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WEST UNIVERSITY OF TIMISOARA
FACULTY OF CHEMISTRY, BIOLOGY, GEOGRAPHY
DEPARTMENT OF BIOLOGY-CHEMISTRY

9th Annual Conference BIOLOGY
Biological processes - theoretical and practical applications

May 20, 2017
Timisoara, Romania

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ASSESSMENT OF THE AMFIBIANS CONSERVATION STATUS FROM MUSNIC AREA, VERENDIN LOCALITY

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Romania, after joining the European Union, has assumed responsibility for the implementation of European legislative foresights in all areas, including environmental protection, which means for the terrestrial and aquatic environment the implementation of the Habitats Directive (92/43 EEC) provisions, refers to the conservation of the species and habitats mentioned in its annexes. According to the Habitats Directive, the second Article, maintaining or restoration the populations from the Annexes II, IV and V, to a favorable conservation status, is compulsory. For this activity to be possible, it is first necessary to know the existing species, namely knowledge of the current conservation status. Thereby, the inventory / monitoring of community interest h abitats andspecies has become a mandatory requirement. The general objective of the paper was to inventory and assess the conservation status of the amphibians from Musnic area in Verendin locality, Caras-Severin county. The scientific queries focused on: (1) inventory of amphibian species; (2) biotic profile analysis; (3) abiotic profile analysis; (4) specific habitats mapping (reproduction, feeding, rest); (5) identification of anthropogenic pressures; (6) assessing the conservation status of amphibian species. The community species of amphibians identified following scientific queries were: Bombina bombina, Bombina variegata, Bufo bufo, Rana esculenta, Rana temporaria, Salamandra salamandra, Triturus cristatus and Triturus dobrogicus. The mathematical processing of gross data taken from the scientific queries area has highlighted the fact that: (1) populations of species Bombina variegata and Rana esculenta had a size of 50-100 individuals (class 2); (2) populations of species Bombina bombina, Bufo bufo, Rana temporaria, Salamandra salamandra and Triturus cristatus had a size of 10-50 individuals (class 1); (3) the size of Triturus dobrogicus population in the query area was 1-10 individuals (class 0); (4). The conservation status of the amphibians species in terms of population of the species and habitat was favorable.

KEY WORDS: *amphibians, inventory, mapping, conservation, anthropic.*

STUDY OF ANTIMICROBIOLOGICAL EFFECTS OF *ARISTOLOCHIA CLEMATITIS L.* AND *PLANTAGO MAJOR* EXTRACTS

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The extract of Aristolochia clematitis (Linnaeus) contains as main substance aristolochic acid, having analgesic, anti-diuretic, anti-inflammatory, anti-microbial, anti-oxidant and anti-parasitical effects. The extracts of Plantago major have a have a strong antiviral, anti-inflammatory and antioxidant activity, probably due to the big content of fenolic compounds (flavonoids and tannins). The antimicrobial effect of the extracts was accomplished using standard bacterial strains: gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Legionella pneumophila). Both ethanol extracts had the concentration of 60% and 80%. Working method: diffusimetric method (Kirby Bauer) which permits determination of minimum inhibitory concentration (MIC) and spectrophotometric method which allows determination of minimum bactericidal concentration (MBC). The antimicrobial effects of the tested extracts recorded low values, where were tested strains which were not influenced. The inhibition rays (for the A. clematitis extract) values decrease as following: Enterococcus faecalis > Salmonella enteritidis > Pseudomonas aeruginosa > Escherichia coli > Staphylococcus aureus > Streptococcus pyogenes > Legionella pneumophila. The inhibition rays (for the A. P. major) values of the tested strains decrease as following: Salmonella enteritidis > Streptococcus pyogenes > Pseudomonas aeruginosa > Enterococcus faecalis > Legionella pneumophila > Escherichia coli > Staphylococcus aureus. The recorded values for the inhibition rates confirm the obtained results for the inhibition rates. The results of tested antimicrobial effect of the extracts show a low potential on the tested bacterial strains.

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COMPUTATIONAL STUDY OF THE POSSIBLE ADVERSE EFFECTS OF CHITOSAN DERIVATIVES USED IN THE PHARMACEUTICAL INDUSTRY

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Chitosan has multiple applications in drug delivery and wound healing processes. Many chitosan derivatives have been obtained in order to increase the solubility and to assure the association of bioactive molecules to chitosan to make it more effective for medical purposes. The aim of this study is to predict the oral bioavailability, toxicity, pharmacokinetics properties and side effects for some of the chitosan derivatives with low molecular weight used in pharmaceutical industry. The derivatives under study reveal low oral bioavailability, small skin penetration coefficient and insignificant gastrointestinal absorption. They are not able to penetrate the blood brain barrier and such as do not affect the central nervous system. Also, they are not predicted to inhibit the cytochromes P450 mainly involved in drug metabolization. All these results illustrate low toxicity and reduced side effects for studied chitosan derivatives.

K**EYWORDS**: *pesticides, wheat crop, pharmacokinetics, adverse effects, toxicity*

COMPUTATIONAL STUDY OF THE TOXICITY OF SOME PESTICIDES USED FOR WHEAT CROPS IN ROMANIA

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The aim of this study is to predict the absorbtion, distribution, metabolization, excretion and toxicity (ADME-Tox) profiles, pharmacokinetics properties, side effects and toxicity for some of the pesticides used for wheat crops in Romania. All these pesticides reveal increased human toxicity, especially when inhaled. Some of them are easily absorbed in the gastrointestinal tract, may penetrate the blood brain barrier and affect the central nervous system. Many of them are also able to inhibit the cytochromes P450 that are mainly involved in drug metabolism negatively affecting people under medication. They also expose a good skin permeation and it may contribute to increasing their toxicity and side effects. These results illustrate the toxicity of pesticides for humans and the increased risk for the occupational exposure.

K**EYWORDS**: *pesticides, wheat crop, pharmacokinetics, adverse effects, toxicity*

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FAST METHOD FOR TESTING BACTERIAL CELL VIABILITY AT THE ACTION OF BIOLOGICALLY ACTIVE COMPOUNDS

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Althaea officinalis, indigenous plant from Europe, West Asia and North Africa, used as a medicinal and ornamental plant, can be used in natural medicine for the treatment of gastric and duodenal ulcers, abscesses, constipation, diarrhea. It is also believed to have antimicrobial, anti-inflammatory, immuno-modulatory, antitussive and other pharmacological effects. Calendula officinalis L. belongs to the Asteraceae family, known as marigolds. The plant has been grown as a medicinal and food herb since the Middle Ages. The extract of C. officinalis contains a large number of carotenoids: flavoxanthin, lutein, rubixanthin, β-carotene, γ-carotenililopen. The antibacterial effect of the extracts of Althaea officinalis and Calendula officinalis has been tested on standardized bacterial strains: Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Legionella pneumophila). The antifungal effect of the extracts was tested on the Candida albicans strain. Spectrophotometric method for determining the bactericidal minimum concentration (CMB) by a cell viability test. On the basis of the inhibition rates, it can be said that the extract of Althaea officinalis has an antibacterial effect on the strains of Streptococcus pyogenes, Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa at the concentration of 577.80 mg / ml and does not show any antifungal effect on the Candida albicans strain . Values for inhibition rates when applying Calendula officinalis extract do not reveal an antibacterial effect on the bacterial strains analyzed, but show antifungal effect on the Candida albicans strain at both tested concentrations. Applying the spectrophotometric method allows for rapid determination (10 hours) of the antimicrobial capacity of plant extracts by determining the minimum bactericidal concentration.

THE ANTIBACTERIAL EFFECT OF *MELALEUCA ALTERNIFOLIA* (TEA TREE) EXTRACTS

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Tea tree oil is the essential product optained from Melaleuca alternifolia, with antiviral, antibacterial, antifungal and anti-inflammatory properties. The antimicrobial effect of the Melaleuca alternifolia extract was tested using standardized bacterial strains: Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Legionella pneumophila). The working methods were: the diffusion method (Kirby Bauer) which allows the determination of the minimum inhibitory concentration (MMI) and the spectrophotometric method to determine the minimum bactericidal concentration (CMB). Based on the inhibition rates, the antimicrobial effect of the TTO extract decreases as regards the strains used, as follows: Salmonella enteritidis > Streptococcus pyogenes > Escherichia coli > Legionella pneumophila > Enterococcus faecalis > Pseudomonas aeruginosa > Staphylococcus aureus. The inhibition rate values determined upon application of the TTO extract confirm the results obtained for the inhibition rays. The obtained results strongly demonstrate the antibacterial properties of the TTO extract and highlight its use for other strains of pathogenic bacteria.

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IN VITRO NEPHROTOXICITY EFFECTS OF ETORICOXIB

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Aim. *Due to its role in the detoxification of xenobiotics, the kidney is one of the target organs in drug-induced toxicity. Drug-induced nephrotoxicity is an important problem of public health. The susceptibility to drug-induced nephrotoxicity is commonly detected only late. Etoricoxib is a selective cyclooxygenase-2 inhibitor. The purpose of our study was to assess in vitro the effect of this drug on human normal renal cells.*

Material and methods. *To assess nephrotoxicity of etoricoxib**,** we used primary podocyte cells from healty donors. Urinary podocytes were isolated after centrifugation of fresh urine, at 700g for 5 minutes. The sediment was resuspended in DMEM / F-12 supplemented with 10% FBS, 1% penicillin/streptomycin and 1% antimycotic and the cells were grown at 37°C with 5% CO2. Urinary podocytes were identified using fluorescence microscopy and primary anti-podocalyxin antibody. After proliferation, $2x10^4$ cells/ml were seeded in 1 ml specific medium in 96 and 24-well microplates. After 24h, medium was removed, and the cells were incubated for 24 h with increasing concentrations of etoricoxib only and etoricoxib/ metamizole. Cell viability was assayed by MTT test, and apoptosis by DAPI staining.*

Results. *In the final urine the cells are absent or are found in a small number. Urinary cells may have hematologic origin (erythrocytes, leukocytes) or epithelial origin (renal epithelial cells – podocytes, urothelial cells, squamous epithelial cells). Podocyte cells may be observed in the urine of healthy persons as a results of normal exfoliation. We analyzed cell morphology in phase-contrast microscopy and fluorescence microscopy to differentiate the podocytes from other types of epithelial cells. Mononuclear cells with characteristic underlying (podocytary foot) and rare binuclear cells have been revealed. Podocalyxin-positive cells were considered to be podocytes. The results of MTT test shows that etoricoxib, in reduced concetrations (25, 50, 100 µM) did not affect cell proliferation. At highet concentration 200 µM, we noticed a weak inhibition on cells multiplication. No modification of the cell phenotype was observed. The number of apoptotic cells were similar with the control goup. There was a stastically significant difference between cells inhibition and drug concentration in the case of combination of etoricoxib and metamizole (10 µM).*

Conclusions. *Althouth the effect of etoricoxib on podocyte proliferation is reduced, the concomitent administration of etoricoxib and metamizole induced cytotoxycity by non-apoptotic mecanism. One strategy to avoid such problems would be the development of drugs with decreased nephrotoxic potential. However, the prediction of nephrotoxicity during preclinical drug development is difficult, and the nephrotoxic potential of newly approved drugs is often underestimated. in vitro models showed that high predictivity can be obtained by using primary or stem cell derived human renal cells in combination with appropriate end points.*

MISMATCH REPAIR GENES IN COLORECTAL CANCER

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Introduction. *HNPCC, also known as Lynch's syndrome, is the most common form of genetic predisposition known for colon cancer. HNPCC is a condition caused by germline mutations of the genes involved in mismatch repair (MMR). MSH2 and MSH1 are proteins involved in DNA repair. They suffer mutations in some forms of cancer. The main objective of the study was to visualize and evaluate the loss of expression of the proteins MSH2 and MLH1 which could reflect the existence of a mutation of the corresponding gene. Another objective of the study was to determine whether the IHC analysis performed after the operation of the lesional and peri-lesional tissues can be used as a prognostic factor for familial cases.*

Material and methods. *The study traced the expression of MSH2 and MLH1 evidenced by immunohistochemical techniques (IHC) in a total of 24 specimens of colorectal carcinoma taken postoperatively.*

Results. *Our immunohistochemical study revealed a normal expression of the MSH2 and MLH1 proteins. In the peri-lesional tissue, IHC analysis revealed the normal expression of MMRs: type MSH2 and MLH1. The IHC study shows the expression decrease / absence of MMR proteins in cancer cells nuclei: MSH2 and 20 MLH1- cases. Literature reports revealed MSH2 and MLH1 genes structural mutations in 50 to 60% of colon cancer cases. Our study reveals in tumor zones: MSH2 expression in cell nuclei in 6 cases (25%); decrease in MSH2 expression in cell nuclei in 2 cases (8.33%); loss of MSH2 expression in cell nuclei in 16 cases (66.66%). In the case of MLH1 in the tumor zones: MLH1 expression in cell nuclei in 4 cases (16.66%); decreased MLH1 expression in cell nuclei in 3 cases (12.5%); expression loss of MLH1 in cell nuclei in 17 cases (70.83%). Peri-tumoral area: MSH2 expression in cell nuclei in 10 cases (41.66%); MSH2 expression decrease in cell nuclei in 4 cases (16.66%); MSH2 expression loss in cell nuclei in 10 cases (41.66%); MLH1 expression in the nuclei of cells in 8 cases (33.33%); MLH1 decreased expression in cell nuclei in 8 cases (33.33%); MLH1 expression loss in cell nuclei in 8 cases (33.33%). Non-lesional zone: MSH2 expression in the non-lesional area in 20 cases (83.33%); decreased MSH2 expression in the non-lesional zone in 2 cases (8.33%); MSH2 expression loss in the non-lesional zone in 2 cases (8.33%); Expression of MLH1 in the non-lesional area in 21 cases (87.50%);MLH1 expression decrease in the lesion area in 1 (4.16%); MLH1 expression loss in the non-lesional zone in 2 cases (8.33%)*

Conclusions. *MMR genes family belongs to tumor suppressors class involved in the repair DNA replication errors. MMR genes complete inactivation determines correcting errors impossibility in a mutant phenotype replication and emergence called RER + phenotype detectable in tumor cells. One of the MMR germ line mutation identification allows HNPCC diagnosis assertion, guides the family to the individual detection and case progress follow-up (for patients and their families). However, the analysis costs being extremely high, strictly or very probably limits some cases molecular analysis. Because it is a simple, fast and inexpensive method, with high specificity and sensitivity, applicable in most tissue biopsies, immunohistochemical analysis may include PCR and sequencing in a considerable cases percentage.*

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IN VITRO ANTIOXIDANT ACTIVITY OF *SPIRULINA PLATENSIS* – PRELIMINARY RESULTS

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Introduction. *Novel approch of the pharmaceutical industry are to develop new therapeutic strategies with low-costs for diseases prevention. Recent research are now being focused on the natural compounds produced by cyanobacteria. Spirulina is one of the most used cyanobacteria in medicine, because spirulina is safe for human consumption, is free of toxin and for the long term dietary supplementation may be consumed without evident toxic side effects. It is known to produce intracellular and extracellular metabolites with diverse biological activities such as antifungal, antiviral, antibacterial and activities. The aim of this study was to highlight the antioxidant and protective potential of Spirulina platensis (SP) aqueous extracts on normal skin cell line UV exposed.*

Material and methods. *Algal extract was obtained by mixing 10 g of dried Spirulina powder to 1000 ml of water for 24 h at room temperature, and centrifuged at 4000 rpm for 10 min. The obtained extract was freed from solvent by evaporation under reduced pressure and then resuspended in the specific cell culture medium to make the solution of known concentration of 50mg /ml. for in vitro assay of SP effects we used HaCat cell line (Human Primary Epidermal Keratinocytes Cells). 1x104 cells were seeded in a specific in 24 wells plate. after 24h the different concentrations of SP extract were added:→ 5 mg/ml (A), 10 mg/ml (B), 15mg/ml (C), and 25 mg/ml (D) the cell was exposed at 50mJ/cm2 UVA+UVB. after 24 h the SP effects were assayed: - trypan blue dye for cell viability; phase contrast microscopy (MCF) – cell morphology, SOD assay – antioxidant activity.*

Results. *UV exposed cells presented morphological alteration compared with non-exposed group. We noticed the change of cell specific forms in UV exposed group. Apoptotic and necrotic lesions were observed. The applied extracts have improuved the cell phenotype. Apoptotic and necrotic alterations were reduced compared with UV exposed group without SP. The 50mJ/cm2 induce the decrease of cell viability. The SP extracts improuve the number of living cells, in a dose-dependent manner. To investigate whether the radical scavenging activity of SP was mediated by antioxidant enzymes, the activities of antioxidant enzymes were examined in HaCaT cells after UV and SP exposure. SOD activity decreased in UV-induced cells compared to the untreated control. SOD activity was enhanced by SP treatment in a dose-dependent manner.*

Conclusions. *50mj/cm2 UVA+UVB-induced treatment reduced the activity of antioxidant enzymes and skin barrier function, while SP increased their activity. These results suggest that SP exerted cytoprotective activity against UVB-induced oxidative stress in HaCaT cells through stimulation of antioxidant enzymes activities.*

MOLECULAR BASES OF HEPATIC REGENERATION

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Introduction. *In recent years, major advances have been made in understanding the molecular and cellular mechanisms underlying liver development and regeneration. Knowing the pathways and molecules involved in tissue regeneration will allow us in the future, by applying modern techniques of cellular and molecular biotechnology, to act at certain levels or on specific molecules so that we can control the balance between proliferation and cell death. The study aims to present the molecular mechanisms of cell death by apoptosis in mouse liver after partial hepatectomy.*

Material and methods. *The studied material was liver fragments taken after necropsy from 30 male mice, 8 weeks old, of the NMRI strain. Partial hepatectomy (HP) was performed after intraperitoneal anesthesia with Ketamine / Xylazine (100 mg / kg Ketamine combined with 10 mg / kg Xylazine). Subsequently, the left and right side lobe of the medial lobe (68% of the total liver mass) were ligated using 3/0 catgut threads. After sacrification, tissue fragments were taken from the liver lobes and fixed in formol to make microscopic preparations. The animals under study were divided into 5 lots each consisting of 6 animals: lot A - the control group - in which partial hepatectomy has not been performed; lot B - animals sacrificed at 18 h after HP; lot C - animals sacrificed at 36 h after HP; lot D - animals sacrificed 7 days after HP lot E - animals sacrificed 2 months after HP*

Results. *Changes in liver weight and cell proliferation kinetics recorded increasing values from 18h after HP to 7th day after HP. At 2 months posthepatectomy, liver mass has values comparable to those measured in the control group. In the control group, as well as in animals sacrificed 2 months after HP, we observed moderate Bax proapoptotic protein immunoprecipitation in the hepatocyte cytoplasm. In the experimental groups, we noticed a non-systemic diffuse immunochromation with a poor intensity of immunoblotting. The pattern of the Bax expression progressively decreased from group B to group D. The immunohistochemical expression of the BCL2 protein was negative in the hepatocytes, both in the control group and in the experimental groups. In the experimental groups, Bcl2 positive lymphocyte immunoexpression was observed, both periportal and intralobular. Bcl2 protein expression was mild at 18h following heepectomy, with poorly dispersed Bcl2 reactive nuclei with poor immunocompromised intensity and moderate at 36h after HP.*

Conclusions. *Hepatic regeneration after partial hepatectomy in mice is achieved on the basis of proliferation of mature differentiated hepatocytes. In the experimental groups, a non-systemic diffuse immunostaining with a poor intensity of the immunocolorization was noted. The pattern of the Bax expression progressively decreased from group B to group D, indicating a low or absent apoptotic activity during the recovery of the liver population. The pattern of Bcl2 expression was evident only in lymphocytes, both periportal and intralobular. The absence of Bcl2 hepatocyte immunoassay indicates that this protein does not participate in the inhibition of the apoptotic process during hepatic regeneration.*