БЪЛГАРСКО НАУЧНО ДРУЖЕСТВО ПО ФАРМАЦИЯ
BULGARIAN SCIENTIFIC PHARMACEUTICAL SOCIETY

VII-МИ КОНГРЕС ПО ФАРМАЦИЯ С МЕЖДУНАРОДНО УЧАСТИЕ

21-24 ноември 2019 г.

хотел „Рила“, к.к. Боровец

С подкрепата на: Български Фармацевтичен Съюз
Supported by: Bulgarian Pharmaceutical Union

7th CONGRESS OF PHARMACY WITH INTERNATIONAL PARTICIPATION

November 21-24, 2019

Rila Hotel, Borovets

Pharmaceutical Altitudes

Dear Colleagues,

On behalf of the Executive Council of the Bulgarian Scientific Pharmaceutical Society and of the Organizing Committee I am pleased to cordially welcome You at the 7th Congress of Pharmacy with International Participation "Pharmaceutical Altitudes" in the lovely resort of Borovetz (Rila Hotel), Bulgaria (21-24 November 2019).

We are organizing the Congress as an exciting venue, whereby leading internationally acknowledged experts in the fields of pharmacy and allied sciences will meet to debate the state-of-the-art scientific advances, and to face the evolving challenges and opportunities in the fast-changing world of Pharmacy, as an integral part of healthcare. Special emphasis is focused on drug design, phytopharmaceuticals, biotechnological drugs and biosimilars, pharmaceutical nanotechnologies and biopharmaceutics, pharmaco-economics and health technology assessment.

Esteemed colleagues, we welcome You to present your own research, to learn from prominent experts, network with key-opinion leaders, enjoy hearing high level lecturers reporting their latest scientific projects or sharing their vision over the evolving challenges in the pharmaceutical science and practice. The agenda of the Congress also includes specialized expert panels with prominent Bulgarian clinical key-opinion leaders, intended to discuss the state-of-the-art advances and the challenges of modern pharmacotherapy.

Cordially Yours,
Prof. Georgi Ts. Momekov, PhD
President of the Bulgarian Scientific Pharmaceutical Society

7th CONGRESS OF PHARMACY

WITH INTERNATIONAL PARTICIPATION

November 21-24, 2019, Rila Hotel, Borovets

Supported by: Bulgarian Pharmaceutical Union

ORGANIZER

BULGARIAN SCIENTIFIC PHARMACEUTICAL SOCIETY

ORGANIZING COMMITTEE

Chairman:
Prof. Georgi Momekov, PhD

Members:
Prof. Danka Obreshkova, Dsci
Prof. Denitsa Momekova, PhD
Assoc. prof. Niko Benbassat, PhD

Secretary:
Lilia Dimitrova, PhD

SCIENTIFIC COMMITTEE

Chairman:
Prof. Nikolay Danchev, PhD

Members:
Akad. Hristo Tsvetanov, Dsci
Prof. Nikolay Lambov, PhD
Prof. Plamen Peykov, PhD
Prof. Valentina Petkova, Dsci
Prof. Genka Petrova, Dsci
Prof. Irini Doychinova, Dsci

БЪЛГАРСКО НАУЧНО ДРУЖЕСТВО ПО ФАРМАЦИЯ
BULGARIAN SCIENTIFIC PHARMACEUTICAL SOCIETY

7th CONGRESS OF PHARMACY

WITH INTERNATIONAL PARTICIPATION

November 21-24, 2019, Rila Hotel, Borovets

ABSTRACTS

Pharmaceutical Altitudes

The abstracts are published as presented by the authors

CONTENTS

POSTER SESSION

back to contents

ARE THE POLYMERIC NANO-CARRIERS ALTERNATIVE TO CANCER THERAPY?

Tsvetanov Ch.

Cancer remains one of the hardest for treatment diseases. Each year, over 10 million people worldwide are affected by cancer. Cancer therapy involves: surgery, radiation therapy, chemotherapy, immunotherapy, stem cell therapy, etc. The use of chemotherapy can lead to the death of healthy cells as well as to body toxicity. The use of nano-carriers in chemotherapy offers the following advantages:

- prevents preliminary drug degradation;
- prevents drug pre-interaction with the biological environment;
- provides effective drug absorption into the tumor tissue;
- controls the location of the drug;
- improves drug penetration into the cell.

The following problems with the use of polymeric drug nano-carriers will be discussed:

- active and passive drug targeting;
- targeting agents;
- types of nano-carriers – their advantages and disadvantages;
- overcoming the membrane protein barrier – resistance of the cells by ejecting the drug;
- the possibility of finding the so-called “magic bullet” – the drug that selectively attacks only the diseased tissue.

CHALLENGES AND OPPORTUNITIES IN PHARMACY PRACTICE AND PROFESSION

Angelovska B.

Faculty of Medical Sciences, ,”Gotse Delchev” University, Shtip, Republic of North Macedonia

Pharmaceutical care is a patient-centered, outcomes oriented pharmacy practice that requires cooperation of pharmacists with patients and other healthcare professionals in order to promote health, to prevent disease, and to assess, monitor, initiate, and modify medication use to assure that drug therapy regimens are safe and effective. The aim of pharmaceutical care is to optimize patients’ health-related quality of life (HRQoL), and also to achieve positive clinical outcomes, within realistic economic expenditures. The requirements for providing safe and effective personalized care are becoming more and more important, taking into account the differences in the socioeconomic structure and demographic specifics.

Despite the constant changes in health care systems, pharmacies still remain an integral part of the health care system. Their unique position in healthcare system is related to the fact that pharmacies are the last place for interaction between patients with healthcare professionals. Preservation of professional independence of

back to contents

the pharmacist in order to maintain their independence from commercial pressure is of paramount importance for the further development of pharmaceutical practice.

In order to accept the current and future challenges, we want to create a future with highly qualified and independent pharmacists able to give continuous support of every individual patient, of public health and also of the healthcare system.

References:

1. Principles of Practice for Pharmaceutical Care [Internet]. Home. 2018 [cited 2018 Apr 15]. Available from: https://www.pharmacist.com/principles-practice-pharmaceutical-care

2. mHealth, Use of mobile health tools in pharmacy practice, FIP-YPG report, 2019, https://www.fip.org/files/content/publications/2019/mHealth-Use-of-mobile-health-tools-in-pharmacy-practice.pdf

3. Pharmaceutical Group of European Union EUROPEAN COMMUNITY PHARMACY BLUEPRINT for optimisation of health outcomes to individual patients and value for health systems across Europe, Brussels,Belgium, 2012, www.pgeu.eu

4. The FIP Community Pharmacy Section, Vision 2020, https://www.fip.org

5. Pharmacists' role in harm reduction, Draft report of a FIP Bureau Working Group FIP, 3-2-2017, https://www.pharmaceutical-journal.com/

6. Essentials of Pharmacy Management, The role of business in pharmacy practice, https://www.pharmpress.com/files/docs/EPM2e_samplechapter.pdf

7. IRISH PHARMACY UNION, Vision for community pharmacy in Ireland, https://ipu.ie/wp-content/uploads/2018/04/PwC_IPU-report.pdf

8. PGEU Weekly Newsletter, 10th - 21st June 2019, https://www.ema.europa.eu/en/news-events/publications/newsletters

9. https://www.fip.org/worldpharmacistsday

10. VISION 2020 PARTNERSHIP FOR CARE Pharmacists and Doctors working together Pharmaceutical Society of New Zealand Inc. (PSNZ) 2019, https://www.psnz.org.nz/Folder?Action=View%20File&Folder_id=86&File=Partnershipforcare2020PharmacistsandDoctorsworkingtogether2014VisionPrinter.pdf

CYTOCHROME P450 METABOLISM IN BRAIN: ROLES AND IMPLICATIONS IN NEURODEGENERATIVE DISEASES

Dragoni S., Chiaino E., Frosini M., Fernandez-Abascal J., Valoti M.

Dipartimento di Scienze della Vita,
Università di Siena, 2 Via Aldo Moro, 53100 Siena, Italy

The cytochrome P450 (CYP) family is involved in different steps of therapy from drug efficacy and dose requirement to adverse drug reactions and direct toxicity. Most of the studies have been conducted in the liver which is the major organ involved in drug metabolism due to the high concentration of CYPs in the endoplasmic reticulum of hepatocytes. However, the CYP families involved in xenobiotic metabolism are also expressed in extrahepatic tissues (i.e., intestines, brain, kidney). Since the expression of the majority of the isoforms appears to be low compared to the predominant expression in the liver, and their role in the overall total body clearance is lower, the basal expression and up-regulation in peripheral tissues

back to contents

can significantly affect local disposition of drugs or endogenous compounds and thus modify the pharmacological/toxicological effects or affect the distribution of xenobiotics in the human body. In the brain, the overall level of CYP is ~0.5 – 2% of that in liver microsomes and could play a role in tissue- and/or cell-specific sensitivity to certain drugs or xenobiotics (Ferguson and Tyndale, 2011). There have been a number of suggestions that environmental toxins may play a role in the pathogenesis of neurodegenerative disorders by directly damaging neurons or through bioactivation of some toxic compounds via CYPs. This can be an important aspect as the mechanisms involved in Parkinson's disease are thought to be multifactorial in nature and elucidation of how CYP may predispose the brain to subsequent damage caused by exogenous toxins is important. CYP isoforms are generally associated with the endoplasmic reticulum however, some CYP enzymes have been found in the mitochondria of a number of different brain areas including the substantia nigra. Therefore CYP and CYP-induction will result in neurotoxicity/neuroprotection due to enhanced metabolism of exogenous substrates. In our experiments, we have demonstrated that CYPs is inducible in vivo and in vitro models (Marini et al, 2007, Fernandez-Abascal et al., 2018). Moreover, in SH-SY5Y cells the induction of CYPs was able to reverse the damage of mitochondria complex I and the apoptosis promoted by the neurotoxin MPP+. These data suggest that cytochrome P450 can play an important role in the regulation of molecular mechanisms involved in neurodegeneration.

Ferguson CS, Tyndale RF. Cytochrome P450 enzymes in the brain: emerging evidence of biological significance. Trends Pharmacol Sci. 2011:708-14.

Marini S, Nannelli A, Sodini D, Dragoni S, Valoti M, Longo V, Gervasi PG. Expression, microsomal and mitochondrial activities of cytochrome P450 enzymes in brain regions from control and phenobarbital-treated rabbits. Life Sci. 2007;80910-7.

Fernandez-Abascal J, Ripullone M, Valeri A, Leone C, Valoti M. β-Naphtoflavone and Ethanol Induce Cytochrome P450 and Protect towards MPP^+ Toxicity in Human Neuroblastoma SH-SY5Y Cells. Int J Mol Sci. 2018; 19. pii: E3369.

CURRENT PHARMACOLOGY AND PHARMACOTHERAPY OF MULTIPLE SCLEROSIS

Danchev N., Kostadinova I.

Faculty of Pharmacy, Department of Pharmacology, Pharmacotherapy and Toxicology, Medical University of Sofia, Bulgaria

Corresponding author: nikolai.danchev@gmail.com

Multiple sclerosis (MS) is a neurodegenerative disease of the central nervous system, characterized by demyelination of the axons. It affects many young people in active age between 30-35 years. There are four main clinical forms of MS and the most common is the relapsing-remitting form. The disease requires expensive treatment, making it a socially significant disease. Over the last 15-20 years, many first- and second-line therapies have been developed. The first-line therapy includes application of interferons Interferon beta1b and Interferon beta 1a and the copolymer

back to contents

of the four amino acids - lysine, alanine, tyrosine and glutamic acid (INN glatiramer acetate). There are presented the pharmacodynamics, pharmacokinetics, clinical application and adverse effects of drugs for multiple sclerosis used in Bulgaria. The monoclonal antibodies Natalizumab and Alemtuzumab used as a novel therapy approach to multiple sclerosis are also discussed. There are presented the results of our own investigations of peptide derivatives of 4-aminopyridine.

ALZHEIMER'S DISEASE – OPPORTUNITIES OF PHARMACOTHERAPY

Kostadinova I., Danchev N.
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
Corresponding author: Ivanka Kostadinova, PhD
e-mail: i.kostadinova@pharmfac.mu-sofia.bg

Alzheimer's disease (AD) is a chronic, progressive, neurodegenerative disease, in which the combination of various risk factors and mechanisms leads to a deterioration of brain cortical functions, memory deficits, learning and also behavioural changes. AD is the most common cause of dementia in people over 65 years of age, representing 50-60% of all cases of dementia. There are many hypotheses about AD, including abnormal deposit of amyloid β (Aβ) protein in the extracellular spaces of neurons, formation of twisted fibers of tau proteins inside neurons, cholinergic neuron damage, inflammation, oxidative stress. Approved medicines for AD are mainly related to the cholinergic hypothesis. In this regard, first-line drugs are inhibitors of the acetylcholinesterase enzyme - Galantamine, Rivastigmine and Donepezil. For moderate to severe AD, N-methyl-D-aspartate receptor antagonist Memantine is used. Due to the large number of side effects, new drugs are currently being studied, some of which are in Phase 3 of clinical trial such as the beta secretase inhibitor Elenbecestat.

PHARMACOLOGIC INFLUENCE ON HYPERTHERMIC REACTION IN EXPERIMENTAL SEROTONIN SYNDROME MODELS

Koleva K., Bogdanov G., Nikolov R.
Department of Pharmacology, Pharmacotherapy and Toxicology,
Faculty of Medicine, Medical University, Sofia

Aim of the study: Serotonin syndrome is observed as a result of an overdose of serotonergic drugs or interactions with the combined administration of two or more drugs that increase the intrasynaptic concentration of serotonin (5-hydroxytryptamine). The aim of the present study is to establish the effects of diazepam, vigabatrin, olanzapine and risperidone on the hyperthermic reaction in a serotonin syndrome in rats.
Materials and methods: The animal models of serotonin syndrome used in our experiments were induced by co-administration of both 5-hydroxy-L-tryptophan and

back to contents

clorgyline or fluoxetine and tranylcypromine. Body temperature experiments were conducted at ambient temperature of 24 ± 1° C. The body temperature of the animals was measured with thermistor probes (TX-8) and monitored on a multichannel recorder Iso-Thermex 16. The thermistor probes were lubricated and inserted rectally to a depth of 6 cm.

Results: Pretreatment with diazepam (5 mg/kg, i.p.) or vigabatrin (300 mg/kg, i.p.) decreased hyperthermia in an experimental model of the serotonin syndrome. Pretreatment with olanzapine (5 mg/kg, i.p.) or risperidone (1 mg/kg, i.p.) diminishes or blocks the development of hyperthermic response in a serotonin syndrome model.

Conclusion: Pretreatment of rats with the GABA ergic drugs or 5-HT2 receptor antagonists prevents hyperthermia in an experimental serotonin syndrome. The results obtained in present study suggest involvement of interactions between GABAergic and serotoninergic systems in the processes of thermoregulation.

THE SYNERGISTIC EFFECTS OF CANNABIDIOL AND EPIRUBICIN ON HL-60 CELL LINES AND FREE RADICAL DAMAGES

Zhelyazkova M.[1], Baltadzhieva K.[2], Momekov G.[2], Hristova-Avakumova N.[2], Hadjimitova V.

[1]Bulgarian Drug Agency, Sofia, Bulgaria
[2]Medical University of Sofia, Bulgaria

Aim: Chemotherapy is one of the successful therapeutic approaches for cancer but still there is an urgent need for novel treatment in relation to drug resistance and toxicity. Interest in the anticancer properties of Cannabidiol (CBD) - the main non-psychoactive cannabinoid received from hemp of the *Cannabis* plant has been renewed. We hereby present our novel results for its potential interactions with cytostatic drugs where data has been limited.

Materials and methods: The anticancer properties of CBD in combination with epirubicin were assessed by a standard MTT-test and the Chou Talalay method. Evaluation of the effect of CBD on the chemiluminescent response in hypochlorite containing system has been done. A spectrophotometric estimation of CBD influence on the "% of molecular damage" under experimental conditions of ferrous induced oxidative molecular damage of deoxyribose was accomplished.

Results: CBD has anti-proliferative effect on sensitive and resistant to doxorubicine HL-60 cell lines (IC_{50} = 6,6 and 7.5 mmol/L), and it induces a concentration-dependent cell death. The combination realizes a more pronounced anti-proliferative effect (combination index more than 1). In the samples containing CBD has been observed higher CL-signal and absorbance at 532 nm compared to the controls, which indicate prooxidant effect and increased "% of molecular damage". It was demonstrated that CBD-driven increase in ROS production is needed for realizing the programmed cell death.

Conclusion: The highlighted data suggest the possibility for simultaneous application of CBD and conventional cytostatic treatment which may be a beneficial option for lympho-proliferative or resistant cancer.

Key words: cannabidiol, epirubicin, anticancer effects, free radicals, pharmacological interactions

back to contents

TREATMENT OF DYSLIPIDEMIAS WITH A FOCUS ON ATHEROSCLEROTIC DISEASE

Georgiev B.
National heart hospital

Dyslipidemia is defined as changes in lipid parameters - elevated total and LDL-cholesterol (hypercholesterolemia), only elevated triglycerides (hypertriglyceridemia) or elevated total LDL-cholesterol and triglycerides - mixed dyslipidemia. Isolated low HDL-cholesterol can also be observed.
Hypercholesterolemia is a major risk factor for atherosclerotic disease, which is the first one for myocardial infarction and in the top three of the risk factors for stroke and peripheral arterial disease.
The drug treatment of dyslipidemia includes statins, fibrates, nicotinic acid, inhibitors of dietary cholesterol absorption like ezetimibe, which can be small molecules, antibodies, antisense drugs, etc.

BENEFICIAL INFLUENCE OF CHRONIC AGOMELATINE TREATMENT ON DESYNCHRONIZED DIURNAL RHYTHMS OF HORMONAL RELEASE, BEHAVIOR AND SLEEP ARCHITECTURE IN A RAT MODEL OF MELATONIN DEFICIT

Tchekalarova J., Kortenska L., Ivanova N., Atanasova M., Stoynova T., Nenchovska Z., Mitreva R., Marinov P.

Aim: The aim of the study was to explore the effects of agomelatine on desynchronized circadian rhythms of different hormones, behavior and sleep/wake cycle in a rat model of melatonin deficit.
Materials and methods: Wistar rats, kept under either basal conditions (12:12 LD cycle+vehicle), chronic constant light CCL+vehicle and CCL+agomelatine (40 mg/kg/day for 21 days) were exposed to a battery of behavioral test for anxiety and depressive-like behavior or equipped with electroencephalographic (EEG) and electromyographic (EMG) electrodes. The expression of plasma melatonin, corticosterone levels and BDNF in the hippocampus and frontal cortex (FC) was tested by ELISA test.
Results: The CCL induced depressive-like (anhedonia and hopelessness) responses during the subjective dark/active phase were positively affected by agomelatine. In addition, this melatonin analogue restored the impaired circadian patterns of plasma melatonin and decreased the level of BDNF in the hippocampus but not that of corticosterone. The CCL-exposed rats were characterized by impaired

back to contents

sleep/wake cycle with reduced slow-wave sleep (SWS) and delta power but increased rapid eye movement (REM) sleep and theta power. Agomelatine restored the diurnal rhythm of sleep/wake cycle and sleep architecture.

Conclusion: The potential beneficial effect of this novel atypical antidepressant to resynchronize the diurnal rhythms of comorbid depression, sleep/wake cycle and sleep architecture accompanying melatonin deficit suggests the key role of the hormone melatonin and BDNF in the mechanism of action of agomelatine.

Acknowledgement: This research was supported by the National Science Fund, Bulgaria (grants No. DN 03/10/2016; No. DN 12/6).

HEART FAILURE THERAPY ACCORDING TO THE LATEST CLASSIFICATION FOR EUROPE

Georgiev B.
National heart hospital

Heart failure is a syndrome, not a disease that is characterized by fatigue, shortness of breath, congestive symptoms in an effort or rest, as a result of heart disease. The latest classification of heart failure includes diseases with or without ventricular pumping dysfunction. However, in order to be clasified as patients with heart failure, patients should exhibit symptoms or signs of heart failure reduced by less than 40% left ventricular (LV) ejection fraction (EF) or fraction over 40% with elevated natriuretic peptides and at least structural heart disease or diastolic dysfunction.

Therapies have undergone a change in recent years, and in some of the phenotypes, not all is clear about the effects of different therapeutic strategies.

In heart failure with reduced LVEF (<40%) therapy is initiated with a drug that suppress the renin-angiotensin-aldosterone system (PAAS) - ACE-inhibitor or angiotensin-receptor blocker, beta blockers and, if symptoms persist, a mineralocorticoid receptor antagonist. The diuretics are administered concomitantly in the presence of congestive symptoms and the dose is adjusted according to the clinical manifestation. It should be noted that only some ACE-inhibitors have an indication for use in left ventricular systolic dysfunction - captopril, enalapril, lisinopril, ramipril, trandolaril, only 3 ARBs have this indication - candesartan, valsartan, losartan and from MPA - both spironolactone and eplerenone can be used. Of the beta-blocker group, only cardioselective beta-blockers bisoprolol, metoprolol succinate, nebivolol, and carvedilol (not selective but with additional vasodilator properties) are used with reduced LVEF. In the next step, ivabradine or aRNI (after stopping the ACE-inhibitor or ARB) can be added to conventional therapy. Many other drugs such as digoxin, nitrates have no place in conventional therapy of heart failure with suppressed LVEF.

In heart failure patients with LVEF > 40% or with a normal fraction, many of the medications administered are free of clinical evidence of benefit. With adequate treatment for underlying cardiovascular disease, their prognosis can be improved.

back to contents

AGRIMONIA EUPATORIA L. LOWERS LIPID ACCUMULATION IN DIFFERENTIATED HUMAN ADIPOCYTES

Vasileva L.[1], Koycheva I.[1,2], Balcheva-Sivenova Z.[1,2], Aneva I.[3], Georgiev M.[1,2]
[1]Center of Plant System Biology and Biotechnology, Plovdiv, Bulgaria
[2]Group of Plant Cell Biotechnology and Metabolomics, Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv, Bulgaria
[3]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Aim: Excessively increasing numbers of overweight and obese individuals are reported worldwide. The research interest in plant-derived natural compounds as potential drug candidates revives [1]. *Agrimonia eupatoria L.* (AGE) is reported in Bulgarian ethnobotanical herbal combinations for weight reduction. In the present study we hypothesized that the extract of *A. eupatoria* would modulate the processes of differentiation and lipogenesis in human Simpson-Golabi-Behmel syndrome (SGBS) pre-adipocytes cell line. The SGBS cells serve as an in vitro model of adipocytes differentiation and metabolism [2].
Materials and methods: Differentiation was induced in SGBS pre-adipocytes upon confluence. After initiation of differentiation the SGBS cells were repeatedly treated with AGE extract in doses of 6.25, 12.5, 25, 50 and 100 µg/mL. Staining with Oil Red O was performed in mature adipocytes to quantify total lipid content. Total cell lysates were extracted for determination of certain protein levels by Western blotting. Additionally, the phytochemical profile of AGE extract was analyzed by NMR-based metabolomics.
Results: The application of doses below 25 µg/mL AGE extract markedly lowered the lipid content in SGBS adipocytes compared to the control samples.
Conclusion: The obtained data indicate that the extract of *A. eupatoria* is worth further exploration as a potential source of bioactive leads that interferes with the lipid storage functions of mature adipocytes.
Acknowledgements: This project for establishment of CPSBB has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement № PlantaSYST – SGA/CSA: 739582 – under FPA: 664620. The SGBS cell line was kindly provided by Prof. Dr. Martin Wabitsch (University of Ulm, Germany).
References:
1.Vasileva L., Marchev A., Georgiev M. (2018) Causes and solutions to “globesity”: The new fa(s)t alarming global epidemic. Food and Chemical Toxicology 121: 173-193.
2.Klusoczki A., Vereb Z., Vamos A., Fischer-Posovszky P., Wabitsch M., Bacso Z., Fesus L., Kristof E. (2019) Differentiating SGBS adipocytes respond to PPARγ stimulation, irisin and BMP7 by functional browning and beige characteristics. Scientific Reports 9(1): 5823.

back to contents

IMBALANCE OF ESSENTIAL AND TOXIC METALS IN CHILDREN WITH AUTISTIC PECTRUM DISORDERS

Ciurinskiene S., Savcheva M.

Autism is a neuro-developmental syndrome, with symptoms' manifestation as early as the first year, which affects 1 out of 68 children (WHO, 2016). A good number of researchers (Yasko, Amy, 2009; Walsh, William, 2011) have proven the relation between autism and the inability of the organism to excrete toxic metals, due to disturbances in the methylation cycle. During the last 10 years, a great deal of attention has been paid to the theory of metabolic deficiencies/detoxification. According to this theory, autism is a result of disturbed detoxification, due to lack of antioxidants, glutathione, metallothionein.

Objective of the study: 1. To identify the predominant toxic metals, most common in Bulgarian children with autism spectrum disorders (ASD).

2. To find out whether there are increased toxic metals in children with ASD, different from mercury and aluminium.

Materials and methods: The study spans over 53 children at the age of 1 – 13. 47 boys and 6 girls, were tested for toxic metals overload, from January 1st 2017 to March 30th 2018.

The level of toxicity was identified, using a hair metal elements test.

The behavioural symptoms were tested with CARS2.

Results: 53 out of 53 children with developmental disorders turned out positive for increased level of one or more toxic metals. The severity of the autistic symptoms, however, correlated not with the extent of increase, but with the numbers of the detected metals – the more the number of the different metals were, the heavier were the symptoms.

Conclusions: Our study supports the metabolic deficiency theory, which occurs as a result of disturbed detoxification, caused by variations of several genes like GPX1, GSTP1, SOD2 etc., which in turn leads to intoxication with metals and bad metabolites, accompanied by gut and brain inflammation.

Key words: autism, toxic metals, detoxification

back to contents

EFFECTS OF LEPTIN ON NADPH-DIAPHORASE ACTIVITY IN THE *NUCLEUS PARAVENTRICULARIS* AND *NUCLEUS ARCUATUS* OF THE HYPOTHALAMUS IN RATS

Hristov M.[1], Landzhov B.[2], Yakimova K.[1]

[1]*Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Medicine, Medical University, Sofia*

[2]*Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University, Sofia*

Aim of the study: To investigate reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase activity in the *nucleus paraventricularis* and *nucleus arcuatus* of the hypothalamus after systemic administration of leptin in rats.

Materials and methods: The study was carried out on 6 male Wistar rats with body weight 350 g, divided into 2 groups of 3 rats each: first group - injected i.p. with leptin (0.5 mg/kg) and second group (control group) - injected with saline (0.2 ml/100g). The animals were anesthetized 45 minutes later with thiopental (40mg/kg) and transcardial perfusion was performed using 4% paraformaldehyde. Serial coronal sections were cut on a freezing microtome (Reichert-Jung) at a thickness of 40 μm. Sections were then stained with the NADPH-d-technique using 0.1–0.2 mg/ml of nitroblue tetrazolium, 1 mg/ml β-NADPH and 0.3–0.5 % Triton X-100 in 0.1 M TRIS–HCl buffer (pH 7.4) at 37° C for 30 – 60 min. Data were statistically assessed by one-way analysis of variance (ANOVA) and presented as mean ± standard error of the mean (SEM).

Results: Systemic administration of leptin resulted in a significantly higher NADPH-d positive cell number in *nucleus paraventricularis* compared to that observed in the control animals. In contrast to saline-treated rats, where *nucleus arcuatus* was unstained, leptin-treated rats have shown a few NADPH-d positive neurons and many NADPH-d positive tanycytes with well visible basal processes, that make contact with blood vessels and neurons in *nucleus arcuatus*.

Conclusion: We have shown that systemic administration of leptin resulted in an increased NADPH-diaphorase activity in the *nucleus paraventricularis* and *nucleus arcuatus* of the hypothalamus in rats. We suggest that *nucleus paraventricularis* and *nucleus arcuatus* may be important centers in the hypothalamus for the leptin action, mediated by increased NO synthesis.

back to contents

COMPARATIVE STUDY OF THE PROTECTIVE EFFECTS OF *NIGELLA SATIVA* OIL, CURCUMIN, AND HYDROXYTYROSOL AGAINST DEXTRAN SULFATE SODIUM-INDUCED COLITIS IN MICE

Simeonova R.[1], Zheleva-Dimitrova D.[2], Balabanova V.[2], Benbassat N.[2], Savov I.[3], Gevrenova R.[2]

Faculty of Pharmacy, Medical University, Sofia

Aim: This study aimed to investigate the possible protective role of *Nigella sativa* oil (NSO) vs curcumin (CUR) and hydroxytyrosol acetate (HTA) against Dextran Sulfate Sodium (DSS) induced oxidative damage and impairment of colons from mice.
Materials and methods: High-resolution gas chromatography-mass spectrometry (GC-HRMS) was used for the analysis of the NSO. Colitis was induced by a 5-day oral DSS (2.5%) administration. The levels of nitric oxides (NO), C-reactive protein (CRP), malondialdehyde (MDA), reduced glutathione (GSH), the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), as well as the activity of myeloperoxidase (MPO), were measured spectrophotometrically. IL-6 levels were analyzed using electrochemiluminescence immunoassay "ECLIA".
Results: Palmitic, linoleic, and oleic acids were detected. Colitis was discerned by a reduction in body weight, presence of blood in the stools, reduced colon weight and length. Colon inflammation was also manifested by an increased level of inflammatory markers IL-6, CRP, NO, MPO activity, and disturbed oxidative stress markers GSH, antioxidant enzymes activity (CAT, SOD, and GPx), and MDA quantity. All the investigated compounds markedly attenuated colonic inflammation by inhibiting MPO activity and by decreasing the augmented levels of IL-6, NO and CRP. The compounds improved colon anti-oxidant defence machinery by decreasing the quantity of MDA, a marker of lipid peroxidation and increasing the level of GSH and antioxidant enzymes activity. The investigated compounds ameliorated colon injury and inflammatory signs as visualized by histopathological examination.
Conclusion: Results revealed that NSO produced a comparable therapeutic effect as the positive controls CUR and HTA.

URIC ACID IS A RISK FACTOR FOR IN INCIDENT AN PATIENTS WITH UREMIA

Zylbeari L., Haxhirexha K., Haxhirexha A., Dika-Haxhirexha F., Bexheti Z., Ahmeti-Lika S., Zylbeari G.,

Faculty of Medical Sciences, University of Tetovo, Tetovo

Low serum uric acid (SUA) is a mortality risk factor in incident hemodialysis patients with a high comorbidity burden and hypoalbuminemia. Hyperuricemia is also common in uremic patients where it has been reported in up to 60-75% of patients. Studies performed in hemodialysis (HD) patients confirmed that hyperuricemia is also associated with a significantly increased mortality risk in the uremic patients. In this study we hypothesized that a low serum uric acid level is a mortality risk factor associated with clinical markers of MIA syndrome, inflamatio-oxidative stress, in a

back to contents

cohort of patients treated with long-term hemodialysis. We found low uric acid levels conferred a greater than 2-fold risk for death, whereas elevated uric acid was not associated with increased mortality risk. We therefore undertook a detailed analysis to better understand the potential mechanism by which a low uric acid level might confer increa-sed risk in this population. We found that in our patients, low uric acid levels were associated with markers of malnutrition and a high comorbidity burden, which is consistent with the hypothesis that oxidative stress might contribute to the increased mortality in these patients. Twice a week every month, pre- and postdialysis blood samples from each of the patients was collected. The core indicators were urea reduction rate (URR), serum hematocrit and S-albumin levels. The target value for URR was ≥68%, that of serum hematocrit was ≥36 g% and that of S-albumin was ≥3.5 g/dl. For patients that had core indicator values that fell below the target values, the appropriate laboratory test was repeated on the following Monday or Tuesday. Prescription adjustments were made following NK/DOQI (National Kidney Disease Outcome Quality Inititatve) guidelines through a Continuous Quality Improvement program, using established protocols. The mean values of the core indicators for each patient over the entire period of study were employed for final analysis. In conclusion, our results suggest that uric acid is similar to other cardiovascular risk factors in that there is a reverse epidemiology pattern in HD patients. This relationship is largely explained by the association of low uric acid levels with comorbid conditions and markers of protein energy wasting that are suggestive of higher oxidative stress. A reverse epidemiology of classic cardiovascular risk factors was observed in hemodialysis patients with a high comorbidity burden. Further studies are necessary to understand if the low serum uric acid level is the cause or consequence of these conditions. In conclusion, our results suggest that uric acid is similar to other cardiovascular risk factors in that there is a reverse epidemiology pattern in HD patients. This relationship is largely explained by the association of low uric acid levels with comorbid conditions and markers of protein energy wasting that are suggestive of higher oxidative stress.
Keywords: hypoalbuminemia, hemodialysis, serum uric Acid (SUA)

MANAGEMENT OF RENAL ANEMY

Zulbeari L., Haxhirexha K., Haxhirexha F., Bexheti Z., Ahmeti-Lika S.
Medical Faculty, University of Tetove

Abstract: One of the most common complications in uraemic patients treated with chronic hemodialysis (HD) is the anemia that is still wound up in the early stages of the disease. National Kidney Foundation (NKF) and KDOQI-Kidney Disease Outcomes Quality Initiative renal anemia is treated when concentrations of Hb for women e <12 g/dl whereas in men e <13.5 g/dl. (1,2). Chronic renal disease according to NKF and KDOQI is separated into 5 different phases based on the glomerular filtration rate (Glomerular Filtration Rate-GFR (3,4). According to the recommendations of NKF and KDOQI, about 90% of patients with ESRD with glomerular filtration GFR, below 25-30 ml/min, has anemia with low concentrations of Hb <10 g/dl (5). The most common cause of this type of anemia is the lack of

back to contents

erythropoietin (EPO) which is produced principally in the bubble - the cuts and is the basic factor for the stimulation of erythropoiesis. The introduction of erythropoiesis stimulating agent (ESA) for the treatment of renal anemia at the end of 1980 was a major breakthrough in the field of medicine (6). In our work, all anemic patients were treated with ESA at a dose of 2000-4000 IU subcutaneously (s.c.) after the end of the HD session. Out of the total number of 120 patients at 100 (83%) there was anemia that showed a high sensitivity to the ESA preparation, while 20 (17%) patients had normal Hb, Er, Htc values and were not treated with ESA.

Material and methods: In the cross-sectional study, the GH cells included N0=240 examiners of which 120 were HD treated patients while 120 were healthy individuals serving as a control group. Of the 120 patients treated with HD-54 (45%) were women while 64 (55%) were males with an average median of 59.50 ± 14/30 years old, treated over 12 in the Nephrology Clinic in Skopje and in Tetovo Hospital Clinic. The control group of healthy examiners (volunteer blood donors) was also 54 (45%) female and 64 (55%) males, and was similar to the group of patients according to age (58.0 ± 13.80 years), gender and national relevance. Statistical analysis: The statistical methods used were: average arithmetic value and standard deviation: X ± SD, Studentov t test, Anonova Two-Factor. The results obtained from the Hb, Er, Htc examinations were processed and presented in the form of charts, tabulations and in the knowledge-grams processed in a standard statistical program (Statistic for Windows, version 6.0A, Stat. Softinc Tulsa, OK USA.

Results: Before starting ESA therapy, the mean values of Hb in the examined female females were: 5.20±1.15 mmol/l, while male male patients were = 5.60 ± 1.10 mmol/l, the Er number of females was: 2.90 ± 0.86 x 1012/l, while masculine were = 3.20 □ 0.90 x 1012/l. The values of (Htc in females were = 0.24 ± 0.04 while in males-0.28 ± 0.06. After treatment with ESA the desired target for Hb ("target Hgb≥6.80mmol/l") was postponed after the sixth week of 46 female patients out of the total number of 54 females with Hb> 6.80 mmol/l, while male target Hb> 6.80 mmol/l reached 58 patients in the total number of 66 meshes, number Er females reached: 3,50 ± 0.86 x 1012/l, while masculine was 3.80±0.90 x 1012/l. Htc values in females reached up to 0.30 ± 0.02 while in males 0.32 ±0.04. The sFe deficiency was compensated by applying the Amp.Ferri (III) Oxidum Sa-ccharatum intravenously in 150 ml NaCl. Comparison of the winning values of patients with psoriatic treatment with ESA and after treatment showed a statistically significant difference for p <0.0001.

Conclusion: Our results have shown that the application of ESA therapy is the most comprehensive and effective way of correcting and treating renal anemia and timely preventing left ventricular hypertrophy.

Key Words: renal anemy, Erythropoesis stimulating agent (ESA), Hemodialysis

Literature:

1. Obrador GT, Roberts T, St Peter WL, et al. Trends in anemia at initiation of dialysis in the United States. Kidney Int 2001; 60:1875.
2. Hariharan S, Yang H. Introduction. Am J Kidney Dis. 2006; 47:S1.
3. 2005 Annual Report. ESRD clinical performance measures project. Am J Kidney Dis 2006; 48:S33.

back to contents

4. Revised European best practice guidelines for the management of anaemia in patients with chronic renal failure. Nephrol Dial Transplant 19 (Suppl. 2):ii1 –ii47, 2004.
5. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J: Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988–1994). Arch Intern Med 162:1401 –1408, 2002.
6. National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis39 (Suppl. 1):S1 –S266, 2002.
7. National Kidney Foundation: Clinical practice guidelines and clinical practice recommendations for anemia in chronic kidney disease in adults. Am J Kidney Dis5 (Suppl. 3):S1 –S108, 2006.
Author's address: Prof. Dr. Lutfi Zylbeari; e-mail: dr-luti@hotmail.com

MOLECULAR GENETIC ANALYSIS OF TOXIC MICRO-DERIVATIVES IN DRINKING WATER

Ilieva V.[1], Georgieva T.[2], Kondeva-Burdina M.[1]
[1]Faculty of Pharmacy, Medical University, Sofia
[2]GMO Laboratory, National Center of Publish Health and Analysis

The aim of the study: The aim of the researches is to evaluate the risk of cyanobacteria's toxicity and toxins in samples of the above-mentioned Bulgarian dams. Goals are to be discovered potentially toxic or pathogenic micro-organisms in order to protect the health of the population.
Materials and methods: For the research are used molecular-genetic methods such as polymerase chain reaction in real time (RT-PCR). Initially, bacterial DNA was isolated by DNA extraction with pre-set lysis-buffer for isolating bacterial DNA from the food matrices, processed for water samples, followed by RT-PCR. From multiply, genes are determined the amount and toxicity of cyanobacteria in the available samples. RT-PCR allows being observed the change of the fluorescence in each cycle in real time.
Results: In this research, samples were tested from the following dams: Studena, Ptchelina, Uzungerel, Mandra, Durakulak, Vaia, Bistrista and Iskar. Those dams are the main water source, so they are important for the population. In the result of the conducted experiments is found that positive results for the presence only of cyanobacteria in Vaya dam and Durankulak dam – the presence of cyanobacteria and the toxin produced by them microcystin B; for Mandra dam are found only positive samples for cyanobacteria.
Conclusion: RT-PCR is an important tool for the control of drinking water. Blue-green algae are an important indicator of drinking water, and this requires more rigorous control and the introduction of molecular-genetic methods for this as reference.
Correspondent author: Viktoria Ilieva, viki.ilieva1991@gmail.com; 0896828312

back to contents

BONE REMODELLING EFFECTS OF SOPHORAE FRUCTUS EXTRACT AND CURCUMIN (CURSOL®) ON GOSERELINE-INDUCED OSTEOPOROSIS IN RATS

Chakuleska L.[1], Shkondrov A.[2], Krasteva I.[2], Manov V.[3], Zlateva-Panayotova N.[4], Marinov G.[4], Simeonova R.[1], Danchev N.[1]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[3]Department of Internal Noninfectious Diseases, Pathology and Pharmacology, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria
[4]Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria
Corresponding author: Lidija Chakuleska
Telephone: 00359 8 86742922
E-mail: lcakuleska@gmail.com

Introduction: Numerous natural compounds present in dietary and medicinal plants have been proven useful in the prevention and treatment of osteoporosis (OP). *Sophora japonica L*. (Fabacaeae) is a source of natural isoflavones as genistein and daidzein that could help alleviate the risk of fracture in osteoporotic patients. Curcumin is known for its anti-inflammatory action.
Aim: The aim of the present study was to investigate the effects of Sophorae fructus extract (SFE) combined with highly soluble form of curcumin (Cursol®) on the skeletal system of rats with estrogen deficiency induced by subcutaneous implantation of a gonadtropin releasing hormone (GnRH) analogue goserelin (GR) (Zoladex®).
Materials and methods: The experiments were carried out on 3-months-old Wistar rats, divided into four groups: control, estrogen deficient rats (ED), ED and supplemented with the combination of SFE (100 mg/kg/day) and Cursol® 2 g/kg/day, orally for 30 days, ED and supplemented with the positive control estradiol (EST) (40 µg/kg/day). Serum and urine bone turnover markers, bone radiological and histopathological changes were studied.
Results: GR treatment has a dramatic impact on the biochemical biomarkers and on the cancellous bone at the proximal tibia. The combination of SFE and Cursol® restored the biochemistry and histopathological changes in the chemically castrated animals. These effects were commeasurable with EST.
Conclusion: These findings provide information about the bone protective effects of these natural substances against GR-induced bone loss, which could be developed as candidates for prevention of drug induced osteoporosis.

back to contents

NEUROPSYCHIATRIC COMORBIDITIES IN EPILEPSY AND THE POSSIBLE BENEFICIAL EFFECT OF MELATONIN – THE EVIDENCES FROM META-ANALYSES

Gateva P.[1], Bakalov D.[2]
[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Medicine, Medical University, Sofia
[2]Department of Pathophysiology, Faculty of Medicine, Medical University, Sofia
Corresponding author: P. Gateva +359 889 428 105,
e-mail: pandreeva_gateva@outlook.com

In one third of patients with epilepsy the antiepileptic treatment is without success. A possible reason for this could be the high neuropsychiatric comorbidity. Melatonin is an endogenous indoleamin with chronobiological, antioxidant and neuroprotective properties. It is registered in Europe both as a food supplement and as a medication for short-term treatment of insomnia in adults and for treatment of children with autism or Smith-Magenis syndrome.
Our aim was: (1) to analyze the literature for the possible involvement of the melatonin system in the comorbidity in epilepsy, and (2) to analyze data from evidence-based medicines about efficacy and safety of melatonin.
Materials and methods: Searching in data-bases Medline and Google Scholar, we identified and analyzed qualitatively meta-analyses published until May 2019 about neurological and psychiatric comorbidity of epilepsy with focusing on the melatonin co-medication.
Results: The most common neuropsychiatric comorbidities in epilepsy are autism, depression with suicide, dementia, chronic pain, psychogenic nonepileptic seizures and multiple sclerosis. High level of evidence exists only for data about the efficacy of melatonin co-medication in cases with desynchronized biorhythms and insomnia. Some of the planned meta-analyses could not be realized due to the insufficiency of information. In general, data about the safety of melatonin are favorable.
Conclusion: Conduction of well-planned clinical trials with patients with epilepsy and neuropsychiatric co-morbidity, evaluating the role of melatonin is needed. Usage of melatonin by children imposes more detailed evaluation of the safety profile.
Acknowledgment: Supported by Bulgarian Science Fund DN 13/16 21.12.2017.

SIGNIFICANCE OF ANTIBIOTIC RESITANCE IN CLINICAL PRACTICE AND MEASURES FOR LIMITING IT TO HOSPITALS

Hristova M., Eneva-Dimitrova M.
"Nadezhda" MHAT for Women's Health

According to the World Health Organization antibiotic resistance is one of the biggest threats for human health. Globally it is related to longer hospital stay, increased medical cost and increased morbidity and mortality. A multidisciplinary approach is necessary in hospital practice in order to limit antibiotic resistance including prevention of infection, rational prescribing of antibiotics after isolation of the

back to contents

microorganism and determination of its sensitivity and antibiotic stewardship. Every hospital has to create antibiotic policy which corresponds to the national and international guidelines for treatment of bacterial infections as well as the scope of activities of the hospital. The aim of the current work is to determine the impact of the introduction of an antibiotic policy in a hospital on the frequency and severity of bacterial infections. A number of clinical cases of the emergency and progress of antibiotic resistance in hospital settings were reviewed. Based on the WHO recommendations on antibiotic resistance and the antimicrobial prescribing guideline of NICE, a hospital antibiotic policy was made. The policy aims at insuring proper antibiotic use and limiting antibiotic resistance. The changes in the prescription and application of antibiotics and the spread of resistant bacteria were examined before and after the implementation of the antibiotic policy. The results are presented as reduction of the prescription of antibiotics, reduction of the cost of treatment and influence on the spread of resistant microorganisms.

NANOCOMPOSITE CRYOGEL CARRIERS FOR THE DELIVERY OF POORLY WATER-SOLUBLE DRUGS

Petrov P.[1], Momekova D.[2]

[1]Institute of Polymers, Bulgarian Academy of Sciences, 103 A "Akad. G. Bonchev" Str., 1113, Sofia, Bulgaria; e-mail:ppetrov@polymer.bas.bg

[2]Faculty of Pharmacy, Medical University, 2 "Dunav" Str., 1000, Sofia, Bulgaria

Aim: The present study aims at developing nanocomposite cryogels comprising polymeric core-shell micelles for loading and sustained release of hydrophobic drugs.

Materials and methods: Hydroxypropyl cellulose; hydroxyethyl cellulose; Pluronic P65; Pluronic F127; pentaerythritoltetraacrylate; (4-benzoylbenzyl) trimethylammonium chloride; N,N′-methylenebisacrylamide; curcumin; canabidiol; photo-crosslinking.

Results: Polysaccharide cryogels comprising either stabilized or dynamic polymeric micelles were fabricated by combination of cryogenic treatment and photo-crosslinking. The effect of micelle content on gel fraction yield, swelling degree, cryogel morphology and mechanical properties were evaluated. Two different methods of loading of poorly water-soluble drugs into nanocomposite cryogels were studied. Finally, the encapsulation efficiency and drug release profile of cryogel carriers were determined.

Conclusions: Novel nanocomposite carriers were developed by embedding stabilized or dynamic core-shell micelles into polysaccharide cryogels. The tested systems exhibited sustained release profile and excellent physicochemical characteristics which make them applicable for topical delivery of hydrophobic drugs.

Acknowledgments: This work was supported by the Bulgarian Ministry of Education and Science (Grant DO1-217/30.11.2018) under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM # 658 / 14.09.2018

back to contents

THERMORESPONSIVE POLY (N-ISOPROPYLACRYLAMIDE) COPOLYMER NETWORKS FOR GALANTAMINE HYDROBROMIDE DELIVERY

Georgieva D.[1], Kostova B.[1], Ivanova-Mileva K.[2], Ivanova S.[2], Rachev D.[1], Christova D.[2]

[1]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, 2 "Dunav" Str., 1000 Sofia, Bulgaria
[2]Institute of Polymers, Bulgarian Academy of Sciences, "Akad. G. Bonchev" Str., Bl. 103-A, 1113 Sofia, Bulgaria

Aim of study: The aim of the present study was to synthesize poly (N-isopropylacrylamide) (PNIPAAM) copolymer networks and evaluate their potential as drug carriers for galantamine hydrobromide (GH) delivery.
Materials and methods: PNIPAAM copolymer networks were synthesized using two methods: free radical polymerization and redox polymerization. Poly (ethylene glycol) di-acrylate (PEGDA) was used as a crosslinker. The obtained networks were characterized by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction, the swelling kinetics were studied and in vitro drug release experiments were conducted.
Results: PNIPAAM copolymer networks were synthesized and it was established that there was a difference in swelling kinetics of the networks obtained by the two methods. Networks prepared by redox polymerization swell in a bigger extent than those obtained by free radical polymerization. This affects GH-loading, which is higher for the first ones. GH release was monitored, revealing that the networks released up to 100% of the active substance within 4 hours. This rapid release was due to the increased solubility of GH, which was in amorphous state in the networks, which was proven by X-ray diffraction.
Conclusions: PNIPAAM copolymer networks were synthesized and their potential as GH delivery systems was investigated. The studies conducted suggest that the networks have potential as GH drug delivery systems.

back to contents

IN VIVO STUDY OF ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF KETOPROFEN BIGEL FOR DERMAL APPLICATION

Peneva P.[1], Apostolova E.[2], Kassarova M.[1]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv
[2]Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University, Plovdiv
Corresponding author: Petya Peneva, tel. 0886358683, e-mail: pharmpeneva@abv.bg

Aim of the study: The aim of the present study was to determine *in vivo* the antinociceptive and anti-inflammatory activity of carbopol hydrogel/sorbitan monostearate-almond oil bigel with ketoprofen for dermal application.
Materials and methods: The bigel formulation was prepared from a carbopol hydrogel and sorbitan monostearate-almond oil oleogel in a 70/30 ratio. *In vivo* experiments were performed with 82 male Wistar rats (weighing 180 to 400 g). A test for acute dermal toxicity was conducted, antinociceptive activity was determined by hot plate test, anti-inflammatory activity was recorded with carrageenan induced edema. The animals with which the anti-inflammatory activity has been detected have been tested for anti-hyperalgesia effect. The obtained results were compared to a commercial product.
Results: The bigel formulation with ketoprofen exhibits a well-expressed antinociceptive effect. It shows better anti-inflammatory activity and anti-hyperalgesia effect in the first hour of the study compared to a market product hydrogel. The test for acute dermal toxicity showed that the bigel formulation could be considered as safe.
Conclusion: As a result of the in vivo experiments, it was found that the bigel formulation could be considered as safe and effective dosage form for dermal application.

SOLUBILITY OF FENOFIBRATE IN HUMAN GASTRO-INTESTINAL MODEL

Katev V., Vinarov Z., Radeva D., Tcholakova S., Denkov N.
Faculty of Chemistry and Pharmacy, Sofia University, Sofia, Bulgaria
e-mail: vk@lcpe.uni-sofia.bg

Aim: Many new drug candidates are poorly water soluble, which results in low and/or variable intestinal absorption. One of the modern approaches to solve this problem is by using lipid-based drug delivery systems (LBDDS). The LBDDS contain lipids, surfactants and co-solvents. The aim of the study is to clarify the effect of natural surfactants which are encountered in the food, GI-tract or they are lipolysis products, on the fenofibrate solubility by using a GIT *in-vitro* model.

back to contents

Materials and methods: We studied fatty acids, phospholipids and monoglycerides with different hydrophobic chain length and saturation. The solubility of fenofibrate was determined by high-performance liquid chromatography (HPLC).
Experimental results: A maximum in the fenofibrate solubility was observed with increasing chain length and in the presence of double bond in the molecule. Highest solubility was achieved when a phospholipid was used. We measured intermediate solubility when we used monoglycerides and lowest when using fatty acids.
Conclusion: The relationship between the structure of natural surfactants and fenofibrate solubility was clarified by using an *in-vitro* digestion model.
References:
1. Z. Vinarov, V. Katev, D. Radeva, S. Tcholakova, N. Denkov. Drug. Dev. Ind. Pharm., 44 (2018) 677-686.
2. Z. Vinarov, V. Katev, N. Burdzhiev, S. Tcholakova, N. Denkov, Molecular Pharmaceutics, 15 (2018), 5741−5753.

IMPACT OF SURFACTANT-BILE INTERACTIONS ON THE SOLUBILITY OF HYDROPHOBIC DRUGS

Vinarov Z., Tcholakova S., Denkov N.
Department of Chemical and Pharmaceutical Engineering,
Faculty of Chemistry and Pharmacy, Sofia University
e-mail: zv@lcpe.uni-sofia.bg

Aim: Up to 90% of the new molecular entities have poor aqueous solubility, which leads to low oral absorption. One of the solubility enhancement techniques is solubilization in surfactant solutions. [1-4] However, surfactant micelles can interact with the bile salts in the intestines, thereby changing drug solubility. [5] The aim of our study is to clarify how the drug and surfactant structure determines drug solubilization, and to demonstrate the impact of bile salts on micellar drug solubility.
Materials and methods: We studied a total of 17 surfactants and 6 hydrophobic drugs. Drug solubility was determined by HPLC. The size of colloidal aggregates and the surfactant-bile interactions were investigated by DLS and DOSY ^{1}H NMR.
Results: The solubility of all studied drugs increases with the increase of the surfactant hydrophobic chain length. Very high solubilization was determined for pairs of oppositely charged surfactant and drug molecules, due to the electrostatic attraction. Drug precipitation was observed when drug-loaded ionic surfactant micelles were introduced in bile solutions. In contrast, the drugs remained solubilized in mixtures of nonionic surfactants + bile. ^{1}H DOSY showed that mixed bile salt + surfactant micelles with low drug solubilization capacity were formed for the ionic surfactants, whereas separate surfactant-rich and bile salt-rich micelles were found to coexist in the nonionic surfactant + bile mixtures.
Conclusion: The relationship between surfactant and drug structure, and the solubilization capacity was clarified. It was shown that surfactant-bile interactions can have a dramatic effect on drug solubility, which can affect the oral drug absorption.

back to contents

References:

1. Improving Ibuprofen Solubility by Surfactant-Facilitated Self-Assembly into Mixed Micelles. K. Stoyanova, Z. Vinarov, S. Tcholakova. J. Drug. Deliv. Sci. Tec. 36 (2016) 208–215.
2. Effect of Surfactant Molecular Structure on Progesterone Solubilization. Z. Vinarov, P. Dobreva, S. Tcholakova. J. Drug. Deliv. Sci. Tec., 43 (2018) 44–49.
3. Albendazole Solution Formulation via Vesicle-To-Micelle Transition of Phospholipid-Surfactant Aggregates. Z. Vinarov, G. Gancheva, V. Katev, S. Tcholakova, in Drug. Dev. Ind. Pharm. (2018).
4. Micellar Solubilization of Poorly Water-soluble Drugs: Effect of Surfactant and Solubilizate Molecular Structure. Z. Vinarov, V. Katev, D. Radeva, S. Tcholakova, N. Denkov. Drug. Dev. Ind. Pharm., 44 (2018) 677–686.
5. Effect of Surfactant–Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media. Z. Vinarov, V. Katev, N. Burdzhiev, S. Tcholakova, N. Denkov, Mol. Pharm. 15 (2018) 5741−5753.

CALORIMETRIC STUDY ON SOLVENT MEDIATED INTERACTIONS OF INDOMETHACINE WITH POLY(N-ISOPROPYLACRYLAMIDE)-GRAFT-POLY(ETHYLENE OXIDE) COPOLYMER

Valkova I.[1]*, Michailova V.[2]

[1]Department of Chemistry, Medical University, Sofia
[2]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia
Corresponding autor: Tel: +359 2 9236 599, e-mail: ivalkova@abv.bg

The application of NSAID indomethacine (IMC) has been limited because of its hydrophobicity. This limitation can be overcome by associating it with a delivering carrier. Thermoresponsive poly(N-isopropylacrylamide)-graft-poly(ethylene oxide) (PNIPAM-g-PEO) is attractive for such applications as it forms core-shell nanostructures close to physiological temperature. It can also micellize in aqueous ethanol solutions and that can be used to enhance drug solubilization and loading within PNIPAM chains.

Isothermal titration calorimetry (ITC) is one of the most reliable techniques that permit direct thermodynamic characterization (standard free energy, enthalpy and entropy) of associative processes in complex media. To examine the effect of the organic solvent on the IMC-PNIPAM interaction we conducted ITC measurements in phosphate buffer (PBS) pH 7.4 and its mixtures with ethanol.

It is found that in PBS the interaction between IMC and PNIPAM is mainly hydrophobic since the entropy changes are all positive and the contribution of $-T\Delta S°$ to $\Delta G°$ is larger in magnitude than that of $\Delta H°$.

The picture completely changes when PBS solutions of PNIPAM are titrated with ethanol and IMC/ethanol solutions. The interactions are accompanied by large negative changes in enthalpy and a significant entropy decrease, suggesting that the hydrogen binding of ethanol to PNIPAM energetically dominates the hydrophobic IMC-PNIPAM interactions. We also noticed that the heat flow is larger for the IMC-

back to contents

containing systems that could be attributed to the ability of IMC to promote ethanol accumulation within the PNIPAM chains. This finding is also confirmed by the negative values of ΔH° and ΔS° from the IMC/ethanol-PNIPAM/ethanol titration in which the IMC and PNIPAM solvation shells are in equilibrium with the tested solvent composition. Combining all the above results, the most likely contribution to such enthalpy and entropy changes is the mutual synergistic effect of IMC and ethanol on their complexation with PNIPAM.

Acknowledgements: This research was carried out with the financial support of Medical University of Sofia, Grant-Project 8273/21.11.2018, Financial contract D-77/23.04.2019.

IN VITRO CARDIOPROTECTIVE EFFECTS OF DOXORUBICIN ENCAPSULATED IN CHITOSAN-ALGINATE PARTICLES

Yordanov Y.[1], Tzankov B.[2], Tzankova V.[1], Frosini M.[3], Valoti M.[3], Yoncheva K.[2]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

[2]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

[3]Department of Life Sciences, University of Siena, Siena, Italy

Corresponding author: Yordan Yordanov

e-mail: yyordanov@pharmfac.mu-sofia.bg; tel: 359878676339

Aim: Doxorubicin (DOX) is a potent and widely used chemotherapeutic drug, but its application is often related to severe dose-dependent cardiotoxicity by oxidative stress mediated mechanisms. Encapsulation of DOX in nanoparticulate drug delivery systems could reduce the side effect of doxorubicin. The aim of the present study was to compare the cytotoxic effects of free and encapsulated in chitosan alginate particles doxorubicin on two cell lines *in vitro*. The effects of encapsulated DOX on glutathione (GSH) antioxidant system were also evaluated.

Materials and methods: The cytotoxic effects of free and encapsulated doxorubicin were evaluated on H9c2 cells (cardioblasts) and on L5178 MDR1 (multidrug resistant lymphoma cells). The cytotoxic effects were evaluated by means of the MTT – test and Alamar blue metabolic assays. The effects on the levels of total glutathione and the GSH/GSSG ratio were investigated by means of a modification of Tietze's enzymatic-recycling assay.

Results: The encapsulated DOX showed decreased cytotoxicity on H9c2 cardioblasts, while the cytotoxic effects were retained on lymphoma L5178 MDR1 cells. The treatment with encapsulated DOX (sub cytotoxic concentrations) caused reduction in GSH oxidation compared to free DOX. The GSH/GSSG ratio was optimal in cells, treated with non-loaded chitosan-alginate particles, which shows the lack of pro-oxidant activity and a positive safety profile of the drug carrier.

Conclusion: Chitosan-alginate nanoparticles are a promising drug carrier that effectively encapsulated doxorubicin retained its cytotoxic effects on multidrug resistant L5178 lymphoma cells and exerted an in vitro cardioprotective effect on H9c2 cells.

back to contents

MEMBRANE PERMEABILITY OF FENOFIBRATE SOLUBILIZED IN SURFACTANT MICELLES

Krastev D.[1], Vinarov Z.[1], Müllertz A.[2], Tcholakova S.[1]

[1]Department of Chemical and Pharmaceutical Engineering, Faculty of Chemistry and Pharmacy, Sofia University, Bulgaria

[2]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

Aim of the study: The main factors governing the oral bioavailability of pharmaceuticals are their solubility and permeability. In previous articles we have showed [1-2] that the solubility of fenofibrate can be enhanced significantly (over 100 times) in aqueous solutions with the help of surfactants. In continuation of this research, the main goal now is to determine the effect of surfactant type on the permeation rate of fenofibrate.

Materials and methods: To measure these effects we have used a cell-free in vitro permeability system µFlux (Pion Inc.), with continuous in situ optical fiber monitoring of the drug concentration in both the donor and the acceptor compartment during the experiment. 11 surfactants with different hydrophobic chain length (from C-10 to C-18) and different charge of the hydrophilic head group were studied.

Results: The results show that there are significant differences between ionic and non-ionic surfactants. After the end of the experiment, the drug concentrations in the acceptor compartment were three to four times higher with ionic surfactants (SDS and TTAB) compared to nonionic surfactants (Tween and Brij).

Conclusion: The obtained results demonstrate that the permeation rate depends significantly on the type of surfactant used for drug solubilization. Additional experiments with broader selection of surfactants and permeation experiments with phospholipid bilayers are planned, in order to clarify the main mechanism that governs the transmembrane transport of drugs solubilized in surfactant micelles.

References:

1. Z. Vinarov, V. Katev, D. Radeva, S. Tcholakova, N. Denkov. Drug. Dev. Ind. Pharm., 44 (2018) 677.
2. Z. Vinarov, V. Katev, N. Burdzhiev, S. Tcholakova, N. Denkov, Mol. Pharm. 559 (2018) doi: 10.1021/acs.molpharmaceut.8b00884.

back to contents

IN VITRO SAFETY AND ANTIOXIDANT ACTIVITY EVALUATION OF CAFFEIC ACID PHENETHYL ESTER LOADED COPOLYMER MICELLES

Aluani D.[1], Tzankova V.[1], Yoncheva K.[2], Bankova V.[3] Atanasova M.[4], Grancharov G.[4], Petrov P.[4]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[2]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[3]Institute of Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4]Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria

Caffeic acid phenethyl ester (CAPE), one of the components of propolis, possesses broad biological activities as antioxidant, anti-inflammatory, anticancer, etc. However, the higher lipophilicity of CAPE limits its in vivo applications, since the low water solubility hinders both the formulation of stable drug dosage form and its bioavailability.

Aim: Safety evaluation and antioxidant activity of a newly developed CAPE loaded poly (ethylene oxide)-b-poly (ε-caprolactone)-b-poly (ethylene oxide) (PEO-PCL-PEO) micelles *in vitro*.
Methods: *In vitro* toxicity screening of the newly developed PEO-PCL-PEO copolymer micelles on human endothelial Ea.hy926 cells. The viability of the cells was assessed by MTT assay and lactate dehydrogenase (LDH) leakage. The antioxidant properties of pure and micellar CAPE were determined in a model of H2O2-induced oxidative damage in Ea.hy926 cells.
Results: The treatment of Ea.hy926 cells with empty micelles showed slight cell viability decrease at the highest studied concentrations (125 and 250 μg/ml). In contrast, the treatment with pure CAPE (17 and 34 μg/ml) caused a statistically significant decrease of cell viability by 38% and 50% (***$p<0.001$) vs untreated control group. LDH release was increased by 32% and 41% (***$p<0.001$) *vs* untreated controls at both tested concentrations. A similar trend was observed with the CAPE-loaded micelles on endothelial cells. The results from H2O2-induced oxidative damage showed similar antioxidant effects of pure and micellar CAPE.
Conclusion: The presented data showed a beneficial safety profile of PEO-PCL-PEO micelles and suggested that the CAPE loading in copolymer micelles preserved its antioxidant properties *in vitro* in human endothelial cells.
Acknowledgments: The financial support from the National Science Fund of Bulgaria (Contract DN-09/1-2016) is greatly acknowledged.
Corresponding author: Denitsa Aluani
email: denitsa.aluani@gmail.com tel: 359888869426

back to contents

PREPARATION AND CHARACTERIZATION OF DOXYCYCLINE LOADED NIOSOMES FOR OCULAR DELIVERY

Gugleva V.[1], Momekova D.[2], Titeva S.[1], Rangelov S.[3]
[1]Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical Univeristy Varna, Bulgaria
[2]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[3]Institute of Polymers, Bulgarian Academy of Sciences, Sofia, Bulgaria

Aim of the study: Niosomes are vesicular systems, formed from the self-assembly of non-ionic surfactants and cholesterol in aqueous media. In ocular therapeutics doxycycline is known to be effective in management of matrix metalloproteases-mediated ocular surface diseases. The aim of this study was to prepare and evaluate niosomes for ophthalmic drug delivery of doxycycline hyclate.
Materials and methods: Niosomes were prepared using various surfactants (Span 20, Span 60, Span 80, Tween 60) and cholesterol in different molar ratios by thin film hydration method followed by extrusion and reverse phase evaporation method. Niosomes were characterized by transmission electron microscopy, dynamic light scattering, Fourier-transform infrared spectroscopy, doxycycline entrapment efficiency and *in vitro* release performance. Ocular irritation test was also conducted.
Results: Niosomes composed of Span 60 and cholesterol at 6:4 molar ratio prepared by thin film hydration and extrusion method are characterized with diameter 163 nm, monomodal size distribution and entrapment efficiency of 52%. Niosomes prepared by reverse phase evaporation method from Span 60 and cholesterol in molar ratio 7:3 are characterized with larger size (720 nm) and higher entrapment efficiency (app.59%). *In vitro* drug release studies indicated sustained doxycycline release up to 20th hour. Both niosomal formulations were non-irritant and excellently tolerated by the eye.
Conclusion: On the ground of the excellent physicochemical characteristics, *in vitro* release studies and occular irritancy test can be concluded that the elaborated niosomes are promising nanocarriers for ocular delivery of doxycycline hyclate.

DEVELOPMENT OF ADVANCED DRUG DELIVERY SYSTEMS BASED ON MESOPOROUS SILICA NANOPARTICLES

Popova T., Tzankov B., Voycheva C., Spasova I., Avramova K., Kovacheva D., Yoncheva K., Lambov N.
Faculty of Pharmacy, Medical University, Sofia

The aim of the study: Bicalutamide (BLT) is one of the most widely used agents in androgen ablation therapy. Due to its low aqueous solubility BLM is included into class II of biopharmaceutic classification system (BCS). Considering this the aim of the present study is to develop mesoporous silica nanoparticles as drug delivery systems with improved therapeutic outcomes.

Materials and methods: For the need of the present study the active substance was included into two different types mesoporous silica nanoparticles – MCM-41 and SBA-16 characterized with good potential for the functionalization, high drug loading capacity as well as improved stability. FTIR, N2-physisorption, XRD, TGA and TEM were used for physico-chemical characterization of obtained formulations. *In-vitro* dissolution studies were also performed in order to determine the release kinetics of bicalutamide.
Results: The results from performed analysis confirmed no changes in mesoporous structure after drug loading procedure. Moreover, the obtained nanoparticles had negative surface charge and average diameter less than 500 nm. In-vitro dissolution tests demonstrated differences into release kinetics of an active substance from obtained mesoporous silica nanoparticles compared to the dissolution behavior of a pure substance.
Conclusion: In the current study mesoporous silica nanoparticles were successfully obtained and loaded with model active substance with low aqueous solubility and pronounced cytostatic effect. The changes of the release kinetics of bicalutamide were probably due to the high surface area and changed crystallinity of an active substance, owing to the inclusion into mesoporous silica nanoparticles.

ANALYSIS OF THE STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF ADVANCED THERAPY MEDICINAL PRODUCTS

Shopov G., Hristov E.
Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria
Corresponding author: George Shopov
Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski" Sofia University
1 "James Boucher" Blvd., 1164 Sofia, Bulgaria
e-mail: jorj9shopov@yahoo.com

Introduction: Nowadays of rapid development of biotechnology and genetic engineering, advanced therapy medicinal products are emerging as a new class of innovative medicinal products. Advanced therapy medicinal products are divided into three main types: tissue engineering medicinal products, gene therapy medicines and somatic cell therapy. Aim: In a comparative perspective, we analyze mechanisms of action of the various types of advanced therapies, evaluate their structural and functional characteristics, with an emphasis on viruses as vectors and carriers of therapeutic effect of the so-called "oncolytic viral therapies".
Materials and Methods: Through graphic analysis, static and dynamic 2D and 3D models we will present cellular and tissue constructs involved in building this type of medicinal products as well as their mechanisms of action.
Results and Conclusion: In the development of this area of medicinal products, science is a matter of the future and enormous hopes for the treatment of severe traumas, rare diseases, genetic and oncologic diseases.
Key words: Medicinal products, advanced therapy, oncolytic viruses

back to contents

IMMUNOGENICITY PREDICTION OF BACTERIAL PROTEINS BY RANDOM FOREST ALGORITHM

Zaharieva N., Doytchinova I., Dimitrov I.
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
tel.: +359 2 9236537

Immunogenicity is defined as the ability of a substance to produce an immune response in the host. Bacterial proteins are the most common substances associated with the etiology of infectious diseases. The in silico prediction of protein immunogenicity is the first step in the process of vaccine design and development.

Ten years ago, a server for immunogenicity prediction of proteins of bacterial, viral, fungal, parasite and tumor origin, named VaxiJen (http://www.ddg-pharmfac.net/vaxijen/VaxiJen), was developed in our Lab. The models for immunogenicity prediction were derived by partial least square (PLS) discriminant analysis on sets of known immunogenic and non-immunogenic proteins. The primary structures of proteins were encoded by z-descriptors and transformed into uniform vectors by auto- and cross-covariance (ACC) calculations.

Recently, the set of bacterial immunogens was updated and now it contains 317 proteins from 47 species (http://www.ddg-pharmfac.net/vaxijen/ VaxiJen/database. fasta). A mirror set of non-immunogenic proteins from the same species was collected. The amino acids in the primary structure of each protein were represented by five E-descriptors. The first descriptor (E1) reflects the hydrophobicity of amino acid; the second (E2) – size; the third (E3) – helix propensity; the forth (E4) correlates with the relative abundance of amino acid; and the fifth (E5) is dominated by β-sheet propensity. The resulting protein strings were transformed into uniform vectors by ACC.

The new dataset was used to derive models by PLS discriminant analysis and by random forest algorithm (RF). The predictive ability of the derived models was assessed by receiver operating characteristics curves (ROC-statistics) after 10-fold cross-validation. The RF model showed better overall performance than the PLS model. The area under the ROC curve (AUC) and the prediction accuracy are 0.834 and 0.752, respectively, for the RF model *vs* 0.702 and 0.732 for the PLS model.

back to contents

PROSPECTIVE STUDY OF ADVERSE DRUG REACTIONS IN THE BULGARIAN POPULATION OF PATIENTS WITH INFLAMMATORY JOINT DISEASES TREATED WITH BIOLOGICAL MEDICINAL PRODUCTS

Parvova I.[1], Rangelov A.[2], Hristov E.[2], Rashkov R.[1], Getov I.[3], Ognianov S.[2]

[1]Department of Internal Medicine, Clinic of Rheumatology,
"Sveti Ivan Rilski" University Hospital, Medical University, Sofia, Bulgaria
[2]Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria
[3]Department of Social Pharmacy, Faculty of Pharmacy,
Medical University, Sofia, Bulgaria
Corresponding author: Iva Parvova MD, PhD, Fepartment of Internal Medicine, Clinic of Rheumatology, "Sveti Ivan Rilski" University Hospital",
Medical University, Sofia, Bulgaria
13 "Urvich" str., 1612 Sofia, Bulgaria
e-mail: ivaparvova@mail.bg
Tel: 00 359 894 463 171

Aim: To analyze Adverse Drug Reactions (ADRs) in patients with inflammatory joint diseases eligible to treat with biological medicinal products (BMP). Materials and **Methods:** A single-center, observational, open-label, prospective, non-interventional, pharmacoepidemiological study of clinical cases of ADRs in the Bulgarian population of patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA), treated with BMP between March 2015 and October 2016. The study was conducted on a protocol basis and after signed informed consent. Patients are treated with: Etanercept, Adalimumab, Golimumab, Certolizumab, Rituximab. The statistical analysis was made with SPSS version 16.0.
Results: 53 patients were screened, 5 did not meet the inclusion criteria; 47 enrolled, 5 - withdrawn from the study, 42 - analyzed. Disease distribution: RA-40.5% (n=17), PsA-19% (n=8), AS-40.5% (n=17). Women - 52% (n=22), male - 48% (n= 0). 76% of patients was treated with Adalimumab and Etanercept. 17% of patients (n=7), biological treatment was discontinued due to serious ADRs. 3 of them were 3rd grade of severity, 4 – 4th grade. The largest relative share occupied by ADRs with grade of severity 1 and 2, as 1 being 63%. The total number of reported and confirmed ADRs is 160. 3 ADRs meet the definition of SUSAR; 30 was unexpected; 127 - suspected. The total incidence of ADR was measured at 4.37 ADR/patient.
Discussion: We have established a very high incidence of ADRs that is inappropriately higher than pre-authorization data for the analyzed BMP. The most common cause of discontinuation of biological therapy in patients with inflammatory joint disease is the onset of ADR.
Keywords: inflammatory joint diseases, adverse drug reactions, biological treatment, prospective study

back to contents

MONOCLONAL ANTIBODIES PATHWAY

Marinov L., Nikolova I.
Faculty of Pharmacy, Medical University, Sofia

Monoclonal antibodies (mAbs) are one of the most important classes of therapeutic proteins that are used for the treatment of various oncological, inflammatory and autoimmune diseases. Their development began more than 40 years ago. The first authorized mAb, in 1986, was muromonab-CD3, followed by chimeric (Abciximab) in 1995 and the first fully human antibody – Necitumumab, registered in 1999.
During the period 2005-2017, around 880 monoclonal antibodies have started clinical trials, of which more than 80 have a marketing authorization. There is a tendency to increase their number in each subsequent year - from 12 in the early 1990-s to 70 in 2014. In 2015-2017, there has been an increase in the number of molecules that have started clinical trials to over a 100 per year. In 2013, the first biosimilar mAb, Infliximab, was registered in Europe. Approximately 20% of all registered mAbs have an oncological indication, 17% haematological, 13% dermatological, 11% rheumatologic, 10% gastroenterological and pulmonary, 7% are related to orthopedics, 4% to cardiology, 3% to ophthalmology and immunology, 1% with nephrology and lymphology. In 2018, 12 new mAbs have been authorized for use in Europe and US; 9 of them have indications different from cancer, including three for migraine prevention and one for HIV treatment.
Monoclonal antibodies market is expected to almost double in 2023 compared to 2018 and reach over 63 billion USD. In addition to cancer and autoimmune diseases, new fields for the development of mAbs include obesity, diabetes, Alzheimer's disease, bacterial infections and skin diseases.

OUR CONTRIBUTION IN DEVELOPING OF INNOVATIVE POLYBACTERIAL IMMUNOSTIMULATORS FOR IMMUNOTHERAPY AND IMMUNOPROPHYLAXIS

Schekerdjiiska S., Georgiev N., Schekerdjiiski R.
NATSTIM Ltd

Aim: Creation and production of polybacterial immunostimulators - products of natural or synthetic origin, resulting from the development of modern biotechnology with different chemical characteristics and mechanism of action.
Developing of low-dose polybacterial immunostimulators based on the established and widely used in medical practice - Respirax, Urostim.
Creation and implementation of products for immune-prophylaxis to enhance the body's natural resistance to various bacterial and viral infections, to support the treatment of other pathogenetically related diseases such as malignancies. It is also known the negative effect on the immune system of the increasing pollution of the environment and especially of the air, the increasing radiation, which further directed our efforts in the creation of products related to immunostimulation and immune-prophylaxis.

back to contents

Materials and methods: The detailed and extensive clinical trials conducted with Respivax, Urostim, in the last years and their positive impact on the various mechanisms of the immune system, as well as the long-standing experience of their uses, have led to the creation of various so-called "Target" polybacterial immunostimulators for oral administration to stimulate the natural mechanisms of the immune system and to assist in the prophylaxis of infections of the respiratory and urogenital systems.

Results: Based on the long-standing experience in creation of combined antioxidants and immunomodulator products, we developed a new formula which contains a patented immunosupporting complex and a specific list of essential antioxidants and micronutrients (Respisim, Pharynostim, Immuno-Uroprostanorm, etc.).

Conclusion: IMUNOSTIMULATORS are particularly suitable for the prophylaxis and treatment of:

- Influenza and other viral respiratory infections and subsequent secondary bacterial infections;
- Infections of different organs and systems induced by antibiotic and chemotherapeutic resistant strains;
- Infections with different localization in patients with hypersensitivity reactions to antibiotics and other antibacterial agents used;
- Lack of antibiotics for specific treatment of viral infections;
- In the complex therapy of malignancies;
- In the control of diseases and conditions associated with suppressed immune system resulting from the action of harmful factors in the working environment.

*The products quoted above are commonly developed with NCIPD, BUL BIO-NCIPD and NATSTIM Ltd

.

JOINT EUROPEAN LEGISLATION ON HEALTH TECHNOLOGIES ASSESSMENT – WHERE THE COUNTRIES COULD COLLABORATE

Petrova G.

Faculty of Pharmacy, Medical University, Sofia

Introduction: The Proposal for a Regulation on health technology assessment and amending Directive 2011/24/EU for joint health technologies assessment (HTA) raised a numerous discussions in society.

Goal: The current work presents the results from the study of the historical development of the collaboration between the European countries in the area of HTA and the possibilities of its further enlargement.

Methods: Historical analysis of the development of European legislation and regulatory projects in the area of HTA during 1994-2019.

Results: Collaboration in the area of HTA is 20 years old and starts with the financing of the project EUR-ASSESS of the European commission in 1994-96, followed by other projects as ECHTA/ECHAHI and tree years lasting EU Joint Action project EUnetHTA. The objectives of these projects were to create a joint framework for effective creation and development of joint HTA, which further to be used from the

back to contents

European regulatory institutions. During the latest 3 years some countries developed regional collaboration in medical and health technologies, pricing and reimbursement areas as are the BeNeLuxAlr, Valletta, FINOSE and Visegrad initiatives. The HTA Network was created with art. 15 of the Directive 2011/24/EU. In 2019 the European commission approved the new project HTx (Next generation HTA) which gathers together 16 European universities and governmental institutions. The Medical university of Sofia is part of this project.

Conclusion: Having in mind the successful initiatives, the joint assessment of the efficacy and safety of new health technologies is more recommendable. Endorsement of joint standard for evaluation will increase the requirements towards the HTA at national level; decrease the duplication of work and eliminate the doubling ad collection of data. This will increase the objectivity and transparency of decision-making for public finding of new technologies. At national level will remain the cost-effectiveness and budget impact evaluation of new health technologies.

ANALYSIS OF THE CHANGES IN REGULATORY POLICY OF PRICING AND REIMBURSEMENT OF MEDICNAL PRODUCTS IN BULGARIA

Savova A., Nikolova I., Terezova S., Zidarova B., Vasileva M., Apostolova D., Danchev N.

Faculty of Pharmacy, Medical University of Sofia; National Council on Pricing and Reimbursement of Medicinal Products

Goal: To analyse the recent changes in the regulatory basis of pricing and reimbursement of medicinal products in Bulgaria.

Materials and methods: Regulatory analysis of two normative documents – Law on medicinal products in human medicine (Law) and Regulation on conditions, rules, and order for regulation, and registering of the prices of medicinal products for human use (Regulation) in 2019.

Results: Based on the changes in the Law the Regulation on pricing and reimbursement new procedures have been introduced, namely for health technology assessment (HTA) and therapeutic effect monitoring for advanced medicinal products included in the Positive Drug List (PDL). The requirement for at least one positive HTA evaluation from governmental institutions in UK, France, Germany, and Sweden is also introduced for applying for inclusion into the PDL.

The order for conditions and criteria for monitoring of the therapeutic effect of advanced therapies, as well as the authorized health care establishments is defined. Number of reference countries for external prices comparison is decreased from 17 to 10 and they all became obligatory. With the changes in the Tariff for taxes according to the Law for medicines new governmental taxes are endorsed for HTA process funding. As the Annex to the Regulation are explained in details instructions for preparation and evaluation of HTA dossiers.

Conclusion: The changes in the Regulation on pricing and reimbursement of medicinal products aimed at optimising the decision making process for listing into

back to contents

the PDL. They introduce one new contemporary European approach for health technologies assessment and prices control of medicinal products in Bulgaria.

METHODOLOGICAL AND TECHNOLOGICAL APPROACH TO IMPROVING THE STATISTICAL ACCURACY OF DIAGNOSTIC TEST AND METHODS

Mitov K., Tachkov K.

Department of Organization and Economics of Pharmacy, Faculty of Pharmacy, Medical University, Sofia

Goal: To develop a methodologic and technological approach for reducing the risk of errors when diagnosing patients with reference intervals, which are not fixed and can change with time.
Methods: A cyclical, stepwise, programming approach and recommendations on its implications on an individual and laboratory level has been proposed. This approach is based on the development of a register for systemic collection, accumulation and analysis of data from clinical tests.
Results: Establishing reference intervals for a given diagnosis, although useful, hide risk of several statistical errors, not always considered – errors in poor laboratory data, errors due to a lack of a representative sample of measurements on an individual level, errors attributable to the sensitivity and specificity of the applied method, errors due to a change in a reference interval. To objectify the diagnostic process, tree types of data are required, which guidelines do not offer: data on mean value, data on variation and data on variability of clinical markers. An individual level solution would be the automatization of data collection and creation of control diagram, comprising an individual's clinical parameters and tendencies for a given time period. On a laboratory level, the creation of a register with all tests carried out, stratified by type of test would allow calculation of a population interval and variations. On a national level this would improve diagnosis by calculating well-defined, population-based reference intervals.
Conclusion: In the field of diagnostics, modern electronic technologies for continuous collection, accumulation and analysis of clinical records, offer possibilities for improving early diagnosis of high-risk individuals.

back to contents

COST-EFFECTIVENESS ANALYSIS OF THERAPY FOR ACROMEGALY – A CASE WITH PEGVISOMANT

Kamusheva M.[1], Rusenova Y.[1], Vandeva S.[2], Mitov K.[1], Petrova G.[1]
[1]Department of Organization and Economics of Pharmacy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Clinical Center of Endocrinology and Gerontology, Medical University, Sofia
Corresponding author: Maria Kamusheva,
e-mail: mkamusheva@pharmfac.mu-sofia.bg, Tel. Number: 0886428154

The aim of the study: To assess the cost-effectiveness of pegvisomant in comparison to octreotide for patients with acromegaly in Bulgaria.
Materials and methods: Cost-effectiveness analysis is performed from the perspective of National Health Insurance Fund (NHIF) and includes: determination of effectiveness expressed as change in the levels of insulin-like growth factor 1 (IGF-1) (nmol/l) on the basis of real-world data extracted from existing acromegaly patients registry in Bulgaria, calculation of the annual costs for each alternative (octreotide and pegvisomant), estimation of incremental cost effectiveness ratio (ICER), application of one-way sensitivity analysis and presentation of the results through the Tornado diagram.
Results: The ICER is 18 438.38 BGN per 1nmol/l reduction in IGF-1, which expresses the additional money that should be paid per one additional unit of result. The incremental ratio is lower than the threshold of 3xGDP (gross domestic product per capita) defined by the World Health Organization. The key parameters are varied within the intervals +/-5% for effectiveness and +/-5%, +/-15% and +/-30% for the cost. The increase of pegvisomant effectiveness with 5% leads to most significant change in ICER as it receives the lowest possible ICER value of 9 089,07 additional money per 1nmol/l additional reduction in IGF-1 in comparison with octreotide.
Conclusion: Pegvisomant leads to better therapeutic outcomes and higher pharmacotherapy costs for acromegaly patients. It is a cost-effective alternative from the NHIF perspective. The reduction of pegvisomant annual expenditures with 30% and 15% and the increase of its effectiveness with 5% have significant impact on ICER value.

BREAST CANCER LANDSCAPE MAPPING IN BULGARIA

Dimitrova M., Petrova G.
"Organization and Economy of Pharmacy" Department,
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
Corresponding author: Maria Dimitrova,
e-mail: mdimitrova@pharmfac.mu-sofia.bg, 02 9235 568

Aim of the study: To evaluate the screening, diagnostic, health care services and therapy practices in breast cancer (BC) and their availability in Bulgaria.
Materials and methods: Demographic, epidemiological, clinical, therapeutic and economic profile of the disease was created using the publically available data bases of the National Cancer Register, National Health Insurance Fund and National

Council of Prices and Reimbursement of Medicines. Data were processed through descriptive statistics.

Results: BC is the most common cancer among women in Bulgaria (26.8%) and according to expert data about 65% are diagnosed in I-II stage, however, there is no mechanism for controlled action of early detection yet. The average 5-year survival rate is 72% for stage I-II and 27% for the advanced breast cancer (ABC) but is still under the average for the European Union. The applied methods for diagnosis and monitoring of BC follow the international guidelines with the majority of them covered with public expenditures. The pharmaco-therapeutic guideline complies with the ESMO and NCCN recommendations and patient access to therapy is relatively fast through the inclusion of medicines in the Positive Drug List (PDL). Currently only one CDK 4/6 inhibitor (ribociclib) for ABC has no market access granted yet but it has positive HTA decision and is about to be included in PDL.

Conclusion: BC continues to place substantial economic and social burden. Stricter follow of the international guidelines and some improvements in the health policies should be placed in order to increase the early detection.

ANALYSIS OF PRIMARY OUTPATIENT DATA FOR *OFF-LABEL* USE OF MEDICINES IN NEUROLOGY

Drenska M.[1], Elitova S.[1], Grigorova V.[2], Naseva E.[3], Getov I.[1]

[1]Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Acibadem City Clinic Cancer Center, Sofia, Bulgaria
[3]Faculty of Public Health, Medical University Sofia, Bulgaria
Corresponding author: Drenska Maria;
e-mail: maria.drenska@gmail.com

Objective: The *off-label* use of medicines is a common practice that covers a wide range of therapeutic areas in both adults and children. So far, the extent of the *off-label* use among the Bulgarian population has not been studied. The aim of this nested, retrospective, non-interventional, single center study is to provide data on the frequency, type and the most common situations in which *off-label* medicines are prescribed in daily neurology practice in Bulgaria.

Methods: The data on prescriptions of 360 neurology outpatients, treated in one-year period, were recorded and provided for analyses. The Summaries of Product Characteristics (SmPC), published on the Bulgarian Drug Agency website, were used as reference documents for assessment of prescriptions. Descriptive statistics, with absolute frequencies, means and standard deviation, were used to analyses the processed data.

Results: The results from this study show that most neurology patients (63%) were exposed to *off-label* use. Most of the medicines prescribed *off-label* (90%), were used for another therapeutic indication then the one listed in the authorized product information. Certain medicines were found to be used *off-label* in 100% of prescriptions.

Conclusion: Although the study is limited to one center, it deserves attention as it reveals many different aspects of the *off-label* use in neurology patients in Bulgaria.

back to contents

Further studies based on a larger number of medical centers are needed to establish more accurate data on *off-label* prescribing in neurology patients on national level.

RISK-MINIMIZATION MEASURES IN THE EU: REVIEW AND ANALYSIS 2017-2018

Getova V.[1], Staynova R.[2], Getov I.[3]
[1]Bulgarian Drug Agency
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv
[3]Faculty of Pharmacy, Medical University, Sofia

Aim: The current study aims to research, systemize and analyze the pharmacovigilance referral procedures which took place in the EU for two years' period 2017-2018. The purpose of the study is to point out the qualitative characteristics of the procedures and to summarize future development and practical aspects of pharmacovigilance.
Materials and methods: For the purpose of the study PRAC decisions and minutes for all procedures which started and completed within 2017-2018 were extracted and systemized. The systemization was based on several criteria including legal basis of procedure, scope, proposed risk-minimization measures and whether Bulgaria is concerned or not.
Results: The results from the study showed that the scope of the majority of referrals included medicinal products available on the Bulgarian market. PRAC's recommendations are most frequently focused on narrowing patient population and therapeutic indications and enhancing safety monitoring during the use of medicines. National competent authorities play major role both as initiators of referrals and national safety monitors.
Conclusion: The conducted analysis shows that conducting PRAC safety referral procedure is a complex and dynamic process where real-life clinical data is valuable source of information. The suggested risk-minimization steps are mainly focused on the enforcement of measures already existing in the risk-management plan of the products. They also require a constant follow-up monitoring for efficacy. National competent authorities are leading participants in organizing the work and composing the agenda of the committee.

back to contents

COST-EFFECTIVENESS OF PHARMACOLOGIC TREATMENT IN PRIMARY OPEN-ANGLE GLAUCOMA AND "DRY EYE" SYNDROME IN REAL THERAPEUTIC CONDITIONS

Tashkov K., Vasilev A., Kostova S.

Aim: The goal of this study is to assess the level of disease control with conventional therapies, prescription habits, and evaluate the cost-effectiveness of therapy in Primary Open-Angle Glaucoma (POAG).
Materials and methods: A prospective, observational study was carried out in the Ophthalmology clinic in "Alexandrovska" Univeristy Hospital. Data collected included type of therapy, control of POAG, and measurements of control of "dry-eye" through TMS, NIBUT Ave, and ST). Therapy costs were calculated based on retail prices for both reimbursed and non-reimbursed products. A "decision tree" model was constructed with the probabilities of prescribing a particular product and the cost-effectiveness of treatment was evaluated. Deterministic sensitivity analysis was conducted to test robustness.
Results: The probability of prescribing a formulation with preservatives was two-times higher than preservative-free products (p=0.69, and p=0.31). In the monotherapy group, lower costs were observed in the preservative containing products, whereas in the combination group, preservative-free products had a lower yearly cost of therapy. Both preservative-containing and preservative-free products were equally prescribed as combination products (p=0.5, p=0.46, respectively). The highest incremental cost-effectiveness ratio (ICER) was for NUBIT (8318,9 BGN), whereas for TMS and ST the ICER was 75.40, and 115.38 BGN, respectively. Varying the parameters within ±30% showed that all values were below the threshold of 20 000 BGN, thus are cost-effective.
Conclusion: Using products with preservatives hides a risk of developing "dry-eye" syndrome. Treatment with preservative-free products is cost-effectives, thus, physicians should carefully monitor patients and choose the appropriate medication carefully.

ASSESSMENT OF MEDICINAL THERAPY IN NEONATOLOGY

Petkova V., Antova V.
Faculty of Pharmacy, Medical University, Sofia
tel. 029236593, e-mail: vpetkova@pharmfac.mu-sofia.bg

Aim of the study: Study of prescription drugs in the Department of Intensive Neonatology of "Maichin Dom" SHATOG for 2018.
Materials and methods: A retrospective study was carried out by type and quantity of the medicines prescribed in the Department of Intensive Neonatology of "Maichin Dom" SHATOG for 2018. An analysis was performed on the following criteria: the licensing status, pharmacological groups, which have in SPC indications and dosage for use in newlyborns, number and type of prescription drugs specially formulated for neonatal therapy.

back to contents

Results: The total number of prescribed medicinal products for 2018 is 98. Of these, only three were specifically designed to treat neonates. The largest proportion of prescribed medicinal products were antibiotics - 22 or 22.45% of all prescribed medicinal products. In the current study, the percentage of drugs with *off-label* use was 59.18% in total.
Conclusion: Although there are already legislative initiatives in the US and the EU to promote drug use studies in neonates, the proportion of *off-label* drugs used in this group remains high.

PILOT STUDY AND ASSESSMENT OF PHARMACIES' SERVICES AND PATIENTS' SATISFACTION IN BURGAS REGION

Burgazliev H.[1], Krumov S.[2], Luizov A.[3], Getov I.[4]
[1] Medical College, University „Prof Assen Zlatarov" Burgas
[2]National Center of Public Health and Analyses
[3]Burgas Free University
[4]Medical University Sofia, Faculty of Pharmacy

Aim of the study: This study aims to investigate and measure the satisfaction of visitors in community pharmacies in the Burgas region, Bulgaria from the provided pharmaceutical activities and services, and the expectations for improvement.
Material and methods: A methodical approach used was indirect individual anonymous poll with questionnaire file card of pre-trained field interviewers (in 30 community pharmacies on the territory of Bourgas region) during the period 01.5 - 30.6.2019. The questions are closed, incl. with more than one response and Likert Scale assessment, basic demographic information was also collected. Participation of respondents - visitors to the pharmacy is voluntary, and written consent is not required due to the nature of the study, such as medical-social, non-interventional and anonymous. A nested population based non-systematic sample was formed on the basis of the total number of visitors and the SPSS v.24.0 statistical software was used to process the primary information.
Results: The total number of respondents is expected to be over 500, with a high degree of validity of completed questionnaire file cards. Data is in the process of collection and processing, but preliminary results show a high level of satisfaction, expectations and specific guidelines for improving the range of activities, services and service organization in community pharmacies.
Conclusion: Consumer satisfaction and expectations are the focus of marketing research and are particularly important for community pharmacies in the strong market competition. In addition, data using a standardized study approach may be useful at macro, micro and regional level.

back to contents

ANALYSIS OF THE REGULATORY CHANGES AND PREPARATION OF THE INDIVIDUAL PRESCRIPTIONS IN THE HOSPITAL PHARMACIES

Georgieva S.
Medical University of Sofia, Medical College; "Alexandrovska" University Hospital

Goal: To analyse the changes in the regulatory framework in the area of hospital pharmacy in Bulgaria and related changes in the preparation of the individual prescriptions for inpatients.
Methods: Regulatory analysis of the Law for medicinal products for human medicine; Regulation of the order, rules and organization of work in the pharmacies; Regulation on prescription and dispensing of medicines, and Guidelines for good pharmaceutical practice. Retrospective analysis of the individual prescriptions for inpatients in "Alexandrovska" University Hospital for the period 2016-2019.
Results: Significant changes in the LMPHM and related regulations led to changes in the type and volume of the individual prescriptions for inpatients. A drastic decrease is observed for the classic magisterial prescriptions, mainly dermatological ones, and increase in the number of individual prescriptions for direct application for patients with malignant diseases. In recent years is also observed an increase in the number of prepared radiopharmaceuticals following pharmacopeia prescriptions. On average 400-450 patients per month receive radiopharmaceuticals while the classic forms are around 20 per month.
Conclusion: The regulatory changes necessitate new knowledge from the hospital pharmacists, as well as reorganization of the work, and new equipment to comply with the requirements of the good pharmaceutical practice.

POSSIBILITIES FOR OPTIMIZING THE EDUCATION OF PHARMACY STUDENTS BY USING A SPECIALIZED ELECTRONIC PLATFORM - SWOT ANALYSIS BASED ON THE EXPERIENCE IN MU-VARNA

Todorova A.[1], Grigorov E.[1,2], Petrova G.[1], Kolev J.[1], Kumanov I.[1], Naydenova D.[1]
[1]„Organization and Economics of Pharmacy" Department, Faculty of Pharmacy, Medical University, Varna
[2]National Center of Public Health and Analyses

Introduction: The Blackboard Learning platform has been introduced in 2014 and plays an important role in the training and education of the students from the Medical University, Varna. The main priority is not to shift the attendance classes, but to provide new opportunities for teachers and students to use digital content and additional educational resources.
Objective: To analyze from the point of view of the Pharmacy specialists the strengths and weaknesses, the prospects and the difficulties that accompany the training of the students with the help of the electronic platform.

Methods: Using the classic technique of building a SWOT table, we show and address the individual elements of this type of analysis - strengths and weaknesses, opportunities and threats.
Results: The Blackboard electronic platform provides additional educational opportunities to improve communication with students, facilitating access to learning resources. It also has innovative options for presenting the material through videos and interactive exercises, enhancing the learner's knowledge and skills, increasing motivation and active participation in traditional lectures and seminars.
On the other hand, for teachers, it is an effective tool for tailoring the learning process and taking into account the specificities of organizing the training in each discipline, as well as tracking the success rate. Requires training and improvement of the teachers' digital competences. A drawback may be the risk of a possible collapse and the associated loss of information.
Conclusion: Rapidly developing information and communication technologies pose a number of challenges to education related to the pedagogical and organizational aspects of their application. Public needs and expectations in the context of their widespread penetration of life are increasing, which requires them to be introduced into the learning process to optimize training.
Keywords: training, pharmacists, SWOT analysis, lecturers

STUDY OF THE ATTITUDE OF PHARMACISTS IN BULGARIA TOWARD THE REMUNERATION OF VALUE-ADDED PHARMACY SERVICES

Balkanski S.[1], Simeonova J.[2], Gitev I.[1], Getov I.[1,3]
[1]Bulgarian Pharmaceutical Union
[2]Faculty of Pharmacy, Medical University, Pleven
[3]Faculty of Pharmacy, Medical University, Sofia

Aim: The aim of the study is to evaluate and analyse the current state of the value-added pharmacy services (VAPS) offered in the community pharmacies in Bulgaria and the pharmacists' attitude toward their remuneration.
Materials and methods: A cross-sectional study was carried-out in 2018. A web-based questionnaire was developed consisting of 15 questions and sent to all members of Bulgarian Pharmaceutical Union. Data was processed by SPSS v.24.0. The number of cases falling in each range of categorical variables and percentage were displayed.
Results: Over 51% of the pharmacies in Bulgaria offer VAPS but mainly measuring of blood pressure (67.4%) and of blood glucose (12.9%). About 60% of community pharmacists in the country are willing to perform other VAPS. Most pharmacists (98.3%) support the idea of introducing a remuneration fee for VAPS. According to 49.8%, the fee should be over BGN 10 and 30.9% - up to BGN 5. With the increase in the number of patients in the pharmacy, the share of pharmacists who are disapproving of the idea of introducing a remuneration fee for blood sugar measuring - from 9.3% in pharmacies serving up to 50 patients on average per day up to 52% at pharmacies serving over 500 patients / day ($\chi 2=12.127$; df=6; p=0.059).

back to contents

Conclusion: The study shows that the pharmacy can offer other services, different from consultation and dispensing of medicinal products. Such additional services in the pharmacy would have added value from societal, healthcare and economic point of view.

THE FIRST FEMALE PHARMACISTS IN BULGARIA - WHAT DOES IT MEAN TO BE AMONG THEM? DREAMS OR REALITY?

Stoyanova S.[1], Hristov E.[1], Andreevska K.[1], Delyiski T.[1], Ognyanov S.[1], Dimitrova Z.[1], Burgazliev H.[2]

[1]Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria
[2]College of Pharmacy, "Prof. Assen Zlatarov" University, Bourgas, Bulgaria
Corresponding author: Stefka Stoyanova, Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski" Sofia University, 1 "James Bourchier" blv., 1164 Sofia, Bulgaria; e-mail: stefkasd@mail.bg

For many years, a pharmacist has been a typical male profession and has been practiced by women exceptionally. However nowadays, the pharmacy becomes one of the most preferred women's specialties for professional realization. Have we considered who are the first Bulgarian ladies who have managed to fight for the right to be pharmacists, assistant pharmacists and master pharmacists in times of full patriarchy? Even in the textbooks of the History of Pharmacy in Bulgaria there are not enough verifiable data and reliable information about the first Bulgarian women who have been practicing in the field of pharmacy.

Aim: To investigate who are the first female pharmacists in the history of pharmaceutical science and practice of Bulgarian origin and practiced in the Bulgarian lands.

Materials and methods: We conducted extensive search and analysis of historical information and documents by reviewing electronic sources, literary data, searching in historical museums in the country, the Archives State Agency, the Institute of History at the Bulgarian Academy of Sciences, the "Sveti Kiril I Metodii" National Library and personal archives.

Results and conclusion: We have found data on three worthy Bulgarian women, an example of women who have made their dream a reality - to be a Master of Pharmacy and have contributed to the development of pharmacies in a number of Bulgarian cities - Raina Aleksova, Anna Belizarova Yakovova and Penka Hristova Sarafova. Despite the few sources and the lack of living witnesses of that distant and romantic historical period, we believe that the three worthy Bulgarian ladies described as the first Bulgarian women made a professional breakthrough in pharmacy in its various practical applications! Their names, lives, deeds, efforts and achievements must not fall into oblivion, just the opposite - they must be remembered and respected!

Key words: Master pharmacist, pharmacist student, first Bulgarian woman Master of Pharmacy

back to contents

COMPARATIVE ANALYSIS OF THE PRICING AND REIMBURSEMENT SYSTEMS BETWEEN ITALY AND BULGARIA

Cannavale C.[1], De Rosa A.[1], Yordanov E.[2], Hristov E.[2]
[1]Department of Pharmacy, "Federico II" University of Naples, Italy
[2]Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria
Corresponding author: Emanuyl Yordanov, Master of Pharmacy, Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski" Sofia University,
1 "James Boucher" blv., 1164 Sofia, Bulgaria
e-mail: emanuil.iordanov@gmail.com

Introduction: The pricing and reimbursement approaches in the EU Member States are unified by Directive 89/105 / EEC of 21 December 1988. The Directive lays down common principles such as - time limits for taking a decision, legal remedies, disclosure of information, introducing tacit consent, etc. However, specific mechanisms are the subject of purely national measures, making the pricing and reimbursement picture of the various Member States extremely varied.
Aim. To make a comparative analysis of the regulatory measures for pricing and reimbursement of medicinal products applied by the governments of Bulgaria and Italy.
Materials and methods: Of all commonly agreed pricing and reimbursement measures that regulate and control the cost of pharmaceuticals at macro and micro levels, we looked at four main: positive drug lists, internal reference pricing, health technology assessment, and innovative schemes such as risk sharing agreements. We used documentary method, analysis and synthesis, comparative method and statistical methods.
Results and discussion: The pricing and reimbursement systems of the two countries are fundamentally different. In the period since 2000, legislation (laws, regulations, guidelines), representation and composition of different types of bodies and commissions, switching to the NHIF system and other measures concerning the system of pricing and reimbursement of medicinal products and patient access to them in Bulgaria have been amended and supplemented more than 35 times, which characterizes Bulgarian pricing and reimbursement policies as permanently regulatory unstable and volatile. Unlike Bulgaria, Italy's pricing and reimbursement system has long traditions, based on well-established principles and guarantees stability in the availability and affordability of medicinal products to the population.
Key words: Medicinal products, prices of medicnes, reimbursement.

back to contents

INFLUENCE OF PHARMACEUTICAL CARE AND PROFESSIONAL PHARMACIST'S CONSULTATION ON THE PATIENT

Kachulev K.[1], Simova T.[2], Enchev D.[1]
[1]Faculty of Public Health, Medical University, Sofia
[2]TSC Directorate, Bulgarian Drug Agency
For correspondence: Konstantin Kachulev, +359 878 287 705,
e-mail: kkachulev@gmail.com

Aim of the study: To investigate and assess the impact on the patient of the decision to introduce an electronic (e) recipe and e-dossier, through the expert assessment of the pharmacists (Phs) working in the pharmacy and through the subjective assessment of the patients.

Materials and methods:

Patient poll

From 10.2016. until 01.2017. a cross-sectional study of patients was conducted through a survey (609 questionnaires).

A qualitative criterion for selecting respondents in the study is to meet the definition of a patient or to use prescription or OTCs purchased in the pharmacy.

Pharmacists' Survey

A cross-sectional study was conducted using a rapid data collection method - 15 days across the country through one of the drug distributors in Bulgaria.

Results: 71% of the patients consider e-carrier data storage to be safer, and this would facilitate pharmacy work by 59%, according to the Phs.

68.3% consider safer storage of their personal data and their disease data, and agree this to be done by introducing e-dossier and e-recipe.

Relatively large is the proportion of people who cannot assess the risks and benefits of the proposed policies.

The question "Do you approve the introduction of an e-dossier and an e-prescription?" 436 (72%) of the patients and 322 (87%) of the MF respond with "Yes".

Conclusion: The institutions responsible for implementing these policies need to be more proactive in informing healthcare users.

Patients have confidence in e-dossier and e-recipe, and according to the Phs putting them into practice would make pharmacy work easier.

back to contents

HEALTH RELATED QUALITY OF LIFE – A PILOT STUDY IN HEALTHY VOLUNTEERS

Asipova N.[1], Hristov E.[1], Delyiski T.[1], Parvova I.[2], Yordanov E.[1], Andreevska K.[1], Ognianov S.[1], Burgazliev H.[3]

[1]Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski" Sofia University, Bulgaria
[2]Department of Internal Medicine, Medical University, Sofia, Bulgaria
[3]College of Pharmacy, "Prof. Dr. Assen Zlatarov" University, Bourgas, Bulgaria
Corresponding author: Nasie Asipova, Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski" Sofia University, 1 "James Bourchier" blv., 1164 Sofia, Bulgaria
e-mail: nasi_12@abv.bg

Key words: HRQoL, SF-36v2, domains, health status, healthy volunteers. The broad definition of health proposed by the WHO more than 50 years ago states: "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity." Most healthcare providers and professionals have adapted this broad view of health and now include measures for the overall impact of the disease and its treatment. The most common tool used is Short Form-36. SF-36 consists of 36 questions for a multi-purpose study of the patient's overall health status developed by the WHO.

Aim of study: The aim of the study is to create experimental conditions for selection of healthy volunteers by assessing the health status of pharmacy students through self-assessment of their condition, refracted through the HRQoL tool SF-36, in four main domains: physical, social and behavioral functioning, mental health, and general perceptions of health.

Materials and methods: We interviewed pharmacy students from the Faculty of Chemistry and Pharmacy at "Sveti Kliment Ohridski" Sofia University using SF-36v2 Questionnaire. The research included 26 students aged between 22 and 25, distributed equally between men and women, after they duly signed an informed consent form and were informed of the conditions for completing the questionnaires. Data is processed statistically with the specified program.

Results and discussion: The results obtained showed serious deviations from the norms of the functioning of the "healthy volunteers" in the four functional domains and posed a number of questions as to how to select control groups of healthy volunteers for randomized controlled clinical trials.

back to contents

ANALYTICAL STUDY OF PRODUCTS CONTAINING CANNABIDIOL

Andonova L.[1], Ivanov E.[2], Pencheva I.[1], Konstantinov S.[2]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical Univercity-Sofia, 2 "Dunav" str., Sofia 1000, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., Sofia 1000, Bulgaria
Corresponding author: Lily Andonova, PhD
e-mail: ldimitrova@pharmfac.mu-sofia.bg
Tel: +359 2 9236 543

Cannabidiol (CBD) is the major, biologically active, but psycho-inactive component of the glandular hairs of Cannabis sativa. CBD exhibits diverse pharmacological activities such as anticonvulsive, sedative, hypnotic, antipsychotic, antiinflammatory and neuroprotective.
Aim: With respect to quality control optimization solutions the aim of this study is to develop a simple, precise, accurate and reproducible HPLC method for assay of CBD obtained with certain forms of extractions and purifications in food supplements.
Matherials and methods: HPLC method with isocratic elution and UV detection, supplements containing CBD.
Results: Analytical parameters reproducibility, linearity, accuracy, LOD, LOQ, specificity and system suitability test were developed and optimized.
Conclusion: The proposed method could be applicable for identification, purity and assay tests of food supplements containing CBD extract according to EU regulation concerning food authorizations.

APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR STATINS ANALYSIS IN BIOLOGICAL MEDIA

Maslarska V., Smerikarova M., Bozhanov S.
Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Purpose of the study: Statins are widely used to treat hypercholesterolemia in patients with established cardiovascular disease as well as those with a high risk of developing atherosclerosis. The aim of the present study is to summarize the information in the available literature as well as to systematize the main approaches in the analysis of statins in biological media by high performance liquid chromatography.
Materials and Methods: Sample preparation methods, optimal chromatographic conditions and various types of detectors were considered.
Results: High Performance Liquid Chromatography has been successfully applied in analyzing statins in various biological fluids such as plasma, serum and urine. Most methods allow quantitative determination of statins at level of ng/ml, making them suitable for therapeutic monitoring.

back to contents

Conclusion: There is a continuing interest in the development of new bioanalytical methods for the separation and quantification of statins in biological media. The characteristic specificity and sensitivity, as well as the variety of chromatographic columns and detectors, make high performance liquid chromatography a method of choice for statin assay in biological samples.

SYNTHESIS AND PRELIMINARY SCREENING FOR CYTOTOXICITY AND BIOLOGICAL EFFECTS OF SERIES PYRROLE HYDRAZONES

Tzankova D., Aluani D., Peikova L., Georgieva M.
Department of Pharmaceutical Chemistry,
Faculty of Pharmacy, Medical University, Sofia
Department of Pharmacology, Pharmacotherapy and Toxicology,
Faculty of Pharmacy, Medical University, Sofia

The aim of the study: The applicability of newly synthesized molecules as potential drugs and prototypes for future optimizations of the structure was evaluated with theoretical *in silico* approaches, predicting their pharmacokinetics and drug-like properties. The obtained values were compared to Lipinski's criteria, recommending the following boundary conditions for some molecular parameters: *hydrophobic parameter* Log P < 5, *molecular weight* MW < 500, *number of proton acceptors* n (O, N) < 10 and *number of proton donors* n (OH, NH) < 5. In addition the evaluated structures were subjected to an attempt to predict the possible pharmacological effect against included in the web based software Molinspiration Cheminformatics drug targets, divided into the following six classes: GPCR ligands, ionic channels modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and enzyme inhibitors.
Materials and methods: Series evaluated N-pyrrolyl hydrazide-hydrazones was subjected to a preliminary *in silico* evaluation of their hepatootxicity through the predicting software ProTox II. The obtained results were further compared to the results of *in vitro* evaluation on the cell viability against HepG2 cell line of the tested compounds, determined through a MTT-test.
Results: The performed theoretical evaluation on drug likeness is clear, that the investigated molecules violate Lipinski's Rule on 2 parameters – hydrophobicity and molecular weight, which is a prerequisite of impaired pharmacokinetics. As most probable pharmacological effects was predicted, that the newly synthesized compounds would have high effects as enzyme inhibitors and GPCR ligands.
Conclusion: The *in silico* evaluation of the hepatotoxicity determined the target compounds as low toxic. These predictions were confirmed by the results from the performed *in vitro* experiments.

back to contents

IN VITRO INTERACTION OF 5-AMINOOROTIC ACID AND ITS GALLIUM(III) COMPLEX WITH THE SUPEROXIDE RADICAL, YIELDED BY POTASSIUM SUPEROXIDE AND XANTHINE/XANTHINE OXIDASE MODEL SYSTEMS

Todorov L.[1*], Kostova I.[1]
[1] *Pharmeceutical Faculty, Medical university – Sofia*
[*] *lozantodorov@yahoo.com*

Aim of the investigation: The superoxide radical ($O_2^{\bullet-}$) is involved in many normal and pathological bioreactions in living organisms. Gallium(III) salts and complexes are known for their anticancer properties. The Gallium(III) complex (GaAOA) with 5-aminoorotic acid (HAOA) has demonstrated better *in vitro* antioxidant properties than the ligand itself. The aim of the current investigation is to assess the interaction of HAOA and GaAOA with $O_2^{\bullet-}$.
Materials and methods: The effects of HAOA and GaAOA on the in vitro accumulation of superoxide and free radicals were estimated using potassium superoxide (KO_2) and xanthine/xanthine oxidase (X/XO) as model systems. Luminol-dependent chemiluminescence (LDCL) was utilized to estimate the radicals scavenging activity. The specific activity of xanthine oxidase was estimated by way of UV spectrophotometric measurement.
Results: Both HAOA and GaAOA, reduced the LDCL in presence of KO_2-generated $O_2^{\bullet-}$ in a concentration-dependent manner. Within the experimental error limits, both compounds were scavengers of superoxide radical generated by the model system X/XO.
Conclusions: Data suggested better antioxidant effect of GaAOA than that of HAOA. Evidently all three ligands of GaAOA participated in the scavenging of superoxide. The effects in rat blood plasma were more complex, considering the chemical and biochemical complexity of this model system.
Acknowledgement: The authors gratefully acknowledge the financial support of the Grand Commission in the Medical University of Sofia (Grant No. Д-130/2019).

DETERMINATION OF Cd AND Pb IN PRODUCTS OF BULGARIAN OIL-BEARING ROSE (*ROSA DAMASCENA MILL*)

Bozhanov S.[1], Smerikarova M.[1], Maslarska V.[1]
[1]*Chemistry Department, Faculty of Pharmacy, Medical University, Sofia*

Aim of the study: Determination of Cd and Pb in rose oil, rose water and rose concrete of Bulgarian oil-bearing rose (*Rosa damascena Mill*.)
Materials and methods: Direct Electrothermal Atomic Absorption Spectrometry (ETAAS) was applied after minimum sample pretreatment - dissolution in a suitable organic solvent.
Results: The optimal parameters (atomizers, modifiers, temperature programs) as well as the detection limits of the elements (Cd - 0.08 ng/g and Pb - 1.2 ng) were

back to contents

determined. The relative standard deviation was within 2-9%. Samples from the areas of Pavel Banya and Zelenikovo were analyzed.
Conclusion: Further researches are needed to clarify the sources of trace elements as well as to track their content at the different stages of the production process.

INTRODUCTION OF THE QUARZ CRYSTAL MICROBALANCE METHOD IN THE PHARMACEUTICAL ANALYSIS: DETERMINATION OF DRUG CONTENT IN POLYMERIC CARRIERS

Kolev I.[1], Ivanova N.[1], Marinov M.[2]
[1]Faculty of Pharmacy, "Prof. Dr. Paraskev Stoyanov" Medical University, Varna, 84 "Tzar Osvoboditel" Blvd., 9000, Varna, Bulgaria
[2]Freelance Electronics and System Design Engineer, 54 "Chajka" str., 9000, Varna, Bulgaria

Aim of the study: The quartz crystal microbalance (QCM) method provides a wide range of possibilities for tracking chemical or physical phenomena occurring with a mass variation in nanoscale order. In this study, we propose a version of the QCM method with application in the pharmaceutical analysis, and in particular regarding drug assay in polymeric matrix-type carriers.
Materials and methods: A series of polymeric mixtures of drugs with increasing concentrations were applied to a sensitive quartz resonator in the form of solutions. Evaporation was initialized to obtain homogeneous layers suitable for calibration of the analytical method. Homogeneous solutions of drug-delivery micoparticles, synthesized with known drug concentrations, were used as test analytes. A variation in the sample mass was initiated by passing acidic gas into a pressurized system storing the loaded resonator. The drug content analysis was based on the occurrence of irreversible changes (related to chemisorption of the irradiating gas) in the analyte mass.
Results: The proposed method demonstrated better accuracy when compared to a conventionally used pharmacopoeial spectral assay.
Conclusion: The QCM method was proven to be accurate and precise and thus according to our research, possess the potency for the routine pharmaceutical control.

VIRTUAL SCREENING AND HIT SELECTION OF NATURAL COMPOUNDS AS ACETYLCHOLINESTERASE INHIBITORS

Atanasova M*., Dimitrov I., Doytchinova I.
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
**corresponding author: matanasova@ddg-pharmfac.net; phone: +359 2 9236 599*

Acetylcholinesterase (AChE) is one of the classical targets in the treatment of Alzheimer's disease (AD). AChE is a serine protease that catalyzes the hydrolysis of acetylcholine (ACh) to choline and acetate in neuromuscular and brain synapses.

back to contents

Loss of cognitive abilities, a typical symptom in patients with AD, is due to prolonged loss of Ach because of hyperactivity of AChE and hyperactive N-methyl-D-aspartat (NMDA) glutamate receptors. Inhibition of AChE slows down the hydrolysis of ACh and increases its levels. Thus, the cognitive abilities are improved.
The AChE inhibitor galantamine (GAL), an alkaloid from *Galanthus* genus with multitarget mechanism of action, is the most appropriate drug for treatment of AD. GAL inhibits AChE and interacts with nicotine receptors leading to improvement in the cognitive brain function. Additionally, it increases phagocytose and clearance of amyloid-β peptides in the brain in rats and rodent models.
In the present study, 12 ZINC databases of natural compounds (almost 150 000 compounds) were screened virtually for affinity to AChE by molecular docking with GOLD v.5.2.2 at the following settings: ChemPLP scoring function, rigid protein and flexible ligand. Next, the compounds were screened for blood brain barrier (BBB) permeability, drug-likeness and lead-likeness by SwissADME tool. The complexes with ChemPLP score > 70 were inspected visually. Finally, the synthetic feasibility or the available vendors were assessed and 20 hits were selected for in vitro tests.

DESIGN AND DOCKING OF CURCUMIN-GALANTAMINE HYBRIDS AS ACETYLCHOLINESTERASE INHIBITORS WITH DUAL-SITE BINDING

Lukarski A.[1], Atanasova M., Stavrakov G., Philipova I., Doytchinova I.
[1]Faculty of Pharmacy, Medical University, Sofia, Bulgaria
Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Science, Sofia, Bulgaria
**Corresponding author: e-mail: nasko_lukarski@abv.bg; phone: 02 9236599*

Alzheimer's disease (AD) is the leading cause of dementia in the elder people (> 65 y.o.) affecting more than 25 million people worldwide. The major pathological changes caused by AD are amyloid-beta (Aβ) peptide plaques, formation of neurofibrillary tangles, loss of cholinergic neurons and oxidative stress. For this reason, there is an increased demand for new drug candidates, aimed at reduction of both the oxidative stress and the formation of amyloid plaques.
Curcumin is a natural polyunsaturated phenol compound which has antioxidant and anti-inflammatory properties. An inhibitory activity of curcumin against the acetylcholinesterase (AChE) and Aβ formation has also been demonstrated, making it an ideal candidate for design of its hybrids with galantamine.
In the present study 192 galanthamine-curcumin and curcumin-like compounds were designed to bind to the active site of AChE (CAS) and the peripheral anionic site (PAS). The aim was to increase the binding affinity to AChE and the inhibition of the formation of Aβ plaques. The hybrids were docked with state-of-the-art GOLD software, using a protocol previously optimized for affinity prediction of galantamine derivatives. With two web-based servers, the blood-brain barrier (BBB) permeability of the hybrids was predicted, which is a prerequisite for their activity.
All structures theoretically display a higher affinity towards the enzyme in comparison to galantamine, used as reference. For 156 of them was predicted good BBB

back to contents

permeability. A synthetic strategy was considered for the preparation of 17 selected representatives for which in vitro tests are foreseen.

DEVELOPMENT OF RP-HPLC METHOD FOR IDENTIFICATION OF THE PRODUCTS OF HYDROLYSIS OF PROPANEHYDRAZONE, CONTAINING A XANTHINE FRAGMENT IN ITS STRUCTURE

Peikova L., Dineva A., Georgieva M., Zlatkov A.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia

Hydrazones and oximes are well known derivatives, containing the double carbon-nitrogen bond, characterized with its liability to hydrolysis.
An important functionality of a given molecule, containing such liable groups, determining its biological activity is their stability during storage, their chemical stability and their stability under close to physiological conditions.
The aim of the study: The purpose of this study is to identify the hydrolysis products through a developed RP-HPLC method. For this reason the following parameters of the chromatographic system were varied: column type, mobile phase contents, column temperature and flow rate, as factors determining the efficiency of the chromatographic procedure. The change in the discussed parameters affected mainly the retention times and the chromatographic peak's symmetry.
Materials and methods: As most appropriate were determined the listed chromatographic conditions: flow 1.0 ml/min; temperature of the column 25ºC; Column Purospher®STAR RP-18 endcapped (125x 4.0mm), 5µm; UV-detector with fixed wavelength at 272 nm; mobile phase: methanol : water : acetonitril: glacial acetic acid = 52:46.5:1:0.5 v/v/v/v and injection volume of 20 µl.
Results: The developed chromatographic procedure was validated according to guidelines as expressed in European Pharmacopoeia 7.0 and ICH and applied for determination of the hydrolytic stability of a model compound. The method was found to selective, linear and repeatable.
Conclusion: With the validated RP-HPLC procedure were identified and isolated the products of degradation of the studied 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purine-7(6H)-yl)-N'-(3-fluorobenzylidene)propanehydrazide under close to physiological conditions –pH of 2.0, 7.4 and 9.0 and temperature of 37oC. In addition the storing stability and chemical stability was also investigated in media of pH 13.0. The results show, that the tested compound is stable in neutral and weak alkali media of pH 7.4 and 9.0 and liable to hydrolysis in pH 2.0 and temperature of 37oC, as well as in strong alkali media of pH 13.0.

back to contents

DEVELOPMENT OF CHYRAL RP-HPLC METHOD FOR IDENTIFICATION OF OPTICAL ISOMERS IN THE STRUCTURE OF 2-(1,3-DIMETHYL-2,6-DIOXO-2,3-DIHYDRO-1H-PURINE-7(6H)-YL)-N'-(3-FLUOROBENYLYDENE)PROPANHYDRAZIDE

Dineva A., Peikova L., Georgieva M., Zlatkov A.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia

The aim of the study: The development of methods for separation of enantiomers is a procedure which requires the application of powerful and specific techniques and methodologies, including the introduction of new highly selective and sensitive columns, which is a prerequisite of high costs and prolongs analysis. The application of chyral columns will lead to decrease in the costs and the time of the analysis.
Materials and methods: In the current research we aimed to develop a single stage, easy, selective and precise RP-HPLC method for simultaneous separation and identification of geometrical isomers of a synthesized in our laboratory xanthine hydrazone, containing an asymmetrical center in its structure.
Results: For development of the RP-HPLC method the influence of the type of the column, the mobile phase contents, the temperature of the column and the flow rate was investigated. The change in the followed parameters affected mostly the retention time and the shape of the obtained peaks. It was seen that the application of a hyral column gave best effectiveness of the chromatographic system.
Thus the next experiments were performed on a chromatographic system containing: chyral column ACE®Equivalence™ C18 (250x 4.6mm), 5µm; UV-detector with fixed wavelength 272 nm; mobile phase: methanol: water: phosphate buffer pH=7.4 = 50:46:4 v/v; temperature of the column – 25 °C; flow – 1 ml/min and injection volume – 20 µl.
Conclusion: The developed chyral RP-HPLC method was validated according to the European Pharmacopoeia 7.0 and ICH guidelines and used for the successful separation of the two enantiomers of the newly synthesized 2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purine-7(6H)-yl)-N'-(3-fluorobenzylydene) propanehydrazide.

back to contents

SESQUITERPENE LACTONES FROM SONCHUS PALUSTRIS L. (ASTERACEAE, CICHORIEAE)

Shulha O.[1], Çiçek S.[1], Piccolella S.[2], Rárová L.[3], Strnad M.[3], Pacifico S.[2], Zidorn C.[1]

[1]*Pharmazeutisches Institut, Abteilung Pharmazeutische Biologie, Christian-Albrechts-Universität zu Kiel, 76 Gutenbergstraße, 24118 Kiel, Germany*

[2]*Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, 43 Via Vivaldi, 81100 Caserta, Italy*

[3]*Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany ASCR & Palacky University, 27 Šlechtitelů, 78371 Olomouc, Czech Republic*

In our continuing search for new bioactive compounds in the Cichorieae tribe of the Asteraceae family, we have studied the formerly not investigated European species *Sonchus palustris* L.

Standard chromatographic techniques were used for natural product isolation and 1D and 2D NMR, together with HRMS were used for structure elucidation. In addition, some of the pure compounds were assayed *in vitro* for cytotoxicity.

Seven previously undescribed sesquiterpene lactones, three known sesquiterpene lactones (ixerin D, 15-p-hydroxyphenylacetyllactucin, and 15-p-hydroxyphenylacetyllactucin-8-sulfate), and two known quinic acid derivatives (3-O-feruloylquinic acid and 3,5-di-O-caffeoylquinic acid) were isolated from *Sonchus palustris* L. roots. Four formerly unknown compounds were elucidated to be 3β,14-dihydroxycostunolide-3-O-β-D-gluco¬pyranosyl-(2-O-p-hydroxyphenylacetyl)-14-O-p-hydroxyphenylacetate, 15-p-methoxyphenyl-acetyllactucin, 15-p-methoxyphenylacetyllactucin-8-sulfate, and 8-p-hydroxyphenylacetyllactucin-15-sulfate. Additionally, three undescribed conjugates of lactucin and a eudesmanolide type sesquiterpenic acid were characterized. Five compounds [3β,14-dihydroxycostunolide-3-O-β-D-glucopyranosyl-(2-O-p-hydroxy¬phenylacetyl)-14-O-p-hydroxyphenylacetate, ixerin D, 15-p-hydroxyphenylacetyllactucin, 15-p-methoxyphenylacetyllactucin, and 15-p-hydroxyphenyl-acetyllactucin-8-sulfate] were additionally tested for their antiproliferative activity. 15-p-hydroxy-phenyl¬acetyllactucin and 15-p-methoxyphenylacetyllactucin showed cytotoxicity against CEM, MCF7, and HeLa cells with IC50 values ranging from 3.9 ± 0.2 to 34.6 ± 2.8 μM, while 3β,14-dihydroxycostunolide-3-O-β-D-glucopyranosyl-(2-O-p-hydroxy¬phenylacetyl)-14-O-p-hydroxyphenylacetate, ixerin D, and 15-p-hydroxy-phenylacetyllactucin-8-sulfate were significantly less active (> 50 μM).

Our new data supports previous observations that the Cichorieae are a group particularly rich in bioactive compounds, mainly from the class of sesquiterpene lactones.

back to contents

HERBAL MEDICINE DEVELOPED AT THE ALL-RUSSIAN RESEARCH INSTITUTE OF MEDICAL AND AROMATIC PLANTS. ACHIEVEMENTS AND PERSPECTIVE

Mizina P.

Federal State Budgetary Institution "All-Russian Scientific Research Institute of Medical and Aromatic Plants" (FSBI VILAR)

Moscow, 7 Grin str., e-mail: mizina-pg@yandex.ru; Tel.8 903 503 32 01

FSBI VILAR turned 88 years. This is a multidisciplinary scientific institution, its structure includes 4 research centers:

- The Center of Medical Plant Growing with two departments (natural resources, agrobiology and selection) and the Botanical Garden of medical plants;
- Center of Chemistry and Pharmaceutical Technology with three departments (phytochemistry and standardization, experimental technology, biotechnology);
- Center of Medicine (preclinical research center) with two departments (experimental and clinical pharmacology, toxicology) and a laboratory of microbiological research;
- Research Center of Biomedical Technologies with two departments (biomedical problems, special works).

During its 88-year history of development of FSBI VILAR, working on the principle “From plant to drug”, has created more than 100 medical herbal preparations of multidirectional pharmacological effect. Many of them have no analogues to foreign drugs. These are our drugs such as alapinin, flakozid, sanguirytrin, hyporamin, eukalimin, belacechol and others.

However, the relevance of herbal medicines use in practical medicine is currently disputed, which cannot be considered justified. Drugs created on the basis of medicinal plant materials, have softer effect on the human body and fewer side effects compared to synthetic drugs and can be successfully used in practical medicine along with synthetic drugs; their development is important, relevant, necessary and allows to solve many issues in providing the population with affordable, effective and safe medicines.

It is advisable to include herbal medicines in different nosologies into clinical guidelines and treatment standards. It is necessary to make wider use of information on herbal medicines, including it in educational processes at all stages of medical and pharmaceutical workers training. And in this aspect it is necessary to consolidate all scientific and practical workers in medicine and pharmacy.

back to contents

ENZYME MODIFICATION OF POLYSACCHARIDES FROM HERBAL PLANTS AND THEIR BIOLOGICAL ACTIVITY AS PREBIOTICS

Iliev I.
Department of Biochemistry and Microbiology, Faculty of Biology, "Paisii Hilendarski" Plovdiv University, 4000 Plovdiv, Bulgaria
**Correspondence: ilievini@abv.bg, 0888519288*

Aim: The aim of the present work is to investigate the correlation between the chemical structure and prebiotic potential of oligosaccharides from herbal plants Plantago major and Lycium barbarum after their enzymatic hydrolysis with different types of hemicellulases.
Methods: The neutral sugars, uronic acid, total phenolic and protein content were analyzed spectrophotometrically. The monosaccharide composition of polysaccharides and oligosaccharides was studied by HPLC, with RI Detector and YMC Polyamine II (250×4.6 mm) column. Different probiotic strains of genus *Lactobacillus* were used to examine the prebiotic activity of the enzymatically hydrolysed oligosaccharides.
Results: We present a principal sheme of technological process for production of prebiotic oligosaccharides by enzyme hydrolysis of different plant and and herbal polysaccharides, using original method of our laboratory. In the presence of different oligosaccharides and disaccharides as substrates the lactobacilli produce different glycohydrolase enzymes for their metabolism. The utilization of polysaccharide hydrolysates of Plantago major and Lycium barbarum were studied by measuring the cell growth, the production of different end products, and the activity of α-galactosidase, α-glucosidase, and β-xylosidase from the tested probiotic strains. The results showed that L. plantarum strains specifically metabolized the carbohydrate composition when they were cultivated in mMRS medium in the presence of 2% hydrolysates.
Conclusion: Health effects of prebiotics are evolving but currently include benefits to the gastrointestinal tract (for example, inhibition of pathogens, immune stimulation), cardiometabolism (for example, reduction in blood lipid levels, effects upon insulin resistance), mental health (for example, metabolites that influence brain function, energy and cognition) and bone (for example, mineral bioavailability), among others.

INVASIVE ALIEN SPECIES – CHEAP SOURCES OF VALUABLE ESSENTIAL OILS

Kozuharova E.*, Benbassat N., Ionkova I.
Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia

Here we present data on the distribution and invasion level in Bulgaria of *Ailanthus altissima* (Mill.) Swingle, (Simaroubaceae), *Amorpha fruticosa* L. (Fabaceae), *Ambrosia artemisiifolia* L., *Xanthium strumarium* L., *Erigeron canadensis* L., and *Dittrichia graveolens* (L.) W. Greuter (Asteraceae). The high tolerance of various habitat conditions and potent propagation ability of these alien plants promote their

back to contents

aggressive invasive behaviour. Additionally, they not only over-compete the local vegetation but suppress the seed development. In the newly invaded habitats they might not have suitable herbivores to control their populations. The aim of this review study is to attract the attention towards these plants and their potential to be used as cheap sources of valuable essential oils.

A growing body of scientific literature points to the presence of essential oils with various compositions. The main components are limonene, borneol, caryophyllene oxid, α-pinene, germacrene D, *β*-caryophyllene. These essential oils possess different activities such as anti-inflammatory, antimicrobial, antifungal, antiviral, anti-inflammatory, insect repellent, insecticidal and herbicidal activity.

Due to the fact that these are aggressive invasive species, they can provide abundant and cheap resources. Additionally, exploitation of the biomass for extraction of essential oils might contribute to relieving the destructive impact of these species on the natural habitats.

Key words: essential oils, pharmacological activity, management.

Acknowledgements: This work has been carried out in the framework of the Grant Д-79/23.04.2019, Project 8276/20.11.2019 CMS, MU-Sofia

IN VIVO EVALUATION EFFECTS OF FLAVONOID MAURITIANIN, ISOLATED FROM *ASTRAGALUS MONSPESSULANUS* SPP. *MONSPESSULANUS*

Kondeva-Burdina M.[1], Krasteva I.[2], Shkondrov A.[2], Popov G.[3], Manov V.[3]

[1] "Drug Metabolism and Drug Toxicity" Laboratory, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

[3]Department of Internal Uninfected Diseases, Pathology and Pharmacology, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria

In this study, we investigate the in vivo effects of flavonoid mauritianin, isolated from *Astragalus monspessulanus* spp. *monspessulanus*, administered alone and in carbon tetrachloride-induced toxicity.

The effects of the flavonoid were evaluated by: complete blood count and biochemical parameters; malondialdehyde (MDA) production, level of reduced glutathione (GSH) and activity of ethylmorphine-N-demethylase (ENDM) (marker for the isoform CYP3A activity). Histopathological evaluation of the mauritianin effects was made on liver, kidney, brain and spleen. The effects were compared to those of the classical protector and antioxidant silymarin.

The rats were treated 21 days with mauritianin and silymarin (dose 100 mg kg^{-1}/p.o). Carbon tetrachloride (10 % solution) was given once (dose 1.25 mL/kg p.o).

Administered alone, mauritianin didn't reveal statistically significant toxic effects, compared to the control (non-treated rats). In a model of carbon tetrachloride-induced toxicity, mauritianin protects the exam parameters: decreased the MDA

back to contents

production, prevent the GSH level and decreased the activity of EMND. The results were proved by histopathological examinations.
We found that *in vivo* mauritianin revealed protective effects in carbon tetrachloride-induced toxicity, which might be due to possible influence of the metabolism of the toxic agent.

IDENTIFICATION AND MASS FRAGMENTATION ANALYZES OF STEROID ALKALOIDS AND PHENOLIC ACIDS FOUND IN *SOLANUM SCHIMPERIANUM*

Voynikov Y.[1], Zheleva-Dimitrova D.[2], Balabanova V.[2], Gevrenova R.[2]
[1]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Purpose: Phytochemical analyses of leaves extract of the plant *Solanum schimperianum*, Hochst were performed. High-performance liquid chromatography and high resolution mass-spectrometry (LC-HRMS) was utilized for the identification of several steroid alkaloids as well as 21 phenolic acids.
Materials and methods: The leaves of *S. Schimperianum* were harvested in the region of Erkowit, East Sudan. After ultrasonic extraction with 5% acetic acid, the aqueous layer was alkalized and n-butanol was added. The organic phase was evaporated to dryness and reconstituted in methanol. Mass spectrometric analyzes were carried out on a Q Exact Plus mass spectrometer (ThermoFisher Scientific) equipped with an electrospray ionization probe (HESI-II) (ThermoScientific).
Results: Based on accurate high-resolution mass measurements and analysis of isotope distributions and mass fragmentation analyses, several steroid alkaloids bearing spirosolane or solanidane type ring were identified. Furthermore, by targeted analysis, 21 phenolic acids were identified in the plant extract.
Conclusion: The mass fragmentation behavior of steroidal alkaloids and phenolic acids was investigated which allowed their identification and characterization in the leaves extract of *Solanum schimperianum*, Hochst.
Corresponding author: Yulian Voynikov
mobile: 0 888 398 907; e-mail: y_voynikov@pharmfac.mu-sofia.bg

back to contents

LIQUID CHROMATOGRAPHY–HIGH RESOLUTION MASS SPECTROMETRY - EFFECTIVE TOOL IN *GYPSOPHILA* SAPONINS ANNOTATION

Gevrenova R.

Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

Corresponding author: Reneta Gevrenova, tel.+359898641361, e-mail: rgevrenova@gmail.com

The aim of the study: The study aimed to develop a hyphenated platform liquid chromatography - high resolution mass spectrometry (LC-HRMS) for comparison of saponin profiles from *Gypsophila* roots and annotation and dereplication of glucuronide oleanane-type triterpenoid carboxylic acid 3,28-bidesmosides (GOTCABs).

Materials and methods: Saponins were analyzed in a set of accessions including wild (*G. trichotoma* and *G. glomerata*) and in vivo cultivated (*G.scorzonerifolia, G. acutifolia, G. altissima, G. paniculata, G. pacifica, G. oldhamiana, G. zhegualensis*) plants. They were profiled and characterized by classical reverse phase and hydrophilic interaction (HILIC) chromatography coupled with hybrid quadrupole-Orbitrap HRMS.

Results: Fragmentation pathways evidenced from 15 saponin references, high-accuracy MS and MS/MS data, elemental composition, isotopic peak profiles and database searching in an in-house library of GOTCABs were employed to characterize sapogenins and sugar chains. A total of 70 GOTCAB core structures were identified or tentatively elucidated in the assayed species, forming isobaric and positional isomers. They possess gypsogenin, quillaic,oleanolic and gypsogenic acid as sapogenin, substituted at C-3 with O-β-D-galactopyranosyl-(1→2)-[pentosyl-(1→3)]-β-D-glucuronopyranoside. Possible fragmentation pathways for four groups of GOTCABs were suggested. Type I consisted of saponins with C-28 ester-bonded oligosaccharide substituted with acetyl or/and methoxycinnamoyl group(s); type II and III included sulphated, and acetylated and sulphated saponins, while type IV grouped compounds with C-28 unsubstituted tri- to hexa-saccharides. The wild species possessed sulphated GOTCAB, while cultivated accessions displayed a variety of mono- and di-acetylated GOTCABs.

Conclusion: An in-depth depiction of the GOTCAB saponin composition of wild and cultivated *Gypsophila* species was achieved.

back to contents

UHPLC-HRMS BASED STRATEGY FOR THE DEREPLICATION OF CAFFEIC ACID OLIGOMERS AND SAPONINS IN *CLINOPODIUM VULGARE* L. WATER EXTRACT

Zheleva-Dimitrova D., Gevrenova R.

Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

The aim of the study: In this study we focused on a comprehensive characterization of *Clinopodium vulgare* lyophilized water extract (CVE) by ultra high-performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS). A strategy for the dereplication of caffeic acid oligomers and saponins in CVE was developped.

Materials and methods: CVE was analyzed by UHPLC-HRMS system equipped with a hybrid quadrupol-Orbitrap high resolution "Q-Exactive" mass spectrometer coupled with a heated electrospray ionization probe. The mass analyzer scanned over a mass range 100-1500 Da in Full MS-ddMS^2/Top N scan type and negative ion mode. The identification of the studied compounds was based on the accurate masses, MS/MS data and comparison to fragmentation fingerprints observed for the reference standards, and literature data.

Results: Dereplication represents a key step for rapidly identifying known secondary metabolites in complex biological matrices. In this context, UHPLC-HRMS was used via untargeted data-dependent MS/MS experiments; massive amounts of detailed information on the chemical composition of crude *C. vulgare* extract were generated. Two monomers, seven dimers, twenty-three trimers and nine caffeic acid tetramers, as well as five saponins were tentatively identified in CVE using the UHPLC-HRMS based strategy. A variety of clinopodic, salvianolic, isosalvianolic, yunnaneic acids and oleanane-type triterpenoid saponins were found in CVE for the first time.

Conclusion: The obtained UHPLC-HRMS based strategy for the dereplication of secondary metabolites in *Clinopodium vulgare* extract highlights the studied species as a new rich source of water soluble caffeic acid oligomers.

back to contents

PHYTOCHEMICAL AND BIOLOGICAL INSIGHT INTO *TELEKIA SPECIOSA* (SCHREB.) BAUMG. AND *TANACETUM MACROPHYLLUM* (WALDST. & KIT) SCHULTZ BIP. (ASTERACEAE)

Balabanova V.[1], Zengin G.[2], Zheleva-Dimitrova D.[1], Benbassat N.[1], Gevrenova R.[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Biology, Science Faculty, Selcuk University, Konya, Turkey
Corresponding author: Vessela Balabanova, e-mail: vessela.balabanova@gmail.com, Phone:+359 898 249 850

The aim of the study: In this study we aim at investigating the phytochemical and biological profiling of *Telekia speciosa* (Schreb.) Baumg. and *Tanacetum macrophyllum* (Waldst. & Kit) Schultz Bip. (Asteraceae) grown on Vitosha Mt.
Materials and methods: The flower heads of the studied species were assessed using spectrophotometric and ultra high-performance liquid chromatography - high resolution mass spectrometry (UHPLC-HRMS) techniques. The species' antioxidant and enzyme inhibitory activity was evaluated. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazine-6-sulphonic acid) (ABTS) and ferric and cupric reducing power (FRAP and CUPRAC), metal chelating and phosphomolybdenum methods were used for antioxidant activity assays. In addition, cholinesterase, α-amylase and α-glucosidase inhibitory ability of the extracts were tested.
Results: The obtained results revealed that *Flos Tanaceti* has higher antioxidant, α-glucosidase and α-amylase inhibitory activities in comparison with the anthodium of *T. speciosa*, while the later has prominent acetylcholinesterase inhibitory ability. Moreover, a variety of hydroxyisopropanoyl- and hydroxyisovaleryl-esters of caffeoyl- and feruloylhexosides, as well as caffeoyl- and feruloylquinic acids, dicaffeoyl- and caffeoylferuloylquinic acids were tentatively elucidated.
Conclusion: In conclusion, the studied species have significant antioxidant and enzyme inhibitory capacity and could be used as a rich source of bioactive compounds.
Acknowledgements: The study was supported by Grant D-222/12.12.2018 from the Medical Science Council at the Medical University of Sofia, Bulgaria.

back to contents

IN VITRO EFFECTS OF SAPONINS' MIXTURE FROM *ASTRAGALUS GLYCYPHYLLOS* ON ISOLATED RAT BRAIN SYNAPTOSOMES AND MICROSOMES

Voynova M.[1], Shkondrov A.[2], Kondeva-Burdina M.[1], Krasteva I.[2]
[1]"Drug Metabolism and Drug Toxicity" Laboratory, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., Sofia, Bulgaria
[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., Sofia, Bulgaria

Astragalus glycyphyllos L. (Fabaceae) is used in folk medicine as an antihypertensive, diuretic, anti-inflammatory, anti-tumour, laxative, expectorant, etc. In our study, we investigate the effects of saponins' mixture, isolated from *Astragalus glycyphyllos*, a native plant in Bulgarian flora, in different models of neurotoxicity: 6-hydroxydopamine (6-OHDA)-induced oxidative stress on isolated rat brain synaptosomes and non-enzyme-induced lipid peroxidation on isolated rat brain microsomes. The saponins' mixture revealed statistically significant neuroprotective and antioxidant effects in these models, by preservation the synaptosomal viability and GSH level (at synaptosomes) and decrease of malondialdehyde (MDA) production (marker for lipid peroxidation) (at microsomes). These effects might be due to possible scavenger activity of reactive oxygen species (ROS) and preservation of reduced glutathione level – a classical nucleophile, which is a ROS scavenger.

BIOACTIVE COMPOUNDS FROM GARDEN SNAILS *HELIX ASPERSA* WITH APPLICATIONS IN PHARMACEUTICS INDUSTRY AND MEDICINE

Dolashka P.[1], Dolashki A.[1], Velkova L.[1], Atanasov V.[1], Devreese B.[2]
[1]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
**Corresponding author: pda54@abv.bg*
[2] Laboratory for Protein Biochemistry and microbiology, Department of Biochemistry and Microbiology, Ghent University

The hemolymph and mucus of garden snails are a complex multi-component mixture comprising various biochemical and pharmacologically active substances with different masses and properties. Some of them exhibit antibacterial, antitumor activities and have antioxidant and immunomodulatory properties.
The mucus was collected and purified from garden snails *H. aspersa*, grown in Bulgarian farms using patented technology without suffering any snail. The crude extract was separated using Millipore filters by ultrafiltration into different fractions. We have performed *in vitro* studies on the antimicrobial activities of different extracts, obtained from mucus, against Gram+ and Gram- bacteria, as well as fungal strains *A. niger, A. fumigatus* and *C. albicans*. Our results have shown that fractions

back to contents

<10kDa, <20kDa and between 1-10kDa and possess strong antimicrobial activity against the tested pathogens. The fraction 10-50kDa possesses the most significant fungistatic activity on the mycelium growth of *A. niger* strain compared to other tested fractions.
Using tandem mass spectrometry we identified the primary structures of many novel antimicrobial peptides. Most of them contain high level of glycine and leucine residues into the amino acid sequences and belong of a class of Gly/Leu-rich AMPs, but others, contain proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity.
Our results may be considered as basic information for further investigations on bioactive compounds from *H. aspersa* for creating new natural products with potential biomedical applications.
Keywords: garden snails *H. aspersa*, *H. aspersa hemocyanin*, antimicrobial peptides (AMPs), anticancer activity
Acknowledgments: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Program "BioActivMed" approved by DCM № 658/14.09.2018 and FWO VS.076.18N/2018-2020

CLINOPODIUM VULGARE AND ITS MAJOR CONSTITUENT ROSMARINIC ACID, PROTECT AGAINST SCOPOLAMINE-INDUCED OXIDATIVE STRESS IN RAT BRAIN

Nasar- Eddin G[1]., Simeonova R[1]., Zheleva D[2]., Gevrenova R[2]., Danchev N[1].
[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

Introduction: One of the mechanisms involved in the brain toxicity, is the oxidative stress. *Clinopodium vulgare* L. (Lamiaceae) is a perennial herbaceous plant used in the Bulgarian folk medicine.
Aim: The aim of the current study was to investigate the antioxidant and neuroprotective effects of *C. vulgare* extract (CVE) and its main constituent rosmarinic acid (RA), on scopolamine (SC) induced brain toxicity in Wistar rats.
Materials and methods: The animals were divided into four groups: control; group treated with scopolamine (3 mg/kg, i.p., 7 days); animals treated simultaneously with SC and CVE (100 mg/kg, oral gavage for 14 days), and group four – animals treated with SC and RA (20 mg/kg i.p., 14 days). Acetylcholinesterase (AChE) activity, nitrites (NO_x) concentration, and the following antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione-peroxidase (GP_x), as well as the biomarkers of oxidative stress malondialdehyde (MDA) and reduced glutathione (GSH) were measured in the whole brain of the experimental animals.
Results: Scopolamine repeated administration resulted in increased AChE activity and oxidative stress, discerned by an increased lipid peroxidation and formation of NO_x, depletion of GSH levels, and impairment of the enzymatic antioxidant defense system measured in brain.

back to contents

CVE and RA administered along with SC restored the GSH levels and antioxidant enzyme activities near control levels. Both substances reduced the increased AChE activity, MDA, and NO_x brain concentrations and thus exerting neuroprotective action on the SC induced brain toxicity.

Conclusions: Both substances possess antioxidant and neuroprotective activity and could be candidates for further investigations.

The study was supported by Grant Young scientist - 147 / 23.04.2019 from the Medical Science Council at the Medical University of Sofia.

BIOLOGICAL ACTIVITY OF YEASTS AND THEIR USE AS ANTINEOPLASTIC AGENTS

Hristova D.[1], Russinova-Videva S.[2], Zaharieva M.[2], Naidenski H.[2], Konstantinov S.[1]

[1]Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]"Stephan Angeloff" Institute of Microbiology, Bulgarian Academy of Sciences, Sofia and Plovdiv, Bulgaria

Studies on yeast show that they have a broad spectrum of biological activity - immunostimulating, antidiabetic and antineoplastic. Psychrophilic microorganisms need to possess adaptable metabolism thus producing bioactive components with attractive pharmacological properties. In this study we used methanol extracts of two strain yeasts- *Sporobolomyces salmonicolor* AL_{36} and *Cryptococcus luarentii* AL_{65}. They were prepared in bioreactor and in flasks. By using method of growing yeast cells in a bioreactor it is possible to easily control conditions, being independent of geographic location and climatic features of the area, short time to carry out the fermentation processes and low value of the substrates used, are other advantages.

Our study aimed to determine and compare the antineoplastic activity of the Antarctic yeast extract against different tumor cell lines: T-24 and CAL-29 (urinary bladder cancer), HuT-29 and MJ (cutaneous T-cell lymphoma), HD-MY-Z (Hodgkin lymphoma), RPMI-8226 (multiple myeloma). The cytotoxic efficacy was measured using the MTT-assay. Induction of apoptosis was ascertained by nuclear changes, DNA fragmentation, as well as by caspase activation.

Concentration response curves showed IC_{50} values between 28,27µg and 219,60 µg/ml for the different tumor cell lines. Highest sensitivity to the antineoplastic activity of the yeast extracts was registered in multiple myeloma RPMI-8226 cells.

Along with these results with Antarctic yeast extracts we do not expect any toxic side effects. Taken together our data indicate that yeast extracts are very attractive and perspective sources of substances with valuable pharmacological and toxicological profile and could be used to ameliorate the complex treatment of human malignant diseases.

Acknowledgments: A substantial part of the experimental work has been supported by the National Program BioActiveMed D01-217/30.11.2018, sponsored by the Bulgarian Ministry of Education and Science.

back to contents

BIOACTIVE COMPOUNDS ISOLATED FROM MARINE *SNAIL RAPANA VENOSA* WITH POTENTIAL APPLICATION IN MEDICINE

Velkova L.[1], Konstantinov S.[2], Dolashki A.[1], Atanasov V.[1], Dolashka P.[1]
[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Faculty of Pharmacy, Medical University of Sofia, Bulgaria*
Corresponding author: e-mail: lyudmila_velkova@abv.bg

Aim: The priority for the next decades will be focused on discovers and investigation of alternative natural molecules with a new mechanism of action as medicaments for therapy. The object of our investigation is hemolymph of marine snail *R. venosa*, which is a complex mixture from various bioactive substances with potential application in medicine. The aim of this study was to investigate the anti-tumor effect of different fractions from hemolymph of *R. venosa*.
Methods: The hemolymph from *R. venosa*, was separated into several fractions, by ultrafiltration using membranes with different pore sizes. The native molecule of *R. venosa* hemocyanin (RvH) and its two structural subunits RvH1 and RvH2 were isolated and purified from fraction >100 kDa. The isolated components: RvH, RvH1 and RvH2, fraction below 10 kDa and fraction between 10-50 kDa were investigated in vitro for antitumor activity of human cell lines: bladder cancer (T-24) and cutaneous T-cell lymphomas (MJ, Mycosis fungoides and HuT-78, Sézary syndrome).
Results: The results revealed a significant cytotoxic effect of structural subunit RvH2 against bladder cancer (tumor cells line T-24). The most effective inhibition against cutaneous T-cell lymphomas (MJ, Mycosis fungoides) was observed after treatment with fraction, containing bioactive components with Mw between 10-50 kDa from hemolymph of *R. venosa*.
Conclusion: The absence of toxic effects on *R. venosa* extracts makes them promising candidates for topical application in the bladder before and after transurethral resection. Our results may be considered as basic information for further investigations on bioactive compounds from hemolymph of *R. venosa* for creating new natural products with potential biomedical applications.
Acknowledgments: This work was supported by the Bulgarian Ministry of Education and Science under the National Research Program “BioActivMed” approved by DCM № 658/14.09.2018.

POSTER SESSION

back to contents

HOUSEHOLD AND PUBLIC SPENDING ON HEALTH

Mitkova Z*., Petrova G.
Faculty of Pharmacy, Medical University, Sofia
**e-mail: sppmitkova@mail.bg*

Aim of the study: Between 2000 and 2016, global spending on health increased every year. Government and household health expenditures have risen in absolute terms as a result of economic growth. This study explores the trend of household and public health expenditures in Bulgaria during 2010-2017.
Materials and methods: The study is observational and retrospective analysis on household and public expenditures for healthcare and medicines over the period 2010-2017. National statistical Instituteand Annual reports of National Health Insurance Fund were used for data collection. Statistical significance in the changes was tested with t-test.
Results: The results showed that health expenditures per household on average (BGN) haverisen from 447 in 2010 to 642 BGN in 2017. The health expenditure per person has increased from 181 to 280 BGN within the same period. The household costs for medicinal products growth up too (from 177 to 205 BGN per person during 2015-2017).
NHIF costs for reimbursed medicines, dietary foods for special medical uses and medical devices continue to grow latest years. Pharmaceuticals spending is increasing almost twice (from 365 to 798 million BGN) while the costs for medicines, dietary foods and medical devices is increasing from 384 to 821 million BGN between 2010-2017. T-test analysis shows statistically significant differences in costs **($p < 0.0029$).**
Conclusion: Our study confirms the world trend of increasing household and public health expenditures observed last decade. This trend may be due to different factors as population aging, chronic diseases, healthcare system financing etc.

PHARMACY STUDENTS' OPINION REGARDING THEIR KNOWLEDGE OF BIOTECHNOLOGY MEDICINAL PRODUCTS

Tamer D.[1], Grozev J.[1], Todorova A.[2], Kamusheva M.[1], Petrova G.[1]
[1]Faculty of Pharmacy, Medical University, Sofia
[2]Faculty of Pharmacy, Medical University, Varna
Corresponding author: Djanet Tamer; e-mail: janitamer@gmail.com; Tel. 0885204137

The aim of the study: To assess pharmacy students' opinion regarding their knowledge of biotechnological medicines (BM).

Materials and methods: An online questionnaire-based survey among pharmacy students in Bulgaria was carried out. Descriptive statistics, test for one proportion and comparison of proportions have been applied using the statistical program MedCalc.
Results: The number of respondents is 172 out of all targeted 281 (CI 95%, margin of error 5%) pharmacy students from three Faculties of Pharmacy in Bulgaria – Medical University, Sofia (52.3% of all respondents), Sofia University (19.2%) and Medical University, Varna (28.5%). 76.7% of the respondents were women, with the predominance between 20 and 22 years old (67.4%). Statistically significant more students shared that they are familiar with BM legal definition (66.3% ($p<0.0001$)) and the difference between biosimilar and generics (59.9% ($p<0.05$)). However, 71.5% deem that their knowledge of BM is insufficient to consult patients in real-life practice ($p<0.0001$). Therefore, most of them (83.1%) opined that more detailed education on BM is needed ($p<0.0001$) and 62.8% ($p=0.0008$) would consider to take up BM related elective discipline. Significantly more students from Sofia are familiar with BM definition (68.9% vs. 51.02% ($p=0.0269$)) but less of them shared that their level of knowledge is enough (18.6% vs. 51.02% ($p<0.0001$)) in comparison with the students from Varna.
Conclusion: The survey revealed that most of the pharmacy students identify some gaps in their knowledge regarding BM. They obviously would like to become more experienced and fluent on the topic. Therefore, more training, elective disciplines and educational symposiums focusing on BM should be provided for pharmacy students.

QUALITY OF LIFE OF ASTHMA PATIENTS

Milushewa P., Doneva M., Petrova G., Najdenova K., Krusheva B., Usunov S., Dimitrov V.
Faculty of Pharmacy, Medical University of Sofia

Goal: The goal of this study is to analyse the quality of life and asthma control in elderly patients with mild, moderate, severe asthma and asthma with rhinitis.
Materials and methods: Prospective study performed at the clinic of allergology at the "Alexandrovska" University hospital. The quality of life is accessed with unidimensional instrument (EQ5D), questionnaire for the evaluation of asthma therapy - Asthma Treatment Assessment Questionnaire (ATAQ) and test for asthma control (ACT). 30 patients with mild, moderate, severe asthma and asthma with rhinitis are inquired during their visit at the department. The results are processed with t-test and Kruskal Wallis test.
Results: The age of the 30 participants is between 30-70 years. With moderate asthma were 7, 14 had severe and 5 asthma with allergic rhinitis. Patients with asthma above 5 year prevail (n=19), and between 1 and 5 years (n=8). Average quality of life is 0.74 (EQ5D3L). According to the answers of ATCQ 14 patients reported partial control, 9 control and 7 with poor control of their asthma. According to answers to ACT 23 are with poor control (77%). There is a statistically significant relation between the duration of the disease and quality of life for patients with duration 1-5 and above 5 years; duration and control according to ATCQ, but not

back to contents

according to ACT. Statistically significant is the difference between the moderate and severe asthma and quality of life.
Conclusions: Asthma control is insufficient, and the worst are the ATC values. The quality of life is somewhat lower in comparison to the general population in the same age group. Duration and type of asthma influenced negatively the control and quality of life.

IN VITRO EFFECTS OF NEWLY SYNTHESIZED SEMICARBAZIDES/THIOSEMICARBAZIDES OF THEOPHYLLINE ON ISOLATED RAT BRAIN SYNAPTOSOMES

Goliykov T.[1], Kondeva-Burdina M.[1], Mitkov J.[2], Zlatkov A.[2]
[1]"Drug Metabolism and Drug Toxicity" Laboratory, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Aim: This study investigates the in vitro effects of newly synthesized semicarbazides/thiosemicarbazides on isolated rat brain synaptosomes.
Materials and methods: The synaptosomes were obtained by multiple centrifugation, using Percoll gradient. The main parameters, characterized the functional status of synaptosomes: synaptosomal viability and depletion of reduced glutathione (GSH), were measured. The effects were compared to those of Theophylline.
Results: Administered alone, all of the compounds (at concentration 100 μM) revealed statistically significant neurotoxic effects on the synaptosomes, compared to the control (non-treated synaptosomes). With higher toxic effects were compounds JE-5, JE-7 and JE-10. JE-5 decreased synaptosomal viability and GSH level, respectively with 51 % and 49 %; JE-7 – with 55 % and 50 %; JE-10 – with 53 % and 51 %, compared to the control (non-treated synaptosomes). Theophylline, itself, decreased viability and GSH – with 42 % and 44 %, respectively, compared to the control (non-treated synaptosomes).
Conclusion: The newly synthesized semicarbazides/thiosemicarbazides of theophylline show significant toxicity on rat brain synaptosomes.

back to contents

INVESTIGATION AND ANALYSIS OF THE HTA PROCESS AMONG EXPERTS, MEMBERS OF WORKING GROUPS

Nikolova A.[1], Grigorov E.[1], Getov I.[2], Salchev P.[1]
[1]National Center of Public Health and Analyses
[2]Medical University, Sofia

Aim of the Study: Analysis of the HTA process and related procedures on the basis of experience gained 2016-2019 and evaluation of the experts, members of working groups on the introduced regulatory changes.
Materials and methods: An online anonymous questionnaire containing 19 questions, addressed to respondents from two distinct groups was developed in two stages:
a. First stage (24.04 - 10.05.2019) - experts, members of working groups and involved in the HTA process until 31.3.2019;
b. Second Stage (15 May - 31 May 2019) – Physicions, Pharmacists, Patients, Industry Representatives, Patient Organizations, etc.;
Results: The number of responses in the first stage is 89. All respondents have positively answered that they were familiar with the process, knew where they could find information about HTA and had experience in conducting. The evaluation of the experts, who participated in the working groups to the process, is largely positive. 96% consider that HTA works in the public interest and evaluates the organization of work as very good (55%). Half of the experts surveyed are not aware or cannot assess the regulatory changes introduced by 01.04.2019 concerning the HTA process.
Conclusion: From the survey data, it can logically be concluded, that the HTA process is sufficiently well known to the experts who participated in working groups. The share of respondents, who take into account the positive aspects of the introduction of HTA in Bulgaria is high. There is a need for a broader explanation of the changes and their impact on the HTA process in the country.

IN VITRO EFFECTS OF NEWLY SYNTHESIZED HYDRAZIDE-HYDRAZONES BEARING INDOLE MOIETY ON ISOLATED RAT BRAIN MICROSOMES

Yanakieva N.[1], Kondeva-Burdina M.[1], Angelova V.[2]
[1]"Drug Metabolism and Drug Toxicity" Laboratory, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

The aim of the study: A series of new aroylhydrazones **3a–3g** has been synthesized with the aim of evaluating their effect on isolated mouse microsomes.
Materials and methods: The aroylhydrazone derivatives have been synthesized in high yields by direct reaction of substituted 5-C substituted indole-3-carbaldehyde **1a-c** with various hydrazides **2a-d**. The synthesis was performed in ethanol at reflux,

using an established procedure. The structures of the newly synthesized hydrazones **3a–3g** were characterized by IR, 1H-NMR, 13C-NMR, HR-MS spectroscopic methods.
The microsomes were obtained by differential centrifugation. As a marker of lipid peroxidation, the production of malondialdehyde (MDA) was measured.
Results: All compounds revealed statistically significant pro-oxidant effects, compared to the control (non-treated microsomes). With highest pro-oxidant effects are compounds bearing Br- and benzyloxy- 5-substituted indole scaffold: **3a, 3e, 3f** and **3g**. The compound **3a** increased the production of MDA with 65 %, **3e** – with 60 %, **3f** – with 58 % and **3g** – with 66 %. The other three compounds: **3b, 3c** and **3d** had lower pro-oxidant effects.
Conclusion: From the results we can define the relation between the structure of the compounds and their toxicity. Three of the compounds with furyl, 4-chlorophenyl, 2,4-dihydroxyphenyl fragments – **3b, 3c, 3d** are convenient for further investigations.

COST-UTILITY ANALYSIS OF HERNIA MESHES

Doneva M.[1], Sopotenski S.[2], Gerassimov N.[3], Kamusheva M.[1], Petrova G.[1]
[1]Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]"Sv. Sofia" Hospital, Sofia, Bulgaria
[3]"Subra" Pharmacy, Dobrich, Bulgaria

Aim: The present study evaluates the quality of life in a conventional hernia operation where light or standard polypropylene hernia meshes were used. (TiO2, Pariettene, Surgimesh, Microval -PP, Surgipro).
Materials and methods: A total of 68 patients were interviewed with a EuroQoL 5D questionnaire. Patient quality of life is compared one day and 3 months and one year after surgery. A cost-benefit analysis for economic assessment of the quality of the health score was applied. The costs are calculated in case of full reimbursement of hernia meshes by the public fund. Health improvement is measured in quality adjusted life years (QALY).
Results: The results show that three months after the operation, patients did not report problems with motor activity, self-care, and usual activities, but only the presence of pain. 23% of patients with implanted standard meshes and 16% of patients with implanted light meshes experience severe pain. One year after surgery complains as pain, pulling and swelling are reported by 26.92% of patients with implanted standard meshes vs. 26.67% of patients with implanted light meshes. There is no statistically significant difference in the level of pain according to the used meshes and the feeling of a foreign body ($p = 0.41$).
The utility index for both types of meshes increases from 0.53 to 0.904, with the value of QALY equal to 0.475 for light meshes vs. 0.469 for standard meshes. The incremental cost utility ratio is 2978 BGN which is far below the threshold defined as gross domestic product per capita in Bulgaria (7100 euro in 2018).
Conclusion: Patient's quality of life depends on the used meshes but this trend decreases one year after surgery. At the end of the study period, patients assessed their condition as very good. The analysis of the results found that both types of

back to contents

meshes offer good value for money for the Bulgarian healthcare system and could be used for implantation and paid by public funds.

Corresponding author: Miglena Doneva
tel. 02 92 36 584; e-mail: miglena_doneva@ abv.bg

IMMUNOSTIMULATION IN CHILDREN. ROLE OF THE PHARMACIST

Argilashki D., Hadzhieva B., Mihaylova A., Koleva N.
Medical College, Medical University, Plovdiv, Bulgaria

Purpose of the study: Study of the use of immunostimulants and the role of the pharmacist in choosing immunostimulating products.
Materials and methods: Database review - Google Scholar, Pubmed and official sites. Instrumental method - survey. Statistical method for processing data from the survey.
Results: Immunity is resistance of the organism against the physical, chemical and biological pathogenetic factors of the surrounding environment. Immune system in children is less developed and therefore often different forms of immunostimulation are used. It has been established that certain medicinal plants modulate various components of the immune system. Some of the most commonly used as immunostimulants medicinal plants are eldberry, echinacea, garlic, and others. Echinacea exhibits an immunomodulatory effect by increasing phagocytosis, total monocytes, neutrophils and NK cells. Elderberry shows antiviral activity against some viruses that increase morbidity during winter months, including common cold and flu, which is evident in both *in-vitro* and *in-vivo* models.
A survey was conducted consisting of four modules - a passport section, pharmacists' awareness of immunostimulants, attitudes and barriers to pharmaceutical care. The study shows that pharmacists are well aware of the means of immunostimulation, the possibilities for their application in different age groups, and warn of difficulties in providing pharmaceutical care.
Conclusion: The use of traditional medicinal plants and plants products provide great immunomodulation capabilities, and any recommended therapy must be age appropriate. In order to provide high-quality pharmaceutical care, due to increased consumer confidence in the pharmacist, it would be good practice in the pharmacy to have a separate place for consultation.
Key words: Immunity, medicinal plants, pharmacist

back to contents

MEDICINES UTILIZATION PATTERNS AMONG OLDER AMBULATORY PATIENTS IN BULGARIA – A PILOT STUDY

Stoeva J.[1], Tachkov K.[1], Kamusheva M.[1], Petrova G.[1], Brkic J.[2], Fialová D.[2,3]
[1]Department of Organization and Economics of Pharmacy,
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Clinical and Social Pharmacy,
Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[3]Department of Geriatrics Gerontology, 1st Faculty of Medicine,
Charles University, Czech Republic
Corresponding author: Maria Kamusheva,
e-mail: mkamusheva@pharmfac.mu-sofia.bg, Tel. Number: 0886428154

The aim of the study: The aim is to analyze medicines utilization among elderly patients over the age of 65 years.
Materials and methods: It is a prospective, questionnaire-based pilot survey conducted in a community pharmacy setting. The questionnaire was developed for the purposes of comprehensive assessment of older adults in a H2020 project EUROAGEISM, FIP7 program financed by the European Commission (2017-2021) and includes questions about demographic characteristics, social status, results of clinical geriatric scales, diagnostic diseases, medicines and laboratory parameters. The participants were enrolled on the basis of provided signed informed consent. Descriptive statistics was used to present the data.
Results: 31 older patients 65+ (81% female and 17% male) were involved randomly in the study and those between 65 and 69 years prevailed – 39%. 25 out (81%) of 31 interviewed patients were diagnosed with any cardiovascular disease. Logically, cardiovascular medicines (anatomical main group C of the Anatomical Therapeutic Chemical Classification System) consisted 51% of all consumed medicines, followed by group A (alimentary tract and metabolism) (17%) and group N (Nervous system) (17%). Adverse drug reactions (ADRs) (32%) were mainly associated with ACE inhibitors (dry cough), non-steroidal anti-inflammatory drugs (stomach problems) and anticoagulants (bleeding).
Conclusions: The diseases prevalence was in accordance with the national statistical data as cardiovascular disorders are a leading cause for morbidity and mortality among Bulgarian population. Reported adverse drug reactions are probably as a result of polypharmacy and co-morbidity among older patients. The ADRs were classified as mild or moderate, but some of them might be a reason for potentially serious DRPs. Therefore, close monitoring of risky patients group should be provided by the community pharmacists.

The EUROAGEISM H2020 project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 764632.

back to contents

MOLECULAR DOCKING STUDY OF RUSCOGENIN, NEORUSCOGENIN AND DIOSGENIN ON ESTROGEN RECEPTORS

Marinova V.*, Atanasova M., Simeonova R., Krasteva I., Doytchinova I.
Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria
**Corresponding author: vasilena.pm@gmail.com; phone: +359 2 9236 599*

Steroidal saponins with aglycones ruscogenin (RSG) and neoruscogenin (NSG) were found in the underground parts of *Ruscus aculeatus* L. The extract from rhizome of the species is used for treatment of chronic venous insufficiency, varicose veins, hemorrhoids, orthostatic hypotension, colitis, and diarrhea and locally - against inflammation and arthritis. It is known that saponins with steroidal structure mimic human sex steroidal hormones (estrogens and androgens) and might alleviate the risk of fracture in osteoporotic patients. RSG closely resembles diosgenin's structure and differs only in one additional hydroxyl group. The roots and rhizomes of *Dioscorea* spp. are a source of diosgenin (DSG), which has shown a vast range of pharmacological activities as anticancer, cardiovascular protective, anti-diabetes, neuroprotective, immunomodulatory, estrogenic and skin protective effects.
Additionally, DSG inhibits osteoclastogenesis, stimulates osteogenic activity of osteoblasts in vitro and exerts antiosteoporotic effects in rats in vivo. The structural similarity of RSG and NSG to DSG makes them therapeutic **candidate agents for bone protection.**
In the present study we used molecular docking to evaluate the ability of RSG and NSG to bind in alpha and beta estrogen receptors (ERs). The docking was performed by GOLD v.5.2.2 software tool. The docking protocol was optimized in terms of scoring functions, rigid or flexible protein and ligand, presence or absence of structural water. Estradiol (EST) and DSG were used as reference compounds. It was found that RSG and NSG form stable complexes with alpha and beta ERs with binding scores equal to these of EST and DSG. The intermolecular interactions of the complexes were analyzed.

MOLECULAR DOCKING STUDY OF DIOSGENIN, RUSCOGENIN AND NEORUSCOGENIN ON VITAMIN D RECEPTOR

Hyuseinova M.*, Atanasova M., Simeonova R., Krasteva I., Doytchinova I.
Faculty of Pharmacy, Medical University, Sofia, Bulgaria
**Corresponding author: melis.u@abv.bg; phone: + 359 2 9236 599*

A lot of natural compounds present in dietary and medicinal plants could be useful in the prevention and treatment of osteoporosis. Diosgenin (DSG) is an aglycone of steroidal saponins isolated from the roots and rhizome of different *Dioscorea spp*. It is claimed to have osteogenic activity. In fact, this compound is known to possess anti-inflammatory and antioxidant properties. *Ruscus aculeatus* L. (Liliaceae) contains steroidal saponins with aglycones ruscogenin (RSG) and neoruscogenin (NSG). Typically, the rhizome extract of *R. aculeatus* is used to treat varicose veins and hemorrhoids due to its vasoconstrictive effects and anti-inflammatory capability.

back to contents

The structures of RSG and NSG are similar to DSG which makes them candidates as anti-osteoporotic agents.
In the present study the binding of DSG, RSG and NSG to vitamin D receptor (VDR) was studied by molecular docking. GOLD v.5.2.2 software was used for the docking calculations. The docking protocol was optimized in terms of scoring function, ridgid/flexible ligand and protein.
The *in silico* prediction by molecular docking showed that DSG, RSG and NSG are able to bind to VDR and thus they are able to affect the intestinal Ca absorbtion and/or estradiol synthesis. These predictions are in a good agreement with the predictions made by the SwissADME tool.

INFLUENCE OF MINERAL ADDITIVES ON SAPONARIN PRODUCTION BY *IN VITRO* CULTURES OF *GYPSOPHILA TRICHOTOMA*

Shkondrov A., Popova P., Zdraveva P., Krasteva I., Ionkova I.
Department of Pharmacognosy, Faculty of Pharmacy,
Medical University, Sofia, Bulgaria

Aim of the study: The aim was to investigate the influence of mineral additives to the culturing media on the accumulation of saponarin *in vitro* cultures of endangered *Gypsophila trichotoma* Wend. (Caryophyllaceae).
Materials and methods: Shoots, callus and suspension cultures were established. They were cultivated on different media, supplemented or not with mineral additives such as $Ca^{2+,}$ Mg^{2+} and NaCl. Analysis of the quantity of saponarin in each culture was performed by high performance liquid chromatography. The results were compared to corresponding cultures, grown on non-modified media.
Results: For the first time in vitro cultures of *G. trichotoma* were established on nutrition media containing mineral supplements. Significant differences in the quantity of saponarin were observed amongst individual cultures. The results obtained from this study show that the problem is more complex and probably each of these ingredients contributes to the production of flavonoids in the cells.
Conclusion: Although the suspension cultures grown on G48 medium without Mg^{2+} accumulated nearly twice as little saponarin compared to shoots on MS medium, we suggest them as a promising source of this rare flavonoid. The results will serve as a basis for detailed optimization of the growth media composition and could serve to establish high-productivity *in vitro* lines in respect of saponarin.

back to contents

DAVID HOSACK (31.08.1769-22.12.1835) – PHYSICIAN, BOTANIST, EDUCATOR AND SCIENTIST, CREATOR OF THE FIRST BOTANICAL GARDEN IN NEW YORK IN 1801

Dimitrova Z.[1], Andreevska K.[1], Burgasliev H.[2], Madžarov V.[3]
[1]Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Sofia, Bulgaria
[2]Department of Pharmacy,
"Prof. Dr. Assen Zlatarov" University, MK, Bourgas, Bulgaria
[3]Department of Pharmaceutical Sciences, Faculty of Pharmacy,
Medical University, Plovdiv, Bulgaria

Purpose of the study: To acquaint the pharmaceutical society in Bulgaria with the ideas and activities of David Hosack, a brilliant American surgeon and a botanist of world renown and recognition.
Materials and methods: The historical method is applied. The two biographical novels were analyzed for Dr. Huzack of Victoria Johnson and of Christine Roberts. Scientific publications were used by Dr. Huzack in the field of medicine and medicinal plants, letters from his conversations with the most famous botanists from around the world were explored.
Results: Born and raised in New York City during the British occupation, determined to educate himself in medicine and botany in Europe also (London, Paris, Edinburgh), he returned to America to apply the knowledge and skills to the welfare of his beloved hometown and homeland. The young doctor and professor of medicine and botany set a goal above all others - to create the first botanical garden in New York, which manages to realize with its own resources in 1801.
Conclusion: The establishment of Botanical Garden provides not only a good base for the training of students, but there will also be conducted scientific studies on growing species throughout the continent and throughout the world.

TRENDS IN MARKETING AUTHORIZATION ON DRUGS IN EUROPEAN UNION AND TIME FOR MARKET ACCESS ON NEW DRUGS IN BULGARIA FOR THE PERIOD 2017-2019

Panayotova D., Dimitrova M.
"Organization and Economy of Pharmacy" Department,
Faculty of Pharmacy, Medical University, Sofia

The aim of the study: A retrospective analysis of the trends in the issued marketing authorizations (MAa) in the European Union and the market access in Bulgaria for the period 2017-2019.
Materials and methods: A retrospective critical analysis of the European Medicines Agency (EMA) database for MAs and the National Council on Prices and Reimbursement of medicines (NCPR) for the period 2017 - 2019 was carried out. Data on the type of MA procedures issued by the European Commission (EC), type of medicine and therapeutic area were collected from EMA. Data from NCPR were

analyzed to evaluate which of the medicines were granted market access in Bulgaria and when.
Results: About 45% all issued MAs (202) over the observed period are for innovative medicines. A steady trend in marketing authorization of biosimilars is observed – 16 for both 2017 and 2018. However, a decline in MA by centralized procedure for generics (20 for 2017 and 12 for 2018), while orphan designation MA increases – 15 for 2017 and 21 for 2018. Up to May 2019 there are 16 MAs issued. 6 conditional approvals and 4 under exceptional circumstances were issued for 2017-2018, all for orphan medicines. A higher percentage of market access in Bulgaria is observed for the biosimilar medicines – 40% of those that received marketing authorization have been included in PDL.
Conclusion: Market access for new drugs in Bulgaria is a relatively fast, but further research is needed in order to identify the major underlying causes for delays.

DETERMINATION OF THE NUMBER OF CHELATORS CONJUGATED TO TRASTUZUMAB, ANTIBODY BASED RADIOPHARMACEUTICAL, USING A MALDI-TOF MS

Arev M.[1], Džodić P.[2], Dimovski A.[3,4], Davalieva K.[3], Kiprijanovska S.[3], Apostolova P.[1], Drakalska E.[1], Janevik-Ivanovska E.[1]
[1]Faculty of Medical Sciences, "Gotse Delchev" University Shtip, Republic of North Macedonia
[2]Faculty of Medicine, Department of Pharmacy, University of Niš, Nis, Serbia
[3]"Georgi D Efremov" Research Centre for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, Republic of North Macedonia
[4]Faculty of Pharmacy, "St. Cyril and Methodius" University, Skopje, Republic of North Macedonia

The aim of the study: To show that successful conjugations of monoclonal antibody (mAb) trastuzumab (Tr) with different bifunctional chelators (BFCs) prepared for labeling with suitable radioactive isotope (Lutetium177 and Yttrium90), dependent of different molar ratio of BFCs and of an optimal number of chelating groups per molecule of antibody (4-5).
In our study, p-SCN-Bn-DTPA (ratio 1:10, 1:20, 1:50), p-SCN-Bn-DOTA (ratio 1:20), and p-SCN-Bn-1B4M-DTPA (ratio 1:10, 1:20, 1:50) were used as bifunctional chelators to obtain the most appropriate stable formulation of radioimmuniconjugates.
Material and methods: Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) was employed for determination of average number of BFCs. Resuspension solution (30% ACN/70% 0.1 TFA) was added to the samples. An aliquot of 1 µl of the final sample was applied to the well plate template and mixed with 1 µl of matrix (20 mg/ml Sinapinic acid in 50% ACN/50% 0.1 TFA). Acquisition mass range of the instrument is 100-300000 Da.
Results: An optimal number of BFCs was calculated in all samples. There was no significant difference in the number of the attached chelating groups by higher excess of the chelators: p-SCN-Bn-DTPA-Tr [1:10 (5 groups); 1:20 (4.8 groups); 1:50

back to contents

(5.3 groups)]; p-SCN-Bn-DOTA-Tr 1:20 (4.9 groups); p-SCN-Bn-1B4M-DTPA [1:10 (4.9 groups); 1:20 (4.5 groups); 1:50 (4.3 groups)].
Conclusion: MALDI-TOF MS can be considered as important method for determination of successful antibody conjugations with and suitable chelator during the formulation of the ready to use radiopharmaceuticals.
Key words: conjugation, chelators, trastuzumab, MALDI-TOF MS

RISK COMMUNICATION IN COMMUNITY PHARMACIES THROUGH THE PRISM OF SELF-MEDICATION

Lebanova H.[1], Staynova R.[2], Grigorov E.[3]
[1]Department of Pharmaceutical Sciences and Social Pharmacy, Faculty of Pharmacy, Medical University, Pleven, Bulgaria
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
[3]Department of Organization and Economics of Pharmacy, Faculty of Pharmacy, Medical University, Varna, Bulgaria
Corresponding author: Assoc. Prof. Hristina Lebanova
+359 888 71 52 86; e-mail: hristina.lebanova@gmail.com

Aim: The aim of the study was to assess community pharmacists' approach to risk communication on self-medication.
Methods: An anonymous, questionnaire-based, descriptive study was performed. A pre-validated close-ended questionnaire was distributed among random sample of community pharmacists. Data was analyzed using SPSS v.19.
Results: A total of 99 valid questionnaires were collected. 60.6% of the responders claim that patients very often consult them about their self-medication practices but rarely about possible adverse drug reactions and other drug-related problems such as drug interactions. 51.5% of pharmacists consider presenting information about risks as a mandatory part of the pharmaceutical consultation and the majority of them (87.9%) believe that it could affect patients' decisions. According to 18.2% of the responders up to 80% of self-medicating patients do not use their over-the-counter medicines as recommended in the patient leaflet. 63.6% of the responders believe that over-the-counter medical products have high potential for drug misuse and abuse and 12.1% consider them with a substantial potential for drug interactions.
Conclusion: Data suggests that patients consider over-the-counter medicines safe and rarely seek information about possible risks. The conducted pilot study showed that pharmacists should be the primary and pro-active source of information about risks of self-medication products.

back to contents

CARDIOVASCULAR RISK ASSESSMENT IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Mihaleva I.[1], Yoncheva M.[2,3], Andreeva–Gateva P.[1]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Medicine, Medical University, Sofia, Bulgaria

[2]National Center of Public Health and Analyses, Bulgaria

[3]Clinic of Endocrinology, Military Medical Academy Sofia, Bulgaria

Address for Correspondence: Ivelina Mihaleva, MD, Department of Pharmacology, Pharmacotherapy and Toxicology, Medical Faculty, Medical University of Sofia, 2 "Zdrave" str., 1431 Sofia

e-mail: ivelina_mbg@yahoo.com, telephone: + 359 88 3300 599

Insulin resistance and cardiovascular diseases as well as the risk factors that contribute to their occurrence such as dyslipidemia, obesity, are main components of the metabolic syndrome. Different methods for insulin sensitivity estimation exist that are widely used in clinical practice. However most of them do not reflect the metabolic disturbances of an individual. That is why new indices emerge in the practice that estimate insulin resistance and cardiovascular risk using easily accessible anthropometric and functional parameters. These include TyG index, visceral adiposity index (VAI), lipid accumulation product (LAP), atherogenic index of plasma (AIP). Cardiovascular risk assessment in diabetic patients is of great importance and this can be seen in the clinical practice guidelines for type 2 diabetes treatment where the cardiovascular disease status of a patient to great extend determines the therapy.

UHPLC/ORBITRAP MS METABOLOMICS PROFILE AND ANTIOXIDANT PROPERTIES OF *ASPHODELUS ALBUS* ROOTS GROWING IN BULGARIA

Lazarova I.[1]*, Zengin G.[2], Nedialkov P.[3], Gevrenova R.[3]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

[2]Department of Biology, Faculty of Science, Selcuk University, Campus, Konya, Turkey

[3]Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

Corresponding author: Tel.: +3592-9236-513; Fax: +359 2 987 98 74

e-mail address: lazarova@pharmfac.mu-sofia.bg (Irina Lazarova)

The aim of the Study: *Asphodelus* L. (Asphodelaceae) species are traditionally used as anti-microbial, anti-fungal, anti-parasitic, cytotoxic, anti-inflammatory or antioxidant agents. There is limited information on the chemical content of *A. albus* and there is no documented data related to biological activities of the plant. The phytochemistry of the Bulgarian herb wasn't investigated. The aim of the study is to

analyze chemical composition and antioxidant potential of *Asphodelus albus* from Bulgarian origin.
Materials and methods: The total phenolic and flavonoid content and flavonoids of Asphodelus albus crude dichloromethane, ethyl acetate, methanol and water extracts were determined using standard Folin-Ciocalteu method and $AlCl_3$ method respectively. Chromatographic analyses were performed by a Dionex UltiMate3000 RSLC UHPLC (ThermoScientific Co, Waltham, MA, USA) and separations were achieved using a Waters ACQUITY UPLC BEH Shield RP18 column (2.1 x 100 mm, 1.7 µm particle size). MS analyses were performed with an Orbitrap mass spectrometer (Q Exactive Plus, ThermoScientific Co, Waltham, MA, USA). Antioxidant capacity of the extracts were spectrophotometrically screened by different experiments as reduction potentials (by FRAP and CUPRAC assays) radical attenuation (using DPPH and ABTS radicals), metal chelating and phosphomolybdenum.
Results: The antioxidant potential of *Asphodelus albus* was proved. The good correlation between phenolics content, radical-scavenging activity and reducing power was observed. The main individual phenolic compounds, belonging to the groups of flavonoids, anthraquinones and phenolic acids, which are responsible for antioxidant activity were identified.
Conclusion: This investigation highlights *Asphodelus albus* as a new source of bioactive compounds and its possible application as an antioxidant agent.

MONITORING OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY AND NUTRITION HABITS IN PEOPLE LIVING WITH HIV – THE POSSIBLE ROLE OF THE CLINICAL PHARMACIST

Kaneva A., Tashkov K., Dimitrova M., Yancheva N.
Department of Organization and Economy of Pharmacy, Faculty of Pharmacy, Medical University, Sofia

Research objectives: The objective of this research is to evaluate the possible role of clinical pharmacists in the process of highly active antiretroviral therapy (HAART) monitoring in people living with HIV (PLWH), nutrition habits (including dietary supplemens– DSs) and the possible interactions with the assigned pharmacotherapy.
Materials and methods: In the period January 2019 – May 2019 a research was conducted on the prescribed HAART and nutrition habits through a direct anonymous questionnaire with PLWH, at “Prof. Ivan Kirov” University Hospital. Demographics (age, gender), data on pharmacotherapy and concomitant diseases, information on nutrition habits (diet and DSs) of PLWH and sources of information on possible undesired interactions were collected. Results were processed through descriptive statistics.
Results: About 50% of the interviewees take dietary supplements, with some experiencing a potential risk of undesired HAART interactions. More than 70% of people living with HIV are aware that interactions between their assigned HAART and DSs taken are possible, but only 60% of the interviewees believe that this type of drug information should be obtained from the physician. None of the interviewees

back to contents

used the pharmacist as a reliable source of information. Approximately 50% of the people living with HIV use the Internet as a source of information.
Conclusion: HAART monitoring in people living with HIV requires a multidisciplinary approach that should also include a clinical pharmacist with respect to optimizing drug information with regard to the prescribed pharmacotherapy and proper nutrition habits.

STRATEGIES TO IMPROVE MEDICATION ADHERENCE IN CHILDREN - CHALLENGES FOR THE MODERN PHARMACIST

Staynova R.[1], Lebanova H.[2], Georgieva Y.[1]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv
[2]Department of Pharmaceutical Sciences and Social Pharmacy, Faculty of Pharmacy, Medical University, Pleven

Aim: To analyze and assess the role of the pharmacist in improving pediatric medication adherence.
Materials and methods: A systematic review of the scientific literature was carried out to identify studies that described interventions involving pharmacists aimed to improve medication adherence in patients under 18 years of age. The PubMed, Scopus, Embase, and Google Scholar databases have been analyzed using the following keywords: "adherence", "children", "pharmacists", separate and in combination.
Results: The results of 15 publications covering the 2008-2018 period are summarized. The results of the selected studies show that effective communication with pharmacists is considered a way to improve children's knowledge of medicines. Observations in pharmacies, however, show that a very small percentage of children receive direct consultation from the pharmacist, and mainly the parent is informed about drug therapy. Despite this trend, children have a positive interest in learning from the pharmacist how their medicines affect the human body and how they are manufactured.
Conclusion: To ensure effective and safe use of medicines and to enhance children's health knowledge, pharmacists need to develop and implement interactive and training techniques. By creating a partnership with the child's parents and understanding their specific needs, pharmacists can help overcome barriers to adherence and offer ways to resolve them.

back to contents

METFORMIN - THE NEW CONTENDER IN THE TREATMENT OF GESTATIONAL DIABETES MELLITUS

Staynova R.[1], Getova V.[2], Yanachkova V.[3]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv
[2]Bulgarian Drug Agency, Sofia
[3]"Dr. Shterev" Hospital, Sofia

Aim: The aim of the present study is to make a critical review of the safety and efficacy data for metformin with regard to its use in the treatment of gestational diabetes mellitus.
Materials and methods: Data from observational and randomized controlled trials in women with gestational diabetes mellitus were summarized and analyzed. The long-term effects of metformin on the offspring are also analyzed. For the purpose of the study, a systematic review of the scientific literature was carried out using the following databases: PubMed, Scopus and Google Scholar.
Results: The reviewed publications show that there is adequate evidence of efficacy and short-term safety of metformin in the treatment of gestational diabetes mellitus in relation to maternal and neonatal outcomes. Some benefits have been observed with metformin associated with lower maternal weight gain and a lower risk of neonatal hypoglycaemia and macrosomia. In addition, metformin has a number of advantages such as: oral administration, lower cost and better compliance. There is insufficient data on the long-term effects of metformin on the offspring. It is necessary to further investigate the effect of metformin on the programming of the epigenome due to its significant transplacental transfer.
Conclusion: Despite the undeniable benefits, there is need for more research before metformin can be considered as a standard for care in the treatment of gestational diabetes mellitus.

back to contents

SELECTIVE CASPASE INHIBITORS FROM *GYPSOPHILA GLOMERATA* PALL Ex M.B. (CARYOPHILLACEAE)

Sugareva P.[1], Lozanova V.[1], Dimitrova B.[2,3]*, Gevrenova R.[3], Simeonova R.[2], Lozanov V.[1]

[1]Department of Medical Chemistry and Biochemistry, Medical University of Sofia, Sofia, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[3]Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
**Corresponding Author: Bozhana Dimitrova, phone: +359883537717, e-mail: bojana_ruseva@yahoo.com*

The aim of the study: In the present study, we aimed at investigating the potential inhibitory activities of C- and C,O-glycosyl-flavons from the *Gypsophila glomerata* aerial parts against caspases, the main regulators of apoptosis.
Materials and methods: *G. glomerata* aerial parts were extracted with aqueous methanol by sonication. The lyophilized extract was fractionated by flash chromatography. The fraction containing C- and C,O-glycosyl-flavons was additionally fractionated by semi-preparative reverse phase high-performance liquid chromatography (RP-HPLC). Eight main C- and C,O-glycosyl-flavons were obtained with >98 purity and their structures were elucidated by high resolution mass spectrometry and nuclear magnetic resonance spectroscopy. The isolated compounds were screened for potential caspase inhibition activity using the set of 10 recombinant caspases by fluorometric kinetic assay.
Results: Eight pure C- and C,O-glycosyl-flavons were isolated from methanol-aqueous extract from *G. glomerata* aerial parts by combination of chromatographic techniques. The results of caspase inhibitory assays show that 3 of them are potent selective inhibitors of caspase-3 and 4 compounds possess selective inhibitor activity against caspase-7. No inhibition activity had been observed against inflammatory and activation caspases.
Conclusion: The methanol-aqueous extract from *G. glomerata* aerial parts contains several selective inhibitors of caspase-3 and caspase-7 that possess inhibitory potential and could be used to control apoptotic events in mammals.
Acknowledgements: The study was supported by the Project "Young Scientist 2019", №8231/20.11.2018, Contract D-140/2019 from the Medical Science Council at the Medical University of Sofia.

back to contents

RENIN-ANGIOTENSIN SYSTEM INHIBITORS: A POTENTIAL ROLE IN SARCOPENIA

Krasteva G., Lakova E.*, Krastev P., Stavreva G.
Department of Pharmacology and Toxicology, Faculty of Pharmacy
**Department of Physiology and Pathophysiology, Medical Faculty*
Medical University, Pleven
Corresponding author: Genka Krasteva
e-mail: krusteva_med@abv.bg; 064 884282

Sarcopenia is age-related chronic condition characterized by progressive and generalized loss of skeletal muscle mass and function and may lead to higher risk of disability, morbidity and mortality. Current pharmacological strategies are unsatisfactory. Experimental and clinical studies have given interesting insight on role of the renin-angiotensin system (RAS) in the skeletal muscles, suggesting a potential benefit of angiotensin convertin enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) in sarcopenia.
Objectives: We highlight current knowledge of the physiological role of RAS in skeletal muscle and discuss the possibility to treat sarcopenia with RAS inhibitors.
Methods: Review of the literature.
Results: Circulating RAS regulates blood pressure and electrolyte homeostasis. Local, tissue RASs are important in the regulation of the cell growth, regeneration, apoptosis, inflammation and angiogenesis. Regulation of local perfusion by AT-II is fundamental for metabolic activity and function of the musculature. Increase of AT-II levels induces skeletal muscle wasting through enhanced protein degradation and apoptosis and decreased protein synthesis. Recently, the attention was focused on muscle satellite cells (muscle progenitor cells) as main target of RAS activation or blockade. AT-II reduces satellite cell migration, differentiation and growth through AT1R. Observational and experimental studies have confirmed that blockade of the AT-II/AT1R axis may be useful in the treatment of sarcopenia. In the future, the "good arm" of the RAS – AT-(1-7)/Mas receptor and AT2R may also be subject to positive modulation with the identification of effective receptor agonists.
Conclusion: RAS is a reliable target for the prevention and treatment of sarcopenia.
Key words: renin-angiotensin system, satellite cells, sarcopenia

ANTIBIOTICS AND ANTIBIOTIC RESISTANCE – TIME FOR INTERVENTIONS IN THE STUDENTS' EDUCATION?

Keuleyan E.[1,3], Nikolov R.[2,3], Dimitrova D.[3], Ivanova E.[3]
[2]Medical Institute, Ministry of the Interior
[3]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Medicine, Medical University, Sofia
[4]"Y. Philaretova" Medical College, Medical University, Sofia

Aim: Antibiotic resistance /AR/ today reaches enormous dimensions and is proclaimed by WHO as a global threat. On the occasion of 18 November 2018 – The

European Antibiotic Day - we elaborated an educational poster at The "Y. Philaretova" Medical College on the basis of British Society of Antimicrobial Chemotherapy materials. After familiarizing the poster, we conducted an inquiry among students in "Medical technician" specialty. Task of the current work is to analyze the students' knowledge about antibiotics and AR.

Materials and methods: Questionnaire forms consisted of 11 questions with 3 to 5 multiple choice answers. They were distributed to be filled in voluntarily and anonymously. In analysis of the results SPSS and determining relative rate were applied.

Results: From 85 questionnaires distributed 77 were returned (87.5 %). Excellent answers were obtained for the questions concerning the reasons and consequences of development of AR – relative rate between 71 % and 100 %. Lower success rate - 43-95 % - was achieved for questions about mechanisms and epidemiology of AR. Unsatisfactory were the answers of questions concerning the importance of antibiotics for current achievements in medicine and for the perspective if AR will not be reduced – respectively 11 % and 56 %.

Conclusion: The students demonstrated relatively good level of knowledge for AR /apparently supported by the elaborated poster/. Much more serious knowledge about antibiotics and antibiotic resistance are needed for the future medical specialists, incl. by improvement of the educational programs – more hours and multidisciplinary training are necessary.

INVESTIGATION OF THE EFFECTS OF 1,2,3-THIADIAZOLE CONTAINING HYDRAZONE DERIVATIVE ON THE LIVER AND KIDNEY FUNCTIONS IN FEMALE MICE

Angelova V.[1], Simeonova R.[2]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Bulgaria

The aim of the study: It is well documented that isoniazid (INH), a first line drug to treat tuberculosis (TB), is associated with hematological disturbances and hepatocellular-type liver injury, with a marked elevation of hepatic transaminases. In our previous investigations, 1,2,3-thiadiazole containing hydrazone derivative showed excellent antimycobacterial activity against a referent strain *M. tuberculosis* H37Rv (MIC 0.39 μM), low cytotoxicity and selectivity index SI>2000. Additionally, the compound does not have toxic effects when administered by oral or intraperitoneal routes to experimental animals, LD50>2000 mg/kg b.w.

The aim of the present study was to determine the effects of 1,2,3-thiadiazole derivative on the liver and kidney functions in female mice and to compare with INH.

Materials and methods: The compound was administered orally for 14 days at three doses (100, 200, and 400 mg/kg b.w.). The quantity of malondialdehyde (MDA), the level of reduced glutathione (GSH), blood hematological and biochemical parameters were assessed, and urine analysis was carried out. As a positive control INH was used, orally treated at a dose of 50 mg/kg b.w.

back to contents

Results: The investigated compound did not affect the urine and serum hematological and biochemical parameters as INH did. The new antimycobacterial agent did not affect significantly the MDA quantity and maintained its level near to the control values, though lower by 36% ($p<0.05$), compared to the control. At the higher doses, 200 and 400 mg/kg, it depleted the GSH content by 25% ($p<0.05$), compared to the control. However, its level remained 47% ($p<0.05$) higher than in the INH treated animals.
Conclusion: These findings revealed the suitability of the test compound for further investigation as a safe and non-toxic antimycobacterial agent.
Funding: This work was partly supported by the National Science Fund of Bulgaria (research grant contract № DN 13/16 21.12.2017).
Correspondence: Violina T. Angelova
Tel: +359 029236586; e-mail: violina_stoyanova@abv.bg

DEVELOPMENTS AND APPLICATIONS OF CHEMICAL CHARACTERIZATION OF BIOPHARMACEUTICALS

Gjorgieva Ackova D., Smilkov K.
Department of Pharmacy, Faculty of Medical Sciences, "Gotse Delčev" University, Štip, R. North Macedonia

The aim of the study: Understanding the behaviour and function of biomolecules at the molecular level is the key to the discovery and development of new drugs, as well as diagnostic techniques. The characterization of biological drugs, where therapeutic monoclonal antibodies (mAbs) are placed, poses many challenges compared to those of low-molecular mass drugs because of their inherent complexity due to their protein nature. Achievements in this field of science have changed the way that drugs are being designed and developed nowadays.
Materials and methods: Vibrational spectroscopy techniques, like Fourier Transform Infrared (FTIR) Spectroscopy and Raman Spectroscopy (RS) have been applied and helped to determine the secondary structure and possible interactions and modifications of complex protein molecules, as well as protein-ligand complexes.
Results: Our investigation has demonstrated the use of these tools to understand protein-ligand interactions in important immune-complexes with previously no available structural information.
Conclusion: The approach presented here has significant potential for analyzing the structure, stability and possible toxicity of biotherapeutics as well as any other biological molecules which are used as therapeutic/diagnostic agents.

back to contents

EFFECTS OF PIOGLITAZONE ON SERUM LEVELS OF PROINFLAMMATORY CYTOKINES IN EXPERIMENTAL MODEL OF AUTISM

Stefanova Z., Bogdanov G., Kamenova K., Varadinova M.
Department of Pharmacology and Toxicology, Medical Faculty, Medical University, Sofia

Recent studies have demonstrated the involvement of neuroinflammation and increased central and peripheral levels of proinflammatory cytokines in the development of neurological and neuropsychiatric disorders. The combined pharmacotherapy of autism spectrum disorder (ASD) includes atypical antipsychotics, antidepressants, antiepileptics, probiotics, and diet. The effects of PPARγ agonists on behavioral symptoms in children with ASD, as well as their potential anti-neuroinflammatory properties are intriguing.

Aim: To investigate the effects of pioglitazone on serum levels of IL-1β and IL-6 in an experimental model of autism.

Materials and methods: The male offspring of pregnant Wistar rats treated or not with valproic acid were separated from their mothers on the 23rd postnatal day and were divided into 4 groups: 1. Control; 2. With experimental autism; 3. Control, treated with pioglitazone; 4. With experimental autism, treated with pioglitazone. After 21 day-treatment the rats were decapitated, blood was collected and the sera were used to determine proinflammatory cytokine levels.

Results: In the present study there were not observed significant changes in the serum levels of IL-1β between the control and the experimental groups. There was demonstrated a significant increase in the IL-6 levels in the experimental group compared to the control group. The experimental autism group treated with pioglitazone had a considerably lower IL-6 levels were compared to the untreated group.

Conclusion: Beneficial effects of pioglitazone on proinflammatory parameters are demonstrated, suggesting its role in neuroinflammatory and in neurotrophic regulation, respectively.

PRODUCTS CONTAINING EXTRACTS FROM MEDICINAL PLANTS FOR PREVENTION OF OSTEOPOROSIS

Tsvetkova D., Ivanova S.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Osteoporosis is characterized by low bone mass and microarchitectural deterioration with increase in bone fragility. In postmenopausal women the reduction of estrogen leads to elevation of oxidative stress and lipid accumulation, which promote osteoblasts apoptosis.

back to contents

Aim of the study: The aim of the study is the collection, analysis and comparison of content and distribition in different dosages and formulations of the most common manufactured products containing extracts from medicinal plants for osteoporosis.
Materials and methods: The investigation has been made through the electronical database of medical sourses.
Results: The most promising plants for potential prevention of osteoporosis are: *Alchemilla vulgaris, Angelica sinensis, Avena sativa, Camellia sinensis, Coriandrum sativum, Dioscorea macrostachya, Dioscorea villosa, Equisetum arvense* (silicic acids), *Ganoderma lucidum, Fucus vesiculosus* (minerals), *Flax seed, Glycine max* (isoflavones), *Glycyrrhiza glabra, Macafem, Matricaria chamomilla, Medicago sativa, Oenothera biennis, Panax ginseng, Persea americana* (vitamin D), *Petroselinum crispum, Piper methysticum, Populus friemontii, Pueraria lobata, Salvia officinalis, Trifolium pratense* (isoflavones), *Vitex agnus castus, Withania somnifera*. Isoflavones significantly increase bone mineral density and inhibit the bone resorption due to reduce the number of osteoclasts.
The most used products containing extracts from medicinal plants are: *Cimicifuga racemosa* Bionorica Menopret tabl., *Trifolium pratense* caps.; Ayrvedic: Arjuna (*Terminalija arjuna*) caps., Ashwagandha (*Withania somnifera*) caps., *Emblica officinalis* caps., Lakshadi Guggul caps. and multicomponent: Vitabiotics Menopace Plus tabl. USANA Phyto Estrin; True Health – Osteopenia Bone Health System.
Conclusion: An observational study shows different trade form preparations: tablets, capsules, powders, solutions of monocomponent and multicomponent products containing extracts from medicinal plants for osteoporosis.
Corresponding author: Assist. prof. Dobrina Tsvetkova, PhD
tel.: 0877757118, e-mail: dobrinka30@mail.bg

COMPARISON OF DIFFERENT EXTEMPORANEOUSLY COMPOUNDED VEHICLES FOR ORAL LIQUID DOSAGE FORM FOR PEDIATRIC PATIENTS

Pehlivanov I., Andonova V.
Department of Pharmaceutical Technologies, Faculty of Pharmacy,
"Prof. dr. P. Stoyanov" Medical University, Varna

Objectives: To evaluate the stability of Nitrofurantoin suspended in different extemporaneously compounded vehicles after storage at 4ºC and at 25ºC. To formulate an effective, readily available vehicle that can guarantee extended stability and precise dosing.
Methods: Nitrofurantoin was suspended at concentration 10 mg/mL in seven different vehicles compounded of different blends of: Syrupus Simplex; Sorbitol 70%; Methylcellulose 1%; Gummi Arabici 1%; Gummi Xanthani 1%; NaCMC 1%. Samples of 100 mL of every compounded suspension was stored in graded glass bottles at 4ºC and at 25ºC and in dark. Samples were analyzed at the beginning and every 10 days up to the 30th day and every 30 days after. Variations of physical properties such as sedimentation, viscosity, ease of resuspension, color, odor and taste were

back to contents

evaluated visually and organoleptically. Variations in Nitrofurantoin concentration and pH were evaluated with suitable analytical procedure (UV-Vis, HPLC).
Results: To the 30th day, only three of the compounded suspensions exhibit significant physical stability and slight change in taste and odor stored at both temperatures. Two samples stored at 25ºC exhibited Nitrofurantoin concentration greater than 95% and 4 samples stored at 4ºC – concentration greater than 95%. To the 120th day only three of the compounded suspensions, stored at 4ºC, exhibit relatively high Nitrofurantoin concentrations: 88.2%, 92% and 81.1%, respectively.
Conclusions: The suspensions compounded with vehicles of blends of syrups, Xanthan, Croscarmellose and Sorbitol exhibit low to none sedimentation, good uniformity of content and organoleptically are suitable for pediatric administration. Further studies are to be concluded in order to be defined the most stable vehicle and final shelf life.

PLANT SOURCES OF MAURITIANIN AND EVALUATION OF ITS PROTECTIVE POTENTIAL

Shkondrov A., Krasteva I., Kondeva-Burdina M.
[1]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*
[2]*Laboratory of Drug metabolism and Drug toxicity, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria*

Aim of the study: Recent phytochemical research of *A. monspessulanus* subsp. *monspessulanus* (Fabaceae) afforded a kaempferol triglycoside (mauritianin). Only few studies of its pharmacological action have been conducted. The aim of the present study was to investigate extracts from overground parts of several *Astragalus* species in order to reveal the quantity of mauritianin and to isolate it in sufficient quantity for pharmacological research.
Materials and methods: A highly accurate liquid chromatography-mass spectrometry screening method was used for the analysis of the samples. Isolation of the compound from the appropriate plant was achieved by chromatographic means. In addition, a pharmacological study of the possible protective effects of the flavonol in an *in vivo* model of intoxication was conducted.
Results: The flavonoid was found in relatively small number of the species investigated. Its quantity was varied, but a source was established. The isolated compound proved its *in vivo* protective effects, comparable to silymarin.
Conclusion: These findings will serve as a perspective for further investigations of the pharmacological effects of mauritianin.
Acknowledgement: Financial support from Council of Medicinal Science at Medical University of Sofia, contract №D-76/23.04.2019 is gratefully acknowledged.

back to contents

SECONDARY METABOLITES IN *ASTRAGALUS CORNICULATUS*

Stambolov I., Shkondrov A., Krasteva I.
Department of Pharmacognosy, Faculty of Pharmacy,
Medical University, Sofia, Bulgaria

Aim of the study: The aim of this study was to investigate the novel for Bulgarian flora *Astragalus corniculatus* Bieb. (Fabaceae) for the presence of notable secondary metabolites.
Materials and methods: A highly sensitive ultrahigh performance liquid chromatography-mass spectrometry method was applied to reveal the phytochemical content of a methanol extract, obtained from the aerial parts of the species.
Results: Results showed that the species contained hydroxymethyl glutaric acid derivatives of kaempferol and quercetin glycosides as well as the flavonol triglycosides alcesefoliside and mauritianin.
Conclusion: These findings serve as an addition to the information of the phytochemical content of *A. corniculatus* and could be a basis for further studies of this valuable plant.
Acknowledgement: This work was supported by the Bulgarian Ministry of Education and Science under the National Program for "Young Scientists and Postdoctoral Students" Research.

ANTIBIOTIC TREATMENT – OPPORTUNITIES FOR OVERCOMING RESISTANCE

Mihaylova S.[1], Tsvetkova A.[1], Todorova A.[2]
[1]*Medical College, Medical University, Varna, Bulgaria*
[2]*Faculty of Pharmacy, Medical University, Varna, Bulgaria*

Introduction of antibiotics into the clinical practice and their frequent and unreasonable use has led to the occurrence of a large number of antibiotic-resistant pathogens. By 2050 antimicrobial resistance can become a more frequent cause of mortality than neoplastic diseases. Globally, on an annual basis, it causes the death of 700 thousand people, 25 000 of whom are patients in Europe.
Objective: To research and analyse modern tendencies for overcoming resistance of pathogenic microorganisms.
Methodology: Review of literature and analysis of the information from different electronic data bases, Pub Med, Scopus, Medline and others, using key words: ESKAPE pathogens, resistance, CDAs and antibiotic activity.
Results: In 2017 the World Health Organisation published a list of pathogenic microorganisms, for which new antibiotics had to be developed urgently. The priority ones are Carbapenem-resistant, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae*, from the group of so called ESKAPE pathogens, multidrug resistant gram-positive and gram-negative bacteria. Calcium-dependent acidic lipopeptide antibiotics (CDAs) are a potential source of new antimicrobial agents. FDA and EMA allow the use of the cyclic lipodepsipeptide Daptomycin for treatment of complicated skin and soft tissue infections caused by multi-resistant

back to contents

Gram-positive bacteria. Malacidines are a new group of CDAs, made by soil microorganism found in 2018. In 2019 scientists from the Rockefeller University described a new group of CDAs - Cadasides, active against multi-resistant Gram-positive bacteria. *In vitro* research on the effect of these antibiotics continues.
Conclusion: Discovery of new antimicrobial medicines against ESKAPE pathogens is one of the greatest challenges in fighting life-threatening infections and protecting global health.

EVALUATION OF THE ANTICONVULSANT, ANTIDEPRESSANT AND ANTIOXIDANT EFFECTS OF NOVEL MELATONIN DERIVATIVES IN MICE

Tchekalarova J., Ivanova N., Nenchovska Z., Stoynova T., Angelova V., Andreeva-Gateva P., Tzoneva R.
Institute of Neurobiology

Aim: The aim of the present study was to explore the effects of newly synthesized melatonin derivatives, containing aroylhydrazone moieties, for anticonvulsant activity, antidepressant in in-vivo tests in mice as well as in-vitro for their activity against oxidative stress.
Materials and methods: The series of synthetized indole C-3 substituted aroylhydrazones **3a-i** and a positive control melatonin, administered at doses of 10, 30, 60 and 100 mg/kg, were explored 30 min on seizure threshold, measured by the timed intravenous pentylenetetrazole (iv PTZ) infusion; motor activity and anxiety in the open field (OF) and depressive-like behavior in tail suspension test (TST) in ICR mice. Immediately after TST, the mice were decapitated and frontal cortex (FC) and the hippocampus was isolated. The level of oxidative stress was examined by Elisa test.
Results: Three out of six compounds, **3c, 3f** and **3e**, respectively, dose-dependently increased the PTZ-induced seizure thresholds for myoclonic twitch, clonic and tonic seizures comparable to the effect of carbamazepine and melatonin. Their anticonvulsant effect was blocked by the non-selective melatonin receptor antagonist luzindol. These three active compounds as well melatonin showed decreased motor activity and anxiety in the highest doses compared to controls as well as dose-dependent antidepressant activity. The compounds **3e** and and **3f** decreased stress-induced lipid peroxidation and increased superoxide dismutase activity specifically in the FC.
Conclusion: Our previous and present results suggest that **3c, 3f** and **3e** can be considered promising agents with pharmacological activity on melatonin receptors in brain.
Acknowledgement: This research was supported by the National Science Fund, Bulgaria (grants No. DN DH13/16, 21.12.2017; DH13/16, 21.12.2017).

back to contents

TWO NEW PHENOLIC GLYSOSIDES FROM AERIAL PARTS FROM *HYPERICUM CERASTIOIDES*

Nedialkov P., Ilieva Y., Kokanova-Nedialkova Z.
Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia

Aim: The aim of current study is to isolate and identify the unknown phenolic metabolites from methanol extract of aerial parts from *Hypericum cerastoides* (Spach) N. Robson.
Materials and methods: The plant material of *H. cerastioides* was collected July 2018 in Treshtenik locality, near Yakoruda town (south slopes of Rila Mountain), dried, powdered and subsequently extracted with dichloromethane and methanol. The methanol extract was partitioned between water and ethylacetate. The ethylacetate layers was combined, evaporated and subjected to series of chromatographic procedures included column chromatography on MCI gel and Sephadex LH-20. Final purification on semiprep.-HPLC led to isolation of two new phenolic glycosides.
Results: The structures of the isolated compounds were established by means of mass spectrometry and 1D and 2D nuclear magnetic resonance experiments as 4-O-{6-[(2E)-p-hydoxycinnamoyl]-*β*-D-glucopyranosyl}-6-isopropyl-tetrahydro-2H-pyran-2-on and 3-O-{6-[(2E)-p-hydoxycinnamoyl]-*β*-D-glucopyranosyl}-6-methyl-5-hydroxyheptanoate that were given the trivial names hypercerstosides B and C, respectively.
Conclusion: An extensive chromatographic procedure of ethylacetate extract of aerial parts from *Hypericum cerastoides* led to the isolation of two new phenolic glucosides that were given the trivial names hypercerstosides B and C.
Acknowledgements: This work was supported by the Council of Medical Science of Medical University of Sofia (Grand № D-75/03.05.2018).

ANALGESIC ACTIVITY OF MELATONIN AND MELATONIN DERIVATIVES BEARING AROYLHYDRAZONE FRAGMENT

Gateva P.[1], Tzinev A.[1], Mancheva I.[1], Koleva V.[1], Marchev S.[1], Angelova V.[2], Tchekalarova J.[3], Surcheva S.[1], Vlaskovska M.[1]
[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Medical Faculty, Medical University, Sofia
[2]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia
[3]Institute of Neurobiology, Bulgarian Academy of Sciences
Corresponding author: P. Gateva +359 889 428 105, e-mail: pandreeva_gateva@outlook.com

Melatonin is an endogenous indoleamine with chronobiological, antioxidant and neuroprotective properties. Preclinical and clinical data indicate that melatonin has also dose-depending analgesic effect. The aim of this study was to compare the potential analgesic effects of newly synthesized melatonin derivatives with anti-seizure activity demonstrated in our previous experiments.

back to contents

Materials and methods: After synthesis of series of melatonin-containing aroylhydrazones, four of the substances demonstrated anti-seizure activity when testing with the test for maximal electroshock (MES), penthylene tetrazole (PTZ) and 6Hz. Those four substances and melatonin were screened for analgesic activity with the hot plate test and formalin test after intraperitoneal injection of 50, 100, 200 and 300 mg/kg in saline solution in male mice 20-25 g. N=6 for each of groups including controls.
Results: Melatonin demonstrated dose-depending analgesic activity in the formalin test. The dose of 200 mg/kg demonstrated the higher effect, the antinociceptive behavior of the animals was reduced by 80% for both phases of the test. Melatonin did not demonstrated antinociceptive activity in the hot plate test. Two of the tested melatonin-aroylhydrazone derivatives, namely the thiophen substituted and the second indol substituted, demonstrated activity both in the hot plate test - 20% reduction of the antinociceptive activity in doses of 200 and 300 mg/kg, as well as a reduction of the antinociceptive behavior in phase 2 of the formalin test similarly to melatonin.
Conclusion: The two newly synthesized substances with demonstrated antinociceptive activity deserves to be further tested for potential applicability in different pain-related pathological process.
Acknowledgement: Supported by the National Science Fund of Bulgaria DN 13/16, 21.12.2017.

POLYMERIC MICROSPHERES WITH ENALAPRIL MALEATE FOR TASTE MASKING

Georgieva Y., Pilicheva B., Kassarova M.
Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
High-technological Center of Emergency Medicine, Plovdiv, Bulgaria
For correspondence: Yana Georgieva, 0879189804, e-mail: iana_georgieva9@abv.bg

Aim of the study: Development of polymeric microspheres with enalapril maleate (ENA) for taste masking in order to incorporate them into orodispersible tablets.
Materials and methods: Polymeric microspheres were prepared by emulsion solvent evaporation technique. For the preparation of the O/W emulsion, the oil phase (dichloromethane, acetone and the dissolved ENA, Eudragit Epo® and ethylcellulose) was emulsified in the aqueous phase containing Tween 20®. The emulsion was stirred for 6 hours until complete evaporation of the organic solvents. The resulting particles were isolated after filtration and dried at room temperature. For the characterization of the obtained microspheres, UV-spectrophotometric quantitative analysis, IR spectroscopy, optical and scanning electron microscopy, powder X-ray diffraction and thermogravimetric analysis were applied.
Results: Five models of polymeric microspheres were developed by varying the amount of the drug and the polymers. The yield of the microspheres was between

back to contents

39% and 53%. The drug loading ranged from 17% to 40% and the encapsulation efficiency varied from 0.89% to 2%. The average size ranged from 140 μm to 339 μm and the moisture content was below 9%. Scanning electron microscopy showed the spherical shape and the uneven surface of the microparticles. The release of ENA from the obtained models in artificial saliva without enzymes (pH 6.8) showed minimal values by model EMS 3 (ENA: Eudragit Epo®: ethylcellulose 0.2: 0.27: 0.53), an evidence for masking the bitter taste of ENA.
Conclusion: The obtained microspheres with Eudragit Epo® and ethylcellulose are a suitable carrier of enalapril maleate and provide optimal taste masking.

MOST COMMON TOXIC METALS IN CHILDREN WITH DEVELOPMENTAL PROBLEMS IN BULGARIA

Ciurinskiene S., Savcheva M., Kadiyska T.

Autism is a neuro-developmental syndrome, perceived as a multigene disorder with epigenetic influences. There is no single diagnostic marker found.
A number of researchers (Yasko, Amy, 2009; Walsh, William, 2011) have discovered the connection between autism spectrum disorders and the incapability of an organism to excrete metals, due to genetic disturbances in the methylation cycle. Variations of certain enzyme coding genes, which take part in the methylation and urea cycle, as well as sulfation, has been identified. Depending on the gene variations, these enzymes are increased or decreased. As a result, detoxification is disturbed.
Objective of the study:
1. To identify the predominant, most common metals in Bulgarian children with developmental disorders (ASD)
2. To identify whether there is a correlation to variations of increased toxic metals and various genes: GPX1, GPST1, CBS, NOS, NAT2, CBS.
Materials and methods: The study spans over 53 children, at the age of 1 – 13. 47 boys and 6 girls, were tested for toxic metals overload, from January 1st 2017 to March 30th 2018. The level of toxicity was identified, using a hair metal elements test.
The genetic analysis included genetic variants, known to be important and involved in the metabolism (synthesis, transport and degradation) of the neurotransmitters dopamine and serotonine, vitamin deficiencies including vitamin D, B6, B9 and B12, energetic balance of the cell, methylation cycles, detoxification capacity of the cell and histamine intolerance.
The autistic symptoms were tested with the test battery CARS2.
Results: 53 out of 53 children with developmental disorders turned out positive for increased level of one or more toxic metals. The severity of the autistic symptoms, however, correlated not with the extent of increase, but with the numbers of the detected metals – the more the number of the different metals were, the heavier the symptoms.
Conclusions: Our study supports the metabolic deficiency theory, which occurs as result of disturbed detoxification, caused by variations of several genes like GPX1,

back to contents

GPST1, CBS, NOS, NAT2, CBS. etc., which in turn leads to intoxication with metal and bad metabolites, accompanied by gut and brain inflammation.
Key words: autism, toxic metals, detoxification

SYNTHESIS AND PREDICTION OF ADME PARAMETERS OF SOME NEW DERIVATIVES OF BEXAROTENE

Mitkov J.[1], Agova N.[2], Georgieva S.[2], Zlatkov A.[1]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Varna, 55 "Prof. Marin Drinov" str., 9002 Varna, Bulgaria

Aim: The aim of the present work is to obtain and structurally characterize novel hydrazide-hydrazone derivatives of Bexarotene. They will be subjected to an in silico study to predict ADME parameters and substrate / metabolic specificity.
Materials and methods: Structural characterization of newly obtained compounds was performed using instrumental methods.
The prediction of the substrate/metabolic specificity is performed using the SMP web-service. The prediction is based on PASS technology and MNA descriptors (http://www.way2drug.com/SMP/).
Calculation of physicochemical descriptors as well as prognosis of ADME parameters, pharmacokinetic properties and drug nature of the tested compounds were performed using the Swiss-based web application SwissADME (http://www.swissadme.ch/).
Results: The present study describes the synthesis of series new Bexarotene hydrazid-hydrazone derivatives which are subjected to in silico prediction of ADME parameters and substrate / metabolic specificity. All compounds show minimal deviations of Log P, while the other parameters are fully satisfying the Lipinski's rule. All compounds showed minimum aberration in Log P, while other parameters are fully in agreement with Lipinski's rule. All test compounds fall into the group of moderately soluble compounds. These calculations represent the compounds as lipophilic. Higher values of the parameter Pa (probability "to be active") are obtained with respect to the enzymes CYP2C19 and CYP3A4.
Conclusion: It was found to be probable that the molecules are lipophilic and slightly water soluble, so they are predicted as poor bioavailable.
Based on the obtained data we consider the tested compounds to perform most probably as CYP2C19 and CYP3A4 substrates. This can be a cause of drug interaction with certain inhibitors or inducers of those enzymes, such as ketoconazole, grapefruit juice, pioglitazone etc.

back to contents

LAVENDER OIL MICROENCAPSULATION USING *BOSWELLIA SERRATA* RESIN AS A WALL MATERIAL

Katsarov P.*, Penkov D., Pilicheva B., Georgieva Y., Peneva P., Kassarova M.
Department of Pharmaceutical Sciences, Faculty of Pharmacy,
Medical University, Plovdiv
Technological Center for Emergency Medicine, Plovdiv
**Corresponding author: tel. 0899040776, e-mail: plamen.katsarov@yahoo.com*

Aim of the Study: Formulation of lavender oil microcapsules with optimal structural and morphological characteristics and high stability of the encapsulated essential oil, using *Boswellia serrata* resin as a wall material.
Materials and methods: Microcapsules were obtained by a spray drying method using Buchi B-290 Mini Spray-dryer at 140˚C temperature, 95% aspiration and 10% pump rate. Aqueous dispersions of *Boswellia serrata* resin with different concentrations were used, with essential oil: resin ratio of 1:4. Microcapsules shape was observed through scanning electron microscopy. Median diameter and particle size distribution of the models were determined by laser diffraction. Gas chromatography was used to analyze the phytochemical composition of the encapsulated oil. The total amount of lavender oil in the microcapsules after preparation and after 12 months of storage was determined by distillation in a Clevenger apparatus.
Results: Three models of lavender oil microcapsules (M12, M15, M17) were formulated, using aqueous dispersions of *Boswellia* resin with concentration of 12, 15 and 17%, respectively. The microcapsules were spherical in shape, with median diameter 5.4μm - 8.9μm and production yield - 28.11% - 51.25%. Model M15 was outlined as the most promising one, showing the highest yield (51.25%), a total oil content of 8.85% and a satisfactory stability - 19% loss of essential oil after 12 months of storage.
Conclusion: *Boswellia* resin can be successfully used as a wall material for formulation of lavender oil microcapsules. The obtained results may serve as a basis for preparation of solid dosage forms with essential oils.
The study was financed by MU-Plovdiv through university project № НО – 12/2015.

back to contents

IN VIVO DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF DRY LEAF EXTRACT FROM SILVER BIRCH

Penkov D.[1,2], Delev D.[3], Katsarov P.[1,2], Kassarova M.[1,2]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
[2]High-Technological Center of Emergency Medicine, Plovdiv, Bulgaria
[3]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University, Plovdiv, Bulgaria
Corresponding author – Dimitar Penkov, tel. 0883348490, e-mail: dimitar_penkov@abv.bg

Aim: The aim of this work was to investigate in vivo the antioxidant activity of a dry leaf extract from *Betula pendula*, Roth.
Materials and methods: 36 white male Wistar rats were used (weight 200 – 250 g). They were divided into 4 groups of 9 animals each, as follows:
Group I - Saline (0.9% NaCl);
Group II - Trolox 160 mg/kg bw;
Group III - extract 100 mg/kg bw;
Group IV - extract 500 mg/kg bw.
The assay was performed in acute and chronic manner. Test solutions were administered once (in the acute) and daily (in the chronic experiment) for 14 consecutive days. Blood collection was performed. The samples were centrifuged and blood plasma was collected to test the antioxidant activity. The FRAP reagent was prepared as a mixture in a ratio 10:1:1 (acetate buffer pH 3.6:10 mM TPTZ solution:20 mM FeCl3.6H2O solution).
Results: Animals treated with the extract, at doses of 100 and 500 mg/kg, showed a significant increase in serum antioxidant activity compared to the control group ($p<0.001$ and $p<0.0001$ respectively). An interesting trend was observed - higher values in the acutely treated groups compared to the chronically treated. This can be probably explained by the biotransformation and elimination processes, which leads to a rapid decrease in the concentration of biologically active substances, responsible for the antioxidant effect. The reference group treated with the synthetic antioxidant Trolox showed the highest results.
Conclusion: Dry birch leaf extract exhibits a relatively strong antioxidant activity, therefore it can be used as a natural source of antioxidants.

back to contents

SYNTHESIS, DRUG LIKENESS AND TOXICITY PREDICTION OF SOME NEW IMIDAZOLE-THIOALKANIC ACIDS

Mitkov J.[1], Stamova S.[2], Georgieva S.[2], Georgieva M.[1]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Varna, 55 "Prof. Marin Drinov" str., 9002 Varna, Bulgaria

Aim: The aim of the present work is to synthesize and structurally characterize series new imidazole-thioalkanic acids. The obtained structures will be subjected to an in silico evaluation for prediction of their Drug likeness and toxicity ptoperties.
Materials and methods: The structural characterization of newly obtained compounds was performed using instrumental methods.
The Drug likeness prediction was based on the methodology used in the web program OSIRIS Property Explorer Data Warrior, and for toxicity prediction was applied the web based server ProTox II (http://charite.de/protox_II).
Results: The calculated values for the molecular descriptors agree with Lipinski's Rule and MDDR quidelines. In relation to Drug likeness and Drug score parameters were obtained positive values for Drug score, close to 1 and positive values for Drug likeness.
The tested compounds were classified as Predicted Toxicity Class 4 – low toxic, with toxicity probability above 60%. Concidering the predicted hepatotoxicity properties, the evaluated structures are expected to be non-toxic.
Conclusion: The structures of the newly synthesized compounds was confirmed by instrumental methods. From the obtained results of Drug likeness and Drug score predition may be concluded that the tested thioalkane acids may be concidered as Drug like molecules.
The performed *in silico* prediction of the Drug Induced Liver Injury (DILI) indicated the targed molecules as non-hepatotoxic. Their classification as Predicted Toxicity Class 4 presents them as low toxic compounds.

DEVELOPMENT OF TECHNOLOGICAL METHOD TO PRODUCE NANOSTRUCTURED LIPID CARRIERS BASED ON NATURAL LIPID

Dobreva M., Stefanov S., Andonova V.
Department of Pharmaceutical Technology, Faculty of Pharmacy, Medical University, Varna

The aim of the study: Development of technological method and investigation of crucial technological parameters, during preparation of nanostructured lipid carriers (NLC) based on natural oils, as novel drug delivery systems.
Materials and methods: Solid and liquid lipids including Compritol 888 ATO, Cera alba and borage oil were used as components of the lipid matrix. Combination of nonionic emulsifiers (Polysorbate 80, Span 80, Poloxamer 407) were selected for stabilization of the particles surface. NLC were prepared using high-shear

back to contents

homogenization method with Ultra- turrax T25 equipment, followed by ultrasonication.
The obtained NLC have been characterized in terms of particle size, polydispersity index and zeta potential (Zetasizer Ultra), also stability study of dispersions was conducted.
Results: The composition of dispersions selected for characterization were 10% lipid phase with 5% surfactant. Solid lipid: liquid lipid ratio forming the lipid matrix was established at 3:1. Different combinations of lipids were melt at temperatures, above their melting points. For Compritol-borage oil (CB) blend that was 85°C and for Cera alba- borage oil (CAB) blend – 75°C. High-shear homogenization at 10 000 rpm for 5 minutes was applied for mixing the dispersion. Depending on surfactants used, NLC prepared with CB lipid matrix have size from 215 nm to 328 nm. Their average zeta potential was around zero (1.18 till -2.65). The NLC comprised of CAB blend show similar size (278 nm -314 nm), but much higher zeta potential (-24.5 to -34.2).
Conclusion: The described technological method is suitable for preparation of NLC offering beneficial characteristics of prospective drug delivery systems.

FOOD SUPPLEMENTS FOR THE MAINTENANCE OF NORMAL LIVER FUNCTION ON THE BULGARIAN PHARMACEUTICAL MARKET – IN COMPLIANCE WITH THE EUROPEAN RECOMMENDATIONS FOR HEALTH CLAIMS AND POSSIBLE INTERACTIONS WITH MEDICINES FOR LIVER DISEASES

Balkanska A., Kondeva M., Dimitrova M.
Faculty of Pharmacy, Medical University, Sofia

Research objective: To evaluate the clinical and laboratory indicators of the second most common active substances in food supplements (FS), used for maintenance of normal liver function (MNLF), for possible interactions with medicines for variety of liver diseases.
Materials and methods: A critical analysis of the European regulation (EO) No 1942/2006 was conducted to evaluate the requirements for health claims for the FS for MNLF and their inclusion in international recommendations and pharmacotherapeutic guidances. The most delivered FS for liver function from wholesalers to pharmacies for MNLF were analysed for the dosage of the active substance. Subsequent evaluation of potential interactions with medicines for treatment of liver diseases published in the literature was performed.
Results: Hepatoprotectors are included in the international guidelines and pharmacotherapeutic guidances as an option to maintain normal liver function. The second most frequent active substance in the liver FS, after sylimarin, is choline (citrate/bitartanat). Health claim for MNLF may be used only for foods/food supplements which contain at least 82,5 mg of choline per 100 g /100 ml. To present no significant interactions were found in the literature between choline and medicines for the treatment of variety of liver diseases.

back to contents

Conclusion: The hepatoprotectors are part of the pharmacotherapeutic guidances as a recommendation to maintain the health of the liver while curing liver diseases. More detailed quality analysis and evaluation of the FS use for liver health, are about to be done with evaluation of eventual interactions in patients with liver diseases.

A NEW SYNTHETIC ROUTE OF OBTAINING OF 5-METHYL-5-BENZYL HYDANTOIN. X-RAY ANALYSIS AND THEORETICAL CALCULATIONS

Buyukliev R.[1], Nikolova R.[2], Shivachev B.[2], Cherneva E.[1], Bakalova A.[1]

[1]Department of Chemistry, Faculty of Pharmacy,
Medical University of Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Institute of Mineralogy and Crystallography,
Bulgarian Academy of Science, 107 "Acad. G. Bonchev" str., 1113 Sofia, Bulgaria

The aim of the study: It is known from the literature that the 5,5-disubstituted hydantoins have anti-convulsive, antipyretic, antibacterial properties. The compound 5-methyl-5-benzyl hydantoin was obtained as early as 1932. The goal of the study is to describe various synthetic route of the obtaining of 5-Methyl-5-benzyl hydantoin and to investigate its structure by X ray analysis.
Materials and methods: 5-Methyl-5-benzyl hydantoin has been synthesized and characterized by single crystal X-ray diffraction, IR and NMR spectrometry. Quantum-chemical computations were used to investigate the structure of the compound based on DFT at the B3LYP/6-311++G** level of theory.
Results: The X-ray diffraction analysis revealed that the compound is crystallized in the monoclinic space group of the Pn (N°7) system. In addition, theoretical calculations based on the DFT/B3LYP/LANL2DZ method were performed. A good consistency was found with the experimental results.
Conclusion: A different synthetic pathway of the compound was realized. The crystal structure of 5-methyl-5-benzyl hydantoin has been determined by X-ray diffraction analysis and also by elemental analyses, IR, ^{1}H and ^{13}C NMR spectra. A good agreement has been found between calculated and experimental structural parameters. The theoretical wavenumbers showed very good agreement with the experimental values.

back to contents

THE EMPLOYMENT OF STUDENTS FROM PHARMACEUTICAL SPECIALTIES IN BULGARIA – A PILOT STUDY

Gergov K.[1], Georgiev J.[1], Burgazliev H.[2], Hristov E.[1], Ognianov S.[1]
[1]Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria
[2]Department of Pharmacy, Medical College,
"Prof. Dr. Assen Zlatarov" University, Bourgas, Bulgaria

Aim: The primary objective is to analyze employment, job-seeking, work habits and preferences of students in pharmaceutical specialty in higher education institutions in Bulgaria. The secondary objectives: To analyze employment opportunities, legal assessment, analyze available practices, identify the main problems in reconciling learning with work.
Materials and methods: We conducted a pilot study in two centers in Bulgaria. The data collection was done using the "direct individual poll" method. The questionnaire containing 28 questions - open and closed type. Target group is 130 students - pharmacy students from "Sveti Kliment Ohridski" Sofia University, Faculty of Chemistry and Pharmacy and bachelor-pharmacists from the Medical College, "Prof. Dr. Assen Zlatarov" University, Bourgas. The data are processed with the SPSS software.
Results and discussion: The pilot study proves that the majority of pharmacy students work during their studies - 86.4%. The main incentive to start work is financial. 56.8% are employed on a labor contract and nearly one third are employed without any contract. In group of assistant pharmacists - 45.3% of respondents work, financial reasons 66.7% are leading again. 86.2% have an employment contract but 67.9% are insured on a minimum wage basis. Mostly, students work in their specialty, in particular to the retailing of medicinal products - as trainees in pharmacies. Students express difficulties in the process of reconciliation of work and training, negative attitude towards the legal framework and a distinctive negative attitude towards the main field of realization for the master pharmacists - the work in the pharmacy.
Key words: Student employment, pharmacy students, assistant pharmacists, pharmacy.
Corresponding author: Konstantin Gergov, Faculty of Chemistry and Pharmacy, "Sveti Kliment Ohridski Sofia University, 1 "James Boucher" blvd., 1164 Sofia, Bulgaria; e-mail: konstantingergov@gmail.com

back to contents

DESIGN, SYNTHESIS, THEORETICAL AND CYTOTOXIC INVESTIGATION OF NEW Pt(II) COMPLEXES WITH 3-METHYL-8-THIA-1,3-DIAZASPIRO[4.5]DECANE-2,4-DIONE

Bakalova A.[1], Buyukliev R.[1], Michailova R.[2], Momekov G.[2], Cherneva E.[1]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
Corresponding author: Adriana Bakalova, tel. +359 897086067, e-mail: adrigebk@abv.bg

The aim of the study: Platinum complexes are still the leading drugs in chemotherapy of cancer. More than thirty Pt(II) and Pt(IV) complexes are in clinical investigation in different stages. The mixed ammine/amine platinum complexes with chlorido ions as leaving groups have demonstrated better antiproliferative activity against cisplatin-resistant cells in vitro and are less toxic than cisplatin. The synthesis of other types of platinum complexes containing different leaving groups, such as iodido ions also showed higher activity than their chlorido analogues and they exceeded the activity of the platinum-based drugs.

Materials and methods: The new compounds have been studied by elemental analyses, IR, ^{1}H and ^{13}C NMR spectral analyses and DFT calculations, using the B3LYP/ LAN2DZ level of theory. The most probable conformations were constructed and optimized. The geometry of the complexes is square-planar. The cytotoxic effects of the compounds were studied vs. the referent antineoplastic agent cisplatin against two human tumour cell lines using the standard MTT-dye reduction assay for cell viability.

Results: The platinum complexes with chemical formulas *cis*-$[PtL(NH_3)Cl_2]$ and *cis*-$[PtL_2I_2]$, where L is 3-methyl-8-thia-1,3-diazaspiro[4.5]decane-2,4-dione were synthesized and studied. The physico-chemical analyses show that the coordination of the ligand with platinum ion is realized through the sulfur atom in the both of complexes.

Conclusion: The platinum complexes showed higher cytotoxic activity on chronic myeloid leukemia K562 and acute lymphoblastic leukemia REH than the ligand but they have lower cytotoxic activity than the reference drug cisplatin.

Acknowledgment: Thanks are due to Medical Science Fund at the Medical University – Sofia (Grant №84/23.04.2019) for the financial support.

back to contents

MATERIOVIGILANCE IN THE NORTH MACEDONIA-REGULATION AND HARMONIZATION

Angelovska B., Drakalska Sersemova E., Stefanovska K.
Faculty of Medical Sciences, ,
"Gotse Delchev" University, Shtip,
Republic of North Macedonia
corresponding author: e-mail: elena.drakalska@ugd.edu.mk

Aim of the study: The purpose of this study is: to analyze the recommendations of the WHO, EU, FDA, PGEU and their implementation in developed countries and RM, to realize the aim of harmonizing the vigilance system, to define the specifics on the classification, the quality standards of materiovigilance that guarantee safety for using the medical devices and legal support that it provides, the opportunities and the degree of harmonization of the vigilance system in Macedonia compared to other countries.
Materials and methods: In order to achieve the set goals, we reviewed relevant literature sources of primary and secondary literature, documents and recommendations of the WHO, EU, FDA, PGEU and specific regulatory authorities for nomenclature, classification, standardization and quality assurance. We made a comparison of the regulation systems of materiovigilance in America, Australia, England, India and Macedonia.
Results and discussion: The obtained results show that there is a high degree of harmonization of regulatory systems of the Republic of Macedonia with other members of GHTF which guarantees a high level of efficiency, security and accuracy, partly limited by the possibility of parallel imports.
Conclusion: Medical devices equally as medicines, are an important segment for normal functioning of the health system and an integral part of pharmacy practice. We conclude that implementation of the system of materiovigilance is in accordance with the recommendations of the WHO, EU and specific bodies established for that purpose.

WHETHER *AMORPHA FRUTICOSA* FRUIT EXTRACT POSSESS GENOTOXIC AND MUTAGENIC EFFECT ON *SACCHAROMYCES CEREVISIAE* OR NOT?

Todorova T.[1], Parvanova P.[1], Dimitrova M.[1], Ionkova I.[2], Krasteva I.[2], Shkondrov A.[2], Mitrovska Z.[1], Kozuharova E.[1], Chankova S.[1]
[1]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia*

Aim of the study: To reveal the potential genotoxic and mutagenic effect of *Amorpha fruticosa* fruit extract.
Materials and methods: Mature fruits of this invasive plant were collected in October 2018 and botanically identified by Ekaterina Kozuharova. Aqueous extract was prepared following a standard procedure. The dried material (300g) was

pulverized, sieved (3mm) and percolated exhaustively with dichloromethane (4.5l). The defatted plant substance was further extracted with 80% methanol (12l), then resulting extract filtered, evaporated on a rotary evaporator and lastly lyophilized.
Cell suspensions (1x107cells/ml) of *Saccharomyces cerevisiae* strain D7ts1 in the end of exponential growth phase were treated with *Amorpha fruticosa* extract for 30 min and Zeocin as a positive control. Seven extract concentrations in the range of 1µg/ml - 1000µg/ml were applied for preliminary evaluation of the potential genotoxicity. Further, 10, 100 and 1000µg/ml were tested by Zimmermman's test using the following endpoints: cell survival for genotoxic and mitotic gene conversion, reverse mutations and mitotic crossingover - for mutagenic effects.
Results: The intensity of the spots was similar among all the samples treated with *Amorpha* and the untreated ones suggesting lack of genotoxicity. Data obtained by Zimmermman's test revealed that the survival after the treatments was comparable to the negative control - untreated cells. No effect was obtained regarding the genetic events - convertant and revertant frequencies as well as total aberrants.
Conclusion: Based on the results presented here, *Amorpha fruticosa* fruit extract at the studied concentrations does not possess genotoxic and mutagenic effect on *Saccharomyces cerevisiae*.
Acknowledgements: This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № D01-230/06.12.2018).

ANXIOLYTIC EFFECT OF THE NEW ANTICONVULSANT LACOSAMIDE COMPARED TO TOPIRAMATE IN THE PILOCARPINE-MODEL OF TEMPORAL LOBE EPILEPSY IN RATS

Shishmanova-Doseva M.[1], Peychev L.[1], Mincheva M.[2], Boyuklieva R.[2]
[1]Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University, Plovdiv
[2]Pharmacy Student, Faculty of Pharmacy, Medical University, Plovdiv

The aim of the study: was to investigate the effect of Lacosamide (LCM) and Topiramate (TPM) on anxiety in naïve rats and in animals subjected to Pilocarpine (Pilo)-induced status epilepticus (SE).
Material and methods: Male Wistar rats were randomly divided into 6 groups (n=8) and SE was evoked by a single administration of Pilo (320 mg/kg) i.p. in half of them. During the next one month all animals were daily treated per os as follows: 1st group (C-veh)–with saline, 2nd Pilo-veh, 3rd (LCM) – LCM 30 mg/kg, 4th (Pilo-LCM) – LCM 30 mg/kg, 5th (TPM) – TPM 80 mg/kg and 6th (Pilo-TPM) – TPM 80 mg/kg. The test was performed in the Elevated-plus maze which consisted of two open and two enclosed arms and a central platform. It was measured (1) number of entries in the open arms; (2) time spent in the open arms; and (3) anxiety index.

Results: Both groups Pilo-LCM and Pilo-TPM had higher numbers of entries in the open arms ($p < 0.01$, $p < 0.05$) and spent longer time in them compared to the Pilo-veh group ($p < 0.001$). LCM animals had also higher numbers of entries in the open arms in comparison with C-veh animals ($p < 0.05$). A lower anxiety index was demonstrated for the groups treated with different anticonvulsants and subjected to status epilepticus comepared to Pilo-veh animals ($p < 0.001$).
Conclusion: Both antivonvulsants LCM and TPM produce anxiolytic effects in a model of temporal lobe epilepsy while LCM decreaces anxiety in naïve rats as well.

A SURVEY OF OPINION AND READINESS TO APPLY SEASONAL FLU SHOT VACCINATION

Hristova H.[1], Getov I.[2]
[1]Sofia university "St. Kliment Ohridski", Faculty of chemistry and pharmacy, Bulgaria
[2]Medical university, Faculty of pharmacy, Sofia, Bulgaria

The aim of the study: The aim of the study is to examine the attitudes of visitors in the pharmacies in regards to the seasonal flu shot vaccination in Bulgaria in relation with the introduction of a national immunization program for people over and above the age of 65.
Materials and methods: For the purposes of the survey we used two methods - a direct individual survey with a questionnaire and a statistical overview. The questionnaire with 10 multiple option-answers was filled by a random selection of respondents - visitors of pharmacies in the period between January to February 2019.
Results: The study found that 81% of the respondents (108 individuals) were not vaccinated during the current season and they don't do it on annual basis.
When presented with the option to vaccinate at the expense of the NHIF, 50% of the interviewed share that they are more likely to be immunized.
The majority of the interviewed say that they place their trust in their GPs about the vaccine, and that if they would need more information they would ask them.
For 61% of the interviewed, the flu shot vaccines are safe, but the main reason that prevents them from being vaccinated is unwanted reactions - 83%.
Conclusion: Individuals in the group of the age of 65 and above are not used to, nor they are positively predisposed towards flu vaccination.
Conclusions on flu vaccine coverage should not be made based on a sales basis.
The opinion of the respondents is equally distributed between the intention to vaccinate at the expense of the NHIF, however the majority agrees to the assumption that this cost should be covered from public funds.
The main reason preventing the respondents of getting a flu shot vaccination is post-vaccination undesired reactions, and the primary and preferred source of information is their GP and then the Magister Pharmacist.

back to contents

ANALYTICAL STUDY OF ACTIVE INGREDIENTS IN MODEL MIXTURES CONTAINING EXTRACTS FROM MEDICINAL PLANTS *CRATAEGUS* SP., *TRIBULUS TERRESTRIS* L. AND *SIDERITIS SCARDICA GRIZEB*

Mitkov S.[1]*, Yordanov D.[2], Najdenov V.[3], Petrova Z.[4], Pencheva I.[5]

[1]"INOLAB" Ltd, "Mladost"1, Sofia 1797, Bulgaria

[2]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Science, 9 "Acad. G. Bonchev" Blvd., Sofia 1113, Bulgaria

[3,4]" Medical Point" Ltd, "Biala Buka" str., Sofia 1220, Bulgaria

[5]Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str.,Sofia 1000, Bulgaria

The aim of the study: The medicinal plants Hawthorn (*Crataegus* sp.), Tribulus (*Tribulus terrestris* L.) and Mountain tea (*Sideritis scardica Grizeb*.) are well-known and have long been used in medicine. They have a beneficial effect on cardiovascular function, antioxidant protection, sexual activity, tone and muscle strength. The subject of the present work is the development of a HPLC procedure for the identification and quantification of pharmacologically active flavonoids and procyanidins in model mixtures of medicinal plants.
Materials and methods: HPLC method with UV detector, model mixtures of medicinal plants.
Results: Tests for identification and assay of the active ingredients contained in mixtures of extracts of *Crataegus* sp.,*Tribulus terrestris* L. and *Sideritis scardica Grizeb*. have been developed. Selectivity, reproducibility, accuracy, linearity, LOD and LOQ were determined.
Conclusion: Validated methods for the identification and assay of biologically active flavonoids and procyanidins in model mixtures of plant extracts are intended to serve in the process of quality control as required by the European Pharmacopoeia.

METHODOLOGICAL AND TECHNOLOGICAL APPROACH TO IMPROVING THE STATISTICAL ACCURACY OF DIAGNOSTIC TEST AND METHODS

Mitov K., Doneva M., Tachkov K.

Faculty of Pharmacy, Medical University, Sofia, Department of Organisation and Economics of Pharmacy

Goal: To present a local version of an automated system for generating, validating, evaluating and archiving exams, for the purposes of educating, professional analysis and socio-cultural education, as well as emphasize the benefits of improving, and objectifying the evaluation process, which would overall lessen the burden.
Materials and methods: A local version of the system was developed with Visual Basic for Excel. Its development was based on the professional experience of the authors, as well as world-wide experience, and the benefits offered by technological advances. To minimize the probability of exam passes to be due to random chance, a function was developed on the basis of the properties of the normal distribution. A

web-version on a local server would allow the integration of a separate module of a validated system in an educational facility to help objectify the exam process.
Results: The local version of the system is an excel file, allowing any computer with MS office to access it. It has a user-friendly interface, whereby the grading scale can be set by the examiner or generated automatically. The function is based on the properties of the normal distribution. This minimizes the possibilities of a successful test to be due to random guessing of answers. Upon completion of the exam, the system offers the possibility of statistical analysis and archiving of results and grades. The system presented here has already been implemented in several departments at the Faculties of Pharmacy and Medicine.
Conclusion: Utilization of a local version of this automated system allows generating, evaluating, grading and archiving grades for the purposes of education, professional analysis and accumulation of knowledge in real-world practice. Its implementation greatly eases the burden of assessing, experienced by educators, and adds objectivity to the process.

CHALLENGES IN THE DIAGNOSIS OF DISEASES THROUGH REFERENCE INTERVALS AND THEIR CHANGES

Tachkov K., Savova A., Mitov K.
Faculty of Pharmacy, Medical University of Sofia, Department of Organisation and Economics of Pharmacy

Goal: To present a local version of an automated system for generating, validating, evaluating and archiving exams, for the purposes of educating, professional analysis and socio-cultural education, as well as emphasize the benefits of improving, and objectifying the evaluation process, which would overall lessen the burden.
Materials and methods: A local version of the system was developed with Visual Basic for Excel. Its development was based on the professional experience of the authors, as well as world-wide experience, and the benefits offered by technological advances. To minimize the probability of exam passes due to random chance, a function was developed on the basis of the properties of the normal distribution. A web-version on a local server would allow the integration of a separate module of a validated system in an educational facility to help objectify the exam process.
Results: The local version of the system is an excel file, allowing any computer with MS office to access it. It has a user-friendly interface, whereby the grading scale can be set by the examiner or generated automatically. The function is based on the properties of the normal distribution. This minimizes the possibilities of a successful test to be due to random guessing of answers. Upon exam completion, the system offers the possibility of statistical analysis and archiving of results and grades. It has already been implemented in several departments at the Faculties of Pharmacy and Medicine.
Conclusion: Utilization of a local version of this automated system allows generating, evaluating, grading and archiving grades for the purposes of education, professional analysis and accumulation of knowledge in practice. Its implementation

back to contents

eases the burden of assessing, experienced by educators, and adds objectivity to the process.

COMPARISON BETWEEN PEDIATRIC AND ADULT SUSPECTED NEUROLOGICAL AND PSYCHIATRIC ADVERSE DRUG REACTIONS(ADRs) OF MELATONIN REPORTED TO THE EUROPEAN MEDICINES AGENCY (EMA) AND US FOOD AND DRUG ADMINISTRATION (FDA)

Bakalov D.[1], Andreeva-Gateva P.[2], Hadjiolova R.[1]

[1]Pathophysiology department, Medical University, Sofia, Bulgaria
[2]Pharmacology and toxicology, Medical University, Sofia, Bulgaria

Aim: We are using the ADR databases of EMA and FDA to compare the distribution of melatonin's neurological and psychiatric ADRs between pediatric and adult population. The results will serve as basis for future research involving melatonin use in specific populations.
Methods: All ADR reports received in EMA and FDA up to 14 may 2019 were analyzed for overall numbers, age and gender. The nature of the ADRs and the most frequently reported drug events were evaluated using Medical Dictionary for Regulatory Activities (MedDRA).
Results: Adult reports of ADRs in were more common than those in children under the MedDRA classification 'nervous system disorders', 'psychiatric disorders'. In pediatric population most common ADRs reported were somnolence, agitation, headache, insomnia and anxiety, and in people over 18 years old – headache, dizziness, somnolence and insomnia. For children, in most of the cases melatonin was reported as used off-label, and even though most of the cases were resolved without sequel, there were few cases reported of un-resolved effects.
Conclusions: The present study focused on the neurological and psychiatric ADR reports of melatonin in some of the largest databases. It confirmed that reports of reactions in children were different to those in adults, not only in terms of reactions but also more concentrated around limited sets of reaction types and drugs. It showed that future research of melatonin in pediatric population is needed, because the current SmPCs lack such information, and in the last few years the cases of melatonin use in children is increasing.
Keywords: melatonin, ADR, children, pharmacovigilance.
This work is supported by the Bulgarian Ministry of Education and Science under the National Program for "Young Scientists and Postdoctoral Students" Research.

back to contents

OLMESARTAN TABLETS – DRUG RELEASE OPTIMAZITION BY GRANULATION IN FLUIDIZED BED APPARATUS

Tzankov S.[1], Ivanova I.[2]
[1]*Faculty of Pharmacy, Medical University, Pleven*
[2]*"Tchaikapharma" High Quality Medicines Inc.*

Olmesartan medoxomil (angiotensin-II-antagonist) is practically insoluble in water drug. This makes a problem with drug`s release from tablets. The micronization of the substance is a widely used method for drug release optimization. A special equipment is needed for the micronization process. The aim of this study is to enhance dissolution rate of olmesartan by a simple method without using special equipment. The literature search suggested the method of dissolved active substance spraying in fluid-bed apparatus as an appropriate tool for this purpose. Most often, during the process, the drug substance is in an amorphous form with high dissolution rate.

The dissolution media phosphate buffer (pH 6,8) was established as discriminative during the studies of drug release in medias with different pH values. It was defined for studding of olmesartan release.

The technological process was developed for granulation in fluid-bed apparatus. The suitable co-solvent, which completely dissolve olmesartan (such as methanol and acetone), and binding polymer (hydroxypropyl cellulose) were chosen. At the same time, granules without dissolving of active substance were produced using conventional technology.

The comparison of drug release from tablets, produced by the both technological methods, demonstrates significant and rapid dissolution rate from tablets produced by dissolving of olmesartan in granulated solution. This dissolution profile is similar to those of the originator Olmetec. The final assessment can be done after the completely evaluation of finished product stability.

In conclusion, the performed experiments prove the possibility for optimization of drug release from Olmesartan tablets by using a simple granulation method in fluid-bed apparatus, without need of other special equipment.

back to contents

DEVELOPMENT AND OPTIMISATION OF THE PROCESS OF DECARBOXYLATION OF TETRAHYDROCANNABINOIC ACID, FOLLOWED BY FTIR DETECTION OF TETRAHYDROCANNABINOL IN MEDICINAL CANNABIS

Cocovska I.[1,2], Demirovski Kockova K.[1], Trajanovska Faizova V.[1], Maksimova V[2].

[1]Oaza Alakaloidi, Shtip, Gladno pole, bb, Tarinci, POB 2000, Shtip, Republic of North Macedonia,

[2]"Gotse Delchev" University, Krste Misirkov, bb, POB 2000, Shtip, Republic of North Macedonia

Aim of the study: Conversion of tetrahydrocanabinoic acid (THCA) to tetrahydrocannabinol (THC), is the main reaction essential for the use of cannabis, *Cannabis indica* L. buds in medicinal purposes. Therefore, the aim of this study was to develop and optimize the thermal treatment of Cannabis buds, required for the reaction of decarboxylation of THCA to THC followed by FTIR technique.
Materials and methods: Cannabis buds were heated on aluminum shelves, programmed by heating oven POL EKO SL 400. Temperature program was strictly monitored and changes were recorded on every 5 minutes. Mid Infrared spectra were collected for each sample and temperature, respectively, using Perkin Elmer Spectrum Two™ coupled to an UATR accessory in spectral range of 400-4000 cm-1.
Results: Changes in the infrared spectra indicated that the required elimination of water and appropriate conversion of THCA to THC were established, as the reaction progressed. Reaction of decarboxylation has occurred in temperature range from 85-120° C. Conversion of THCA to THC contributed for obtaining of high concentrations of THC which ranged 38-80% *w/w*. The progress of the decarboxylation enhanced the obtaining of THC.
Conclusion: Monitoring of the process of decarboxylation with FTIR technique presents a fast and plausible method for controlling the conversion of acidic forms of cannabinoids to their neutral forms. The thermal treatment has shown a high yield of THC up to 80% w/w. It should be stressed out that these two methods could be ideally suited to everyday analysis because of their high performances.
Key words: cannabinoids, *Cannabis indica*, decarboxylation, FTIR

TECHNOLOGICAL APPROACHES FOR EXTEMPORANEOUS PREPARATION OF ORAL PEDIATRIC FORMULATIONS WITH SILDENAFIL CITRATE

Dimitrov M., Borinarova M.

Faculty of Pharmacy, Medical University, Sofia

e-mail: mdimitrov@pharmfac.mu-sofia.bg, tel. +35929236526

The aim of the study: The aim of the study is to analyze and critically evaluate potential technological approaches for extemporaneous preparation of pediatric oral formulations containing sildenafil citrate for pediatric personalized therapy.

back to contents

Materials and methods API: Oral pediatric formulations with sildenafil citrate based on active substance-raw material (API) and by dispensing of pre-formulated model tablets were prepared with utilization of suitable carriers.
Results: Literature search on the possibilities and limitations for preparation of oral sildenafil citrate formulations for pediatric practice have been collected and analyzed. Various approaches to the extemporaneous preparation of oral formulations with sildenafil citrate have been applied and evaluated. The main factors influencing the stability of the prepared formulations have been investigated and evaluated.
Conclusion: All oral formulations tested remained unchanged with respect to the appearance at selected storage conditions: at room temperature of 25° C ± 5° C and 5° C ± 2° C in the refrigerator within the design of the experiment. The quantitating content of sildenafil citrate in all prepared oral formulations remains above 95% w / v. The microbiological quality of the formulated preparations as well as the stability after the first opening of the package within 10 consecutive days for the water-carrier formulations and 14 days for the formulations with sugar or fructose syrup were confirmed.

DEVELOPMENT OF MESOPOROUS SILICA PARTICLES AS A DRUG DELIVERY SYSTEM FOR QUERCETIN

Voycheva C., Tsankov B., Popova T., Spassova I., Kovacheva D., Yoncheva K., Lambov N.
Faculty of Pharmacy, Medical University, Sofia

Purpose: The flavonoid quercetin is widely studied for its antioxidant and anti-inflammatory properties. However, poor water solubility, low stability and short half-life may limit its use in therapy and skin care products. The purpose of this work is exploring the possibility of influencing the process of dissolving quercetin, through its inclusion in two types of mesoporous silica nanoparticles, in particular MCM-41 and HMS.
Materials and methods: The loading of both particle types with quercetin was determined by thermogravimetry. The basic physico-chemical characteristics of the particles, such as size, polydispersity index and zeta-potential, were determined. Their specific structure was examined using transmission electron microscopy. In vitro dissolution tests was performed in buffer with pH 5.5 (Apparatus 5 Paddle, USP 37), in view of the subsequent incorporation of the systems into a dosage form for topical administration.
Results: The results of the dissolution tests showed differences in the quercetin release profiles of the two particle types. HMS particles release quercetin significantly slower than MCM-41 particles, in particular the dissolution rate is twice as low as that seen with MCM-41. However, in both cases, a higher dissolution rate was established when quercetin was included in the mesoporous particles, compared with dissolution of unencapsulated quercetin.
Conclusion: The inclusion of quercetin in mesoporous silica nanoparticles could be an alternative approach to solving problems with its dissolution.

back to contents

EFFECTS OF ANTHOCYANINS^{R} ON FRUCTOSYLTRANSFERASE Lm17 ACTIVITY

Bodlev S., Vasileva T., Iliev I.*, Nikolova M.

Department of Biochemistry and Microbiology, Faculty of Biology, "Paisii Hilendarski" Plovdiv University, 24 "Tzar Assen" str., 4000 Plovdiv, Bulgaria

**Correspondence author: e-mail: ilievini@abv.bg, +35932261479*

The aim of the study: The aim of the present study was to investigate the effect of different anthocyaninsR on the enzyme activity of fructosyltransferase Lm17.

Material and methods: A spectrophotometric method for detecting enzyme activity was used as well as a HPLC assay to determine the type and concentration of anthhocyanins^{R}.

Results: When using the anthocyanins delphinin-3-galactoside, delphinine-3-glucoside and cyaniding-3-glucoside, as well as anthocyaninsR as acceptor of glycosidic molecules and sucrose as a donor, the rate of the transferase reaction of fructosyltransferase Lm17 is monitored. Ratios between the acceptor (anthocyaninsR) and the substrate are used, varying very widely from 1:80 to 1:1. More than 85% inhibition of fructosyltransferase Lm17activity is achieved by using an A / S ratio of 1: 2.5, the acceptor being a mixture of the delphinidin-3-galactoside, delphinidin-3-glucoside and cyanidin-3-glucoside =1.3:1.3:1 and 5% sucrose. Fifty percent inhibition of enzyme activity is reached at a ratio of 1:4 and a 2% substrate output concentration and an acceptor combination of delphinin-3-galactoside, delphinine-3-glucoside and cyanide-3-glucoside.

Conclusion: The study found that the enzyme fructosyltransferase Lm17 can successfully be used to modify the structure of anthocyanins^{R} (a mixture of delphinin-3-galactoside, delphinine-3-glucoside and cyaniding-3-glucoside) that could show new biological activities. This could be critical to improve their pharmacokinetic parameters.

Acknowledgements: The authors express their gratitude to the National Science Fund, Bulgarian Ministry of Education and Science for the financial support in this study made in connection with the project DM16/2.

back to contents

PHENOLIC COMPOUNDS WITH RADICAL-SCAVENGING, ANTI-α-GLUCOSIDASE AND PROLIPASE ACTIVITIES FROM THE AERIAL PARTS OF *HYPERICUM CERASTOIDES* (SPACH) N. ROBSON

Kokanova-Nedialkova Z., Nedialkov P., Ilieva Y.
Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

The aim of the study: Seven phenolic compounds (coumaroylquinic acid 1, myricetin-3-O-glycoside 2, myricetin-3-O-galactoside 3, hypercerastoside A 4, methyl ester of chlorogenic acid 5, hypercerastoside B 6 and hypercerastoside C 7) isolated from the aerial parts of *Hypericum cerastoides* (Spach) N. Robson. were tested for radical-scavenging, anti-*α*-glucosidase and prolipase activities.
Materials and methods: DPPH and ABTS methods were used for the determination of radical-scavenging activity. Anti-*α*-glucosidase and prolipase activities were established by LC-MS.
Results: The highest DPPH activity possessed compounds 2 (84.32%), 3 (82.70%) and 5 (84.57%), compared to the radical-scavenging activity of Vit C (59.44%) and Trolox (88.33%). All compounds displayed ABTS activity. The highest activity possessed the glycosides of myricetin respectively compounds 2 (97.23%) and 3 (96.04%). Their radical-scavenging activity were higher than that of Vit C (66.21%) and Trolox (94.16%). The results from the *in vitro* investigations showed that compounds 1 (IC_{50} = 44 μM), 3 (IC_{50} = 206 μM) and 6 (IC_{50} = 371 μM) possessed similar activity to acarbose (Glucobay)(IC_{50} = 206 μM), a known anti-diabetic drug used to treat diabetes mellitus type 2. Five of the seven isolated compounds, respectively substances 1-3, 5 and 6, exhibited prolipase activity. The highest activity was expressed by the myricetin glycosides, compounds 2 and 3, which stimulated the activity of the enzyme lipase extensively five times when were applied at a concentration of 200 μM.
Conclusion: The research of the aerial part of *Hypericum cerastoides* confirmed the scientific hypothesis that the title plant contains phenolic compounds with radical-scavenging, anti-*α*-glucosidase and prolipase activities.
Acknowledgements: This work was supported by the Council of Medical Science of Medical University of Sofia.
(Grand № D-75/03.05.2018).

BASIC STUDY OF PHARMACEUTICAL BIOECONOPHYSICAL ASPECTS OF DRUG ADMINISTRATION

Petrov M., Petrova V.
"Galantus" Pharmacy, Bourgas
0892230778, e-mail: galantusgratus@abv.bg

Aim of the research: The modern stage of the development of pharmacy and medicine in general involves a massive integration of different scientific directions into a new scientific integrative system such as pharmaceutical bioeconophysics [1],

[2] which is an integrative scientific system of pharmacy, biophysics, human physiology and the economical sciences. In accordance with this new concept, the aim is the study of the obtaining of a quantitative law of the cost of one mole of drug substance in interaction with different types of organ receptors and this law contains such parameters as molar mass, half-life time, the mass of drug's form (tablets, capsule, solution, etc.), with the application of the microeconomical econophysical model of the "ideal gas" [3] and that could serve as an estimation of the cost of one mole of new synthesized molecules.

Means and methods: The drugs that enter the human stimulate certain receptors, ion channels, act on enzymes or transporters proteins. As a result, they cause the human body to react in a specific way by creations of complexes formed of receptors and drugs molecules or other products of chemical reactions. The human being is a complex biological system that can be studied by biophysical methods and interacting with social and economical systems of societies by bioeconophysical methods respectively [1], [2].

Generalizing what has been said above, we can conclude that ***pharmaceutical bioeconophysics can be defined as a new multidisciplinary philosophical scientific orientation that studies the general laws of the evolution of the economical processes of pharmaceutical supply for the maintaining of social and physiological healthy equilibrium state of Human being by the application of physics - mathematical and statistical methods of the integrative system of philosophical, biophysical and pharmaceutical sciences.***

The econophysical studies that include the principle of Pareto were reflected in [3] which shows that each stock article of pharmaceutical products is characterized by so-named econophysical temperature that represents the capacity of the generating power of average turnovers (average revenues) during one day of one stock article and respectively for each rating marketing groups A, B, C, X, Z of the stocks these values of temperatures are K_A=21; K_B=13; K_C=8; K_X=5; K_Z=3 that coincide with the numbers of Fibonacci which stay on the basement of so-named "Golden ratio" of Nature's structures, biological structures and economical systems. [3], [4], [5]. The studies of [3] apply the physical model of the "ideal gas" of the pharmaceutical stocks and this model is related to the marketing state in the conditions of hyper competition. The sold and reserve inventory of stocks is described by the equation of marketing state [3]:

$$< P >_p \, N_{tot} = N_{art} \cdot K \qquad (1)$$

here $< P >_p$ is the average price of one pharmaceutical product; N_{tot} - total amount of products of the inventory; N_{art} - total amount of the names of articles; K - the value of econophysical temperature and for the full big ensemble of stocks this value is $K = 5{,}65$ which is calculated on the base of K_A=21; K_B=13; K_C=8; K_X=5; K_Z=3 by the consideration of the peculiarities of ABC analysis and this value $K = 5{,}65$ is a worldwide constant that is independent on national currencies [3]. The value $K = 5.65$ is such a constant that describes the equilibrium of the system, stability and harmony by statistical point of view of big ensemble of constituent components of the system.

back to contents

This equation of the marketing state (2) has a best analogy with the equation of Mendeleev – Clapeyron of the state of ideal gas and this equation has a profound deep meaning and could stay to the basis of analyzes of the administered doses of the drugs.

Econophysics operates with economical and social structures that carry out activity and this activity is described specifically by this equation of state of "ideal gas" [3]. Referring to biological structures such as the human being and the drug administration, then this equation can be prescribed as:

$$K \cdot N_{art} = < P >_m \cdot N \qquad (2)$$

where N is the total amount of administrated drug's molecules; $< P >_m$ is the average price of one molecule; N_{art} is the amount of various types of drug's articles approximately administrated at the same moment of time; $K = 5.65$. The case when one article is administrated then $N_{art} = 1$. This equation (2) could be stated as the equation of pharmaceutical bioeconophysical state of Human being.

As a result of administration of doses a series of biochemical processes between molecules of the drug and receptors take place. According to the thermodynamical laws of the conservation of energy a work is performed. This work is the emitted energy as the result of chemical reactions between receptors and drug's molecules.

The first law of thermodynamics is known as such formulation: It is impossible to have a thermal machine in the first kind, the unique result of which is the production of work without the consumption of energy from external environment [3]. Transposed to bioeconophysics this formulation has such explanation: The human being will not resist statistically for a long time basing only on the proper internal energy. Finally the internal energy will be consumed and new energy as the form of food or drugs (if it is necessary drugs) is necessary to maintain the equilibrium. The first law of thermodynamics quantitatively is written as:

$$\Delta U = Q - A \qquad (3)$$

The variation of the internal energy of the system ΔU is the result of the heat Q transmitted to the system and the work A done by the system. The drug and receptor interaction occurs at approximately constant temperature and the process can be considered isothermal process. The respective work A' that is within the limits of activation energies of receptors by the creation of complexes of ligands with receptors is considered as the performed work upon the system at isothermal process is calculated as:

$$A' = -A = -\int_{N}^{N_{1/2}} < P > dN = K \cdot N_{art} \cdot \int_{N_{1/2}}^{N} \frac{dN}{N} = K \cdot N_{art} \cdot \ln 2 \qquad (4)$$

here $N_{1/2}$ is the amount of molecules corresponding to the moment of half-life time. A biological half-life or elimination half-life is the time it takes for a substance (drug, radioactive nuclide, or other) to lose one-half from initial amount of molecules of its pharmacologic, physiologic, or radiological activity. In a medical context, the half-life may also describe the time that it takes for the concentration of a substance in blood plasma to reach one-half of its steady-state value (the "plasma half-life").[6]

The formula of half-life time of elimination [7] is: $$T_{1/2} = \frac{\ln 2}{\lambda} \qquad (5)$$

Here λ is drug's elimination rate constant.

Substituting (5) into (4), then: $A^{/} = K \cdot N_{art} \cdot \lambda \cdot T_{1/2}$ (6)

On the other hand the value $A^{/}$ represents the total energy of all receptors. The total amount of all receptors on cell's surface is $N_{tot_{rec}}$ and the energy of one receptor is $< E >_{rec}$, then the total energy is $A^{/} =< E >_{rec} \cdot N_{tot_{rec}}$ and substituting in (6) will give:

$$< E >_{rec} \cdot N_{tot_{rec}} = K \cdot N_{art} \cdot \lambda \cdot T_{1/2} \quad (7)$$

Substituting (2) into (7) will give: $< E >_{rec} \cdot N_{tot_{rec}} =< P >_{m} \cdot N \cdot \lambda \cdot T_{1/2}$ (8)

Finally, the price of one molecule is: $< P >_{m} = \frac{< E >_{rec} N_{tot_{rec}}}{N \cdot \ln 2}$ (9)

The total amount *N* of administrated molecules can be expressed by the amount of moles ν

$$\nu = \frac{N}{N_A} = \frac{m_{form}}{M} \quad (10)$$

here m_{form} is the mass of one tablet, or one capsule, etc.; *M* – the molar mass; N_A – number of Avogadro. Then: $N = \frac{m_{form}}{M} \cdot N_A$ (11)

Substituting (11) into (9) will give: $< P >_{m} = \frac{< E >_{rec} N_{tot_{rec}} \cdot M}{m_{form} \cdot N_A \cdot \ln 2}$ (12)

The respective expression for the price of one mole $< P >_{mole} =< P >_{m} \cdot N_A$

$$< P >_{mole} = \frac{< E >_{rec} N_{tot_{rec}} \cdot M}{m_{form} \cdot \ln 2} \quad (13)$$

The expression (13) shows that if the molar mass is increased then the price of one mole is increased at the constant value of $m_{form} = const$ and respectively for *M=const* the price of one mole is decreased if the mass m_{form} is increased. The aim is how to know or to find the values of $< E >_{rec}$ and $N_{tot_{rec}}$. Exact values for each parameter $< E >_{rec}$ and $N_{tot_{rec}}$ will not be found, but the factor $< E >_{rec} N_{tot_{rec}}$ is found by the dependence of $\ln(< P >_{mole}) = f\left(\ln\left(\frac{M}{m_{form} \ln(2)}\right)\right)$ that is a linear dependence. So:

$$\ln(< P >_{mole}) = \ln B + \ln\left(\frac{M}{m_{form} \ln(2)}\right) \quad (14)$$

here $B =< E >_{rec} N_{tot_{rec}}$.

back to contents

Results:

1	2	3	4	5	6	7	8	9	10	11	12
name of product	Molar mass; gr/mole	Mass of one molecule; gr	Mass of drug's form	Amount of molecules in one drug's form	Price of product	Price of one drug's form	Price of one molecule	Amount of mole in one form	Price of one mole	logarithm of column 9	logarithm of column 10

The following table it is necessary to fill in the data as follows:

The graphic of the price of one molecule as a function of amount of molecules in one drug's form is represented in Fig. 1, a . The same similar dependence is for the price of one mole as a function of amount of moles (Fig. 1, b).
These dependences of Fig. 1 as if imitating the microeconomic dependencies of demand, the lower prices the demand is bigger and vice versa.

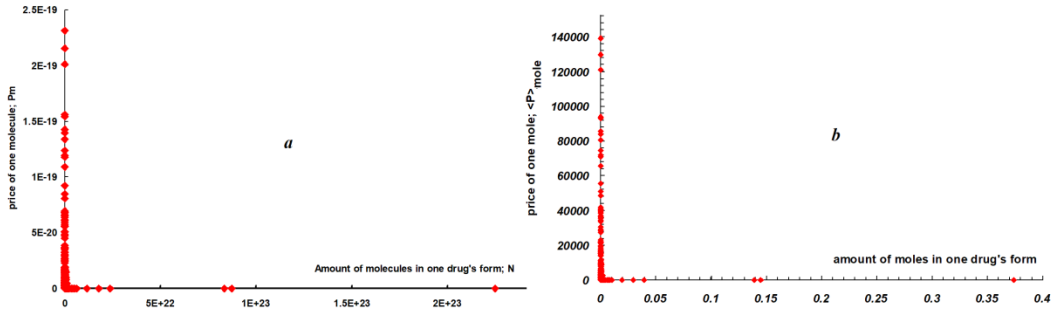


Fig. 1 The dependence of the price of one molecule as a function of amount of molecules in one drug's form (a) and similar for moles (b)

The main aim is to check the expression (14). The respective graphic of the expression (14) is presented on Fig. 2. The value of $\ln B = -2.5452 \Rightarrow B = e^{-2.5452} = 0.078$

So: $B = < E >_{rec} \cdot N_{tot_{rec}} = 0.078$

If the total amount of receptors is of order $10^{20} \to 10^{25}$ then the energy of activation of one receptor is of such order: $< E >_{rec} = 12.82 \cdot 10^{-20} \to 12.82 \cdot 10^{-25} (joule)$.

The respective value of energy for one mole of receptors is:

$E_{mole} = < E >_{rec} \cdot 6.02 \cdot 10^{23} = 12.82 \cdot 10^{-20} \cdot 6.02 \cdot 10^{23} \approx 76.92 \cdot 10^{3} (Joule) \approx 77 (kJ)$ that is of order of data presented in [8].

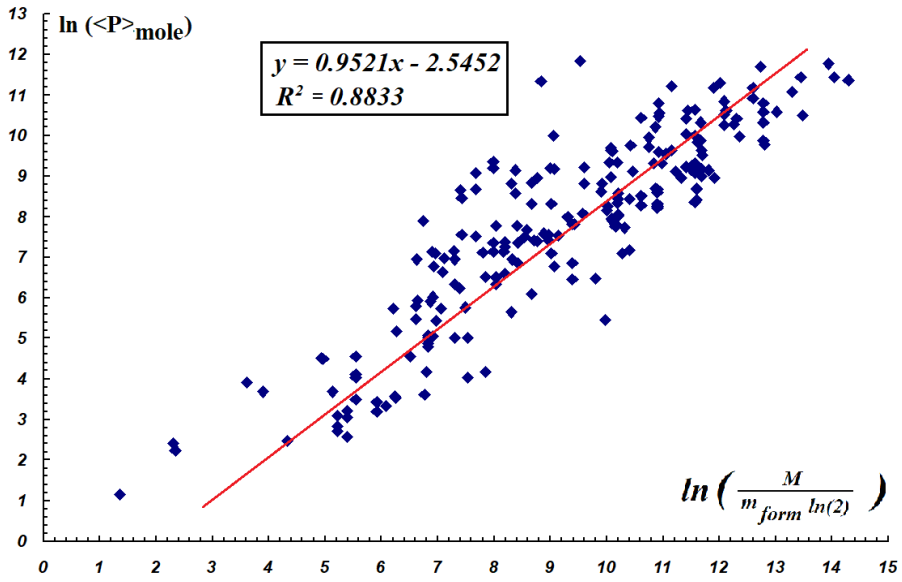


Fig. 2 The dependence of $\ln(< P >_{mole}) = f\left(\ln\left(\frac{M}{m_{form}\ln(2)}\right)\right)$.

So, the expression (13) will be written simply as:

$$< P >_{mole} = \frac{< E >_{rec} N_{tot_{rec}} \cdot M}{m_{form} \cdot \ln 2} = \frac{0.078 \cdot M}{m_{form} \ln 2} = \frac{0.1132 \cdot M}{m_{form}} \qquad (15)$$

The expression (15) could be written also by the coefficient $B_0(t)$ that in general depends in time because of the evolution of prices each five-seven years.

$$< P >_{mole} = \frac{B_0(t) \cdot M}{m_{form}}; \quad B_o(2019) = 0.1132 \qquad (16)$$

For example, metamizole natrium with molar mass 333.34 gr/mole and the tablets with 500 mg, then:

$$< P >_{mole} = \frac{0.1132 \cdot M}{m_{form}} = \frac{0.1132 \cdot 333.34}{0.5} = 75.46(BGN)$$

If the aim is to estimate the cost of one gram, then the expression (16) will be divided by the molar mass M, and: $< P >_{gr} = \frac{B_0(t)}{m_{form}}$.

For this example of metamizol natrium the calculation of the price of one gram gives:

$$< P >_{gr} = \frac{B_0(t)}{m_{form}} = \frac{0.1132}{0.5} = 0.22(BGN).$$

Another example, cetirizine dihydrochloride of the tablets Zyrtec 10 mg with the molar mass 388 gr/mole and the tablets with 10 mg, then:

$$< P >_{mole} = \frac{0.1132 \cdot M}{m_{form}} = \frac{0.1132 \cdot 388}{0.01} = 4392.16(BGN)$$

The respective cost of one gram of cetirizine dihydrochloride

$$< P >_{gr} = \frac{0.1132}{m_{form}} = \frac{0.1132}{0.01} = 11.32(BGN)$$

Conclusions: This young bioeconophysical science puts forward cardinal tasks of in-depth study with the integration of different separate scientific fields. It seeks ways to explain the role of man in the complex social and economic system as well as not only internal but also external factors that influence the state of human health. The pharmacokinetics and pharmacodynamics of the administered doses have an integration initiation in this new scientific system, which gives an appreciation not only for drug interaction with the respective receptors, but also for a way of estimating the cost and what is the possible duration of time of prophylaxis and treatment could be. It is a possibility to operate with the energy, energy of activation of the receptors of cell's membranes and to include into the quantitative laws of description. Money also is the form of energy in econophysics and somehow it is related to the processes with production of pure physical energy. The alive systems step by step are described not only by biophysical methods but also by bioeconophysical ones operating with the first law of thermodynamics that in this paper the quantitative expression of first law of thermodynamics for human being somehow includes parameters as half – life time, energy of activation of receptors, prices of molecules, etc. Usually, the modern tendency of econophysics is to incorporate in one quantitative expression a lot of parameters from various parts of researched methods that have some logical interconnection.

Like any other science that always undergoes transformation during history, bioeconophysics remains as an open way of discussion and performance of suggested models.

Bibliography:

1. Jurgen Mimkes, (2016), "Bio-econo-physics: Synthesis of Natural and Social Sciences?" The 7th Biophysical Economics Conference at the University of District of Columbia, Washington DC.

2. Yuichi Tei, Gheorghe Savoiu, 2018. "From Bioeconomics to Bioeconopysis in the Context of (Bio)Diversity and Modern Morality, "The Audit Financiar journal, Chamber of Financial Auditors of Romania, vol. 20(49), pages 754-754, August.

3. Mihai Petrov, "The Thermodynamical Processes of the Model of Ideal Gas for the Description of the Activity of the Microeconomical Systems", European Journal of Business and Management, Vol 10, No 26 (2018).

4. Jason Marshall, "What is the Golden Ratio and how is it Related to the Fibonacci sequence?" May 5, 2010, https://www.quickanddirtytips.com.

5. Justin Kuepper, "Fibonacci and the Golden Ratio", May 25, 2019, articles/technical/04/033https://www.investopedia.com/104.asp.

6. "Drug half life", https://www.drugs.com/article/drug-half-life.html.

7. Seng Kok-Yong and Lee Lawrence , "Drug Distribution and Drug Elimination", http://dx.doi.org/10.5772/59929.

8. Deliang Chen, Numan Oezguen, Petri Urvil, Colin Ferguson, Sara M. Dann, Tor C. Savidge,
"Regulation of protein-ligand binding affinity by hydrogen bond pairing", Science Advances 25 Mar 2016:Vol. 2, no. 3, e1501240, DOI: 10.1126/sciadv.1501240.

back to contents

INFLUENCE OF PHARMACEUTICAL CARE AND PROFESSIONAL PHARMACISTS' CONSULTATION OF THE PATIENT

Kachulev K.[1], Simova T.[2], Enchev D.[3]
[1]Faculty of Public Health at Sofia Medical University
[2]TSC Directorate, Bulgarian Drug Agency
[3]Faculty of Public Health, Medical University, Sofia

Aim of the study: To investigate and evaluate the influence of the pharmaceutical care and the professional pharmacist's consultation on the patient. Patients' and pharmacists' interviews with subsequent comparison of the results.

Materials and methods:

Patient poll:

From 10.2016 until 01.2017 a cross-sectional study of patients was conducted through a survey (609 questionnaires).

A qualitative criterion for selecting respondents in the study is to meet the definition of a patient or to use prescription or OTCs purchased in the pharmacy.

Pharmacists' Survey:

A cross-sectional study was conducted using a rapid data collection method - 15 days across the country through one of the drug distributors in Bulgaria.

Results: The largest percentage of patients (42%) reported that their pharmacists spent between 3 and 6 minutes for counseling. 75% of patients said they had access to pharmacist's care. 72.41% (441 out of 609) from the respondents have access to a pharmacy and access to pharmaceutical care.

The pharmacists can spend an average of 8.32 minutes for the patient's pharmaceutical care. More than half of the pharmacists say that doctors send their patients to their pharmacies for pharmaceutical advice and care, which is a good trend.

Conclusion: There is a difference between the concept of pharmaceutical care and the routine work of the pharmacists, but the time and the attention that pharmacists spend on the patients' problems is a good start for the introduction of the concept into practice. However, the time that the pharmacists can spend, at this stage, is extremely inadequate.

For correspondence: Konstantin Kachulev, +359 878 287 705,
e-mail: kkachulev@gmail.com

back to contents

PILOT STUDY OF THE ATTITUDES OF MASTER PHARMACISTS AND PATIENTS TO THE APPLICATION OF PHARMACEUTICAL CARE IN REAL PHARMACEUTICAL PRACTICE

Georgiev S., Asipova N., Lazarova G., Kadiev A., Andreevska K., Hristov E.
Faculty of Chemistry and Pharmacy,
"Sveti Kliment Ohridski" Sofia University, Bulgaria

Purpose: In Bulgaria, pharmaceutical care is not regulated by legislation as part of the pharmaceutical profession, but it is included in pharmacy curricula. The primary objective is to evaluate patients 'and pharmacists' attitudes about the need for pharmaceutical care. Secondary aims are to assess the level of knowledge of pharmaceutical care and their practical relevance.
Materials and methods: Pilot survey conducted among master pharmacists and patients in Bulgaria. We developed a questionnaire containing 16 questions in different directions - defining the term pharmaceutical care, target patient groups, disease groups, specific pharmaceutical activities. We processed the results with descriptive statistical methods.
Results: discussion and conclusion. The study included more than 300 respondents (pharmacists and patients) between 18 and 75 years old. Respondents are stratified by demographic indicators and availability of medical education. A share of 2/3 of the patient group considers that there is a need for pharmaceutical care, most often for patients with chronic illness, cardiovascular disease, endocrine disorder (diabetes), respiratory disease, patients in elderly and others. There is a predominant view that patients should not pay for the care they receive from pharmacists, although about 60% of respondents say that receiving pharmaceutical care determines which pharmacy they visit. Approximately 30% of the surveyed leading factor remains the price of medicines. Some of the pharmacists surveyed are restrictive to the provision of additional services if there is no adequate funding from the state or the NHIF.

THE HIGH TECH IN STRESS MONITORING AND MANAGEMENT

Manasiev E., Grigorov E., Kochev S.
NCPHA, Medical University, Varna

This study aims to find the possibility to include the high tech in the measurement of the stress level. The general assumption is that the stress is more like negative issue, which blocked, lead to discomfort and the various pathological diseases. It may be concluded that more of activities are related with the programs for stress reduction and in general it avoidance. On the other hand some new studies show that the stress is the single solution for the enhancement. In this topic the studies are mainly related with the subject which is visible easy to be measured like music, sports, some form of arts. There was just few or nothing related with the working daily routine.
In our study we made research in the current practice in stress measurement and was looking for different solutions where the high tech was included. Along with the

standardized tests for stress measurement we researched some more high tech method including biofeedback sensors for body temperature, heart rate, heart rate variability, electromagnetism of the different body parts, blood and monitoring of hormones like cortisol and adrenalin.
We also tested with biofeedback wrist bands a team of professional athletes where we were able to provide feedback when the optimal stress level is achieved and to able to manage it.
Regarding the research and the experiments we came to conclusion that there is a way for the broad study to be made with the topic how we can managed and control the level of stress with the new high technology and machine learning for some activities.

PREPARATION OF COMPLEX CHITOSAN-ALGINATE NANOPARTICLES LOADED WITH EXTRACT FROM *ASTRAGALUS GLYCYPHYLLOS* AND INVESTIGATION OF ANTITUMOR EFFECT ON GRAFFI TUMOR-BEARING HAMSTERS

Georgieva D.[1], Kostova B.[1], Krasteva I.[2], Shkondrov A.[2], Georgieva A.[3], Toshkova R.[3]

[1]1Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[3]Bulgaria Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, 25 "Acad. G. Bonchev" Str., 1113 Sofia, Bulgaria
**Dilyana Georgieva: diljana1977@abv.bg, phone number 02 9236 571*

Aim of study: The aim of the present study was to obtain complex chitosan-alginate nanoparticles (NP) loaded with defatted extract from *Astragalus glycyphylos* L. and to assess their antitumor effect on Graffi tumor-bearing hamsters.
Materials and methods: NP were prepared by adding the extract into a chitosan solution in 1% acetic acid under mechanical stirring for 24 hours, after that adding dropwise Tripolyphosphate pentasodium (TPP) solution. A solution of sodium alginate was used to coat the chitosan NP with alginate. The samples were left at room temperature for 24 h until complete formation of the NP. In order to demonstrate the antitumor effect several biometric markers were studied, such as transplantability (%), survival rates (%), mean survival time, mortality (%), and tumor size (mm).
Results: Extract-loaded NP were obtained by a previously developed method. Conducted *in vivo* experiments showed antitumor activity. Lower mortality and prolonged mean survival time were observed. Reduction of the rate of growth as well as the size of the tumors was established.
Conclusions: NP with *A. glycyphylos* were prepared and their antitumor potential tested. The applied experimental therapy with the complex chitosan-alginate NP

back to contents

showed protective effect expressed in delayed tumor growth and prolonged mean survival time. This is a basis for further research on improving the obtained systems.

ESTIMATION OF MOLECULAR PROPERTIES OF DIHYDROXYBENZALDEHYDE BASED HYDRAZONES

Nikolova-Mladenova B.[1]*, Ivanov D.[1]
[1]Department of Chemistry, Faculty of Pharmacy, Medical University, 2 "Dunav" str., 1000 Sofia, Bulgaria
**e-mail: borianank@abv.bg*

Hydrazones of the type R'-CH=N-NH-CO-R obtained by the condensation of aromatic aldehydes and various hydrazides display a broad range of pharmacological properties

The aim of the study: The current study reports in silico evaluation of molecular parameters and biological activity of 2,3-dihydroxy-, 2,4-dihydroxy- and 2,5-dihydroxybenzlaldehyde based hydrazones. The important molecular properties were calculated to reveal how the presence and positions of two OH-groups affect the lipophilicity of the compounds.

Materials and methods: The hydrazones were designed by inserting of two OH-groups at different positions in aldehyde moiety and various hydrazide moieties. The molecular properties of the compounds, important for drug pharmacokinetics in the human body, were assessed by method based on group contribution. The appropriate molecular parameters include molecular weight, lipophilicity, hydrogen bond donors/acceptors, solubility and other related properties.

Results: All dihydroxy-substituted hydrazones are small flexible molecules with balanced lipophilicity which suppose good permeability of the compounds over the cellular plasma membranes.

Conclusion: The obtained results revealed that all compounds are potential candidates for future drug discovery study.

Acknowledgements: Thanks are due to Medical Science National Fund at the Medical University – Sofia (Grant D-83/23.04.2019) for the financial support.

SYNTHESIS AND CYTOTOXIC ACTIVITY OF DIHYDROXY-SUBSTITUTED HYDRAZONES

Nikolova-Mladenova B.[1]*, Momekov G. [2]
[1]Department of Chemistry, Faculty of Pharmacy, Medical University, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, 2 "Dunav" str., 1000 Sofia, Bulgaria
**e-mail: borianank@abv.bg*

Aroylhydrazones obtained by condensation of aromatic aldehydes and hydrazides show diverse biological activities including a high anticancer activity. Numerous

hydrazones with different substituents have been synthesized in order to discover new active compounds.
The aim of the study: The study reports synthesis, spectral characterization and pharmacological investigations of 2,3-dihydroxy-, 2,4-dihydroxy- and 2,5-dihydroxy-benzlaldehyde based hydrazones.
Materials and methods: The hydrazones were prepared by the condensation between 2,3-dihydroxy-, 2,4-dihydroxy- and 2,5-dihydroxybenzlaldehyde and different hydrazides in ethanol. The compounds were characterized by elemental and thermo-gravimetric analysis, IR, NMR and mass spectroscopy. The cytotoxic activity was assessed using the MTT-dye reduction assay on leukemic and cancer human cell lines.
Results: The molecular formulas of the hydrazones were determined on the basis of the elemental, thermo-gravimetric and mass analyses. The IR, ^{1}H-NMR and ^{13}C-NMR spectral data confirmed the structures and proved the formation of hydrazone bond. The bioassay results demonstrated that the compounds exhibit concentration-dependent cytotoxic effects at low micro molar concentrations. The obtained IC_{50} values were lower than these of the referent cytotoxic drug melphalan.
Conclusion: The results revealed that compounds are potential candidates for future drug discovery study.
Acknowledgements: Thanks are due to Medical Science National Fund at the Medical University – Sofia (Grant D-83/23.04.2019) for the financial support.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TINIDAZOLE AND SOME PROTON PUMP INHIBITOR

Smerikarova M.[1], Bozhanov S.[1], Maslarska V.[1]
[1]Faculty of Pharmacy, Department of Chemistry, Medical University, Sofia

Aim of the study: A reversed phase high performance liquid chromatographic method was developed and validated for the determination of Tinidazole, Esomeprazole and Lansoprasole in synthetic mixture.
Materials and methods: Separation was achieved on a C18 column (LiChrospher® (250 x 4 mm, 5 µm)). Isocratic elution is carried out at a flow rate of 1.0 ml/min at ambient temperature. Mobile phase containing Acetonitrile: Phosphate buffer (pH=7.6±0.1) (40:60 v/v) was used. Ultraviolet detection was performed at 280 nm.
Results: The retention times of Tinidazole, Esomeprazole and Lansoprasole were found to be 5.36, 11.73 and 17.69 min, respectively. The method was validated for analytical parameters specificity, linearity, precision, and accuracy, limit of detection and limit of quantitation. The response was linear and R^2 was 0.9995 for both Tinidazole and Esomeprazole and 0.9994 for Lansoprazole. The concentration ranges were selected according to the prescribed doses of each drug and were 12.5 - 100 µg/ml, 5.0 – 40.0 µg/ml and 7.5 – 60.0 µg/ml for Tinidazole, Esomeprazole and Lansoprazole respectively. The relative standard deviation was found to be not greater than 2 %.

back to contents

Conclusion: The results of the studies showed that the proposed chromatographic method is simple, rapid, precise and accurate, which can be applied for the routine analysis of Tinidazole and proton pump inhibitors - Esomeprazole and Lansoprazole in quality control practice.

MULTYFUNCTIONAL POLYMER NANOCARRIERS FOR EFFICIENT MITOCHONDRIA-TARGETING

Momekova D.[1], Babikova D.[2], Ugrinova I.[3], Momekov G.[4], Dimitrov I.[2]

[1]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

[2]Institute of Polymers, Bulgarian Academy of Sciences, 103A "Akad. G. Bonchev" str., 1113 Sofia, Bulgaria

[3]Institute of Molecular Biology, "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, 21 "Akad. G. Bonchev" str., 1113 Sofia, Bulgaria

[4]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria

Aim: Over the past decade, subcellular drug targeting of biologically active molecules to and into cell organelles, has gained significant interest and is referred to as a "new frontier" or "third level" of drug targeting. The aim of the present study is the design and preparation of a novel mitochondria targeted multyfunctional polymer nanocarriers with a biodegradable hydrophobic core, a polycationic middle layer possessing the buffering capacity needed for the nanovehicle's endosomal escape, modified with pendant subcellular targeting ligands, and PEG outer shell, detachable in slightly acidic conditions, bearing cell targeting ligands.

Material and methods: Amphiphilic poly(D,L-lactide)-b-poly(N,N-dimethylaminoethyl methacrylate)-b-poly(Ethylene oxyde)(PLA-b-PDMAEMA-b-PEO-TPP+) block copolymers ; curcumin; acute myeloid leukemia HL-60, multidrug-resistant subline HL-60/DOX and human urinary bladder EJ carcinoma cells; NMR; GPC; DLS; TEM; MTT assay; apoptosys assay, NF-κB (p65) inhibition assay, Fluorescent microscopy.

Results: The block copolymer self-associated into multifunctional nanosized particles with an average diameter of 46 nm, which were successfully loaded with the hydrophobic natural anticancer drug curcumin. The multifunctional nanocarrier exhibited superior ability to trigger programmed cell death and more pronounced NF-κBinhibitory effects on the chemosensitive cell line HL-60 and its drug-resistant variants as compared to the free drug, and more importantly as compared to a non-functionalized nanocarrier of similar composition. Furthermore, the cellular internalization and targeted subcellular delivery to mitochondriaof multi- and non-functionalized nanocarriers were visualized, revealing the clear advantage of the former.

Conclusion: The results obtained are promising for further investigation of the multifunctional micelles as nanocarriers for enhanced subcellular targeted drug delivery.

back to contents

ANTINEOPLASTIC ACTIVITY OF SAPONINS FROM TWO BULGARIAN *ASTRAGALUS* SPECIES

Krasteva I.[1], Shkondrov A.[1], Mihaylova R.[2], Konstantinov S.[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia, Bulgaria
[2]Laboratory of Experimental Chemotherapy, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, Sofia, Bulgaria

Aim of the study: *Astragalus glycyphyllos* L. (Liquorice Milk-Vetch) is used in Bulgarian folk medicine as an antihypertensive, diuretic, anti-inflammatory, etc. Phytochemical investigation of the plant led to the isolation of six partially identified saponins, askenoside C and F, epoxycycloartane saponin lactone. *A. glycyphylloides* DC. (Pseudo Liquorice Milk-vetch) is morphologically similar to *A. glycyphyllos*. One oleanane-based saponin was isolated from the aerial parts recently were reported as well. The aim was to estimate the antineoplastic effects of purified saponins' mixtures, obtained from the two species on a panel of malignant cells of human origin (cutaneous T-cell lymphoma, myeloid and lymphoid leukaemia and urinary bladder cancer).
Materials and methods: Saponins mixtures were obtained from n-butanolic extracts of *A. glycyphyllos* and *A. glycyphylloides* by chromatographic techniques and the content of the main saponins was evaluated by LC-MS. Various human malignant cell lines were used and their viability was assessed using MTT test.
Results: Results showed that saponin fractions from both species possessed concentration dependent antitumor efficacy and could be regarded as sources of pharmacologically active compounds according to the estimated IC_{50} values (between 15.5 and 440 μg/ml). Interestingly some saponins' mixtures showed higher activity in MDR1 and BCR-ABL expressing tumour cells, which are expected to be less sensitive and are even resistant towards classical cytostatics.
Conclusion: Taken together our experimental data indicate that Astragalus plants are valuable sources of antineoplastic saponins which need further detailed pharmacological evaluation.
Acknowledgement Financial support from Ministry of Education and Science, program № DO1-217/30.11.2018 is gratefully acknowledged.

STABILIZATION OF DOXORUBICIN BY ENCAPSULATION IN CHITOSAN-ALGINATE NANOPARTICLES

Yoncheva K.[1], Tzankov B.[1], Spassova I.[2], Kovacheva D.[2]
[1]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, 1000 Sofia, Bulgaria
[2]Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences

Aim of the study: Doxorubicin is an anthracycline antibiotic, possessing a broad-spectrum of anticancer activity, including breast, ovarian, lung, bladder cancer etc.

However, its photodegradation is a challenge for pharmaceutical technology. Encapsulation of doxorubicin in nanoparticles could represent a useful approach for preventing its photodegradation. The present study is dedicated on the encapsulation of doxorubicin in chitosan-alginate nanoparticles and its impact on the stability of the drug.

Materials and methods: Doxorubicin was loaded in chitosan-alginate nanoparticles by ionic gelation method. The size, dispersity and zeta-potential of the resulted drug loaded nanoparticles were examined by photon correlation spectroscopy and electrophoretic laser doppler velocimetry. Scanning electron microscopy, thermogravimetric and X-ray analyses were also applied for the characterization of doxorubicin loaded nanoparticles.

Results: The drug loaded chitosan-alginate nanoparticles possessed mean diameter around 300 nm and negative zeta-potential. The scanning electron microscopy revealed a spherical shape of the nanoparticles. The encapsulation of doxorubicin prevented the degradation of the drug under light exposure.

Conclusion: Chitosan-alginate nanoparticles are considered to be an appropriate carrier for doxorubicin delivery providing efficient protection and stability of the drug against photodegradation.

3'-METHYL-4-THIO-1H-TETRAHYDROPYRANSPIRO-5'-HYDANTOIN AND ITS Pt(IV) AND Pd(IV) COMPLEXES AS NOVEL POTENT XANTHINE OXIDASE INHIBITORS

Cherneva E.[1], Tomović K.[2], Šmelcerović Ž.[3], Šmelcerović A.[4], Buykliev R.[1], Bakalova A.[1]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Department of Pharmacy, Faculty of Medicine, University of Niš, 81 "Dr. Zorana Đinđića" blv., 18000 Niš, Serbia
[3]Center for Biomedicinal Science, Faculty of Medicine, University of Niš, 81 "Dr. Zorana Đinđića" blv., 18000 Niš, Serbia
[4]Department of Chemistry, Faculty of Medicine, University of Niš, 81 "Dr. Zorana Đinđića" blv., 18000 Niš, Serbia

The aim of the study: Xanthine oxidase (XO) is a versatile molybdoflavoprotein, involved in the metabolism of purines, catalyzing the oxidative hydroxylation of hypoxanthine to xanthine, and xanthine to uric acid, with generation of reactive oxygen species. Not only uric acid produced by XO catalysis, but also free radicals are responsible for pathological consequences of XO overactivity. Allopurinol, potent competitive XO inhibitor, has widely been used in the therapy of gout, with latter approved febuxostat and topiroxostat. There are efforts towards the discovery of new XO inhibitors.

In this study, 3'-methyl-4-thio-1H-tetrahydropyranspiro-5'-hydantoin (**1**) and its Pt(IV) and Pd(IV) complexes (Fig. 1) were evaluated for inhibitory activity against XO *in vitro*.

back to contents

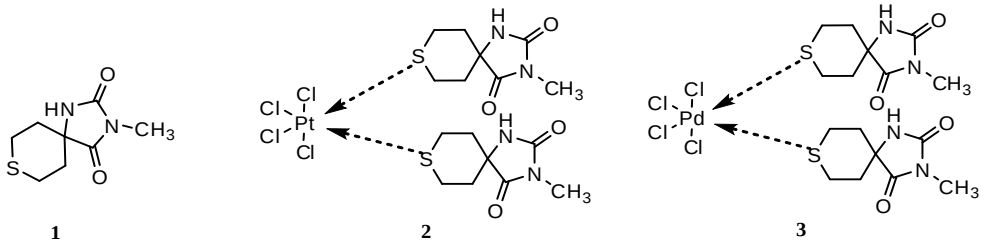


Figure 1. Structures of the assayed compounds

Materials and methods: The synthesis of 3'-methyl-4-thio-1H-tetrahydropyranspiro-5'-hydantoin and its Pd(IV) complex was performed as described in the previous study. *In vitro* evaluation of the inhibitory potential of 3'-methyl-4-thio-1H-tetrahydropyranspiro-5'-hydantoin and its Pt(IV) and Pd(IV) complexes (**1-3**) on bovine milk XO was based on spectrophotometric measurement of uric acid formation at 293 nm, using allopurinol as positive control.

Results: 3'-methyl-4-thio-1H-tetrahydropyranspiro-5'-hydantoin (**1**) and its Pd(IV) complex (**3**) did not show significant inhibitory activity on XO *in vitro* at concentrations below 150 μM, while its Pt(IV) complex (**2**) inhibited the enzyme with IC_{50} value 19.33 ± 5.80 μM. IC_{50} value of allopurinol was 1.70 ± 0.51 μM.

Conclusion: 3'-methyl-4-thio-1H-tetrahydropyranspiro-5'-hydantoin Pt(IV) complex was shown as potent inhibitor of XO *in vitro*. This complex with some structural freshness in comparison to standard and reported XO inhibitors represents a candidate for further evaluation.

Acknowledgments: The financial support of this work by Medical Science Fund at the Medical University, Sofia (Grant №84/23.04.2019), Science Fund/Chemical Technology and Metallurgy University (Grant 11523), Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. OI 172044) and Faculty of Medicine of the University of Niš (Internal project No. 4) is gratefully acknowledged.

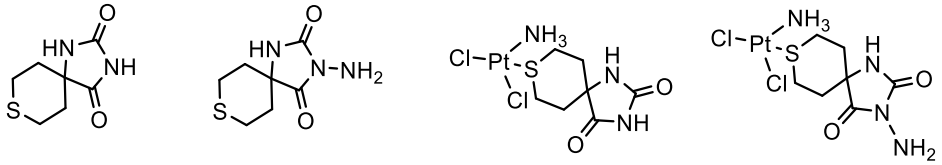
back to contents

XANTHINE OXIDASE INHIBITORY ACTIVITY OF THIOCYCLOHEXANESPIRO-5'-HYDANTOIN AND 3'-AMINOTHIOCYCLOHEXANESPIRO-5'-HYDANTOIN AND THEIR Pt(II) COMPLEXES

Cherneva E.[1], Šmelcerović Ž.[2], Tomović K.[3], Šmelcerović A.[4], Buykliev R.[1], Bakalova A.[1]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University, 2 "Dunav" str., 1000 Sofia, Bulgaria
[2]Center for Biomedicinal Science, Faculty of Medicine, University of Niš, 81 "Dr. Zorana Đinđića" blv., 18000 Niš, Serbia
[3]Department of Pharmacy, Faculty of Medicine, University of Niš, 81 "Dr Zorana Đinđića" blv., 18000 Niš, Serbia
[4]Department of Chemistry, Faculty of Medicine, University of Niš, 81 "Dr Zorana Đinđića" blv., 18000 Niš, Serbia

The aim of the study: Xanthine oxidase (XO), a metalloflavoprotein enzyme, catalyzes hydroxylation of hypoxanthine and xanthine to uric acid in purine catabolism producing reactive oxygen species, what leads to hyperuricemia and oxidative damage of the tissues where overactivity of XO is present. Allopurinol, purine-based XO inhibitor, has widely been used in gout treatment. Additionally, representatives of non-purine inhibitors, febuxostat and topiroxostat, have been approved. There is a need for new non-purine XO inhibitors. In this study, thiocyclohexanespiro-5'-hydantoin (**1**), 3'-aminothiocyclohexanespiro-5'-hydantoin (**2**) and their Pt(II) complexes(**3** and **4**) (Figure 1) were evaluated for inhibitory activity against XO *in vitro*.



1 **2** **3** **4**

Figure 1. Chemical formulas of the studied compounds

Materials and methods: The synthesis of compounds **1-3** was performed as described in the previous studies. Bovine milk XO was used for *in vitro* inhibition test based on the spectrophotometric determination of uric acid formation at 293 nm. Allopurinol was used as a positive control.

Results: In comparison to ligands (**1** and **2**) which did not show significant inhibitory activity at concentrations below 150 μM, their Pt(II) complexes (**3** and **4**) inhibited XO acitivity *in vitro* with IC_{50} values 110.33 ± 26.38 μM and 115.45 ± 42.43 μM, respectively. IC_{50} value of allopurinol was 1.70 ± 0.51 μM.

Conclusion: Thiocyclohexanespiro-5'-hydantoin and 3'-aminothiocyclohexanespiro-5'-hydantoin Pt(II) complexes might offer a starting point for optimization of new XO inhibitors structurally different from reported inhibitors so far.

Acknowledgments:

back to contents

The financial support of this work by: Medical Science Fund at the Medical University, Sofia (Grant №84/23.04.2019); Science Fund/Chemical Technology and Metallurgy University (Grant 11523), Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants No. OI 172044) and Faculty of Medicine of the University of Niš (Internal project No. 4) is gratefully acknowledged.

PREPARATION OF 5-METHYL-5-(4-PYRIDYLMETHYL) HYDANTOIN

Buyukliev R.[1], Burdjiev N.[2]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, 2 „Dunav“ str., 1000 Sofia, Bulgaria

[2]Department of Organic Chemistry and Pharmacognosy, Faculty of Chemistry and Pharmacy, Sofia University, 1 „James Bourchier“ blv., 1164 Sofia, Bulgaria

Aim: The aim of the study. It is known from literature that the 5,5-disubstituted hydantoins have anti-convulsive, antipyretic, antibacterial properties. The metal complexes of these hydantoins with platinum and palladium have anti-tumor properties, with some activity being close to that of the cisplatin drug. In previous studies, we have investigated platinum and palladium complexes with various substituted 5-methyl-5- (4-pyridyl) hydantoin derivatives. To complete the series with pyridine hydantoins, we also wanted to synthesize 5-methyl-5- (4-pyridylmethyl) hydantoin.

Materials and methods: It has been found, however, that the synthesis of the necessary ketone-methyl-4-pyridylmethyl ketone does not proceed as described in the literature, therefore we have applied new approaches to the synthesis of this compound. They proved to be successful.

Results: Based on the newly synthesized ketone, the desired product 5-methyl-5- (4-pyridylmethyl) hydantoin was successfully obtained. The reaction time for optimal yields of the products was determined. Structures of newly synthesized compounds were confirmed by elemental analysis, IR, ^{1}H and ^{13}C NMR spectra.

Conclusion: The study of newly synthesized compounds for antitumor activity is forthcoming. New platinum and palladium complexes are also underway, with DFT calculations for their stability as well as their pharmacological test for anti-tumor activity.

back to contents

POSITIVE IMPACT OF THE NOVEL MELATONINERGIC DRUG PIROMELATINE ON ANXIETY IN EXPERIMENTAL MODEL OF PRENATAL STRESS

Ivanova N., Nenchovska Z., Kortenska L., Mitreva R., Stoyanova T., Tchekalarova J.

Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Aim of the study: The unstable emotional status of a pregnant mother is a risk factor for the developing psychiatric disorders in the offspring later in life. The aim of the current investigation is to explore the therapeutic effects of the melatoninergic compound Piromelatine on anxiety disturbances in offspring with a history of prenatal stress (PNS).

Materials and methods: Different types of stressors were applied on pregnant mothers of Sprague Dawley rats, starting from the 7th gestation day until the 21st day /birth/. Treatment and experimental studies were carried out on offspring male sexually mature rats of the prenatally stressed mothers. Chronic treatment with Piromelatine at a dose of 20 mg/kg, or vehicle for the matched controls, were applied intraperitoneally, for a period of 21 days. During the treatment period several behavioral tests were conducted to assess the anxiety-like behavior: Elevated plus maze (EPM), Open field (OF) and Light/Dark Test (LDT).

Results: The prenatally stressed offspring showed decreased motor activity, less time spent in the aversive central zone and higher anxiety index compared to the controls in the OF and the EPM tests, which were corrected by Piromelatine treatment. The melatoninergic drug abolished the PNS decrease of the number of crossings and time spent into the light area, shown in the LDT.

Conclusion: Current data support the fact that Piromelatine might affect positively the anxiety-like behavior in offspring caused by PNS.

Acknowledgement: This research was supported by the National Science Fund, Bulgaria (grant No. DN KP- 06-H21/10).

back to contents

THE INVASIVE ALIEN SPECIES *AMORPHA FRUTICOSA* – A CHEAP SOURCE OF VALUABLE ESSENTIAL OILS

Kozuharova E.[1]*, Benbassat N.[1], Ionkova I.
Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia

The high tolerance of various habitat conditions and potent propagation ability of *Amorpha fruticosa* L. (Fabaceae) promote its aggressive invasive behaviour. The aim of this study is to evaluate 1) several populations of this plant by the potential yield of the fruit, 2) the approximate yield of the essential oils, and 3) composition of the essential oil. The potential yield of fruit is evaluated based on extrapolations of weight and number of fruits per infrutescence, number of infrutescences per plant and number of individuals per population. Steam extraction of the essential oil was performed and GS/MS analysis of the composition of the essential oil.
Key words: essential oils, composition, yield
Acknowledgements: This work has been carried out in the framework of the Grant Д-79/23.04.2019, Project 8276/20.11.2019 CMS, MU-Sofia.

CLINICAL PHARMACY- OCCURRENCE AND DEVELOPMENT

Grekova D.
Department of Pharmaceutical sciences, Faculty of Pharmacy, Mesical University, Plovdiv

Aim: To present and analyze historically the occurrence and development of clinical pharmacy.
Materials and methods: It has been used documentary analysis and review of existing scientific literature.
Results: Clinical pharmacy is defined as a service provide by pharmacists to ensure that patient's rational drug therapy is safe, appropriate and cost-effective.
The main issues that stimulate the need for the introduction of clinical pharmacies are:
- reduce the number of extemporal prescription prepared in the pharmacy;
- a rapid and continuous increase in the number of pharmaceutical products produced by the pharmaceutical industry;
- increasing the need for professional, competent and drug-independent pharmacovigilance, dosing, pharmacodynamics, pharmacoeconomic and pharmacoepidemiology, improving the patients` adherence with therapy and agreement the instructions given by the physician and pharmacist;
- growth in drug-induced disease and mortality.
The term "clinical pharmacy" has its roots in US-practiced hospital pharmacy in the early 1960s. In the United Kingdom, clinical pharmacy is developing similarly to practice in the United States and Australia. In many Asian countries like Japan, India, Singapore, Thailand and Malaysia, there is a great interest in clinical pharmacy for more active inclusion of pharmacists in the health care system.

back to contents

Conclusion: Clinical pharmacy has its long history. The key to its future development will be patient demand for safe and effective drug therapy. There are opportunities for expanding the clinical services provided by pharmacists. Patient consultation is a major component of the practice of clinical pharmacists.

STUDY ON THE ACUTE TOXICITY OF NEWLY SYNTHESIZED ISOQUINOLINE PRECURSOR

Saracheva K.[1], Dimitrova D.[2], Nikolova S.[3], Gledacheva V.[4], Stefanova S.[4], Krastev A.[4]

[1]Department of Pharmacology and Drug Toxicology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria
[2]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University, Plovdiv, Bulgaria
[3]Department of Organic Chemistry, Faculty of Chemistry, „Paisii Hilendarski" University, Plovdiv, Bulgaria
[4]Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria

Aim of the study: The aim of this study is to determine the acute toxicity of a newly synthesized isoquinoline precursor 2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide (NIQP) in mice.
Materials and methods: Seven experimental groups of male ICR white mice were used. All of the mice were treated per orally as following: first group (control) with 0.1 mL/10g body weight dimethyl sulfoxide (DMSO), used as a solvent; second group with 500 mg/kg NIQP; third group with 1000 mg/kg NIQP; fourth group with 1500 mg/kg NIQP; fifth group with 2000 mg/kg NIQP; the sixth one with 3000 mg/kg NIQP and the seventh group with 4000 mg/kg. The Lethal Dose, 50% (LD_{50}) were defined by Chiendu et al., (2013) method.
Results: No behavioral signs of acute toxicity and mortality were identified for the control group or the first four experimental groups (500, 1000, 1500, and 2000 mg/kg NIQP). Mice treated with a dose of 3000 mg/kg did not have any disturbances but after 24 hours, mortality was reported in two of them. Mortality wasn't observed in animal tests even in the highest dose used of 4000 mg/kg. Based on the results obtained by the method of Chiendu et al. (2013) we found that the LD_{50} of 2-chloro-N-(1-(3,4-dimethoxyphenyl)propan-2-yl)-2-phenylacetamide was 3500 mg/kg.
Conclusion: Compounds with LD_{50} of over 5000 mg/kg are with a high level of safety. The newly synthesized compound has higher LD_{50} and lower acute toxicity than the well-known in the practice isoquinoline Papaverine hydrochloride with LD_{50} 750 mg/kg.

back to contents

MODERN TRENDS IN REFORMING THE PHARMACEUTICAL INDUSTRY

Nedelcheva Y.
Bulgarian Drug Agency

The aim of the study: to present the new reality in which the pharmaceutical industry operates. The effects of the global financial crisis are shaping new challenges for reforming the pharmaceutical industry.
Materials and Methods: The research covers the external environment factors and pharmaceutical industry reforms for the period 2008-2019. Modern methodologies have been used, endorsed in the international practice for analysis of the pharmaceutical industry.
Results: Reforms in Bulgaria are similar to those in the international pharmaceutical industry. Leading factor for reforms are the effects of the global financial crisis and increased state involvement through regulatory policy in the pharmaceutical industry.
Conclusion: The external environment is leading in reforming the pharmaceutical industry in Bulgaria. The wave of globalization and the effects of the global financial crisis have increased the role of regulatory policy. Finding a balance between effective and affordable medicines is giving way to growing social expectations from the pharmaceutical industry. Reforming is about adapting regulatory policy to the new reality.
Keywords: Bulgaria, regulatory policy, new reality

ACTIVITY-GUIDED IDENTIFICATION OF ANTICANCER LIGNANS IN JUNIPER EXTRACTS

Nedialkov P.[1], Ivanova D.[2]*, Tashev A.[3], Kokanova-Nedialkova Z.[1], Ilieva, Y.[1], Angelov G.[2], Richardson K.[4], Dosmann M.[4], Atanassova T.[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Medical University, Sofia 1000, Bulgaria
[2]Institute of Chemical Engineering, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
[3]Department of Dendrology, University of Forestry, Sofia 1756, Bulgaria
[4]Arnold Arboretum, Harvard University, Boston 02130-3500, MA, USA
**Corresponding author e-mail: dianadoc@abv.bg, mobile phone: +359 884 43 13 52*

Aim of the study: Podophyllotoxin (PPT) is a precursor for the synthesis of the drugs etoposide, etopophos, teniposide, used in the therapy of lung, breast, ovarian, testicular, stomach, bladder, pancreatic, brain cancer, leukemias etc. *Sinopodophyllum hexandrum* (Royle) T. S. Ying (Himalayan mayapple) and *Podophyllum peltatum* L. (American mayapple) are sources of PPT, however they are already endangered species because of their intensive exploitation. Thus, alternative sources of PPT are necessary. We aimed this study at a comprehensive identification of lignans in the extracts of more than 20 juniper representatives,

back to contents

originating from different continents of the world, as potential sources of anticancer compounds.
Materials and methods: Junipers were delivered from the Arnold Arboretum, Harvard University, USA, and Bulgaria. MTT tests were performed on NB4, K-562, BV-173, T-24, HT-29 cells. Lignans were identified by ultra-high performance liquid chromatography, coupled to high-resolution mass spectrometry (UPLC-HRMS).
Results: A plenty of lignans, such as podophyllotoxin, deoxypodophyllotoxin, beta-peltatin, anhydropodorhizol, yatein, matairesinol etc. were found in various concentrations in the most efficient anticancer extracts of *J. sabina* var. *balkanensis*, *J. virginiana*, *J. × media*, *J. scopulorum*, *J. communis* and their cultivars, as it was determined by UPLC-HRMS.
Conclusion: The identified anticancer junipers produce a plenty of lignans that set the pattern for their future exploitation as potential sources of compounds for the combination therapy of cancer.
Acknowledgements: This research is financially supported under contract №37-2016 with the Arnold Arboretum, Harvard University, and contract №DN 07/25 (2016) of the Bulgarian Fund for Scientific Research.

BIOCOMPATIBILITY AND CYTOTOXICITY OF SILICA-BASED BONE SUBSTITUTE MATERIALS

Dimova T.
Departament of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University, Varna, 55 „Prof. Marin Drinov“ str., 9002 Varna, Bulgaria
e-mail: biotan@abv.bg

Objectives: The aim of the study is to evaluate the biocompatibility and cytotoxicity of bioactive glasses (BG) using the osteosarcoma cell line MG63. In this study, was used BG in the system $70SiO_2$-$25CaO$-$5P_2O_5$ (BG0) with and without Ag_2O for bone tissue regeneration. BGs have been created as fillers for the treatment of bone defects in dental practice.
Materials and methods: BG in the SiO_2-CaO-P_2O_5 and SiO_2-CaO-P_2O_5-xAg_2O system (x=1,2 and 4wt%) were synthesized from TEOS, CaO, H_3PO_4, $AgNO_3$. Cell viability was measured by means of a 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Absorption is measured at 590 and 630 nm reference to ELISA-Reader. The statistical analysis of the results was carried out with an ANOVA test and Bonferoni procedure ($p<0.05$). The morphological alterations of the MG63 were observed directly by a phase contrast microscope.
Results: The synthesized silicate-based materials contain carbonate bound hydroxyapatite type B, silver and wollastonite (BG3). The relatively high content of 4 wt% Ag_2O incorporated in the BG3 has an important effect in the improvement of the proteins for this type of materials. The results of this study indicate that BG0 has no toxic effects, whereas BG1, BG2 and BG3 cause minimal toxic effects. BG stimulate MG63 cells to differentiate into osteogenic media.

back to contents

Conclusion: BG0 and BG containing silver have toxic effects on the cell line MG63. They cannot be used as biocompatible materials at concentrations higher than 1μg/cm^2. BG containing up to 1 μg/cm^2 of Ag_2O stimulate cellular mineralization and osteogenic differentiation of MG63 cells.

IN VITRO TOXICITY EVALUATION OF H2S-DOX IN HUMAN HEPATOCARCINOMA HEPG2 CELLS

Tzankova V.[1], Yordanov Y.[1], Aluani D., Saponara S.[2]

[1]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University, 1000 Sofia, Bulgaria

[2]Department of Life Sciences, University of Siena, Siena, Italy

Aim: Doxorubicin (DOX) is a potent broad-spectrum antineoplastic antibiotic, isolated from Streptomyces species. It is widely used as a single agent or in combination with other anticancer drugs in treating of hematological cancers and solid tumors, lymphomas, and sarcomas. In attempt to overcome the frequent drug-related toxicity and multiple drug resistance (MDR), H2S-releasing doxorubicin derivative (H2S-DOX) was obtained by combining DOX with appropriate H2S donor substructures[1,2]. Our special attention was focused on in vitro toxicity evaluation of H2S-releasing doxorubicin derivative (H2S-DOX) as potentially safe and target specific drug carrier.
Methods: The initial toxicological evaluation of the resulting compound was performed on human hepatocarcinoma HepG2 cells by incubating H2S-DOX (0.01-50 μM) for 48 and 72 h. In vitro cytotoxicity evaluation was performed by LDH-release and MTT-test assay; IC50 was also calculated.
Results: Differently from DOX, most of the products were less toxic on HepG2 cells. Thus, the IC 50 of H2S-DOX calculated at 48 and 72 h incubation were 4.3 and 2.9 μM, compared to 0.2 and 0.1 μM for DOX.
Conclusion: The initiam in vitro hepatotoxicity screening of H2S-DOX on human hepatoma cells HepG2 shows better safety profile of newly synthetized H2S-releasing anthracycline compared to DOX, and opens the possibility for future detailed in vitro and in vivo pre-clinical assessments of this perspective compound.
Acknowledgements: This publication is based upon work from Cost Action CA17104 (STRATAGEM), supported by COST (European Cooperation in Science and Technology).
References:
1Chegaev, Konstantin, et al. "H2S-donating doxorubicins may overcome cardiotoxicity and multidrug resistance." Journal of medicinal chemistry 59.10 (2016): 4881-4889.
2Buondonno, Ilaria, et al. "Endoplasmic reticulum-targeting doxorubicin: a new tool effective against doxorubicin-resistant osteosarcoma." Cellular and Molecular Life Sciences 76.3 (2019): 609-625.
Corresponding author: Virginia Tzankova
e-mail: virginia_tzankova@yahoo.com; tel: +359887930982

back to contents

MARKET LANDSCAPE AND SOME ASPECTS OF REAL WORLD EVIDENCE OF THE NON-PSYCHOACTIVE PHYTOCANNABINOIDS

Kirilov B.[1], Zhelyazkova M.[1], Petkova-Georgieva E.[2]
[1]Bulgarian Drug Agency, Sofia, Bulgaria
[2]Medical University, Plovdiv, Bulgaria

Aim: Real world evidence has demonstrated the potential to validate the therapeutic value of pharmaceutical products and help customize product development in a more patient-centric manner. In our study we conducted a survey with experts in the area (academia, industry and other associations) to solicit their opinions on trends in the cannabinoid market of products received from industrial hemp of the *Cannabis plant*.
Methods: We used the European Pharmacovigilance Issues Tracking Tool (EPITT) functionalities to communicate on the regulatory matters and government policies of the non-psychoactive cannabinoids. To add on, we closely monitored the databases of suspected adverse reactions, including national databases, EudraVigilance, the database of the WHO Programme for International Drug Monitoring (VigiBase), for such products. Our secondary sources include a detailed analysis of marketing strategies for cannabidiol as a dietary supplement and a poll assessing the current situation of the legal market of such products in Bulgaria.
Results: We provide an analysis of the current status of the market of the medicinal products Sativex® (Nabiximols, containing THC and CBD1:1) and Epidiolex® (Cannabidiol) in relation to their approved and unapproved indications. A detailed publication analysis of close to 500 articles that have been published in the last five years, highlighted the key focus areas of ongoing real world evidence-based research activity in the pharmaceutical and life sciences industries.
Conclusion: Our poll and analysis help to explore the potential of real world evidence in the different stages of product development of the non-psychoactive cannabinoid market.
Key words: phytocannabinoids, medicinal drugs, dietary supplements, Cannabidiol, marketing strategies

THE COMBINATION TREATMENT OF TUMOR-CELL LINES WITH EPIRUBICIN AND ARTEMISININ, AND MECHANISMS OF THEIR SYNERGISTIC EFFECTS

Zhelyazkova M.[1], Momekov G.[2], Hristova-Avakumova N.[2], Hadjimitova V. [2]
[1]Bulgarian Drug Agency, Sofia, Bulgaria
[2]Medical University, Sofia, Bulgaria

Aim: Clinical studies involve cotreatment of Epirubicin (EPI) with taxanes, platinum drugs, nitrogen mustard analogs, fluoropyrimidines, and Vinca alkaloids. Well known are the drug–drug interactions with trastuzumab or taxanes that result in cardiotoxicity. However, in this study we demonstrate for the first time the ability of artemisinin (ART) to modulate the response of EPI.

back to contents

Materials and Methods: In our experiments with sensitive and resistant to doxorubicin HL-60 cell lines synergistic effects of the combination of ART and EPI were obtained using MTT assay and the method of Chou Talalay. We assessed the scavenging potential of EPI in stable free radicals (ABTS and DPPH) systems and we tested its protection effect under conditions of Fe(II) induced peroxidation in sample, containing lecithin and deoxyribose as oxidisable substrate.
Results: ART significantly enhances the damage induced by EPI, thereby achieving greater efficacy in the resistant cell line. EPI possess the capability to decrease the concentration of both used radicals – ABTS and DPPH. Having in mind the fact than in both systems containing biologically important molecules we used the same mechanism of oxidative damage we observed different effects that could be attributed to dissimilarity in the interaction between the tested substance and the oxidisable substrate.
Conclusion: These data gives us a reason to conclude that the free radicals induced by EPI and ART participate in the genomic damage of DNA, but the mechanism of cardiotoxicity is associated predominantly to the lipid peroxidation and depends on the applied concentration.
Key words: Epirubicin, Artemisinin, combination indices, synergism, lecithin-obtained and deoxyribose damages

A STUDY OF PARTICIPATION OF PHARMACISTS AND THEIR ROLE IN THE PROCESS OF PHARMACEUTICAL MANUFACTURING IN BULGARIA

Stefanova T.[1], Petrova G.[2], Grigorov E.[2,3]
[1]Student, Faculty of Pharmacy, Medical University, Varna, Bulgaria
[2]Department of Organization and Economics of Pharmacy, Faculty of Pharmacy, Medical University, Varna, Bulgaria
3National Center of Public Health and Analyses, Sofia

Introduction: Over the last decades, the pharmaceutical industry has become one of the vast growing high-tech industries in the world. Main global policies influence on the pharma-market forming a new concept of drug policy.
Aim: The aim of the current study is to investigate and analyze registered manufacturers/importers of medical products who have received a marketing authorization in Bulgaria from the Bulgarian Drug Agency and to define the share of pharmacists on leading positions in the field as production managers or qualified persons.
Methods and materials: This study is based on the analysis of Bulgarian and foreign literature sources and on the results of analysis of official data from BDA published on its internet page. For segmentation of the different manufacturers/importers of medical products, we use the six economic regions in Bulgaria. The relevant data were assessed with SPSS analysis for defining of relations and consequences.

back to contents

Results: The registered manufacturers/importers who have received marketing authorization licenses from the national authority are 48. In this study, manufacturers/importers are considered from the point of view of the economic zones in Bulgaria. In South-East Bulgaria, 35 MAH or 72.9% of the respondents are located. The remaining areas are represented by one or more manufacturers/importers. An exceptionally strong impression is attributed to the number of persons qualified(MAH) – 6 pharmacists for the South-West economic region and only 1 for Central-North economic region.
Conclusion: The results on this study show meager appurtenance of pharmacists in the process of supply and manufacturing of the medicines. This number is extremely insufficient and the pharmaceutical industry should consider attracting and stimulating more of the qualified pharmacist.
Key words: MAH, pharmacist, import, manufacturing

PHARMACOVIGILANCE OF MEDICINES FOR OVARIAN HYPERSTIMULATION. ANALYSIS FROM PATIENT PERSPECTIVE

Stoev S., Lebanova Hr., Dimitrova J., Getov I.

Aim: Current study aims to evaluate the type, incidence and severity of suspected adverse drug reactions (ADR) during controlled ovarian hyperstimulation (COH). Patients awareness of System for spontaneous reporting of ADRs has been evaluated.
Materials and Methods: Statistical analysis of data, generated by completion of validated questionnaire during direct interview with 659 patients for a study period of 14 months, has been performed.
Results: Almost 40 % of participants reported a suspected ADR as a consequence of stimulation hormone application. Mean number of reported ADRs per patient is 1.27. In 90% of cases of an experienced ADR, participants would evaluate the adverse effect as mild. Suspected ADRs have led to therapy termination in 3.8 % of investigated cases of adverse reaction. More than 2/3 of experienced ADRs are associated with local injection site reaction(63.1%), followed by reports of headache (9.8%), gastro-intestinal discomfort (5.8%) and nausea (4,7%). Women, underwent therapy for assisted reproduction are not well aware of functional principles of System for spontaneous ADR reporting and are not motivated enough to directly report a suspected ADR.
Conclusion: According to women underwent assisted reproduction treatment, therapy for COH is associated with low incidence of mild ADRs, that are not significant enough for cycle cancellation. Levels of direct reporting of ADRs remains extremely low.

back to contents

WHEN EFFECTIVENESS MEANS SAFETY. ANALYSIS OF CLINCIAL EXPERIENCE WITH DIFFERENT APPROACHES FOR CONTROLLED OVARIAN HYPERSTIMULATION.

Stoev S., Lebanova Hr., Naseva E., Getov I.

Aim: Current retrospective cohort study analyzes 4726 clinical database records of controlled ovarian hyerstimulation (COH) cycles to assess the significance of target effectiveness endpoints from a safety perspective.
Materials and Methods: Following statistical methods were applied: descriptive statistics, Mann-Whitney U test, Kruskal-Wallis test, Pearson chi-square test, binary logistic regression. Comparative analysis of urinary and recombinant stimulations has been performed according to both primary and secondary endpoints as following: incidence of cycle cancelation, clinical regnancy rate, ovarian hyperstimulation syndrome (OHSS), induced plasma values of estradiol, progesterone, luteinizing hormone, oocyte yield, exogenous gonadotrophin exposure, eggs with normal maturation.
Results: Safety surveillance of COH cycles exaggerates adverse drug reactions reporting and OHSS incidence. Induced follicle count, oocyte yield, mean values of estradiol, luteinizing hormone and progesterone, and cancellation rate are valid indicator for stimulation cycle safety as well as COH effectiveness. Discussed endpoint measures are within the “safe window” for all the investigated groups of medicinal products. Urinary group is characterized with higher cycle cancellation rate, luteinisng hormone levels and mean dose of FSH applied.
Conclusion: Suboptimal effectiveness of COH may jeopardize wellbeing of treated couples. Due to the specific therapeutic characteristics of COH, well-established effectiveness endpoints are valid markers for safety of stimulation medicinal products.

EVALUATION OF THE NEUROPROTECTIVE AND ANTIOXIDANT PROPERTIES OF NEW N-ALKYLATED BENZIMIDAZOLE HYDRAZONES WITH POTENTIAL APPLICATION FOR NEURODEGENERATIVE DISORDERS

Anastassova N.[1]*, Aluani D.[2], Hristova-Avakumova N.[3], Kostadinov A.[1], Tzankova V.[2], Kondeva-Burdina M.[2], Hajimitova V.[3],Yancheva D.[1]
[1]Institute of Organic Chemistry with Center of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[3]Department of Medical Physics and Biophysics, Faculty of Medicine, Medical University of Sofia, Bulgaria

The aim of the study: Safety evaluation and neuroprotective activity of newly synthesized N-akylated benzimidazole hydrazones, as well as estimation their ability to act as radical scavengers.

back to contents

Materials and methods: *In vitro* toxicity screening of the newly compounds on human neuroblastoma SH-SY5Y cell line. The viability of the cells was assessed by MTT assay. The anti-radical properties of the hydrazones were determined by assessing their ability to decrease the concentration of the stable free radicals ABTS and DPPH. The neuroprotective properties of the compounds were determined in a model of H_2O_2-induced oxidative damage in SH-SY5Y cells. For comparison, effects of rasagiline and melatonin were determined.
Results: Most of the compounds have IC_{50} values over 200 μM and possess statistically significant protective effects. It should be noted that the neuroprotective effects were more pronounced than those of melatonin and rasagiline. The determined from the concentration-radical scavenging effect C_{50} values for the hydrazones in the ABTS containing system were similar or lower to the one of melatonin. Variable capability to decrease the absorbance of the DPPH containing samples depending of the structural differences between the compounds is being observed.
Conclusion: The new series of N-alkylated benzimidazoles containing methoxy and hydroxyl substituents exhibits low toxicity and a good in vitro safety profile. The improved neuroprotective and antioxidant properties compared to melatonin and rasagilin, makes them were synthesized as promising radical scavengers for neuroprotective application and showed improved neuroprotective and antioxidant properties.
Acknowledgments: The financial support by the National Science Fund of Bulgaria (contract КП-06-М29/4) is gratefully acknowledged.
Corresponding author: Denitsa Aluani
e-mail: denitsa.aluani@gmail.com tel: 359888869426

POLYVINYL ALCOHOL-BASED FILAMENTS AS A FEEDSTOCK MATERIAL FOR THREE DIMENSIONAL PRINTING OF SOLID DOSAGE FORMS

Ilieva S., Dimitrov M.
Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University, Sofia

The aim of the study: Hot melt extrusion (HME) coupled with fused deposition modeling (FDM) three dimensional (3D) printing is a modern approach of producing patient-specific dosage forms. The purpose of this study is to test the feasibility of three grades of polyvinyl alcohol (PVA) for the production of filaments for FDM.
Materials and Methods: Mixtures containing three grades of partially hydrolyzed polyvinyl alcohol (PVA 1 - 13 000-23 000 MW, PVA 2 – 31 000-50 000 MW and PVA 3 - 72 000 MW) and polyethylene glycol PEG 6000 or sorbitol as a plasticizer were extruded as filaments in a single screw extruder (Wellzoom, PRC). The suitable filaments were printed with a 3D printer (Delta Rostock mini G2S pro, PRC). Additionally, the polymers' viscosity was measured with a HAAKE Viscotester iQ

Rheometer (Thermo scientific, USA) and compared with a commercial PVA filament (Shenzhen Lankeda Technology, PRC).
Results: The viscosity of PVA 1 and PVA 2 was comparable to the reference filament. However, the extrudates produced with PVA 1 were brittle, with uneven diameter and could not be fed into the printer. The extrudates containing PVA 2 and PVA 3 had smooth surface and uniform diameter, which allowed to be loaded into the printer. Only PVA 2 proved to be printable, while PVA 3 caused nozzle blockage due to its higher molecular mass.
Conclusion: Filaments containing PVA with 31 000-50 000 MW and a hydrophilic plasticizer were found suitable for the production of 3D printed solid dosage forms.

CARDIOVASCULAR MEDICINES UTILIZATION IN BULGARIA FROM THE NATIONAL HEALTH INSURANCE FUND PERSPECTIVE

Kovachka G., Mitkova Z., Kamusheva M., Petrova G.
Faculty of Pharmacy, Medical University, Sofia
Corresponding author: Gabriela Kovachka,
e-mail: gabriela.kovachka@gmail.com, tel.: 0892096929

Purpose: The goal is to evaluate the reimbursed cardiovascular medicines utilization pattern in Bulgarian ambulatory patients for a 1-year period (2018).
Methods: It is a retrospective, quantitative utilization study analyzing 67 mono-component products acting on CV system paid by the National Health Insurance Fund (NHIF).Medicines utilization is measured in terms of defined daily dose (DDD) using the following formula: DDD/1000/inhabitants/day = ((Sales in mg/ DDD)/(number ofinhabitans×365)×1000). Reimbursable costs have also been compared. Data was gathered from the NHIF official register.
Results: We have found a very high total consumption of CV medicines, measured in DDD/1000/inhabitants/day. The highest utilization is revealed for Ca-channel blockers (49.71) followed by β-blockers (47.59), ACE- inhibitors (46.69), sartans (34.65) and statins (32.40). The lowest consumption is measured for fibrates (0.51), endothelin receptor antagonist (0.01), selective aldosterone receptor antagonist (0.30) and adrenergic alpha2-agonist (0.34). The NHIF pays the highest reimbursement values for Lercanidipine 4 009 363 BGN, Nebivolol 3 971 512 BGN, Bisoprolol 2 642 796 BGN, Torasemide 2 433 471 BGN and Ivabradine 2 286 518 BGN.
Conclusion: The CV medicines utilization is the highest mainly in the groups where new generics and international non-proprietary names (INNs) were registered. The medicinal products, established in the practice, continue to be utilized. Their consumption is relatively low as a result of generic and therapeutic competition in cardiovascular medicines market. Further quantitative and qualitative studies are needed to predict the trends in CV medicines utilization from the NHIF perspective as well as to assess the rationality of their use.

back to contents

ONCOPHARMACOLOGICAL CHARACTERIZATION OF THE ARYLTETRALIN LIGNAN ANTHRICIN

Petrova E., Mihailova R., Atanasova T., Ionkova I., Momekov G.

The antiproliferative activity of aryltetralin lignan anthricin has been studied in a spectrum of human tumor cell lines, wherein the compound exhibits pronounced cytotoxic activity at low concentrations. Pharmacodynamic studies indicate that cytotoxic activity is mediated by induction of apoptosis through caspase-dependent mechanisms. An in silico study of the physicochemical and pharmacokinetic properties of lignan shows that it is characterized by optimal properties as a potential antineoplastic agent.

PHARMACOANALYTICAL INVESTIGATION OF PRODUCTS FOR ALZHEMER TREATMENT

Obreshkova D.
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, MU-Sofia
Sofia, Bulgaria

The current and growing importance of development of new effective products for prevention and slowing of Alzheimer's disease progression is expanded by including substances with antioxidant and antiinflammatory effects after precise characterization.

Aim of the study: Aim of the study was the pharmacoanalytical characterization of products with a beneficial effect on Alzheimer's disease using modern analytical techniques and approaches.

Materials and methods

Materials: Galantamine hydrobromide/Pymadine, Docosahexaenoic acid, Aminoacid Standard, Curcumine, Ol. *Ocimum basilicum L.*, Extr. *Clinopodium vulgare L.*, Extr. *Echium vulgare L.*

Methods: High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Densitometry, UV-Vis-Spectrophotometry, Chemiluminescence, DPPH.

Results:m Repeatability of densitometric and HPLC-methods was confirmed by the absence of statistically significant differences: $\overline{X}_{Rf}$=0.663; $\overline{X}$ t_R=3.179 min. (Galantamine hydrobromide), $\overline{X}_{Rf}$=0.433; t_R=5.272 min. (Pymadine). In HPLC accuracy is: 97.71% (Galantamine hydrobromide); 101.72% (Pymadine). Radical-scavenging effect of the combination (20.19%) is stronger than activity of Galantamine hydrobromide (15.44%). For densitometric analysis of Docosahexaenoic acid: y=1.108+9370.7. For HPLC: L-Glutamic acid: $y=2.10^9.x–458796$; L-Arginine: $y=2.10^8.x–98532$. For TLC: L-Leucine: $y=1.10^6.x+2949.7$; L-Valine: y=916928.x+404.72. Stability of aminoacids in gamma-irradiation was demonstrated by HPLC. Curcumine antioxidant activity was determined by UV-Vis-spectrophotometry and chemiluminescence. Antibacterial activity of Ol. *Ocimum basilicum L.* has been confirmed. Linalol, Linolen, Methylchavikol, Methylcinnamat in Ol. *Ocimum basilicum L.* and phenolcarboxylic acids (Cinnamic, p-Coumaric,

Ferrulic) in *Extr. Clinopodium vulgare L.* and *Extr. Echium vulgare L.* have been analyzed with GC.
Conclusion: Pharmacoanalytical approaches for Galantamine hydrobromide/Pymadine, Docosahe-xaenoic acid, aminoacids, Curcumine, Ol. *Ocimum basilicum L.*, Extr. *Clinopodium vulgare L.*, Extr. *Echium vulgare L.* have been developed and validated.
Corresponding author: Prof. Danka Obreshkova, Dsci; tel.: 0898486767, e-mail: phddanka@yahoo.com

WATER-EXTRACTABLE POLYSACCHARIDE FROM *PLANTAGO MEDIA* L. LEAVES – STRUCTURAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY

Lukova P.[1*], Nikolova M.[2], Delattre C.[3], Gardarin C.[3], Petit E.[4], Michaud P.[3], Iliev I.[2]

[1]Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University - Plovdiv, 4002 Plovdiv, Bulgaria.
[2]Department of Biochemistry and Microbiology, Faculty of Biology, Plovdiv University Paisii Hilendarski, 4000 Plovdiv, Bulgaria.
[3]Université Clermont Auvergne, CNRS, SIGMA Clermont, Institut Pascal, F-63000 Clermont-Ferrand, France.
[4]EA3900 BIOPI Université de Picardie Jules Verne, Avenue des facultés, Le Bailly, 80025 Amiens cedex, France.
**Correspondence: paolina.lukova@gmail.com*

Aim: The aim of the present study was to characterize the chemical content of water-extractable polysaccharides (WEPs) from *Plantago media* L. leaves and evaluate their antioxidant and prebiotic activity.
Materials and methods: The neutral sugars, uronic acid, total phenolic and protein content were analyzed spectrophotometrically. The monosaccharide composition of WEPs was studied by HPAEC and FT-IR. The molecular weight was estimated by SEC-MALLS. *Lactobacillus plantarum* strains (S26, S27, S30) were used to examine the prebiotic activity of the enzymatically hydrolysed WEPs. The antioxidant activity was evaluated by DPPH and FRAP assays.
Results: The HPAEC analysis showed that galacturonic acid (67.36%) was the main monosaccharide of WEPs followed by galactose (11.89%), rhamnose (7.77%), glucose (6.22%) and small amounts of arabinose, xylose and fructose. FT-IR study indicated a strong characteristic absorption peak at 1552 cm^{-1} corresponding to the vibration of COO^- group of the galacturonic acid. The molecular weight of WEPs was estimated to $3.9x10^5$. Results gave evidence that WEPs exhibited DPPH (40.08 ± 1.75%) and FRAP (132.40 ± 2.11μM Trolox equivalent) antioxidant activity. WEPs hydrolyzates stimulated successfully the production of *α*-galactosidase, *α*-glucosidase and *β*-xylosidase activity in the investigated probiotic strains.
Conclusion: The presented results suggested that *P. media* WEPs have been composed of ramified rhamnogalacturonan I and have the potential of biological active natural compounds with antioxidant and prebiotic applications.

back to contents

QUANTIFICATION OF FLAVONOIDS IN *IN VITRO* CULTURES OF *ASTRAGALUS GLYCYPHYLLOS* L.

Popova P., Zarev Y., Shkondrov A., Krasteva I., Ionkova I.
Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav str., 1000 Sofia, Bulgaria

Aim of the study: The aim was to determine the total flavonoid content of ethylacetate extracts from callus cultures of *A. glycyphyllos* cultivated on media with different concentrations of Mg^{2+} and Ca^{2+}. In addition, to determine rutin and camelliaside A quantity in shoots, callus and suspension cultures grown on Murashige & Skoog's medium (MS), supplemented with plant hormones.
Materials and methods: Callus cultures of *A. glycyphyllos* grown in MS media, supplemented with casein, 2,4-dichlorophenoxyacetic acid, kinetin and indole-3-acetic acid with different concentrations of Mg^{2+} and Ca^{2+} were used. Cultures grown on these media were used for determination of total flavonoids content.
Secondly, rutin and camelliaside A were determined in cultures cultivated either on classical MS or on modified ones both in light and in dark regimen.
Flavonoid content in both groups of samples was quantified by LC/MS.
Results: The highest flavonoids content was observed in callus cultures grown on modified medium with twice the amount of Ca^{2+}, while only a little was achieved in callus cultures cultivated on the same medium with twice reduced amount of Mg^{2+}. Camelliaside A and rutin were determined in the highest quantity in shoots.
Conclusion: Flavonol production in *in vitro* cultures can be enhanced by varying the components of culture medium. These findings suggest that *in vitro* cultivated *A. glycyphyllos* could serve as a source of pharmaceutically important metabolites.
Acknowledgement: Financial support from the Council of Medicinal Science at Medical University of Sofia, contract № D-80/2019 is acknowledged.