weeks increased the number of arriving to drinking-bowl in 1.4 times (p<0.02), and possibly increased the number of drunk sucrose and percentage of consumed sugar to total number of liquid with experimental pathology. Preventive and therapeutic use of derivative 2-oxoindoline 18 effectively countered anhedonia, and increase of the number of arriving to drinking-bowl with sucrose 1.4 times (p<0.02) and increase of drunk sucrose 1.3 times (p<0.02) in comparison with control pathology without correction. Also, substance 18 assisted in the advantage of sucrose solution.

Also, during experimental neurosis in the test sucrose consumption advantage, the percentage of drunk water with sugar in comparison with control group was decreased. After 4 weeks of neurosis, diazepam intake increased the number of arriving to drinking-bowl in 1.4 times (p<0.02), and possibly increased the number of drunk sucrose and percentage of consumed sugar to total number of liquid in comparison with such during experimental pathology. Preventive and therapeutic use of amide of 2-oxoindoline-3-glyoxylic acid with substance 18 after 4 weeks of neurosis modeling effectively withstood anhedonia. During this period the substance possibly assists in the advantage of sucrose among water.

So, experimental neurosis is accompanied with the development of typical emotional and behavioral disorders (decrease of quantitative and qualitative indices of sucrose consumption). Preventive and therapeutic use of amide of 2-oxoindoline-3-glyoxylic acid effectively corrected the development of taste disorders in sucrose consumption and it didn’t exceed diazepam.

Experimental neurosis caused the development of anhedonia. Therapeutic and preventive use of the substance 18 effectively decreased the manifestations of anhedonia after experimental neurosis in rats. Such results can be essential for further investigation of monoaminergic mechanisms of the derivatives of 2-oxoindoline-3-glyoxylic acid during neurotic pathology in animals.

References:

Key words: derivative of 2-oxoindoline-3-glyoxylic acid, rats, experimental neurosis.

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RELIABILITY OF URINE AS SOURCE FOR BIOLOGICAL INFORMATION FOR RISK ESTIMATION FOR PROSTATE MALIGNANCY

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Prostate diseases, including prostatitis, benign prostatic hyperplasia and carcinoma are some of the most common diseases that occur in mainly Caucasian men after fifty years of age. All of these diseases are characterized by similar symptoms, such as frequent urge to urinate, intermittent urination, incomplete urination, increased frequency of nocturnal urinating and erectile dysfunction. Because of similar symptoms between benign prostatic hyperplasia and various different types of cancer precise diagnostic is based on the biopsy of prostate tissues and its result. In practice, the diagnostic screening starts from the serologic analyses, and digital rectal examination. Digital rectal examination is informative for detection of the enlargement of the prostate. Blood tests include determination of PSA (Prostate Specific Antigen) concentration. Conventional screening and diagnostic procedures in prostate diagnostics rely on elevated PSA concentration which is not specific only for prostate cancer (Hessels et al. 2003) and frequently leads to unnecessary invasive biopsy. It is estimated that approximately 50% of persons who underwent tissue biopsy did so based on false positive PSA value (Rigau et al. 2013). PSA, is also known as Gamma - seminoprotein, which belongs to the kallikrein-related peptidase protein family and is secreted by epithelial cells of the prostate, and has a role in the production of seminal liquid that is secreted during ejaculation and allows spermatozoids to move freely. Genetic precursor of PSA is named kallikrein - 3 or shortened KLK3 gene. Limitations of the biopsy procedure caused further search of the easy noninvasive method for screening and differential diagnostics of prostatic diseases (Mengual et al. 2016; Drake et al. 2009; Bryzgunova et. al. 2015). The aim of the study was to evaluate methods of isolation of total RNA from urine samples and to assess the performance of relative gene expression analysis with such RNA as template using Real time PCR. GAPDH (glyceraldehyde 3 - phosphate dehydrogenase) gene was used as Gene of interest. Also the study was aimed at identification of critical procedural factors associated with the quality and quantity of isolated genetic information.

Materials and methods. Urine samples were collected from a total of 162 participants, patients of the Urology Clinic of CCUS (Clinical Center of University of Sarajevo). The patients underwent standard clinical diagnostic procedures for prostate cancer (transrectal examination, biochemical PSA measurement from blood, needle
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Results. RNA was isolated from 133 out of 162 collected urine samples which makes 82% success rate for RNA extraction from urine. The average concentration of total RNA was 4.60 ng/ul with highest concentration of 96 ng/ul. Reverse transcription reaction was done for RNA samples with minimum concentration of 1 and maximum concentration of 96 ng/ul.

GAPDH had a minimum Ct value of 15, the average Ct value was 24, and the maximum measured Ct value was 33. Taking into consideration possible instability of RNA molecule which is high even in the samples of blood and tissue samples which are considered to be ideal, possibility of successful extraction of 82% can be considered as high. Urine, as a byproduct of many physiological reaction, is a very demanding materials for this type of isolation due to the presence of a large number of various compounds affecting RNA degradation.

Conclusions. Although the extraction of total RNA from the urine obtain relatively low concentration, it is possible by reverse transcription to get quality enough cDNA to contend with different types of analysis. According to our estimates, critical step in extraction of total mRNA from urine is quick transport to the laboratory where extraction will be carried. Also, equally important factor is the stabilization of the urine sample in the laboratory for what we used PBS buffer and centrifugation step prior to extraction. Selected kits for RNA extraction (NucleoSpin® RNA isolation kit, Macherey - Nagel GmbH & Co., Duren, Germany) and reverse transcription kit (GeneAmp® Gold RNA PCR Core Kit, Applied Biosystems, Ca., USA) proved to be good enough to from a relatively small amount of extracted starting material synthesized quality material for further work and analysis.

Key words: prostatitis, Prostate Specific Antigen, Urine, RNA, gene expression, Real time PCR.

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Markers for cellular response in J774A.1 macrophage cell culture in a model of bacterial lipopolysaccharides stimulation

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Cell cultures appear to be useful instrument in studies of molecular mechanisms of variety biological processes, diseases, action of drugs and natural products etc. Treatment with bacterial lipopolysaccharides (LPS) stimulate inflammatory response in variety cell types and this is a widely used model for studies related to immune function. Oxidative stress is also a component of such treatment. For the purpose of such experiments it is important to have specified markers to identify presence of a cellular response and to determine most appropriate LPS concentration where this response is most prominent. The aim of the study was to test the effect of LPS stimulation on J774A.1 macrophages on expression of selected genes, related to inflammation, antioxidant defense and necroptosis.

Materials and methods. J774A.1 cell line was cultured in standard conditions in complete DMEM medium with 4.5 g/L glucose, L-glutamine and supplemented with fetal bovine serum to final concentration of 10% and penicillin/streptomycin mixture to final concentration of 100U/mL each. To test the effect of LPS application, cells were seeded in 6 well flasks at density 2x10^5. After overnight incubation cells were treated with increasing concentrations of LPS (50, 100, 200 and 300 ng/mL final concentration) in complete growth medium for 20h. At the end of the experiment RNA purification with TRI Reagent and DNase treatment were performed according manufacturers’ instructions. For gene expression analyses Real-Time PCR was applied with KAPA SYBR® FAST qPCR Kit. Gene expression levels were calculated using the 2-ΔΔCt method and expressed as relative units compared to the untreated controls where the level of gene expression of interest was considered to be equal to 1. Inflammatory cytokines IL-1β, IL-6 and TNF-α, inflammatory enzyme COX2, glutamate-cysteine ligase (GCL) and necroptosis-related protein RIP3 were analyzed.

Results. The most prominent response in regard to GCL, TNF-α and IL-6 expression was observed in the group treated with 100 ng/mL LPS where the levels were 9.1 (p<0.01), 3.4 (p<0.1) and 11.5 (p<0.001) times higher as compared to untreated control. IL-1β was affected at most in cells treated with 100ng/mL LPS and the levels were 146.6 (p<0.001) higher than the control. COX2 expression levels were 2.1 (p<0.001) times elevated. RIP3 expression did not change significantly at any LPS concentration applied.

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