis of hydrazide derivatives, *Curr Med Chem*, 19, 569-612, 2012.
[4] S. S. Machakanur, B. R. Patil, A. H. Pathan, G. N. Naik, S. G. Ligade, K. B. Gudasi, Synthesis, antimicrobial and antimycobacterial evaluation of star shaped hydrazones derived from 1,3,5-triazine**,** *Der Pharma Chem*, 4, 600-607, 2012.
[5] N. B. Patel, S. N. Agravat, F. M. Shaikh, Synthesis and antimicrobial activity of new pyridine derivatives-I, *Med Chem Res*, 20, 1033-1041, 2011.
[6] Y. W. Jo, W. B. Im, J. K. Rhee, M. J. Shim, W. B. Kim, E. C. Choi, Synthesis and antibacterial activity of oxazolidinones containing pyridine substituted with heteroaromatic ring, *Bioorg Med Chem*, 12, 5909-5915, 2004.
[7] V. Judge, B. Narasimhan, M. Ahuja, Isoniazid: the magic molecule, *Med Chem Res*, 21, 3940-3957, 2012.

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL DISUBSTITUTED OXADIAZOLE DERIVATIVES

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Today, there are many drugs on market for microbial infections, but also lots of research still keeps continuing in this area on account of the unsatisfactory status of present treatment of microorganisms, drug side effects, toxic effects and the acquisition by the infecting organisms of the resistance to the present drugs.

The 1,3,4-oxadiazole derivatives have been found to exhibit antimicrobial properties [1, 2]. Benzimidazole compounds have also important role on the treatment of bacterial infections. Benzimidazole is structurally similar to purine, and its derivatives could compete with purines. In the view of such information, we design a new series of 2-((5-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-1,3,4-oxadiazol-2-yl)thio)-derivatives.

The reaction of *o*-phenylenediamine and methyl 4-formylbenzoate in DMF under microwave condition, gave methyl 4-(1H-benzo[d]imidazol-2-yl)benzoate **(1)**. The compound **1** was treated with hydrazine hydrate to afford 4-(1H-benzo[d]imidazol-2-yl)benzohydrazide (**2**). The reaction of compound **2** with CS_2 in the presence of NaOH gave 5-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-1,3,4-oxadiazole-2-thiol (**3)**, which was reacted with substituted bromide derivatives to obtain final compounds.

Structures of the compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR and MS spectroscopic data and elemental analyses results. Antimicrobial activities of the compounds against resistant human pathogenic microorganisms were evaluated according to the CLSI methods [3,4]. Final products were tested for their in vitro growth inhibitory activity against human pathogenic *Escherichia coli* (ATCC 35218), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeuroginosa* (ATCC 27853), and yeast as *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), and *Candida parapsilosis* (ATCC 22019). Chloramphenicol and ketoconazole were used as control drugs.

In the series, **4c** was the most active derivative against all tested bacteria, where some of the compounds also displayed good antibacterial and anticandidal activity within the different inhibition extents.

References

[1] P. Karegoudar, MS. Karthikeyan, DJ. Prasad, M. Mahalinga, BS. Holla, NS. Kumari, Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents, *Eur. J. Med. Chem*. 43, 261-267, 2008.

[2] O. Prakash, M. Kumar, R. Kumar, C. Sharma, KR. Aneja, Hypervalent iodine(III) mediated synthesis of novel unsymmetrical 2,5-disubstituted 1,3,4-oxadiazoles as antibacterial and antifungal agents, *Eur. J. Med. Chem*., 45, 4252-4257, 2010.

[3] Clinical and Laboratory Standarts Institue (CLSI). (Formerly NCCLS) Methods for dilution antimicrobial sceptibilitytests for bacteria that grow aerobically, CLSI M7-A7, Clinical and Laboratory Standards Institue, 940 West Valley Road,Wayne, Pennsylvania, USA, 2006.

[4] Clinical and Laboratory Standards Institute (CLSI) 2008, Reference Method for Broth Dilution Antifungal SusceptibilityTesting of Yeast, Approved Standard, CLSI 27-A3, 3rd ed., PA, USA.

SYNTHESIS AND CHOLINESTERASE INHIBITORY POTENTIAL OF NEW SUBSTITUTED PIPERAZINE DERIVATIVES

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Cholinesterase inhibitors are the first choice in the treatments of Alzheimer's disease (AD) that are confirmed by the US Food and Drug Administration (FDA). Their important effect in patient with AD is reported to be maintenance of a higher level of cognitive function compared with placebo over a 6 to 36 months treatment period. Cholinesterase inhibitors block the destruction of the neurotransmitter acetylcholine (ACh) by acetylcholinesterase (AChE) [1]. Piperazine ring possesses two tertiary nitrogen elements that act as proton acceptor. Thus, nitrogen elements convert to quaternary form and can interact with anionic site of AChE by electrostatic attraction. Due to this property of piperazine it is usually sited into chemical structure of new inhibitor candidates of AChE [2].

Preparation of 4-(substituedpiperazine-1-yl) benzaldehyde derivatives

4-Substituted benzaldehyde derivative (10 mmol, 1,075 mL), K_2CO_3 (10 mmol, 1.38 g), piperazine derivatives (20 mmol), and DMF (5 mL) were added into a vial (30 mL) of microwave synthesis reactor (Anton-Paar, Monowave 300). The reaction mixture was heated under conditions of 200 °C and 10 bars for 15 min. After the control of reaction by TLC, the mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

General Synthesis procedure for 1-(3,4-disubstituedphenyl)-3-[4-(substituted piperazine-1-yl)-phenyl]-prop-2-en-1-one derivatives

The compounds synthesized in step 1 (10 mmol), appropriate acetophenone derivative (10 mmol) and potassium hydroxide (10 mmol) in methanol (10 mL) was stirred at room temperature for 12 h. After TLC screening, the resulting solid was filtered, washed with water, dried, and recrystallized from ethanol

Structure elucidations of the final compounds were performed with IR, ^{1}H-NMR, and ES-MS spectroscopic methods and elemental analysis. The anticholinesterase activity on AChE and BuChE were determined by a modification of Ellman's spectrophotometric method [3]. Enzymatic activity test indicated that the compound **2d** possess the highest inhibitory potency against AChE among the tested compounds.

References

[1] L. Kok-Fui, C. Kit-Lam, L. Chong-Yew, Blood brain barrier permeable anticholinesterase aurones: Synthesis, structure and activityrelationship, and drug-like properties. *European Journal of MedicinalChemistry*, 94, 2015.
[2] K. R. Varadaraju, J. R. Kumar, L. Mallesha, A., Muruli, K. N. S. Mohana, C. K. Mukunda, U. Sharanaiah, Virtual Screening and Biological Evaluation of Piperazine Derivatives as Human Acetylcholinesterase Inhibitors. *International Journal of Alzheimer's Disease*, 13, 2013.
[3] G. L. Ellman, K. D. Courtney, V. Andres Jr, R. M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology,* 7(2), 88-95, 1961.

MOLECULAR MODELING AND ACTIVITY STUDIES OVER MYCOBACTERIUM TUBERCULOSIS DNA GYRASE B ATPase ACTIVE SITE

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Today, HTVS (High throughput virtual screening) and docking using computer based algorithms and statistical techniques such as ROC curves (Receiver Operating Characteristic curves) are becoming very popular in design of new ligands which may be useful as drug molecules [1]. X-ray crystallographic data which includes ligand-macromolecule complex together or a homology model which gives chance to examine the interactions are essential for these algorithms and techniques.

DNA gyrase enzyme inhibition is an important and alternative approach, especially for the new compounds that can offer solution in multi-drug resistant Mycobacterium tuberculosis in clinical antimicrobial therapy [2].

Previously, we designed a homology model of M. Tuberculosis DNA GyrB ATPase active site, using “Crystal structure of the 43K ATPase domain of Thermus thermophilus gyrase B in complex with novobiocin” (RSCB Pdb id; 1KIJ) as a template keeping the novobiocin and a water in the hydophobic pocket which assumed to be important in the activity [3].

This study covers; preparation of different datasets by enrichment of 1442716 compounds belonging to ZINC database by known DNA GyrB ATPase active molecules, performing HTVS and evaluation of docking scores by using ROC curves to verify the true positives.

Besides 11 highest scored molecules were tested by “M. tuberculosis Gyrase Supercoiling Assay” and “Gel Base Drug Inhibition Assay” against novobiocin standard for activity, both showing compound having 5-(4-aminophenoxy)-2-(3-aminophenyl)-2,3-dihydro-1H-isoindole-1,3-dione structure have similar activity compared to standard.

Finally, new probable active derivatives of this compound was proposed by examining molecular interaction field (MIF) and distance/interaction probability analysis over obtained pose during docking step. (This study was supported by TUBITAK research grant 110S017).

References

[1] J. Alvarez, B. Shoichet, Virtual screening in drug discovery, Boca Raton, Taylor & Francis, 2005.
[2] B. Berk, D. Us, S. Oktem, Z.T. Kocagoz, B. Caglayan, I.A. Kurnaz, D. Erol, Molecular modeling and antimycobacterial studies of Mannich bases: 5-hydroxy-2-methyl-4H-pyran-4-ones, *Turkish Journal of Chemistry,* 35, 317-330, 2011.
[3] Drlica, K., Malik, M., Fluoroquinolones: action and resistance, *Curr Top Med Chem*, 3, 249-82, 2003.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW PYRAZOLE DERIVATIVES FOR NOVEL THERAPEUTIC OPPORTUNITIES OF HEPATOCELLULAR CARCINOMA

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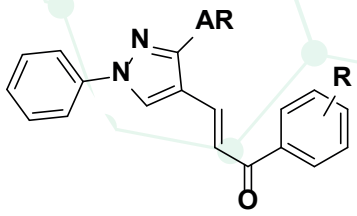
Hepatocellular carcinoma (HCC) is the second most deadly and the sixth most common cancer worldwide. Although chemotherapy is the major treatment method for HCC patients, chemotherapeutic agents are known to have side effects or become ineffective through drug resistance mechanisms of tumor cells. For this reason, it is important to discover non-traditional, efficient and safe chemical agents to be used in treatment of HCC. Combrestatin A-4 (CA-4), is a natural product isolated from the South African bush willow tree *Combretum caffrum*, inhibits the polymerization of microtubules by binding to the colchicine binding site. Pyrazole derivatives of CA-4 are proved to exert an antimitotic activity in human cancer cells by inhibition of tubulin polymerization. In this study, we aimed to define possible anticancer properties of newly synthesized pyrazole derivatives through evaluation of their cytotoxic effect on HCC cell lines and to determine the molecular mechanism underlying this effect. Cytotoxic activities of pyrazole derivatives were analyzed by SRB assay and by real-time cell analyzer on HCC cell lines. Cell cycle analysis through flow cytometry and cell staining methods were used to determine the mechanism by which these derivatives were showing their anticancer effect. Among 42 tested pyrazole derivatives, 14 of them were found to have IC_{50} values below 3 μM. Four of these molecules were chosen to be further studied on each cell line. Flow cytometric analysis of cultured cells treated with these molecules demonstrated that two of these compounds caused time dependent cell cycle arrest at the G2/M phase and also caused apoptotic cell death in both cell lines. Results were confirmed through western blot analysis where active molecules cause PARP cleavage in both cell lines indicating apoptosis and decrease in Cdc2 (CDK1) and CyclinB1 levels addressing cell cycle arrest in G2/M phase (This study was partially supported by Gazi University research grant 02/2012-41).



DESIGN, SYNTHESIS AND EVALUATION OF NOVEL 1,3-DIARYLPYRAZOLES AS DUAL COX/TXS INHIBITORS

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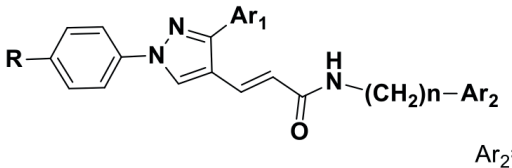
Studies towards better tolerated and potent non-steroidal anti-inflammatory drugs (NSAIDs) with fewer side effects as compared to current NSAIDs has been of interest for many years. The non-selective inhibition of the two-isoforms of COX is thought to be responsible for the gastric side effects associated with the chronic use of NSAIDs. It was thought that more selective COX-2 inhibitors would have reduced side effects. Recently, the research has focused on the development of COX-2 selective inhibitors, which are demonstrated to possess a significantly enhanced gastric safety compared to non-selective NSAIDs. Celecoxib and rofecoxib are two well known selective COX-2 inhibitors belonging COXIB class. However, rofecoxib and other COX-2 inhibitors have been withdrawn from the markets due to their adverse cardiovascular side effects. While a selective COX-2 inhibitor causes to reduction of prostacyclin (PGI_2) amount, thromboxane A_2 (TxA_2) production is still continued in platelets by COX-1 isoform. It is thought that cardiovascular toxicity of coxibs was generated because of imbalance between PGI_2 and TxA_2 levels. In a patient who requires effective anti-inflammatory treatment and has a high risk for both gastrointestinal bleeding and cardiovascular thrombosis, the use of selective COX-2 inhibitors with antiplatelet agent like thromboxane synthase inhibitor is being recommended. This aspect has encouraged the search for dual inhibitor both of COX-2 and thromboxane synthase (TxS) which should display an enhanced anti-inflammatory potency with less side-effect. Compared to COX or TxS pathways as single inhibitors, dual inhibitors shall present at least two major advantages: First, dual inhibitors, by acting on the AA metabolic pathway, possess a wide range of anti-inflammatory activity. Secondly, dual inhibitors appear to be almost exempt from gastric and cardiac toxicity, which are the most troublesome side effects of COX inhibitors. For this purpose we decided to synthesize new compounds possessing both anti-inflammatory and antiplatelet activities. The committed to diarylpyrazol structure, twenty seven new compounds were synthesized and their COX-1, COX-2 and antiplatelet activities were demonstrated. (This study was supported by TUBITAK research grant 110S284).

Ar_1= 3-pyridil or 4-pyridil



Ar_2= imidazole or pyridine

METAL COMPLEXES OF THE 4-DIHYDROXYBORYL-DL-PHENYLALANINE DERIVATIVES

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The elaborations of mechanism of anticancer impression of the platinum and gold complexes may be found in many studies [1,2]. BPA uptake would depend on metabolic status, and preferred backlog in tumor tissue would rely on the comparatively high metabolic activity of tumor cells checked against normal cells [3]. The emerges thought is that Pt(II) and Au(III) complexes of BPA derivatives might have more advances for treatment of malign tumors. Au(III) and Pt(II) complexes of the compounds which based BPA seem like promising cancer therapeutics. In this regard, further studies are continuing in our laboratory as in the past [4]. In this study, different Pt(II) and Au(III) complexes of 4-dihydroxyboryl-DLphenylalanine derivatives whose obtained by condensation with some different ligands were synthesized. The structure clarification of the complexes were implemented by ^{1}H NMR, IR, MS, and elemental analysis. (This study was supported by TUBITAK research grant 110S077).

References

[1] J.J. Roberts, A.J. Thomson, The mechanism of action of antitumor platinum compounds, *Prog. Nucleic Acid Res. Mol. Biol.,* 22:71–133, 1979.
[2] R. Sun, Strategies to Improve the Anti-Cancer Properties of Gold(III) Complexes, *Mod. Chem. Appl.,* 100-102, 2013.
[3] A. Wittig, W.A. Sauerwein, J.A. Coderre, Mechanisms of transport of p-borono-phenylalanine through the cell membrane in vitro, *Radiat. Res.* 153:173–80, 2000.
[4] R.B. Bostancıoğlu, K. Işık, H. Genç, K. Benkli, A.T. Koparal, Studies on the cytotoxic, apoptotic and antitumoral effects of Au(III) and Pt(II) complexes of 1, 10-phenanthroline on V79 379A and A549 cell lines, *J. Enzyme Inhib. Med. Chem.*, 27:458–66, 2012.

FRAGMENT-BASED APPROACHES IN DRUG DISCOVERY

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Fragment-based drug discovery relies on the identification of low molecular weight compounds (MW< 300Da) that bind to a chosen target and attempts to build up drugs from these small molecular pieces and components to achieve the desired biological activity and molecular properties [1]. It combines the empiricism of random screening with the rationality of structure-based design. Though the notion was articulated decades ago, the approach has become practical only recently. First experimental demonstration of the fragment-based approach to drug discovery is “SAR by NMR” (structure-activity relationship by nuclear magnetic resonance) method developed in 1996 by Fesik and his group. The method uses NMR spectroscopy to probe the surface area surrounding a protein’s active site for ligand binders [2,3]. Several other methods followed, using X-ray crystallography, nuclear magnetic resonance (NMR), mass spectroscopy (MS), surface plasmon resonance (SPR), tethering, isothermal calorimetry (ITC), and high concentration assay (HCA) techniques [4]. Multiple new chemical entities developed by the use of experimental fragment-based drug discovery have reached clinical trials and one of them, vemurafenib, was approved for the treatment of BRAF-mutated metastatic melanoma in the United States in August 2011 and the European Union in February 2012 [5]. Currently, there are a large number of drugs in clinical development that were discovered using fragment-based approaches.

Through a literature review we decided that FBDD is living up to its promise of delivering high quality leads with good physical properties and that in future many drug molecules will be derived from fragment-based approaches.

References

[1] A. Alexander, M. Flocco. Fragment-Based Drug Discovery: What has it Achieved so Far? *Curr.Top.Med.Chem.,* 7(16), 1544-67, 2007.
[2] S. Shuker, P. Hajduk, R. Meadows, S. Fesik. Discovering high-affinity ligands for proteins: SAR by NMR. *Science*, 274, 1531-1534, 1996.
[3] D. Erlanson, R. McDowell, T. O’Brien. Fragment-Based Drug Discovery. *Journal of Medicinal Chemistry*, 47(14) 3463-3482, 2004.
[4] S. Howard. Fragment-Based Discovery of Allosteric Ligands. *RSC Drug Discovery Series*, 47, 153-175, 2015.
[5] G. Bollag et al. Vemurafenib: the first drug approved for BRAF-mutant cancer. *Nature Reviews Drug Discovery*, 11, 873-886, 2012.

STUDY ON SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME PYRIDOPYRIDAZINE DERIVATIVES

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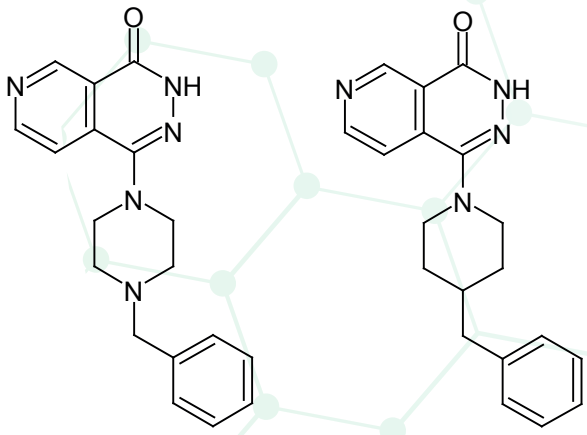
In this study, eight new pyrido[3,4-d]pyridazine derivatives were synthesized and evaluated for their in vitro antimicrobial activity. The chemical structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR, LC-MS and elemental analysis data. The compounds were evaluated for their antibacterial activities against Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 10145, Staphylococcus aureus ATCC 6538, Enterococcus faecalis ATCC 29212 and their clinical isolates, for their antifungal activities against Candida albicans ATCC 1023 and their clinical isolates and for their antimycobacterial activities against Mycobacterium tuberculosis H37Rv. Compound 10 (1-(4-benzylpiperazin-1-yl)pyrido[3,4-d]pyridazin-4(3H)-one) and compound 12 (1-(4-benzylpiperidin-1-yl)pyrido[3,4-d]pyridazin-4(3H)-one) were found to have the highest antimycobacterial activity among the synthesized compounds. However, all compounds were found ineffective against gram-positive, gram-negative bacteria and fungus.



Compound 10 Compound 12

SYNTHESIS AND ANTINOCICEPTIVE ACTIVITY OF MEPERIDINE-LIKE BENZIMIDAZOLE DERIVATIVES

Taner Ercanlı[1], Nur Banu Bal[2], Elif Derya Özdemir[2], Yasemin Dündar[1], M. Orhan Uludağ[2], Bilge Çakır[1], Tuncel Özden[1] and Tijen Önkol[1]

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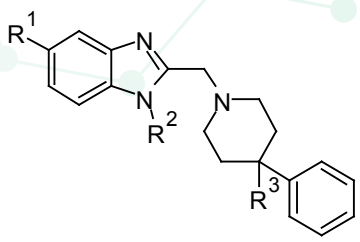
A series of novel benzimidazole derivatives were prepared and characterized by IR, ^{1}H-NMR spectroscopic data and elemental analysis. All the final compounds were screened for their antinociceptive activities with tail flick test. Among the synthesized compounds 3a, 4a, 4c, 8a, 9a exhibited significant antinociceptive activity. Compound 9a was found to have the highest antinociceptive activity at both 60 minute and 120 minute. Additionally, compounds 3a, 4a, 8a and 9a showed naloxone-reversible antinociceptive activity.

Comp.	R^1	R^2	R^3
3a	H	H	CN
4a	H	H	COOH
4c	Cl	H	COOH
8a	H	CH_3	CN
9a	H	CH_3	COOH



Compound 3a-c, 4a-c, 8a-c, 9a

R^1: H, CH_3, Cl; R^2: H, CH_3; R^3: CN, COOH

MICROWAVE SYNTHESIS OF 3-SUBSTITUTED [1,2,4] TRIAZOLO[3,4-*b*][1,3,4]THIADIAZOL-3-YL)METHYL]BENZOXAZOL-2(3*H*)-ONE DERIVATIVES

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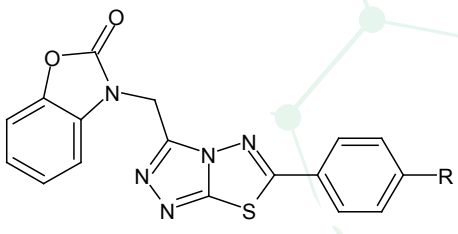
The chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention in recent years owing to their synthetic and effective medicinal importance. Derivatives of 1,2,4-triazole and 1,3,4-thiadiazole condensed nucleus systems found to have diverse pharmacological activities including unique anti-inflammatory, analgesic, antifungal , antibacterial and antiviral properties. Several triazolothiadiazole derivatives have been prepared from different non-steroidal anti-inflammatory agents and found to possess improved pharmacological profile.

On the other hand, the small and simple 2(3H)-benzoxazolone ring is presented in compounds involved in research, aimed at evaluating new products that shows a board spectrum of biological activity such as antimicrobial, antifungal, anticonvulsant, anti-inflammatory and analgesic activities.

Recently most of the reported reactions have been carried out either in sealed vessels or in the solid phase. Microwave irradiation has been also applied to carry out organic synthesis in open vessels using organic solvents. Microwave-assisted reactions using dry media have attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds. The absence of solvent reduces reaction time and improves yield. In our studies, a wide range of organic reactions have been achieved using MWI.

Therefore, these observations prompted us to synthesize new 1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazole derivatives which were attached to position-3 of the 2(3H)-benzoxazolone ring through a methylene bridge. Structure of the compounds synthesized has been elucidated by the aid of IR, ^{1}H NMR and mass spectral data.



R

H
F
Cl
CH_3
OCH_3
CF_3

ANTIDERMATOPHYTIC AND ANTI-*MYCOBACTERIUM* ACTIVITIES OF KOJIC ACID AND ALLOMALTOL DERIVATIVES WITH CYTOTOXICITY

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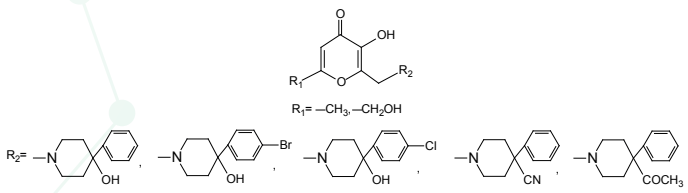
Kojic acid derivatives were synthesized and their extensive bioactivities were determined including anticonvulsant, antibacterial, antifungal, anti-*mycobacterium* and antiviral activities [1-4]. In the present study, the results of a preliminary evaluation of cytotoxicity, antidermatophytic and antimycobacterial activities of kojic acid/allomaltol (2-hydroxymethyl/methyl-5-hydroxy-4*H*-pyran-4-one) derivatives were examined. Chlorokojic acid was prepared with quite significant yield (75 %) by chlorination of kojic acid using thionyl chloride in room temperature. Allomaltol was produced by reduction of chlorokojic acid with zinc dust in concentrated hydrochloric acid [1]. Mannich compounds were prepared by the reaction of appropriate substituted piperidine derivatives with kojic acid/ allomaltol and formaline in room temperature. Hence we will present the results of a preliminary evaluation of antidermatophytic and anti-*mycobacterium* activities of Mannich bases of chlorokojic acid/allomaltol derivatives. *In vitro* antidermatophytic activity of the derivatives against *Microsporum gypseum*, *Trichophyton mentagrophytes var. erinacei* and *Epidermophyton floccosum* were screened as broth microdilution method [5]. Cytotoxicity was evaluated by the maximum non-toxic concentrations (MNTCs) of each sample, which was determined by the method described previously [6] based on cellular morphologic alteration. As for anti-*mycobacterium activity* the breakpoint concentrations (μg mL^{-1}) of the compounds will be determined against standard strains of *M. tuberculosis* H37Rv and *M. avium* (ATCC 15769) clinical isolated strains by using the colorimetric resazurin microtiter assay (REMA) [7].



References

[1] M.D. Aytemir, Ü. Çalış, M. Özalp, Synthesis and evaluation of anticonvulsant and antimicrobial activities of 3-hydroxy-6-methyl-2-substituted 4*H*-pyran-4-one derivatives, *Arch Pharm Pharm Med Chem*, 337, 281-288, 2004.
[2] M.D. Aytemir, B. Özçelik, Synthesis and biological activities of new mannich bases of chlorokojic acid derivatives, *Med. Chem. Res.,* 20, 443-452, 2011.
[3] G. Karakaya, M.D. Aytemir, B. Özçelik, Ü. Çalış, Design, synthesis and *in vivo*/*in vitro* screening of novel chlorokojic acid derivatives, *J. Enz. Inh. Med. Chem.,* 28(3), 627-638, 2013.
[4] M.D. Aytemir, B. Özçelik, G. Karakaya, Evaluation of Bioactivities of Chlorokojic Acid Derivatives Against Dermatophytes Couplet with Cytotoxicity, *Bioorg.. & Med. Chem. Lett.,* 23(12), 3646-3649, 2013.
[5] Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) 2nd Ed, CLSI, M38-A2, Wayne, PA, 2008.
[6] B. Özçelik, I. Gürbüz, T. Karaoğlu, E. Yeşilada, Antiviral and antimicrobial activities of three sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis, Microbiol. Res., 164* ation with clinical *Mycobacterium tuberculosis* isolates by using the microplate alamar blue assay, *J. Clin. Microbiol.*, 36(2), 362-366, 1998.

STRUCTURE-BASED DESIGN AND SYNTHESIS OF NOVEL BRP-7 DERIVATIVES BEARING C(5) POLAR SUSBTITUENTS AS POTENT FLAP INHIBITORS

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LTs derived from arachidonic acid (AA) by 5-lipoxygenase (5-LO) pathway are important lipid mediators having variety of inflammatory and vasoactive actions. This pathway also requires the involvement of 5-LO-activating protein (FLAP), which acts as an AA transfer protein facilitating substrate access for 5-LO, and it may function as scaffold protein for 5-LO at the nuclear membrane where the LT synthetic complex is assembled. Though no FLAP inhibitor has yet reached the market, FLAP is currently considered as a promising and clinically relevant target for pharmacological intervention with disorders requiring anti-LT therapy [1]. Our group has recently identified BRP-7, a non-acidic benzimidazole derivative including the isobutylphenylethyl fingerprint of ibuprofen, as a novel FLAP inhibitor chemotype lacking the typical pharmacophoric features of known FLAP inhibitors (IC_{50}=0.31 μM) [2]. Detailed investigation of the binding interactions of the FLAP x-ray ligand (MK591) with the FLAP active site revealed that Lys-116 at FLAP active site makes salt-bridge interactions with MK591 as well as water-mediated hydrogen bonds. In light of such information, we designed and synthesized novel BRP-7 derivatives bearing polar substituents at C(5) of the benzimidazole ring that could make polar interactions similar to MK591. This presentation will reveal our biological findings showing in vitro potent inhibition of LT biosynthesis and explain the increased in vitro potency with the aid of molecular docking results.

This study was supported by TUBITAK research grant 112S596.

References

[1] D. Pettersen, O. Davidsson, C. Whatling, Recent advances for FLAP inhibitors, *Bioorganic & Medicinal Chemistry Letters,* 2607-2612, 2015.
[2] E. Banoglu, B. Çalışkan, S. Luderer, G. Eren, Y. Özkan, W. Altenhofen, C. Weinigel, D. Barz, J. Gerstmeier, C. Pergola, O. Werz. Identification of novel benzimidazolederivatives as inhibitors of leukotriene biosynthesis by virtual screening targeting 5-lipoxygenase-activating protein, *Bioorg. Med. Chem*, 20(12), 3728-3741, 2012.

STRUCTURE-GUIDED DESIGN OF NOVEL ISOXAZOLE AND BENZIMIDAZOLE DERIVATIVES AS POTENT FLAP AND 5-LO INHIBITORS: INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS

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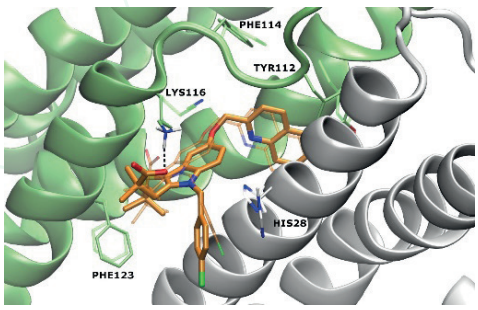
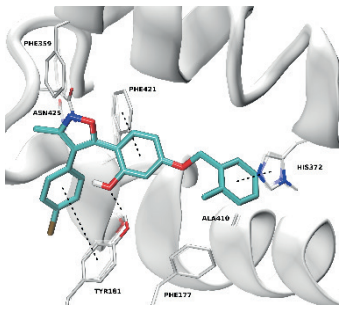
5-Lipoxygenase (5-LO) catalyzes an early step in the biosynthesis of leukotrienes (LT) from arachidonic acid (AA). The efficient utilization of AA by 5-LO for LT synthesis also requires a helper integral membrane protein termed “5-LO activating protein (FLAP)”. It has recently been demonstrated that 5-LO pathway associates with inflammation, allergic reactions, atherosclerosis, cancer and osteoporosis which may lead to development of new therapeutic treatments for pathologies that require anti-LT therapy. Both enzymes (5-LO and/or FLAP) needs to be targeted to inhibit that pathway by the development of novel potent inhibitors. With this aim *in silico* docking, molecular dynamics simulations and QSAR studies are done to extend our view from the previously obtained results by our research group. This studies were applied to the crystal structures of FLAP (PDB Code: 2Q7M, 4.2 Å), and 5-LO (PDB Code: 3O8Y, 2.39 Å). A series of compounds bearing benzimidazole structure were previously synthesized and one of the compounds which showed FLAP inhibitory activity (BRP-7) was chosen for further development. Another series of compounds bearing with isoxazole structure were previously synthesized and tested, resulting in active inhibitors towards both FLAP or 5-LO. Current modeling studies resulted in inhibition of LT biosynthesis in human leukocytes resulted with an IC_{50} value of 0.02 μM without interfering with purified 5-LO for BRP-7 derivatives (zileuton, the only commercially available 5-LO inhibitor has the IC_{50} of 0.8 μM in the same assay). The lead optimization process is still ongoing by combination of the insights from molecular dynamics simulations and in-house FLAP and 5-LO structure activity relationship (SAR) data. (This study is supported by TÜBİTAK with research grant 112S596)





THE ENZYMATIC REACTIVITY, ANTITUMOR AND ANTIBACTERIAL ACTIVITY OF NOVEL N-HETEROCYCLIC ORTHO-QUINOIDAL COMPOUNDS

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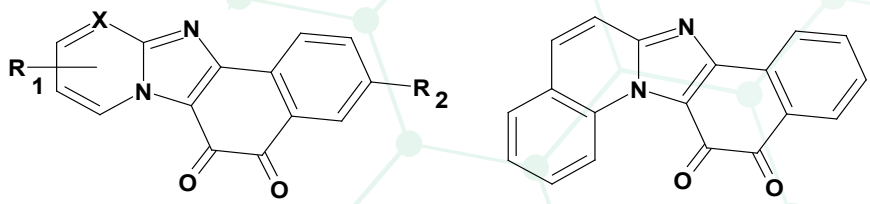
A series of naphtho[1',2':4,5]imidazo[1,2-a]-5,6-diones (NPYPDs), as novel N-heterocyclic ortho-quinoidal compounds, were synthesized. They acted as efficient electron-accepting substrates of NADPH-dependent single- and two-electron transferring flavoenzymes, P-450R (EC 1.6.2.4) DT-diaphorase (NQO1; EC 1.6.99.2), respectively. The enzymatic reduction of NPYPDs was accompanied by their redox-cycling, producing superoxide and peroxide as reactive oxygen species (ROS). The reactivity of NPYPDs towards P-450R (in terms of $k_{cat}/K_{m(NPYPDs)}$ values) spanned in the range of 10^6 - 10^7 M^{-1} s^{-1} and tentatively increased with an increase in their electron-accepting potency. NQO1 catalyzed the reduction of NPYPDs far more efficiently than P-450R, with $k_{cat}/K_{m(NPYPDs)}$ being close to the diffusion control limit (~ 10^8 M^{-1} s^{-1}). NPYPDs showed relatively high cytotoxic activity against human tumor cell lines, A549 and MCF-7; the concentrations of the compounds causing 50 % lethal effect (LC_{50}) varied in the range of 0.1-8.3 μM. Dicumarol, an effective inhibitor of NQO1, partly supressed their cytotoxic activity against both tumor cell lines, showing that the activity of the compounds is partially influenced by NQO1-mediated reactions. Examining the antibacterial activity of NPYPDs on Gram-negative *E. coli* (ATCC 25922) and *S. enterica* (SL 5676) and Gram-positive *S. aureus* (ATCC 25923) bacterial strains, the compounds were found to be potent against *S. aureus* strain; the defined minimum inhibitory concentration (MIC) values of NPYPDs varied in the range of 6-15 μM. (This work is supported by the Scientific Council of Lithuania (the project No. MIP-032/2014)).



R_1 = H, CH_3, OCH_3, F, Cl, Br, I, NO_2, CF_3;

R_2 = H, NO_2, SO_2NH_2;

X= CH or N;

The structural formulas of novel N-heterocyclic ortho-quinoidal compounds

THEORETICAL EXPLANATION OF THE REGIOSELECTIVITY OF HETERO DIELS-ALDER REACTIONS OF 1-AZA-1,3-BUTADIENE WITH DIMETHYLVINYLAMINE USING QUANTUM CHEMISTRY REACTIVITY INDICES

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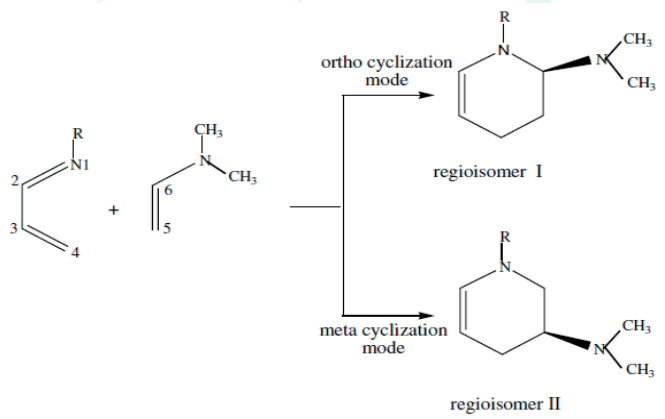
The hetero Diels Alder reaction (HAD) of 1-aza-1,3-butadiene with dimethylvinylamine is an efficient method for the synthesis of nitrogen heterocycles that constitute good intermediates for the preparation of complicated heterocycles.

Experimentally, it has been found that the cycloaddition of 1-aza-1,3-butadiene derivatives (R = H, $(CH_3)CO$, $(CH_3)CO–BH_3$) with dimethylvinylamine gives preferentially the ortho regioisomers [1-7] (Scheme 1). Our aim in this work is to put in evidence the regioselectivity of these HDA reactions using four theoretical models based on the use of reactivity indices. The quantum chemical calculations were performed using the B3LYP/6-31G(d) implemented in Gaussian 09 suite of programs.



Scheme 1

References

[1] Boger DL, Weinreb SN, *Hetero Diels-Alder Methodology in Organic Synthesis*, Academic Press, San Diego, 1987.
[2] Hwang YC, Fowler FW, *J Org Chem* 50, 2719, 1985.
[3] H. Chemouri, W. Benchouk & S. M. Mekelleche *J. Theoret. Comput Chem,* 20, 24, 2006.
[4] P. Pérez, L. R. Domingo, A. Aizman, R. Contreras Elsevier, Amsterdam, Vol. 19, 139-201, 2007.
[5] R. G. Parr, W. Yang W. *Density Functional Theory of Atoms and Molecules*, Oxford University, New York, 1989.
[6] A. Aizman, R. Contreras, *J. Chil. Chem. Soc.*, 49, 107-111, 2004.
[7] K. Chattaraj, S. Duley and L. R. Domingo *Org. Biomol. Chem.*, 10, 2855–2861, 2014.

THE CYTOTOXIC EFFECT OF NEW HYDRAZINECARBOTHIOAMIDES AND 1,2,4-TRIAZOLES ON *DAPHNIA MAGNA* AND *TRITICUM AESTIVUM*

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Researches on obtaining new antitumor drugs answer the needs of improving and developing new therapeutic strategies in diseases with elevated mortality, in order to improve the life quality of patients with cancer and to increase the survival rate. The study of literature highlights the fact that molecules containing a group of hydrazinecarbothioamide or 1,2,4-triazole present a variety of biological actions among which the antitumor action.

The aim of the present work was the evaluation of the cytotoxic effect of new compounds obtained by associating the dibenzo[a,d][7]annulene moieties with pharmacophore fragments hydrazinecarbothioamide or 1,2,4-triazole, using two alternative models on plant and invertebrate organisms.

The synthesis of the new compounds was realized in several steps according to the literature method, starting from 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide [1]. Acylhydrazinecarbothioamides were synthesized by nucleophilic addition of 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide to different arylisothiocyanates. Cyclization of acylhydrazinecarbothioamides in NaOH solution produced the corresponding 1,2,4-triazoles-3(4H)-thiol. The treatment of 1,2,4-triazoles-3(4H)-thioles with alkyl halide, in basic media, produced S-alkylated derivatives.

The cytotoxicity of new compounds was assessed against *Triticum aestivum* L. (Poaceae) and *Daphnia magna* Straus (Daphniidae) [2]. The compounds were tested in serial dilutions using amytriptilyne and the synthesis precursor, 2-(5H-dibenzo[a,d][7]annulen-5-yl)acetohydrazide, as positive controls. Three of the new synthetized compounds induced cytotoxic effects similar to the effect induced by amytriptilyne. The results obtained in this study could help to the identification of new hydrazones derivatives with potential cytotoxic properties. However, further studies are required in order to verify the antitumor properties of the compounds.

This work was supported by University of Medicine and Pharmacy “Carol Davila” Bucharest, project number 28331/04.11.2013.

References

[1] L. I. Socea, T. V. Apostol, G. Şaramet, Ş. F. Bărbuceanu, C. Drăghici, M. Dinu, Synthesis and root growth activity of some new acetylhydrazinecarbothioamides and 1,2,4-triazoles substituted with 5H-dibenzo[a,d]annulene moiety, *J. Serb. Chem. Soc.*, 77(11), 1541-1549, 2012.
[2] G. M. Nitulescu, C. Draghici, O. T. Olaru, New potential antitumor pyrazole derivatives: Synthesis and cytotoxic evaluation, *Int. J. Mol. Sci.*, 14(11), 21805-21818, 2013.

LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY METHOD FOR APREPITANT DETERMINATION AND VALIDATION IN CAPSULES

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Aprepitant, (5-[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2dihydro-3H-1,2,4-triazol-3-one (Fig.1), a potent and selective, brain penetrant neurokinin-1 (NK1) receptor antagonist, has been demonstrated to be effective in the prevention of chemotherapy-induced nausea and vomiting [1]. Previously reported very few methods for aprepitant quantification include HPLC and LC/MS [2, 3].This study is the part of a clinical trial which is about the effectiveness and reliability of aprepitant.

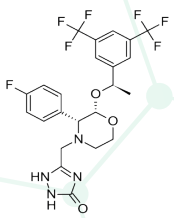

Figure. 1. Structural Formula of Aprepitant

A precise, simple, rapid and accurate reverse phase liquid chromatography- mass spectrometry (LC-MS) method for determination of aprepitant in capsules was developed and validated using ketoconazole as internal standard. The chromatographic determination was achieved by C18 reverse phase column particle size 3µm, 50 x 2.1 mm. The mobile phase consisting of 0.2% formic acid and acetonitrile in the ratio of 50:50 v/v was used. The flow rate was 0.4 ml/min. The MS detection was performed on a single quadrupole liquid chromatograph mass spectrometer (LC-MS) using electro spray ionization, positive mode at m/z 535 for aprepitant. Validation experiments were performed to demonstrate linearity, accuracy, precision, limit of quantitation (LOQ), limit of detection (LOD), and robustness. The calibration curve obtained was linear (r≥0.999) over the concentration range of 0.050-5.00 µg/mL. The results of the study showed that the proposed LC-MS method is simple, rapid, precise and linear, accurate and stability indicating which is useful for the routine determination of aprepitant in bulk drug and in its pharmaceutical dosage form.

References

[1] P. J. Hesketh, S. M. Grunberg, R. J. Gralla, The oral neurokinin-1 antagonists aprepitant for the prevention of chemotherapy-induced nausea and vomiting: a multinational, randomized, double-blind, placebo-controlled trial in patients receiving high-dose cisplatin. The aprepitant protocol 052 study group, *J Clin Oncol*, 21, 4112-4119, 2003.

[2] C. M. Chavez-Eng, M. L. Constanzer, B. K. Matuszewski, Simultaneous determination of Aprepitant and two metabolites in human plasma by high-performance liquid chromatography with tandem mass spectrometric detection, *Journal of Pharmaceutical and Biomedical Analysis*, 35, 1213-1229, 2004.

[3] K. Krishna Chaitanya, D. Gowri Sankar, D. Samson Israel, Narasimha kumar Ch. Validated RP-HPLC method for the quantification of aprepitant in bulk and pharmaceutical dosage forms, *Der Pharma Chemica*, 5(1), 39-46, 2013.

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION OF IRBESARTAN-AMLODIPIN-HYDROCHLOROTHIAZIDE BY UPLC

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Hypertension is one of the most widespread and important health problem that threatens public health. Most frequent cause of death in the world is the organ-system disorders caused by hypertension. Combination of antihypertensive molecules with pharmacologic and physiologic synergism increases the effect of the treatment while decreasing the side effect incidence [1].

Recommendation of European Association of Hypertension is to perform the combination therapy with only one tablet (fixed-dose combination) as long as it is possible [2]. Fixed-dose combination has many advantages. It is pragmatic, provides better and quicker blood pressure control, decreases the side effect frequency, increases patient compliance and decreases the treatment costs [3].

The purpose of this study was to concurrently analyze fixed-dose combination of irbesartan-amlodipine-hydrochlorothiazide; a new, simple, specific, economic and easy to use UPLC (Ultra Performance Liquid Chromatography) method was developed and validated. Chromatographic separation was carried out on Waters Acquity UPLC BEH C 18 column (50 mm×2.1 mm, 1.7 µm). The mobile phase was phosphate buffer (0.01 M) at pH 3.0 / acetonitrile, 63:37 (v/v). The flow rate was 0.5 ml/min and injection volume was 4 µl. The detection was performed at 227 nm in a short runtime of 2.5 minutes. An external method was used for the simultaneous determination of three ingredients. The chromatographic conditions were optimized to obtain good baseline separation and peak shapes. The developed method was validated regarding to system suitability, linearity, precision, accuracy, robustness and solution stability. This study was supported by Republic of Turkey Ministry of Science, Industry and Technology (Project Number: 01640.STZ.2012-2)

References

[1] David A. Williams, Thomas L. Lemke, Foye's Principles of Medicinal Chemistry, 7th Edition, 2012.
[2] http://www.eshonline.org/
[3] Jennifer Frank, MD, *American Family Physician*, 77(9), 1279-1286, 2008.

MOLECULAR MODELING EVALUATION OF NOVEL PIPERAZINE DERIVATIVES OF FLAVONES AS POTENT ANTI-INFLAMATORY AGENT

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In this work a series of novel 6-methoxy-2-(piperazin-1-yl)-4H-chromen-4-one and 5,7-dimethoxy-2-(piperazin-1-ylmethyl)-4H-chromen-4-one derivatives of biological interest were studied. On one side B3lyp/6-31g(d) DFT level of theory calculations were used to obtain their molecular structures, the chemical reactivity parameters (electronegativity, hardness, electrophilicity and Fukui functions) that arise from Conceptual DFT. On other hand Molecular Docking is used to study the biological activity of these series of molecules. The calculated values were compared with the available experimental data for these molecules and discussed in terms of their usefulness in describing anti-inflamatory activities.

PREFORMULATION STUDIES OF IN SITU GELS FOR OCULAR DRUG DELIVERY

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The eye is unique in terms of its anatomical and physiological nature, which make the targeting of drugs to eye tissues one of the greatest challenges in drug delivery [1]. The effective dose administered may be altered by increasing the retention time of medication into the eye by using in situ gels [2]. The aim of this study was to develop in situ gels for ocular delivery. The in situ gels were prepared by mixing weighed quantities of poloxamer 407 and Poloxamer 188 in water using a magnetic stirrer. 20g of the poloxamer solutions were put into a vial that was placed in a temperature controlled stirrer. The solution was heated at the rate of 2°C/min with the continuous stirring. The temperature was determined as gelling temperature, at which the magnetic bar stopped moving due to gelation (Table 1). The osmolality, pH and rheological properties of formulations were measured. The optimum concentrations of the formulation components were determined according to gelation temperature, viscosity and pH values.

Table 1: Gelling temperature and pH of in situ gel formulations

Formulations	Poloxamer 407 %	Poloxamer 188 %	Gelling temperature	pH
F1	15	-	45°C	7.08
F2	15	10	43-38°C	7.15
F3	15	15	40°C	7.31
F4	15	20	35-36°C	7.44
F5	15	23	35-36	7.53
F6	15	25	30-32°C	7.53
F7	18	-	28-29°C	7.08
F8	18	15	42-44°C	7.51
F9	18	18	38-39°C	7.57
F10	18	20	33-34°C	7.53
F11	18	22	32-33°C	7.63
F12	18	25	28-29°C	7.85
F13	20	-	24°C	7.08
F14	20	5	30-32°C	7.07
F15	20	10	33-34°C	7.22
F16	20	15	36-37°C	7.3
F17	20	20	32-33°C	7.34
F18	20	23	32-33°C	7.67
F19	23	7	28-30°C	7.34
F20	25	-	20°C	7.08

Characterization of the new drug delivery systems are major issues to be considered in the formulation stage, especially those intended for ocular administration. The characterizations such as pH, viscosity, gelling temperature of optimum formulations (F6, F10, F11, F14, F17) were appropriate for ocular delivery. In conclusion, this study showed that developed optimum in situ gel formulations could be alternatively used as ocular drug carriers.

References

[1] H. Almeida et al., In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations, *Drug Discovery Today*, 19, 400-412, 2014.
[2] J. Varshosaz et al., Designing of a Thermosensitive Chitosan/Poloxamer In Situ Gel for Ocular Delivery of Ciprofloxacin, *The Open Drug Delivery Journal*, 2, 61-67, 2008.

FLAVONOIDS ISOLATED FROM *OPUNTIA FICUS – INDICA* (L.) MILL.

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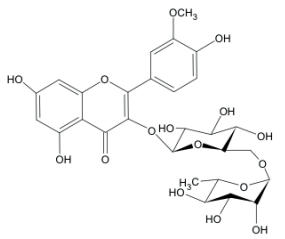
Opuntia ficus indica (L.) Mill. (Cactaceae) is widely known in South Anatolia and used as food industry and to decrease high lipid and blood sugar levels. Besides these effects the antiinfflammatory, antirheumatic, analgesic antioxidant, neuroprotective, antipoliterative anticancer and antigenotoxic effects are known as folk medicine. It is also used in wound healing and burns. For all these purposes the water extracts of the fruits, roots, aerial parts are used [1]. In this study, the fruits of *Opuntia ficus indica* (L.) Mill. collected from Alanya province in September 2014. The juice was squizzed and fleshy parts of the fruits were dried in shade. The dried material was then continuously extracted with methanol:water (50:50) for 48 hours at 50 ºC. The water-methanolic extract was concentrated in rotary evaporatory at 50 ºC under vacuo. The concentrated residue was fractionated with n-hexane, ethyl acetate, and n-buthanol respectively in a separating funnel. To investigate the antioxidant activity of the fractions DPPH were used and Folin-Ciocalteu assay was for their total phenolic content [2]. According to the results the buthanolic and ethyl acetate fractions were rich in phenolics and had high antioxidant activities than n-hexane and phenolic content were found in buthanol fraction. After comparing the amounts of the fractions the buthanol fraction was chosen and applied to polyamide column. A series of adsorption and partition chromatography were applied to phenolic rich compounds obtained from polyamide column. The structural elucidation of the isolated flavonoids was done by TLC and ^{1}H-NMR spectroscopy. The compounds were identified as narcissin (isorhamnetin-3-O-rutinoside) **(1)** and 7-methoxyisorhamnetin-3-O-rutinoside **(2)**, by comparing the data with references [3].



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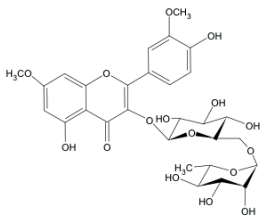

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(1) narcissin (isorhamnetin -3-O-rutinoside)

(2) 7-methoxy isorhamnetin -3-O-rutinoside

References

[1] H. B. Keleş, R. Yücer, S. Çatıkoğlu, M. Bacanlı, N. Başaran, A. A. Başaran, *Opuntia fius indica* Bitkisi Üzerinede Yapılan Farmakognozik Araştırmalar, *Bitkisel İlaç Hammaddesi Bildiri Kitapçığı,* 87, 2014.
[2] U. S. Harput, Y. Genc, I. Saracoglu, Cytotoxic and antioxidative activities of Plantago lagopus L. and characterization of its bioactive compounds, *Food and Chemical Toxicology,* 50, 1554-1559, 2012.
[3] M. Saleem, J. K. Hyoung, K. H. Chang, Secondary metabolites from *Opuntia ficus-indica var. saboten, Phytochemistry*, 67, 1390-1394, 2006.

THE EFFECT OF THE DRUG:POLYMER RATIO ON THE PROPERTIES OF OFLOXACIN LOADED ELECTROSPUN FIBERS

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Electrospinning is recognized as a unique, simple, efficient and versatile method to prepare polymeric fibers with diameter in the range of nanometer to submicron size using electrically charged jet of polymer solutions or melts [1]. Electrospinning also shows great flexibility in polymer materials for drug delivery applications. In the study, Ofloxacin (OFL) loaded poly(ε-caprolactone) (PCL) fibers as a drug delivery system for the treatment of ocular infections were fabricated by electrospinning. The effect of the drug:polymer ratio on fiber morphology, fiber size and drug release were investigated. Morphology and average diameter of electrospun fibers was evaluated by scanning electron microscopy (SEM) images. The physical state of drug in fibers was analyzed by Differantial Scanning Calorimetry (DSC). In the formulations, ratio of OFL: PCL was used as 1:9 (F1), 2:8 (F2), and 3:7(F3). Fiber diameters very near to each other in F1 fibers and drug crystals were not observed indicating that the drug was considerably incorporated into the electrospun fibers. From the image analyses, it was found that the fibers had an average diameter of 520±99.6 nm. On the other hand, F2 and F3 formulations had not very smooth surfaces on which drug crystals were seen. These results have shown that the increase of drug amount in the formulation led to the presence of drug crystals on the surface of fibers. Average diameters of F2 and F3 formulations were 580±252 nm and 371±113 nm, respectively. By decreasing the polymer:drug ratio from 2:8 to 3:7, fiber diameter became smaller due to the lower concentration and chain entanglement of polymer in the spinning solution. It can be seen that drug release profiles were affected by increasing OFL content. F2 and F3 fibers displayed immediate release behavior, more than 62 % and 68 % of the drug were released in the initial 20 min, whereas F1 fiber exhibited slower release, 42 % of the drug was released in the initial 20 min. It is believed that at higher concentration of OFL (F2 and F3), the solved drug in the polymer solution had more tendency to migrate to surface or near the surface of fibers during the electrospinning process. The drug:polymer ratio in the spinning solution had an impact on the average fiber diameter and morphology. The initial amount of the released drug was found to vary as a function of OFL:PCL ratio and polymer composition in the fiber.

References

[1] M. Zamani, M. Morshed, J. Varshosaz,, M. Jannesari, Controlled release of metronidazole benzoate from poly ε-caprolactone electrospun nanofibers for periodontal diseases. *Eur. J. Pharm. Biopharm.*, *75*, 179-185, 2010.

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