# BIOMEDICAL SCIENCES

## PLENARY LECTURE

### INFLAMMASOME SIGNALING IN HEALTH AND DISEASE "BODY ON FIRE"

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## Summary

Inflammasome was discovered by the team of Dr. Jurg Tschopp at the University of Lausanne in 2002. Since then Dr Tschopp and his team succeed to formulate the role of inflammasome in diseases like gout and diabetes type II, discovering how different danger signals might provoke the inflammasome reaction, including viral DNA, muramyl dipeptide, asbestos and silica dioxide. The inflammasomes belong to the Nod-like receptor family of proteins, which is encompassed of around 22 members in humans, each usually consisting of three domains with different functionality – receptor, signalling, and eventually effector one. Some members are receptors only, while others are danger signal aggregators, being able to elicit a pro-inflammatory signalling and even cell death. The role in human pathology of these molecules has become more evident in the recent years, especially after their immune cell only-expression paradigm has been shifted away. Inflammasomes and their signalling perturbations are tightly related to pathogenesis of various autoimmune diseases, cancerogenesis, metabolic syndrome, etc., which makes them potential high therapeutic potential target molecules. Original data will be also presented, showing their role in the male infertility initiation and their role for the reproductive health in general. Their importance for the relation between environment changes and human pathology based on their role as major danger sensor and inflammation response effector in numerous cell types will be summarised.

**Key words:** inflammasome, male infertility, reproductive health

## ORAL PRESENTATIONS

### TISSUE AND LIQUID BIOPSY-BASED RT-PCR METHOD IN A SINGLE INSTITUTION STUDY ON EGFR MUTATIONAL STATUS IN PATIENTS WITH NON-SMALL-CELL LUNG CANCER (NSCLC), TREATED WITH FIRST LINE TYROSINE KINASE INHIBITORS (TKIS)

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## Summary

According to international guidelines, patients with non-small cell lung cancer (NSCLC), whose tumour harbours activating mutations in the EGFR gene, are treated in the first-line setting with TKIs. After a certain period (usually10-12 months), some of these patients progress on that treatment. In two-thirds of the cases, the resistance to first- and second-generation TKIs is based on a secondary mutation in exon 20 of the same gene – T790M. This study aimed to detect the frequency of mutation and adequacy of the method for tissue and liquid biopsy by NSCLC patients progress on first-line TKI treatment. The study included 52 patients with activating

mutations in the EGFR gene - exons 18-21, previously treated with TKIs. After radiological or clinical progression, these patients were tested for T790M mutation. In 14 of the 52 patients, the study was based on DNA extraction, using real-time PCR from formalin-fixed, paraffin-embedded tumour tissue samples. Cobas® DNA Sample Preparation Kit, Roche was used for DNA extraction. In 38 of the 52 cases, blood was collected from patients for DNA extraction in 8ml K2EDTA using cobas® cfDNA Sample Preparation Kit, Roche for circulating free DNA - the method of liquid biopsy. Cobas® EGFR Mutation Test v2 real-time PCR test was used for genotyping and identifying 42 most frequent mutations localized in exons 18-21 of the EGFR gene, including T790M resistant mutation. Activating control mutation was detected again in 24 (46%) of all the patients tested: 13/52 (92%) in tumour tissue samples, 11/52 (28.9%) in plasma samples. T790M mutation was found in 13/52 patients (25%): By 7/14 (50%) after analyzing DNA from paraffin-embedded tumour tissue samples and by 6/38 (15.7%) after the liquid biopsy. In 3 liquid biopsy-based cases neither activating EGFR mutation nor mutation of resistance T790M was detected. Therefore, re-biopsy was performed to get a tissue sample. In all of those 3 cases, an activating mutation was detected, in 1 of them T790M as well. Results of our study match available international data and confirm that the liquid biopsy method is optimal for detecting EGFR mutation status and secondary resistance mutations. By detecting T790M, we select patients for potential treatment with third-generation TKI. The method of liquid biopsy is reliable when at least one activating mutation is detected. In the cases of absence of the control activating mutation, the result is considered unspecified, and it is recommendable to repeat liquid biopsy until a positive result is confirmed or to perform re-biopsy for a new tumour tissue sample.

## APPLANATION TONOMETRY FOR ASSESSMENT OF LEFT VENTRICULAR SYSTOLIC LOAD

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## Summary

Vascular-ventricular coupling is a major determinant of the left ventricular systolic load. Our study aimed to assess non-invasively left ventricular load and its dependency on central arterial hemodynamics. Sixty-five healthy and gender-matched individuals were divided into two groups according to their age: 20y/o and 50y/o. Applanation tonometry was performed using the Sphygmocor device (AtCor Medical). Central pressures and the major indices of pulse wave analysis were computed using Sphygmocor software. Central systolic (120±3 vs. 98±2 mm Hg) and pulse pressures (43±3 vs. 29±1 mm Hg) as well as the augmentation index (AIx75), standardized for heart rate 75 beats per minute (23±3 vs. -6±2%) were significantly higher in the 50y/o group as compared to the young individuals ($p<0.01$, Mann-Whitney test). All these parameters are relevant markers of arterial stiffness and evidenced the development of morphological and functional alterations of the central arterial wall in the middle-aged group. The time-tension index (TTI) computed from the systolic pressure area under the pulse waveform is presumed to represent the left ventricular load and myocardial oxygen demand. TTI was significantly higher in the 50y/o subjects as compared to the 20y/o group (2378±66 vs 1954±73 mmHg s, $p<0.01$). Moreover, we have shown the presence of a significant correlation between TTI and AIx75 ($p<0.01$) in both age groups. This finding confirmed the impact of arterial stiffness on the development of impaired vascular-ventricular coupling. In conclusion, applanation tonometry might be utilized for non-invasive evaluation of the left ventricular load, which is an important parameter of cardiovascular risk.

**Key words:** applanation tonometry, pulse wave analysis, left ventricular load, time-tension index

## ANIMAL MODELS BASED ON TELEMETRY FOR INVESTIGATION OF MECHANISMS OF ACTION OF SELECTIVE CALCIUM INHIBITORS ON PARKINSONIAN DISEASE MODEL 6-OHDA RATS *(IN VIVO)*

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### Summary

Animal model systems allow researchers to investigate the pathogenesis of disease states in ways that would be inaccessible in humans by performing procedures on animals, suggesting a level of harm that would not be considered as ethical to a person. The best animal models of disease are almost similar in aetiology (mechanism of development) and phenotype (signs and symptoms) to the human equivalent. Due to the complex nature of interaction in tracing the pathogenesis of human diseases, they can often be better understood in a simplified (model) system in which the individual parts of the pathological process are isolated and examined. In our laboratory, we use method for telemetrically evaluation of vital signs and parameters from conscious animals (Stellar Telemetry Ltd.) – next generation of implantable telemetry technology. The system allows monitoring of unlimited animals with just one receiver, thus allowing group housing and social interaction studies. Animals are allowed to roam freely and interact in groups with no restriction in number. The Stellar Telemetry system can be implanted into any animal from mice to swine with no additional items required. Stellar Telemetry transmitters allow measurement of activity, pressure (P), electrocardiography (ECG), electroencephalography (EEG), electromyography (EMG), electrooculography (EOG), heart rate and temperature measurement in a hermetically sealed implanted system. All leads feature stable state pressure tipped sensors, eliminating slow frequency response, head pressure and animal movement noise, which are associated with fluid-filled catheters. All mentioned above parameters can be estimated in already made animal models of Cardiovascular or Neurodegenerative disease.

**Key words:** neurodegenerative disorders, animal models, electrocorticography, telemetry

## DYNAMIC SCIENCE STRATIFICATION IN THE FIELD OF BREAST CANCER IMMUNOHISTOCHEMISTRY

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## Summary

Our purpose was to analyze some peculiarities of the dynamic institutionalization and internationalization of breast cancer immunohistochemistry research and identify the outstanding researchers, information sources and scientific institutions in this interdisciplinary field of rising socio-medical importance. In May 2019, a retrospective problem-oriented, title-word based search was performed in Web of Science Core Collection (WoS), MEDLINE and BIOSIS Citation Index (BIOSIS) of Web of Knowledge as well as in Scopus for 2003-2017. The annual dynamics of publications, authors' names, document types, journal and scientific forum titles, institutional nominations, languages of papers and citations received were comparatively assessed. There were 1097 publications abstracted in WoS, 746 in BIOSIS, 664 in Scopus and 591 in MEDLINE. Scientists from 84 countries published articles in 5 languages in WoS, from 71 countries in 15 languages in Scopus, from 65 countries in 4 languages in BIOSIS and in 8 languages in MEDLINE. R. Bhargava, I. O. Ellis and D. G. Hicks presented with most publications. The 'core' journals were Modern Pathology and Laboratory Investigations. The University of Texas System (USA) was the most influential institution. The annual meetings of the United States and Canadian Academy of Pathology contained most conference papers and abstracts. The article by M. E. H. Hammond et al. (J. Clin. Oncol., 2010;28(16):2784-2795) received 1419 citations in WoS, 1567 in Scopus and 300 in BIOSIS. Our results, along with this collection of comprehensive factual information, are of interest for oncologists from smaller countries, institutional and national science managers and journal editors.

**Key words:** scientometrics, breast cancer immunohistochemistry, science internationalization, science institutionalization, information portals

## PLEIOTROPIC EFFECTS OF ANGIOTENSIN-CONVERTING ENZYME

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## Summary

Angiotensin-converting enzyme (ACE) as a component of the renin-angiotensin system (RAS) participates in blood pressure regulation and fluid and electrolyte homeostasis. This metallopeptidase generates vasopressor peptide angiotensin II (Ang II) and inactivates vasodilator peptide bradykinin. ACE has several other peptide substrates. In this review, we discuss the physiological functions of other ACE cleavage products. According to recent findings, ACE is specific in the degradation of Ac-SDKP – a potent antifibrotic agent and negative regulator of blood stem cell proliferation. The profibrotic action of RAS in the myocardium, kidneys and liver is due to Ang II and ACE, and the antifibrotic effect of ACE-inhibitors is a result not only from a reduction of Ang II, but also from the elevation of Ac-SDKP. Another substrate of endopeptidase activity of ACE is Alzheimer amyloid beta-peptide, but the brain RAS is involved in the development of Alzheimer disease( AD) in a more complex manner – by the Ang II effects on AT1 (angiotensin receptors type 1): increased production of amyloid beta-peptide, oxidative stress, inflammation, and reduced acetylcholine release. The beneficial effect of ACE-inhibitors and AT1 blockers against AD is explained by a counterbalanced activation of AT2 (angiotensin receptors type 2). Results from experiments on mice with overexpression of ACE demonstrate the participation of this enzyme in different steps of innate or adaptive immune response – phagocytosis, oxidative response, the bactericidal activity of neutrophils, and antigen processing. Ang II mediates some

of the increased immune effects of ACE, but other responsible peptides are unknown. A better understanding of the pleiotropic effects of ACE could be particularly useful to clarify the results of ACE-inhibitors treatment and the development of novel therapeutic targets.

**Key words:** angiotensin-converting enzyme, fibrosis, Alzheimer, immunity

## KETOGENIC DIETS AND OBESITY

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### Summary

In recent years, the use of ketogenic diets has increased in the treatment of many metabolic diseases, including bodyweight reduction in overweight and obesity. Our objective was to assess the effect of ketogenic diets on weight loss, glycemic control and lipid profile in comparison with low-fat diets. We conducted searches of PubMed, Embase, and Google Scholar to May 2019 for metabolic responses of the organism to low-carbohydrate ketogenic diets. A critical comparative analysis was made between ketogenic diets and low-fat diets, traditionally used in obesity management. The short and long-term metabolic effects of both diets in improving glycemic control, lipid profile and weight loss were analyzed. Dietary interventions with ketogenic diets for weight loss could be used only in particular circumstances.

**Key words:** ketogenic diet, low-fat diet, obesity

## EXPLOITING *SALMONELLA ENTERICA* SEROTYPE TYPHIMURIUM AS AN IMMUNOMODULATORY AND THERAPEUTIC AGENT AGAINST CANCER

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### Summary

Modified bacterial strains maintain good anti-tumour properties and have reduced the potential to cause toxicities to the host. Because of the intrinsic ability of facultative anaerobic bacteria, such as *Salmonella*, to thrive in viable as well as necrotic tissue, bacterial therapy of cancer has several advantages over other therapies. In particular, accumulating evidence suggests that bacterial therapy works by modulating cells of the immune system to inhibit tumour growth. The aim of this presentation is to review the experimental evidence underlying the efficacy of bacterial therapy against cancer and to highlight the alterations within the tumour microenvironment that have been reported following bacterial therapy. The existing literature was reviewed for bacterial anti-cancer therapy, attenuated bacterial strains, tumour microenvironment. A literature search of relevant articles was performed using PubMed, Embase, Web of Science. Many studies have selected attenuated *Salmonella enterica* serotype Typhimurium as the bacterium of choice for cancer therapy. The bacteria are motile, easily genetically modified and grow as facultative

anaerobes in the presence or absence of oxygen. These and other properties allow *Salmonella enterica* serotype Typhimurium to not only target tumours, but also deliver therapeutic agents or immune modulators to the tumour site. *Salmonella enterica* serotype Typhimurium can be used to target and destroy tumours in three specific ways: 1) delivery of anti-cancer agents; 2) sensitizing the immune system to tumour presence; 3) using bacterial toxins to trigger apoptosis. *Salmonella enterica* serotype Typhimurium is a novel therapeutic agent that can be used to enhance anti-tumour immune responses, but further studies are required to achieve minimal host toxicity.

**Key words:** *Salmonella enterica* serotype Typhimurium, anti-tumour immune response, host toxicity

POSTERS

## FADS1 (RS174547 SNPS) GENOTYPES ASSOCIATIONS WITH METABOLIC SYNDROME

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### Summary

Significant interactions between specific risk frequencies of SNPs of FADS genotype in subjects suffering from cardiovascular diseases and type II diabetes with alteration of serum lipids, oxidative stress and inflammatory markers, and glucose homeostasis are presented. This study aimed to investigate the relationship between the single genetic variants of the FADS1 genes and the five components of the metabolic syndrome (MetS). Ninety-eight volunteers (65 with MetS and 33 controls) from Pleven and the region were studied. Determination of gene polymorphisms was performed by PCR using the Gene GET Genomic DNA Purification Kit. Biochemical indices: total cholesterol, HDL, LDL, triglycerides and glucose were measured. Bio-impedancemetry was applied for body composition analysis. No significant differences were found in the relative shares of the MetS and the controls in the rs174547 genotype. In the MetS group, the T allele (major allele) frequency was 0.73, the C allele (MAF-minor allele) frequency was 0.27. Statistically significantly lower mean systolic blood pressure ($p<0.03$) and diastolic blood pressure ($p<0.02$) was found in the male heterozygous C/T carriers. In males with MetS, the C allele effect reduced the triglyceride level ($p<0.06$) and blood glucose ($p<0.078$) marginally. In female controls, who were carriers of the effect C allele, blood glucose levels were significantly lower ($p<0.01$). Female carriers of C/T genotype with MetS had higher HDL-cholesterol values ($p<0.07$). The results showed the protective role of the individual genotype C/T in subjects with metabolic syndrome.

**Key words:** metabolic syndrome, FADS1 (RS174547 SNPS) genotypes

## AGOMELATINE TREATMENT CORRECTS IMPAIRED SLEEP-WAKE CYCLE AND SLEEP ARCHITECTURE THROUGH RESTORATION OF BDNF LEVEL IN THE HIPPOCAMPUS OF RATS EXPOSED TO CHRONIC CONSTANT LIGHT

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## Summary

Exposure to chronic constant light (CCL) has a detrimental impact on circadian rhythms of motor activity and sleep/wake cycles. Agomelatine is an atypical antidepressant showing a chronotropic activity. In this study, we explored whether BDNF in the brain is involved in the mechanism underlying the effects of agomelatine on diurnal variations of motor activity, sleep/wake cycle and sleep architecture in a model of CCL.The rats exposed to CCL showed an impaired diurnal rhythm of motor activity and sleep/wake cycle. While the slow-wave sleep (SWS) and delta power were reduced, a profound increase of rapid eye movement (REM) sleep and theta power was detected during the light and the subjective dark period in CCL rats. However, the duration and number of episodes of the wake were diminished during the subjective dark period in this group. CCL exposure resulted in a reduced level of BDNF in the hippocampus. Agomelatine restored the diurnal rhythm of motor activity, disturbed sleep/wake cycle and sleep architecture, and also decreased the level of BDNF in the hippocampus. These findings support the value of agomelatine as an antidepressant that can adjust circadian homeostasis of motor activity and sleep/wake cycle by correcting the BDNF levels in the hippocampus in a CCL model.

**Key words:** circadian rhythms; EEG; BDNF; agomelatine; rat

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## BENEFICIAL ROLE OF ENDURANCE TRAINING ON DESYNCHRONIZED HORMONAL CIRCADIAN RHYTHMS IN EXPERIMENTAL MODEL OF MELATONIN DEFICIT

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## Summary

Pinealectomy is associated with disturbed circadian rhythms of the endocrine and metabolic functions in the body. Aerobic endurance training is considered a part of complex therapy of many diseases driven by disruptions in circadian dynamics of some physiological indicators, including endocrine and metabolic dysfunction. The present study aimed to explore the influence of systematic endurance training on depressive-like behaviour and associated desynchronized circadian rhythms of melatonin and corticosterone secretion in a rat model of melatonin deficit. Pinealectomy induced a depressive-like behaviour characterized by a decreased grooming in the splash test, diminished preference to sucrose during the light phase in the sucrose preference test and increased immobility in the forced swimming test (FST) of rats. The long-term aerobic training exercises positively affected the depressive-like behaviour in a model of melatonin deficit. It restored the impaired emotional behaviour and increased grooming in splash test, attenuated anhedonia in the active period and hopelessness behaviour in the FST in the group with pinealectomy. Parallel

to the observed positive effect on depression, the exercise procedure corrected the abolished circadian patterns of plasma corticosterone level but not that of melatonin. These findings indicated that the antidepressant-like effect exerted by endurance training might be mediated via correction of the disturbed circadian rhythm of corticosterone release in rats. Therefore, this alternative mode might have a potential therapeutic application in a subpopulation of people with a melatonin deficit.

**Key words:** pinealectomy; aerobic endurance training; depressive behaviour; corticosterone; melatonin; rat

**Acknowledgements:** This work was supported by the National Science Fund of Bulgaria (research grant # No DN 03/10).

## MEMORY-MODULATED EFFECTS OF SUBCHRONICAL CENTRAL ADMINISTRATION OF CANNABINOID RECEPTOR LIGANDS ON OLFACTORY BULBECTOMIZED RATS

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## Summary

The endocannabinoid system plays a role in many physiological processes, including mood, learning and memory. It is also involved in the pathogenesis of anxiety and mood disorders, as well as of neurodegenerative disorders. However, the exact role of cannabinoid CB1 receptors in learning and memory is not well understood. This study aimed to evaluate the effects of cannabinoid ligands, HU 210 (CB1 agonist) and SR 141716A (CB1 antagonist) on learning and memory, applied i.c.v. for seven days on developed depression background. Olfactory bulbectomized rats were used as an experimental model of depression. An active avoidance (shuttle box) and passive avoidance (step-through) tests were used to evaluate learning and memory. OBX rats developed depressive-like behaviour and showed memory deficits. We found that the subchronic treatment of OBX rats with HU 210 and SR 141716A exerted a modulatory effect on performance in both active avoidance (shuttle box) and passive avoidance (step-through) tests. HU 210 ameliorated the memory deficits of OBX rats. However, the scores of the sham-operated controls were not reached. SR 141716A augmented the memory deficits of the OBX rats, the effects being more significant in the shuttle box test. The results point to the possible involvement of CB1 receptors in the learning and memory deficits of OBX rats. Future studies with long-term administration of CB1 antagonist are needed, as it looks promising to bring full recovery of the memory impairments in OBX rats.

**Key words:** CB1 receptor, learning and memory, depression, olfactory bulbectomy, rats

**Acknowledgements:** This study was supported by Grant of the Medical University of Varna 2012/2014.

## SUBCHRONIC CENTRAL ADMINISTRATION OF CANNABINOID LIGANDS MODULATES NOCICEPTION IN BULBECTOMIZED RATS

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## Summary

Endocannabinoid system is involved in neuropsychiatric disorders such as major depression. The bilateral olfactory bulbectomized rat is widely used as an animal model of depression. The removal of the olfactory bulbs produces behavioral, physiological and neurochemical alterations, resembling clinical depression. There are increasing evidence highlighting an important role of the cannabinoid signaling in depression and nociception. The aim of this study was to investigate the effect of CB1 receptor agonist HU 210 and CB1 receptor antagonist SR 141716A administered i.c.v. subchronically (for 7 days) on nociception of rats with model of depression - bilateral olfactory bulbectomy (OBX). Bilateral olfactory bulbectomized rats were used as an experimental model of depression. HU 210 (5 μg) or SR 141716A (3 μg) were infused i.c.v. for 7 consecutive days, starting 15 days after the olfactory bulbectomy. Nociception was examined by applying paw pressure test (analgesy-meter) evaluating the rat pain threshold. On the 7$^{th}$ day, 5 minutes after the last microinjection, the rats were tested in an analgesy-meter and their mechanical evoked pain responses were measured in arbitrary units (AU). Microinjections of HU 210 (5 μg) significantly increased the pain threshold in olfactory bulbectomized rats i.e. exert antinociceptive effect, while SR 141716A (3 μg) exerted nociceptive effect by decreasing the pain threshold. Data point to an involvement of CB1 receptors in depression-like behaviour and nociception in olfactory bulbectomized rats and support the data for the association between depressive disorder and pain pathways.

**Key words***:* CB1 cannabinoid receptors, nociception, olfactory bulbectomy, depression, rat

## EVALUATION OF CYTOTOXICITY AND OXIDATIVE STRESS OF MNPS FOR BIOMEDICAL APPLICATIONS

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## Summary

Magnetic nanoparticles (MNPs) are used in clinical medicine for hyperthermia in cancer therapy, drug delivery or as a medical diagnostic tool, including magnetic resonance tomography. For this purpose, MNPs must possess excellent

biocompatibility, i.e., are not cytotoxic and do not cause oxidative stress. This study was intended to evaluate the cellular toxicity of MNPs in cancerous and non-cancerous cells, as well as the induction of oxidative stress *in vivo*. Cytotoxicity of MCF-7 (breast cancer cells, ATCC®) and MCF-10 (non-cancerous cell line, ATCC®) was studied by MTT test. The MNPs (Chemicell GmbH, fluid MAG-D with an average hydrodynamic diameter of 100 nm) were i.c.v. injected at doses of 10, 100 and 200 μg/kg in ICR mice. The animals were decapitated, and the hippocampi were isolated at 3rd, 24th and 48th h, respectively. The brain homogenates were used to evaluate oxidative stress by ELISA for superoxide dismutase (SOD) and malondialdehyde (MDA) production. The non-toxic concentrations of MNPs for MCF-7 and MCF-10A cells for a co-incubation period of up to 72 h were determined in the range of 100 μg/kg - 200 μg/kg of MNPs. Our *in vivo* study revealed that MNPs in concentrations between 10 μg/kg to 200 μg/kg did not cause oxidative stress in the hippocampus of intact mice. Based on the above results, we conclude that concentration of MNPs in the range of 100-200 μg/kg are well biocompatible and could be used in various experiments as hyperthermia in cancer therapy, drug delivery or as a tool for medical diagnostics.

**Key words:** magnetic nanoparticles, cell viability, oxidative stress, breast cancer cells, hippocampus

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## EFFECTS OF ENDURANCE TRAINING ON DESYNCHRONIZED CIRCADIAN RHYTHMS OF OXIDATIVE STRESS MARKERS IN A RAT MODEL OF MELATONIN DEFICIT

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## Summary

Experimental and clinical studies have demonstrated that melatonin deficit is characterized by disturbed circadian rhythms in regard of a number of various biochemical parameters, including systemic markers of oxidative stress. The direct impact of physical activity on markers of oxidative stress in disease has also been well investigated. We aimed to study the influence of aerobic training with a treadmill on desynchronized circadian rhythms of molecules of oxidative stress in pinealectomized rats. The animals were divided into four groups: sham-operated sedentary rats (sham-sed), sham group with exercise (sham-ex), pinealectomized sedentary rat (pin-sed) and pin rats with exercise (pin-ex). Plasma sample were collected at 4-h intervals for biochemical analysis of oxidative stress. The activity of superoxide dismutase (SOD) the total glutathione (GSH) levels, as well as lipid peroxidation levels evaluated by MDA levels, demonstrated diurnal variations in

the sham-sed group. The peak values of GSH and SOD activity were detected during the dark period, during which melatonin release reaches peak levels. The MDA level rose progressively during the dark period with a normal diurnal fluctuation pattern and shifted in pin-sed rats. Pinealectomy was characterized by a flattened rhythm of SOD activity as well as by shifted and diminished GSH levels. Significantly lower values of MDA were detected 4 hours (22:00 h) after light off in the sham-ex group, as compared to the sham-sed group. In addition, significantly lower MDA levels were detected, at 10:00 h, 18:00 h and 22:00 h in the pin-ex group, as compared to the pin-sed group. The endurance training significantly increased the levels of GSH at dark period onset (18:00 h) and 4 h earlier – the SOD activity in the sham-ex group. A significantly higher SOD activity was detected at onset of light (06:00 h) and four hours later (10:00 h) in the pin group. Our findings indicate that endogenous melatonin plays an important role in diurnal rhythms of components of oxidative stress, while exercise has an antioxidant activity in both pathophysiological conditions and melatonin deficit.

**Key words:** endurance training, melatonin deficit, diurnal rhythms, SOD, GSH, MDA, rat

**Acknowledgements:** This work was supported by the National Science Fund of Bulgaria (research grant # No DM 11/4; DN 03/10).

## THE ADRENERGIC AND THE ENDOGENOUS CANNABINOID SYSTEMS INTERACT IN MODULATION OF HEAT STRESS-INDUCED ANALGESIA

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### Summary

During stress, several physiologic parameters of the body change, pain perception among them. Variations in this so-called stress-induced analgesia (SIA) could be regarded as an indicator of stress reaction development. Several systems interact in the mechanisms of such a reaction. Understanding the stress reaction pathogenesis could give helpful clues for decreasing the potentially harmful effect of stress on the body. Both the adrenergic and the endogenous cannabinoid systems have been demonstrated to take part in stress reaction development, but the exact pattern of their mutual influence has not been elucidated yet. The present study aimed to estimate the effect of the adrenergic and the endogenous cannabinoid systems interaction on SIA after one hour of heat exposure. The experiments were carried out on male Wistar rats, subjected to 38° C for one hour. The $\alpha_2$-Adrenergic agonist Clonidine and $\alpha_2$-adrenoceptor antagonist Yohimbine were administered in different experimental protocols along with CB1-receptor agonist anandamide (AEA) and the CB1-receptor antagonist AM251. The paw-pressure test was adopted for evaluation of pain perception. The results obtained showed that the administration of both AEA and clonidine increased PP-thresholds in intact animals. In the animals after 1 hour of heat exposure, the combination AEA+clonidine increased heat-SIA. Antagonization of CB1- and/or $\alpha_2$-adrenergic receptors abolished the analgesic effect in intact animals and decreased PP-thresholds in animals after 1 hour of heat exposure. Heat-SIA depression was more prominent after yohimbine than after AM251-administration. The adrenergic and the endogenous cannabinoid systems interact in the modulation of heat stress-induced analgesia.

**Key words:** endogenous cannabinoid system, adrenergic system, nociception, stress-induced analgesia, heat stress

**Acknowledgements:** This study was funded (GRANT No15/2016) by the Council of Medical Science, Medical University of Sofia.

## ROLE OF THE OPIOID RECEPTORS IN THE ANTINOCICEPTIVE EFFECTS OF A NEWLY SYNTHETIZED HEMORPHINE PEPTIDE ANALOGUE

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### Summary
In the present study, a new peptide analogue of VV-hemorphin-5 (V1), modified in position 1 by removing one valine and substitution of hydrogen with phosphorus (V2p) was synthesized to investigate its potential analgesic activities in an experimental model of acute and inflammatory pain in adult male ICR mice. We used selective antagonists for delta (naltrindole hydrochloride) and kappa (nor-binaltorphimine dihydrochloride) opioid receptors to evaluate the potential participation of the endogenous opioidergic system in the peptide-induced effects on the nociception. Three doses of the peptide (50, 25 and 12,5 μg/mouse), dissolved in artificial cerebrospinal fluid, were administered intracerebroventricularly before local intraplantar injection of the irritant formalin. Time of licking or shaking the injected hind paw was measured and divided into two phases of pain reaction: acute from 0 to 20th minute, and inflammatory from 20th to 40th after the injection of formalin. The referent peptide V1 showed a significant dose-dependent analgesic effect in both phases of the test. V2p showed an analgesic effect in all three doses used during the acute and inflammatory phases. Delta opioid receptor antagonist abolished the antinociception of V2p during the acute phase, while kappa opioid receptor antagonist significantly diminished the antinociceptive effects of V2p on the inflammatory pain reaction. These data showed that the hemorphin analogue had an antinociceptive activity similar to the original hemorphin, and this effect is mediated by the endogenous opioidergic system due to the participation of delta and kappa receptors.
**Key words:** hemorphin analogues, opioid peptides, analgesic activity, formalin test, mice
**Acknowledegments:** Supported by Grant 80-10-191/2019, Sofia University.

## EXPERIMENTAL STUDIES ON SEROTONIN SYNDROME IN RATS

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### Summary
Serotonin syndrome is likely to be observed as a result of an overdose of serotoninergic drugs or interactions with the combined administration of two or more drugs that increase the intrasynaptic concentration of the 5-hydroxytryptamine (serotonin). The present study investigated the development of serotonin syndrome in rats after administration of serotonergic drugs with different pharmacological mechanisms. We investigated somatic and behavioural manifestations on different animal models of serotonin syndrome, induced by co-administration of serotonergic drugs: 5-hydroxy-L-tryptophan,

clorgyline, tranylcypromine, and fluoxetine. The development of hyperthermia is associated with severe forms of serotonin syndrome and can lead to a fatal outcome. We conducted the experiments at an ambient temperature of 22 ± 1°C. The body temperature of the animals was measured with thermistor probes (TX-8) and monitored on multichannel recorder Iso-Thermex 16. The body temperature was recorded at 30 min intervals following injection of serotonergic drugs. The manifestations of serotonin toxicity observed in the serotonin syndrome models, used in the study, are a headache, paw progression, hind limb ablation, Straub phenomenon, tremor, and hyperthermia. In our experimental studies in rats, typical symptoms of drug-induced serotonin toxicity were identified.

**Key words:** serotonin syndrome, somatic manifestations, behavioural manifestations, hyperthermia, rats

## RIVASTIGMINE AFFECTS MEMORY DISTURBANCES IN RATS WITH ABDOMINAL HYPERTENSION

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**Summary**

Current studies have shown that increased intraabdominal pressure modulates brain perfusion induces hypoxic changes and impairs memory and other cognitive functions. The aim was to investigate whether artificially increased intraabdominal pressure (IIAP) in rats lower their cognitive potential and Rivastigmine can diminish this disturbance. Male Wistar rats (8 per group) were treated with: 1st saline 0.1 ml/100g; 2nd anesthetic (ketamine) + IIAP to 25mmHg (model group); and 3rd anesthetic (ketamine) + IIAP + Rivastigmine 1 mg/kg. The Shuttle-box active avoidance test was made with the parameters observed: number of avoidances, escapes and intertrial crossings. Statistical evaluation was done by ANOVA. In the active avoidance test, the model group decreased the number of avoidances and escapes on the same days of learning and memory tests, as compared to the controls. The group with model and Rivastigmine increased avoidances in 2nd, 3rd, 4th, 5th days learning and on memory test compared to the group with the model. The group with IIAP-model decreased the number of inter-trial crossings on learning and memory retention session, compared to the saline group. The group with IIAP and Rivastigmine increased inter-trial crossings on 3rd, 4th and 5th-day learning, and on memory test compared to the group with model only. Our results allow us to conclude that high intraabdominal pressure impairs learning and memory processes in rats. Rivastigmine improves cognitive functions in rats with intraabdominal and subsequent cerebral hypoxia, probably as a result of an increased level of acetylcholine in synaptic terminals.

## EFFECT OF ATORVASTATIN ON ACUTE INFLAMMATION AND PLASMA LEVELS OF IL-10 IN RATS

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## Summary
Statins possess a wide variety of pleiotropic properties that are independent of their lipid-lowering abilities such as attenuating inflammation, oxidative stress, coagulation, platelet aggregation and neuroprotection. The present study aimed to investigate the anti-inflammatory effect of atorvastatin in a model of acute inflammation. Male Wistar rats were used in the study, divided into four groups. Animals were treated orally for 90 days with atorvastatin 10 and 20 mg/kg bw. The control group was given saline 1 ml/100 g. At the day of the experiment, the fourth group received indomethacin 9 mg/kg bw orally. After the 90 days, paw oedema was induced by carrageenan injection, and the footpad volume was measured at the 4$^{th}$ hour, using a plethysmometer. Blood samples were collected, and plasma concentrations of the anti-inflammatory cytokine interleukin-10 (IL-10) were measured. Indomethacin significantly reduced the extent of the paw oedema at the 4$^{th}$ hour after the carrageenan injection, as compared to the saline-treated group. Administration of atorvastatin in both doses produced significant inhibition of oedema, compared to the saline-treated group. Both statin-treated groups significantly increased levels of IL-10 compared to the saline-treated group. The results from our study suggest that atorvastatin exerts an anti-inflammatory effect, concerning the acute local inflammation and increases the plasma levels of the anti-inflammatory cytokine IL-10.

**Key words**: statins, anti-inflammatory, IL-10, carrageenan-induced paw oedema

## EFFECT OF METABOLIC SYNDROME ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

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## Summary
Metabolic syndrome is a significant public health concern of increasing prevalence. The typical feature of metabolic syndrome – visceral adiposity, is known to be associated with low-grade chronic inflammation. The current experiment aimed to check if the acute inflammatory response would be enhanced by the metabolic syndrome. The present results summarize the results from four different experiments involving 70 male Wistar rats. Half of the rats (the MS group) were subjected to diet-induced metabolic syndrome by feeding them standard laboratory food enriched with 17% lard

and 17% fructose and drinking 10% fructose solution. The control rats (C group) received regular rat chaw and plain water to drink. After ten weeks, all animals underwent testing for acute inflammation by injecting 0.1 ml of 1% carrageenan in saline into the plantar surface of the left hind paw. The volumes of the injected paws were measured 30, 60, 120, 180, 240, and 300 min after the induction of inflammation using a plethysmometer. The rats from the MS group did not increase their body weight but developed significantly more visceral adiposity compared to the C group, including perigonadal, mesenteric, perirenal and retroperitoneal fat pads. The paw oedema, as expressed by the paw volume, was considered a measure of acute inflammation. No differences were observed between the MS and C groups in terms of the paw oedema at any time point. Contrary to the expectations, we found that the metabolic syndrome did not modify acute inflammation despite the presence of increased visceral adiposity.

**Key words:** metabolic syndromes, visceral adiposity, carrageenan-induced paw edema

## SERUM LEVELS OF PROINFLAMMATORY CYTOKINES AFTER COMBINED THERAPY IN AN EXPERIMENTAL MODEL OF AUTISM

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### Summary

In recent years, neuroinflammation has been identified as an essential part in the etiopathogenesis of neurodevelopmental, neurological and psychiatric disorders. Numerous scientific reports discuss the dysregulation of immune parameters that are associated with changes in the microbiota in autism spectrum disorders (ASD). Our previous studies showed beneficial effects of the single administration of probiotic, risperidone or pioglitazone on molecular and behavioural parameters of rats with an experimental ASD. We aimed to investigate the effects of combined administration of probiotic, pioglitazone or risperidone on serum levels of IL-1β and IL-6 in an experimental model of autism. The male offspring of pregnant Wistar rats, treated or not with valproic acid during the pregnancy period, were separated from their mothers on the 23rd postnatal day and were divided into five groups: 1. Control; 2. Experimental; 3. Experimental, treated with a combination of pioglitazone+risperidone; 4. Experimental, treated with a combination of pioglitazone+probiotic; 5. Experimental, treated with a combination of risperidone+probiotic. After 21 days of treatment, the rats were decapitated, blood was collected, and the sera were separated to determine proinflammatory cytokine levels. There was a significant increase in serum IL-6 levels in the experimental group, as compared to the control one. Combined administration of the substances statistically significantly decreased the serum levels of the studied proinflammatory cytokines in all groups with experimental autism. We observed the most considerable decrease in both IL-1β and IL-6 levels in the experimental groups treated with a combination of pioglitazone and probiotic. Our results indicated that combined administration of pioglitazone, probiotic and/or risperidone improves pro-inflammatory parameters in an experimental model of ASD.

**Key words:** neuroinflammation, autism spectrum disorders, proinflammatory cytokines, pharmacotherapy

## INVESTIGATING THE EFFECTS OF THE DOPAMINERGIC AGONIST PRAMIPEXOLE ON LEARNING AND MEMORY IN DIAZEPAM-CHALLENGED RATS

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## Summary
Pramipexole (PMX) is a dopamine receptor agonist used to treat Parkinson's disease. The data concerning the effect of dopaminergic therapy on cognition in Parkinsonian patients is insufficient. Our study aimed to evaluate the effect of pramipexole on learning and memory in rats with diazepam-induced memory impairment. For this purpose, we used male Wistar rats divided as follows (n=8): saline; diazepam group (2.5 mg/kg bw); three experimental groups with diazepam and pramipexole at doses of 0.5;1 and 3 mg/kg bw. Two-way active avoidance test (TWAAT) and locomotor activity tests were performed. The number of conditioned (CR) and unconditioned responses (UCR), vertical and horizontal movements were registered. The diazepam-challenged rats significantly decreased the number of CR during the training session and both memory tests, as compared to saline. However, the decrease in UCR was insignificant. The rats treated with diazepam and pramipexole at doses of 0.5 and 3 mg/kg bw notably increased the CR during short- and long-term memory tests in comparison to the diazepam group. The animals with diazepam and PMX at a dose of 1 mg/kg bw significantly increased the CR during the long-term memory test when compared to both control groups. The lowest dose of PMX significantly increased the UCR during both retention tests, whereas the highest dose increased the number of UCR only during the short-term memory test. The three experimental groups demonstrated improved motor activity compared to both controls. Based on our results, we could conclude that pramipexole improves short- and long-term memory in TWAAT.

**Key words:** pramipexole; learning; memory; diazepam

**Acknowledgements:** The study was financially supported by a grant (SDP-No 11/2015) from the Medical University of Plovdiv, Bulgaria.

## EFFECT OF *HABERLEA RHODOPENSIS* AND HOMOEOPATHIC SILICEA 9CH ON THE WHITE BLOOD CELL LEVELS IN PERIPHERAL BLOOD OF MICE

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## Summary
Bone marrow stem cells are capable of migration into injured tissues – a process with a critical role in regeneration. The number of stem cells in peripheral blood mirrors the specific needs and can be increased by tissue damage or intake of

specific substances. The aim of this study was to evaluate the effect of total extract of Haberlea rhodopensis (HR) and the homeopathic drug Silicea 9CH on white blood cells (WBC), segmented neutrophils (Sg) and stab cells (St) in peripheral blood of mice as an indirect indicator for activated/suppressed proliferation and maturation of stem cells. Thirty male mice were divided into three groups of 10 and treated as follows: 1-st group (control) – Aqua destillata; 2-nd group - total extract of HR, and 3-rd group – aqueous solution of Silicea 9CH. The total count of WBC, St, and Sg cells was evaluated 2 hours after oral administration in samples of peripheral blood. Haberlea rhodopensis induced a significant decrease in the number of WBC in comparison to the control group (9.7±1.0 vs 15.6±1.7; $p<0.050$). Similar results were obtained with Silicea 9CH (12.2±1.4 vs 15.6±1.7). Silicea 9CH reduced the levels of St and increased the number of Sg cells in comparison to the controls. No statistical difference was detected in the cases of Silicea 9CH, St and Sg cells. Our results show that HR and Silicea 9CH could influence the number of stem cells. Besides, Silicea 9CH has the potential to influence the process of WBC maturation.

**Key words:** Haberlea rhodopensis, white blood cells, mice

## CONTEMPORARY DEVELOPMENT IN THE USE OF MEDICINAL CANNABIS: A REVIEW

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### Summary

Following the discovery of a cannabinoid system in the brain, there has been an increased interest in the medical use of cannabis accompanied by an extremely dynamic scientific effort towards the compounds, found in the cannabis plant, namely cannabinoids. This report aimed to provide a brief overview of the contemporary state of knowledge and recent developments, regarding the medical use of cannabis and the respective regulatory frameworks. We summarized information from systematic reviews of evidence from controlled trials and cites directives from international legal frameworks. Medical use of cannabis and cannabinoids implements diverse routes of administration and incorporates a variety of preparations and products with different active ingredients, including medicinal products with marketing authorization, or preparations, such as raw cannabis, magistral and standardized preparations. There are more than one hundred various compounds in cannabis, with tetrahydrocannabinol (THC) and cannabidiol (CBD) being the two most extensively studied. The evidence from controlled clinical trials is rapidly growing and models the use of cannabinoids for many indications, such as anti-emetic, for stimulating appetite, neuropathic pain and spasticity in multiple sclerosis, chronic non-cancer pain, palliative cancer care, childhood epilepsy and depressive disorders. The legal approach to allow the medical use of cannabis or cannabinoids differs a lot within the EU and trends to create a system that provides approval and oversight, limits the use to a restricted set of medical conditions, and often precisely defines the preparations available for patients.

**Key words:** cannabidiol, medical use, tetrahydrocannabinol

## INVOLVEMENT OF OPIOID RECEPTORS IN THE ANALGESIC EFFECT OF A NOVEL PYRROLIC COMPOUND

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## Summary
Pyrrole-based compounds are a promising perspective in the development of new drugs with analgesic and anti-inflammatory properties.2-[3-Acetyl-5-(4-chlorophenyl)-2-methyl-pyrrol-1-yl]-3-methyl-pentanoic acid (compound 3d) is a novel N-pyrrole carboxylic acid, based on the structure of celecoxib. In previous experiments, it demonstrated analgesic activity in animal pain models. The study aimed to assess the involvement of opioid receptors in the analgesic effect of compound 3d after single administration on experimental animals. We divided 49 adult male Wistar rats into seven groups (n=7), treated with saline, compound 3d in doses of 10, 20 and 40 mg/kg b.w., and compound 3d in the same doses with added naloxone 2 mg/kg b.w. intraperitoneally. Paw withdrawal test was employed to assess antinociception and opioidergic involvement. Statistical analysis was performed with IBM SPSS 20.0 software, using One-way ANOVA and Tukey post hoc test. Compound 3d in all tested doses showed increased latency time for paw withdrawal at 1st and 2nd hours of testing, compared to the saline solution control group. The animals treated with naloxone, followed by administration of compound 3d demonstrated lack of such increase in the paw withdrawal time, compared to the control group. Literary data have suggested an involvement of the endogenous opioidergic system in the antinociception exerted by NSAIDs. In our study, compound 3d's analgesic effect observed at the paw withdrawal test disappeared with concomitant administration of the μ-receptor antagonist naloxone. This proved that opioid receptors apparently played a role in the antinociception induced by the compound.
**Key words:** pyrrole, paw withdrawal, naloxone

## SATUREJA GENUS HERBS – AN ALTERNATIVE TO ANTIBIOTIC TREATMENT

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## Summary
WHO data shows that 80% of the world population use phytomedicines for prophylaxis and illness treatment because they are easier to use, and phytomolecules have fewer side effects. Moreover, the widespread use of antibiotics is the leading cause of growing antibiotic resistance (AR), which has become one of the most significant threats to public health. That requires the search and discovery of new molecules with antimicrobial properties. Many herbs are rich in such active ingredients. Since the beginning of the twenty-first century, there has been a growing interest in the plants of the Satureja genus. The studies are focused on the composition of the essential oil and various types of extracts, as well as their antimicrobial, antifungal and antioxidant activity. The major volatile constituents of the essential oil and the extracts are carvacrol, timol, geraniol, lemon, and y-terpene. Their quantitative composition varies, depending on the geographical region and altitude. Different research teams have shown a

pronounced antibacterial activity against more than 20 types of Gram (+) and Gram (-) bacteria including *E. coli, S. aureus, S. epidermidis, L. monocytogenes* and others. Efficacy against pathogenic microorganisms varies depending on the type of extract. The essential oil shows the highest activity, followed by alcoholic extracts. There is also a connection between the composition and the antibacterial activity, which is potentiated by antibiotics. Satureja herbs have shown antifungal activity against eight types of pathogenic fungi, including *C. albicans*. The various active ingredients themselves also exhibit such properties.

**Keywords**: Satureja montana, phytomedicines, antibacterial and antifungal activity

## ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS OF FRUITS AND LEAVES OF DIOSPYROS KAKI (PERSIMMON)

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### Summary

Diospyros kaki, Ebenaceae (persimmon) is a tree originating from China and Japan. Its botanical name comes from Greek "diospyros" (divine fire). It was first introduced in the countries of the Mediterranean and America in the 18th century, and in Bulgaria - around 1935-1940. Its fruits contain microelements, vitamins, organic acids, dietary fibre, triterpenoids etc., while the leaves are rich in flavonoids. The current review aimed to collect information regarding the antioxidant and neuroprotective properties of dry extracts from the leaves and fruits of Diospyros kaki. Data from databases available in Scopus, PubMed and Science direct was used. According to *in vitro* experiments, total flavonoids of persimmon leave contain considerable amounts of catechin. They significantly decrease the level of reactive oxygen species and malondialdehyde, while increasing the activity of catalase, superoxide dismutase and glutathione peroxidase in MC3T3-E1 cells in a dose-dependent manner. The fruit extract contains polyphenols (standardized as gallic acid), proanthocyanidins (catechin), and total flavonoids (quercetin). Their antioxidant effect was measured by ORAC and HORAC assays. Some studies suggest a relation between the antioxidant action of Diospyros kaki extracts and their effectiveness in the additional treatment of neurological disorders such as depression and Alzheimer's disease. Because of its pronounced antioxidant properties, Diospyros kaki has a good potential for complementary therapy of neurological disorders like depression and Alzheimer's disease. More experimental and clinical investigations should be carried on to prove its effects.

**Key words:** Diospyros kaki, dry extracts, antioxidant effect, neuroprotective effect

## AUTOIMMUNE MECHANISMS OF HYPERTENSION

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## Summary

The pathogenesis of essential hypertension is multifactorial, including classical factors such as the renin-angiotensin system, sympathetic nervous system, endothelial system, and renal hemodynamics. Recently, epidemiological and experimental evidence has shown a link between hypertension, inflammation and autoimmunity. In this review, we focused on the immune pathogenesis of hypertension. The immune pathology of hypertension is similar to some autoimmune mechanisms observed in the pathogenesis of systemic lupus erythematosus, psoriasis, systemic sclerosis, and rheumatoid arthritis (T cells activation, overproduction of IL-17, IFN-γ, TNF-α in both experimental and human hypertension). Moreover, the prevalence of hypertension in patients with these autoimmune diseases has significantly increased. The mechanisms of this relation are not enough explored. Emerging evidence supports the concept that immune cells become activated and enter a target organ, including vasculature and kidney. Mediators, released by these cells (reactive oxygen species, cytokines, metalloproteinases, antibodies) promote dysfunction and cause damage to target organs. These inflammatory conditions can cause posttranslational modifications of proteins and the formation of neoantigens, which in some cases stimulate the production of autoantibodies with agonistic activity (agonistic autoantibodies - AA) contributing to hypertension. There is evidence of the presence of β1-adrenergic receptor-AA (in dilated cardiomyopathy), α1-adrenergic receptor-AA (in refractory hypertension and primary aldosteronism), angiotensin receptor type1 (AT1)-AA (in preeclampsia) and endothelin receptor type1 (in systemic lupus erythematosus and systemic sclerosis associated with pulmonary hypertension). Blockade of these autoantibodies prevents hypertension. Efforts to prevent and reverse immune activation may prove beneficial in preventing the long-term consequences of hypertension and its related cardiovascular diseases.

**Key words:** autoimmunity, hypertension, agonistic autoantibodies, cytokines

# DIET-INDUCED METABOLIC SYNDROME IN RATS IS ASSOCIATED WITH ANXIETY AND IMPAIRMENT OF SPATIAL MEMORY

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## Summary

The aim of the present study was to evaluate the spatial memory and anxiety in rats subjected to diet-induced metabolic syndrome. Forty male Wistar rats (160-200 g) were placed in two groups: Control (C) and metabolic syndrome (MS). Rats from the C group received regular rat chow and tap water. MS group received high-fat high-fructose (HFHF) diet and 10% fructose in the drinking water. After ten weeks of dietary intervention, behavioral tests were performed. Spatial memory was evaluated by the place recognition test (PRT). Anxiety was assessed by the open field test (OFT) and elevated by plus maze (EPM) test. OFT also evaluated the locomotor activity. The results from the study were statistically analyzed by Student's unpaired two-tailed t-test. The OFT showed no difference in the locomotor activity between the groups. The rats from the MS group manifested a spatial memory impairment. The time spent in the central zone of the OFT tended to decrease in the MS group. The EPM test demonstrated an anxiogenic effect of the HFHF diet. The present results confirmed our previous finding of HFHF diet-induced anxiety demonstrated in the social interaction test. The metabolic syndrome induced by feeding rats with high-fat high-fructose diet was associated with anxiety and impairment of spatial memory.

**Key words:** rats, metabolic syndrome, spatial memory, anxiety

## EVALUATION OF THE EFFECT OF ANETHOLE ON CARRAGEENAN-INDUCED PAW EDEMA IN METABOLIC RATS

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### Summary

Metabolic syndrome (MS) is a condition associated with low-grade inflammation. Anethole has been reported to have anti-inflammatory activity. The present study aimed to evaluate the effect of chronic anethole administration in a model of carrageenan-induced hind paw oedema in rats with MS. The experimental Wistar male rats were divided into five groups of 10 rats each. The control group received a standard laboratory diet and tap water *ad libitum*. To induce MS, all other groups received high-fat, high-fructose diet (17% lard and 17% fructose added to the standard diet) and 10% fructose solution instead of water, also *ad libitum*. All animals were daily orally treated. Groups Control and MS received sunflower oil, while the other three groups were treated with an oil solution of anethole (62.5 mg/kg, 125mg/kg and 250 mg/kg, respectively). In the tenth experimental week, carrageenan (1.0 mg) was injected into the rat left hind paw. The rat paw volume was measured using a plethysmometer to assess oedema. Measurements were made before oedema induction and 30, 60, 120, 180, 240 and 300 min after carrageenan injection. In MS rats, the paw oedema did not differ significantly from that of the Control group. Anethole treatment with all doses did not cause a significant reduction of oedema compared with both the control and MS groups. The results from the study showed that metabolic syndrome did not aggravate carrageenan-induced oedema. Anethole treatment did not achieve an anti-inflammatory effect.

**Key words:** anethole, metabolic syndrome, rat paw oedema, inflammation

## EFFECTS OF LEU-ENKEPHALIN ANALOGUE ON STRESS-INDUCED ANALGESIA IN RATS

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### Summary

Opioid peptides are a class of neurotransmitters and modulators that serve to regulate neural and endocrine functions involved in the elaboration of adaptive behavior. Many synthetic analogues of endogenous opioid peptides have been investigated to design more potent ligands of opiate receptors. The incorporation of Cys

($O_2NH_2$)$^2$ in the molecule of Leu$^5$-enkephalin in position 2 highly increases the efficacy and does not change significantly the affinity of the analogues to δ-opioid receptors. The aim of this study was to investigate the effect of [Cys($O_2NH_2$)$^2$] enkephalin on stress-induced antinociception. Analgesia was tested using hot-plate (HP) and paw pressure (PP) tests after immobilization (IS), cold- (CS) and hot (HS)-stress. The test and referent substances were applied i.p. immediately after the stress. PP testing after IS, CS and HS showed significantly greater nociceptive effect as compare to the control. [Cys($O_2NH_2$)$^2$] enkephalin and Leu-enkephalin decreased the paw-withdrawal threshold. HP latency was significantly longer after IS, CS and HS stress models and [Cys($O_2NH_2$)$^2$]-enkephalin reduced the latency in the same manner as Leu-enkephalin. The development of stress-induced analgetic response was prevented by Nal. Mechano- and thermoreceptors are involved in different way in effects of Leu-enkephalin and [Cys($O_2NH_2$)$^2$] enkephalin on stress-induced analgesia.

**Key words:** stress-induced analgesia, [Cys($O_2NH_2$)$^2$] enkephalin, immobilization stress, cold stress, hot stress, hot-plate test, paw pressure test

## COMBINED HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STAINING FOR MICROSCOPIC EVALUATION OF DEGENERATION LEVELS IN THE CONNECTIVE TISSUE AND PROPRIOCEPTORS IN HUMAN ANTERIOR CRUCIATE LIGAMENT

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## Summary

Morphology and degenerative changes in the anterior cruciate ligament (ACL) are widely studied. We aimed to test different histochemical and immunohistochemical techniques either alone or in combination to evaluate proprioceptors and degeneration levels in ACL. The research was approved by the Ethics Committee of Medical University Pleven Sixty ACLs were harvested from 57 patients admitted for knee replacement surgery. The samples were processed and stained histochemically and immunohistochemically. Standard and modified protocols for IHC (S100 and NSE) and histochemistry (PAS/Alcian, van Gieson and silver impregnation) were used. The results were analysed and interpreted. Staining with PAS/Alcian in combination with S100 or NSE proved to be useful in identifying proprioceptors, the level of degeneration, and preserving tissue. The combined IHC staining with either van Gieson stain or silver impregnation were found to be of lower value for identification and study of proprioceptors in ACL. Based on our observations, we believe that combined S100 and PAS/Alcian as a reliable method for the identification of neuronal structures and evaluation of degenerative changes.

**Key words:** anterior cruciate ligament, proprioceptors, histochemistry, immunohistochemistry

## IN SILICO PCR PRIMER DESIGNING FOR SPECIFIC IDENTIFICATION OF ALTERNATIVELY SPLICED ISOFORM OF ChAT mRNA IN MICE

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### Summary
As a neurotransmitter, acetylcholine has an important function in the central as well as in the peripheral nervous system. Its biosynthesis from acetyl-coenzyme A and choline is catalyzed by the enzyme choline acetyltransferase (ChAT). In mice, the gene encoding ChAT is composed of 17 exons, three 5'-noncoding (R, N and M), followed by 14 successive coding exons. Seven mRNA isoforms (M, N1, N2, R1, R2, R3 and R4) are transcribed in mice. All these alternative splicing isoforms differ in their 5'-noncoding ends and encode the same protein, the common ChAT. There is a splice variant that lacks coding exons from 5 to 8 that encodes a smaller protein, the peripheral ChAT, localized preferentially in peripheral nerve cells and fibres. PCR is an enzymatic reaction whose efficiency and sensitivity largely depend on the efficiency of the primers. We implemented a bioinformatics approach to design such specific primers and to calculate essential properties as primer length, melting and annealing temperature, GC content (%), primer secondary structures and cross homology by using web-based tools like Primer3, Primer-BLAST and Primer Quest. Due to extreme sequence similarities between different isoforms, the design of a reliable and robust method for measuring their expression proved to be challenging. A complete set of forward and reverse primers targeting the unique exon-exon junctions of all alternatively spliced ChAT isoforms were designed. These unique primers can ensure accurate quantification of all ChAT mRNA isoforms by real-time PCR.
**Key words:** choline acetyltransferase, primer, polymerase chain reaction, bioinformatics

## CAN LIE HISTOCHEMICAL STAINING DEMONSTRATE ISCHEMIA INSIDE AND AROUND THE TUMOR?

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### Summary
Lie staining is traditionally used to demonstrate ischemic zones in the myocardium. Closer observation demonstrates positivity in vascular structures that have smooth muscles in their walls. Aim of the following study was to demonstrate the Lie staining inside breast carcinomas and the staining in the breast parenchyma around the tumour. Twenty cases of invasive breast cancer were additionally tested with Lie staining. Only cases that had an additional spare slide for testing were used. A morphological and molecular

subtype, CD 31 labelled vascular network, Ki-67 index and Lie staining patterns were evaluated and described. Lie staining positivity was observed in the smooth muscle layer of blood vessels walls, mainly around breast carcinomas, but some positivity was also observed in native blood vessels, infiltrated by the tumour. The molecular and morphological type demonstrated some relation to the Lie positivity pattern. Proliferation index and vascularization of the tumour were not unequivocally related to the Lie staining. Lie staining might be considered as a circumstantial marker for tumour ischemia, but additional studies are needed to prove its specificity and sensitivity.

**Key words:** Lie histochemical staining, ischemia, breast cancer

## THROMBOPHILIA IN POLYCYSTIC OVARY SYNDROME PATIENTS: FACTOR V LEIDEN POLYMORPHISM

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### Summary

The aim of the study was to evaluate a genetic influence of carriage of Factor V Leiden (FVL) polymorphism in F5 gene on reproductive problems in overweight/obese patients with polycystic ovary syndrome (PCOS). A total of 109 patients of Caucasian race with polycystic ovaries and 101 healthy control subjects were selected and included in the study. Clinical and laboratory parameters were examined and full history was taken. Single nucleotide polymorphism rs6025 in F5 gene LOCUS 1q24.2 (MIM No 612309) was genotyped in PCOS patients and compared to healthy controls. The data were analysed for correlation with infertility, pregnancy loss in overweight/obese PCOS patients. The results of DNA analysis showed a higher but not significant carriage of FVL in PCOS patients, as compared to the controls (respectively OR=1.701 (0.604± 4.790; p=0.327). Infertility was significantly higher in carriers of FVL polymorphism (25.0% versus 6.2 %, OR=7.533, 95% CI1.545±36.727, p=0.026) and total reproductive failure was higher but not significantly so in FVL carriers (54.5% versus 33.1%, OR=2.438, 95% CI 0.691±8.593, p=0.087). Early pregnancy losses were higher in PCOS non-carriers of FVL polymorphism. The overweight and obese patients were prevailed among the carriers of FVL polymorphism: OR 1.884 and OR 1.969, respectively.The present study provides evidence that FVL genotype is associated with PCOS, although not significantly. In addition, the reproductive problems, and infertility in particular, were much more in PCOS patient carriers of FVL polymorphism than non-carriers.

**Key words:** factor V Leiden, polycystic ovary syndrome, DNA, early pregnancy losses

**Acknowledgements:** The study was conducted with financial support from Medical University – Pleven (Project No 20/2018).

## POTENTIAL CLINICAL APPLICATIONS OF OXIDATIVE STRESS BIOMARKERS

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## Summary

Oxidative stress (OS) is an imbalance between the production of reactive oxygen species in the body and the antioxidant defense system that controls the impact. For a long time, the role of OS has been extensively studied in the aetiology and development of various disease states: neurodegenerative, cardiovascular, oncological, etc. This study aimed to review the recently published literature on the reliable oxidative biomarkers as predictors of disease development and clinical outcome. Scopus, Google Scholar and Science Direct database were used for this purpose. There is a significant increase in the number of publications exploring OS biomarkers, as well as an attempt to systemize them and search for a cross-section between them in order to obtain highly specific and validated clinical data. A wide range of diseases has been associated with elevated protein carbonyls. Since their levels remain stable for hours after venipuncture, they have been considered as one of the most reliable oxidative stress markers. On the other hand, the significance of the frequently studied biomarkers, MDA and 4-HNE, remains controversial. Currently, quantification of F2-isoprostane is considered a reliable indicator of the OS level, but its measurement is a subject of specialized and sophisticated equipment. Isolevuglandins and 3-Nitrotyrosine have also been reported to possess a substantial potential for future clinical applications. In conclusion, further work is required on simultaneous testing of oxidative stress indicators in large controlled patient groups, which could provide an opportunity to establish a reliable set of biomarkers applicable to specific, oxidative stress-associated diseases.

**Key words:** oxidative stress, biomarkers, disease prediction and outcome

## INVOLVEMENT OF NALOXONE AND JTC-801 IN ANALGESIC EFFECTS OF NEWLY SYNTHESIZED NOCICEPTIVE ANALOGS

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## Summary

The present study aimed to investigate the analgesic effects of newly synthesized β2-tryptophan hexapeptide analogues and the involvement of the opioid- and nociceptionergic systems in these effects. The analgesic effect of the four newly synthesized compounds was evaluated in male Wistar rats by paw-pressure and hot-plate tests. All the newly synthesized substances at a dose of 10 μg/kg were injected intraperitoneally (i.p.). JTC-801 (NOP receptor antagonist) at a dose of 0.5 mg/kg and naloxone (opioid receptor antagonist) at a dose 1 mg/kg were injected i.p. The JTC801 injection led to a significant decrease in the pain thresholds of referent substances and analogues 1, 2 and 4, and a significant decrease in HP-latencies of all the investigated compounds. Interestingly, analogue 3 that represents a substitution in the 5th position of a template known to be a NOP-receptors agonist maintained on the 10th min a still manifest analgesic effect, compared to controls and both the referents estimated by PP- but not the HP-test. Naloxone injection abolished the analgesic effect of all the substances investigated, as established by both PP- and HP- tests. Interestingly, the more pronounced decrease both in pain threshold and HP-latency was observed after application of analogue 4 that represents a substitution in a template known to be a μ-receptor antagonist. In conclusion, it may be assumed that hexapeptides, containing β2-tryptophan analogues, expressed analgesic activity through both NOP- and opioid receptors.

**Key words:** analgesia, PP-test, HP-test, β2-

tryptophan hexapeptide analogues, naloxone, NOP receptor antagonist

## ANALGESIC EFFECTS AND SYNTHESIS OF NEW ANALOGUES OF NOCICEPTION

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### Summary

The study aimed to investigate the analgesic effects of newly synthesized analogues of N/OFQ(1-13) and the involvement of the opioidergic system in these effects. The experiments were carried out on male Wistar rats (180-200g) kept under normal conditions at ambient room temperature (22°C). Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed. The changes in the mechanical nociceptive threshold of the rats were measured by an analgesimeter (paw pressure test). The pressure was applied to the hind paw, and the pressure (g) required eliciting nociceptive responses such as squeak and struggle. The response was considered as the mechanical nociceptive threshold. Naloxone (Nal) and nociceptin N/OFQ (1-13) were obtained from Sigma (Germany). Nociceptin analogues were synthesized in the laboratory of Prof. E. Naydenova in the University of Chemical Technology and Metallurgy – Sofia. All drugs were dissolved in saline and injected intraperitoneally. Nal (1 mg/kg, i.p.) was applied 20 min before each peptide (10 μg/kg, i.p.). Our results suggested that the analogues with Orn instead of Lys at the 13th position had a more pronounced analgesic effect than NO/FQ (1-13). The analogues with Orn instead of Lys at the 9th position expressed a significantly less pronounced analgesic effect, compared to NO/FQ (1-13). In the analgesic effects of the newly synthesized, NO/FQ (1-13) analogues were involved the opioidergic system.

**Key words:** nociception, nociceptin analogues, paw pressure test, naloxone

## DECREASING THE ALZHEIMER-RELATED PATHOLOGY IN AB-INDUCED RAT MODEL, TREATED WITH AGOMELATINE

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### Summary

The antidepressant agomelatine attenuates behavioural deficits and concomitant pathology observed in a streptozotocin-induced model of Alzheimer's disease (AD). Male Sprague Dawley rats were treated intraperitoneally (i.p.) with Ago (40 mg/kg) for 30 days, starting after the intracerebroventricular (icv) injection of Aβ. Three groups were studied: sham (icv injection of a vehicle); Aβ (with AD pathology) and Ago-Aβ (AD model rats, treated with agomelatine). Several behavioural tests were conducted: Elevated Plus Maze (EPM); Open Field (OF); Light/Dark Transition (LDT); Radial Arm Maze (RAM), Forced Swimming Test (FST) and Sucrose Preference. Agomelatine treatment

positively affected the behaviour of the Ago-Aβ, making them act in a similar way as the controls during the EPM, OF, LDT, FST and Sucrose preference tests. Statistically significant were their preference to sucrose, indicating lack of anhedonia; the total time they spent in the centre, and the distance they passed during the OF test, showing decreased anxiety; more entries and longer time spent in the open arms during EPM. Treatment with Aβ only made rats stay longer immobile during FST, have higher latency to dark and go less often in the light in LDT test. RAM test did not show any significant difference between Aβ and Ago- Aβ groups. The drug investigations showed its neuroprotective properties against behavioural changes after icv injection of Aβ. Future biochemical and histological experimental studies are required to ascertain that the icv Aβ injected rats react well to agomelatine treatment.

**Key words:** Aβ-induced AD rat model; agomelatine; behavioural tests

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## MESENCHYMAL STEM CELLS PROLIFERATION AND DIFFERENTIATION ON TI6AL4V ALLOY SPECIMENS

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## Summary

Hip replacement is one of the most effective surgical interventions in medicine. Even though the clinical results of arthroplasty are usually excellent, some implants loosen and require revision. Providing conditions that have a positive biological effect on bone-building elements, resulting in a fast, seamless proliferation and differentiation of mesenchymal stem cells (MSC) into osteoblasts could help to resolve this problem. To achieve this goal, the development of an effective in vitro model for studying MSC functions at implant–tissue contact zone is needed. The aim of this study was to evaluate the proliferation and differentiation capacity of human bone-marrow-derived MSC in contact with medical implant-grade titanium alloy. Human MSCs were isolated from bone marrow aspirates obtained from the femoral canal during hip replacement surgeries. At passage 4, MSCs were seeded and cultured on Ti6Al4V alloy specimens and polystyrene plates (control). Cell adhesion and proliferation capabilities were assessed by Alamar Blue assay. MSC differentiation was induced by specific, stimulating factors. Osteogenic and adipogenic differentiation capacity was determined using alkaline phosphatase activity assay, Alizarin red S and Oil red O staining. The results indicate that MSCs can effectively adhere to the surface of titanium alloy specimens and at the same time, maintain their proliferative and osteogenic/adipogenic differentiation potential. It can be concluded that Ti6Al4V alloy surface provides suitable conditions for the proliferation and differentiation of bone marrow MSC and can be used as an in vitro model for studying MSC functions at the bone-implant interface.

**Key words:** mesenchymal stem cells; implant–tissue contact zone; titanium alloy; MSC proliferation; MSC differentiation

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## EXTRACTS FROM *HABERLEA RHODOPENSIS* INFLUENCE THE EXPRESSION OF STRESS RESPONSIVE GENE FOR P21 WITHOUT CHANGING THE ACTIVITY OF RAD51 GENE

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### Summary
*Haberlea rhodopensis* is a famous and rare Balkan endemic plant from a group of extremely resurrecting plants. The alcohol extracts from *Haberlea rhodopensis* consist of many biologically active substances and have shown antioxidant, antigenotoxic and radioprotective capabilities. The presence of high concentrations in the extracts of polyphenols and other scavengers of free radicals was firmly evidenced. However, the delicate molecular mechanisms of their action remain obscure. We have studied the response of HeLa cell line cultures, pre-incubated with different concentrations of *H. rhodopensis* ethanol extracts and then subjected to γ–irradiation. The transcriptional level of several important for DNA repair, cell cycle and apoptosis genes were quantified by RT-qPCR. Data analyses of HeLa cell line cultures, standard RNA extraction protocols, procedures for cDNA synthesis and RT-qPCR reactions were analysed with software programs. Six genes - BRCA1; TP53, PUMA, ATM, RAD51 and CDKN1A(for p21 protein), which take part in DNA repair, cell cycle control and apoptosis were analyzed for changes in expression with and without supplementation with bioactive *H. rhodopensis* extracts, and before and after γ–irradiation. We discuss the dynamics in the expression profile of genes RAD51 and CDKN1A. The alcohol extracts of *H. rhodopensis* possess the potential to change the activity of stress-responsive gene for p21 protein, most probably by eliminating free radicals and thereby reducing oxidative stress. On the other hand, the lack of changes in RAD51 gene expression shows that extract from *H. rhodopensis* differentiate oxidative pathways and fine-tune the cellular response to oxidative stress.

**Key words:** *Haberlea rhodopensis*; alcohol extracts; radioprotection; DNA repair

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## SEX-SPECIFIC DEPENDENCE OF BLOOD GLUTATHIONE PEROXIDASE ACTIVITY IN HEALTHY SUBJECTS

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### Summary
Living cells generate free radicals and reactive oxygen species as a result of continuously running oxidation reactions. At a high rate of formation, reactive oxygen metabolites can cause oxidative damage to DNA, proteins and membrane lipids. Glutathione-peroxidase-1 is an intracellular antioxidant enzyme that controls the levels of organic and inorganic peroxides in the body and maintains a normal thiol redox-balance. The present study aimed to investigate the effect of sex and age on the blood glutathione peroxidase activity of healthy subjects using a spectrophotometric

method. For this purpose, ten clinically healthy volunteers, aged from 26 to 76 years, were studied. The data showed that age was not a statistically significant factor for glutathione peroxidase activity. At the same time, women demonstrated significantly lower glutathione peroxidase activity than men (p=0.032). Future in-depth studies of the blood antioxidant status with a larger number of carefully selected individuals in well-defined age groups are needed to achieve highly reliable experimental results.

**Key words:** antioxidants, blood, reactive oxygen species, glutathione peroxidase, activity

# MICROBIOLOGY, INFECTIOUS DISEASES, EPIDEMIOLOGY AND PARASITOLOGY

## PLENARY LECTURES

### ANTIMICROBIAL STEWARDSHIP – A HEALTHCARE PRIORITY. NEW ANTIBIOTICS

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## Summary

Antimicrobial resistance (AR) currently reaches huge dimensions all over the world. The WHO has recognized it as a global threat requiring priority actions. Medical societies, countries governments and the pharmaceutical industry work together to overcome the increase of AR. This work aims to introduce the Antimicrobial stewardship principles and their implementation in Bulgaria, as well as to overview the new antibiotics. Recently published international literature, together with last-year national developments, were analyzed. There are two main ways to contain AR. The first one – antimicrobial stewardship (AS), aims at better prescribing for patients through rapid clinical and microbiological diagnosis, making an optimal choice of an antibiotic, dose, and duration of treatment. Important pillars in AS are education, control of infection and political engagement. AS will decrease patient morbidity,