XIV CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE MUTAGÊNESE E GENÔMICA AMBIENTAL

Mecanismos celulares e moleculares envolvidos na instabilidade genômica e desafios na manutenção da integridade do DNA

3 - 6 junho de 2019

Dall'Onder Grande Hotel
Bento Gonçalves, RS, Brasil

30 anos

MutaGen-Brasil
Associação Brasileira de Mutagênese e Genômica Ambiental

SCIENTIFIC PROGRAM & ABSTRACTS

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MESSAGE FROM THE PRESIDENT

In this year - 2019 - MutaGen-Brasil completes 30 years of existence. The MutaGen congress is held every two years, and since the founding of SBMCTA in 1989, we had 13 events. The events organized by MutaGen aim to develop, integrate, disseminate and promote research, education and the responsible application of scientific knowledge, as a way to boost the sustainable human and socioeconomic development. In addition, this Association directs special attention to the scientific updating of professionals, to the formation of new researchers in the Area; and to assist in public policies to support human and environmental health risk, seeking to prevent and improve the quality of the environment and life.

This commemorative Congress seeks to rescue the history of our Association, analyzing the direction it has taken in the last decades, as well as promoting discussions about future paths. On behalf of the Organizing Committee of MUTAGEN-2019, I would like to welcome the participants and to express our sincere gratitude to all of you for the opportunity to have this conference. The MUTAGEN-2019 will be a great occasion to learn about the latest findings and research from renowned specialists in Environmental Mutagens and Genomics, as well as to exchange ideas and information with colleagues on the most recent trends.

You are our honoree on the MUTAGEN - 2019! Welcome to MutaGen-2019!

Juliana da Silva
President of the Brazilian Environmental Mutagenesis and Genomics Association
MutaGen - Brasil

MONDAY, JUNE 3rd, 2019

13:00 - 18:00	**Accreditation**		
13:30 - 15:10	**ROOM A - SATELLITE MEETING** **Germ cell mutation: legal requirements and evaluation** **13:30 - 13:50 -** Jill Escher (Recorded interview: Importance in studying germ cell mutations) **13:50 - 14:30 -** David DeMarini (Identification of human germ-cell mutagens) **14:30 - 15:10 -** Francesco Marchetti (Approaches for identifying germ cell mutagens)		
15:00 - 16:00	**ROOM G - Council Meeting**		
15:10 - 15:30	**Coffee break**		
15:30 - 17:00	**ROOM A - SATELLITE MEETING** **Germ cell mutation: legal requirements and evaluation** **15:30 - 16:00 -** Juliana Machado Braz (Legal requirements on genetic toxicology in Brazil) **16:00 - 16:20 -** Izabel Villela (Is Brazil ready to evaluate germ cell mutagens as a legal requirement?) **16:20 - 17:00 - Discussion**		
16:00 - 17:00	**ROOM C** **MUTAGEN SCHOOL 1** **Sharbel Maluf** (Genomic instability in chronic diseases)	**ROOM E** **MUTAGEN SCHOOL 2** **Paula Rohr and Grethel Leon-Mejia** (Monitoring genetic damage in humans occupationally exposed with the micronucleus buccal micronucleus cytome (BMCyt) assay)	**ROOM B** **MUTAGEN SERVICE 1** **Thania Rios Rossi Lima** (Overcoming challenges with different cell cultures in 3D)
18:00 - 18:30	**MALBEC B - Opening Sessionon**		
18:30 - 19:30	**MALBEC B - 30 years of MutaGen - Brasil: Past, present and future** **Catarina Satie Takahashi - João A. Pegas Henriques - Lucia Regina Ribeiro**		
19:30 - 20:30	**MALBEC B - CONFERENCE 1** **David DeMarini** (Environmental mutagenesis: A 50-Year legacy of protecting our genome and our environment)		
20:30 - 22:30	**MALBEC A - Opening Cocktail**		

TUESDAY, JUNE 4th, 2019

08:00 - 09:00	**MALBEC B** **MUTAGEN SCHOOL 3** **Rafael Dihl and Raíne Fogliati** (The use of SMART test to evaluate natural and synthetic products)	**MALBEC C** **MUTAGEN SCHOOL 4** **Bruno do Amaral Crispim and Alexeia Barufatti Grisolia** (Impacts of agricultural and urban activities on genotoxicity using in vivo and in vitro assays)	**ROOM A** **MUTAGEN SERVICE 2** **Deborah A. Roubicek** (Salmonella/microsome - Ames test)
09:00 - 10:00	**MALBEC B - CONFERENCE 2** **José M. Ordovas** (Genomics and epigenomics in personalized nutrition)	**MALBEC C - CONFERENCE 3** **Jean-Sébastien Hoffmann** (DNA polymerase theta in cancer risk and therapy)	
10:00 - 10:15	**MALBEC A - Coffee break**		
10:20 - 12:00	**MALBEC B - SESSION 1** **Natural and synthetic agents: DNA damage and repair** **10:20 - 10:45 - Carlos Renato Machado** (DNA repair in Trypanosoma cruzi: what we know, what we want to know) **10:45 - 11:10 - João A. Pegas Henriques** (New features on Pso2 protein family in DNA interstrand cross-link repair and in the maintenance of genomic integrity in Saccharomyces cerevisiae) **11:10 - 11:35 - Helga Stopper** (Genotoxicity in human hematopoietic stem cells, lymphoblastoid TK6 cells and promyelocytic HL60 cells - influence of cell differentiation state) **11:35 - 12:00 - Alexandre Escargueil** (New compounds and strategies to circumvent resistance to DNA targeting agents in the context of cancer)	**MALBEC C - SESSION 2** **Response to DNA damage (RDD) and genomic instability (Gi)** **10:20 - 10:45 - Leonardo Karam Teixeira** (Cyclin E1 promotes replication stress and chromosome instability in human cells) **10:45 - 11:10 - Francisco Bastos de Oliveira** (Transcriptional response to DNA replication stress) **11:10 - 11:35 - Rodrigo Martins** (Replicative stress response in central nervous system development) **11:35 - 12:00 - Marcus Bustamante Smolka** (Novel mechanisms in homology-directed DNA repair and genotoxin tolerance)	
12:00 - 14:00	**Lunch**		

14:00 - 15:40	**MALBEC B - SESSION 3** **DNA damage, repair and cancer** **14:00 - 14:25 - Nadja Cristhina de Souza Pinto** (Mitochondrial dysfunction in cells with DNA repair defects) **14:25 - 14:50 - Nicolas Hoch** (Protein ADP-ribosylation, genomic stability and human disease) **14:50 - 15:15 - Tirzah Lajus** (A portrait of germline mutation in patients at-risk for breast cancer in Brazil) **15:15 - 15:40 - Elza T. Sakamoto - Hojo** (DNA repair inhibition as a therapeutic molecular target for the sensitization of drug-resistant glioblastoma cells)	**MALBEC C - SESSION 4** **Environmental genotoxicity** **14:00 - 14:25 - Vera Vargas** (Biomarkers of genotoxicity as early protectors of biodiversity and human health) **14:25 - 14:50 - Rafael Dihl** (Genotoxicity of secondary metabolites from cyanobacteria) **14:50 - 15:15 - Cesar Koppe Grisolia** (An integrated approach for genotoxicity assessment of environmental contaminants) **15:15 - 15:40 - Viviane Souza do Amaral** (Natural radiation and their impact on genome instability: An exposome approach)
15:40 - 16:00	**MALBEC A - Coffee break**	
16:00 - 17:40	**MALBEC B - SESSION 5** **Young Researcher Award: Thematic axes 1 and 2** 1. Environmental mutagenesis (environmental monitoring, mutagenesis of natural products, drugs, physical agents, country legislation) 2. Mutagenesis and human and animal health (human and animal biomonitoring, cancer, degenerative diseases, aging) **16:00 - 16:25 - Bárbara Verena Dias Galvão** (ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF MYRCIARIA CAULIFLORA LEAF AGAINST TRYPANOSOMA CRUZI)	**MALBEC B - SESSION 6** **Young Researcher Award: Thematic axes 3 and 4** 3. Genomic instability and DNA repair **16:00 - 16:25 - Franciele Busatto** (TOPOISOMERASE II INHIBITORS-INDUCED LESIONS – AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS)

	16:25 - 16:50 - Francisco da Silva Júnior (RETENE, A NON-PRIORITY HYDROCARBON,IS ABLE TO GENERATE OXIDATIVE STRESS, MUTAGENIC EFFECTS, AND CELL DEATH)	**16:25 - 16:50 - Fernanda Rowies** (MOLECULAR CHARACTERIZATION OF DNA REPAIR IN MOUSE OLFACTORY NEURONS)
	16:50 - 17:15 - Natália Chermont dos Santos Moreira (NEURONAL DIFFERENTIATION AND NEUROPROTECTIVE EFFECTS OF NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITORS DESIGNED FOR ALZHEIMER'S DISEASE THERAPY) **17:15 - 17:40 - Melissa Souza** (THE INFLUENCE OF POLYMORPHISMS IN DNA DAMAGE, TELOMERE LENGTH AND GLOBAL DNA METHYLATION EVALUATED IN OPEN-CAST COAL MINING WORKERS)	**16:50 - 17:15 - Jéssica Lima** (CHRONIC HYPERGLYCEMIA INDUCES DNA DAMAGE ANDOXIDATIVE STRESS WHICH CAN BE DIMINISHED BY DIETARY RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS) **17:15 - 17:40 - Eduardo Kennedy Carrão Dantas** (IN VITRO APPROACHES FOR MUTAGENICITY AND HEPATOTOXICITY INDUCED BY MARKETABLE PRE-WORKOUT DIETARY SUPPLEMENTS)
17:40 - 18:00	**MALBEC A - Poster Session** 1. Environmental mutagenesis (environmental monitoring, mutagenesis of natural products, drugs, physical agents, country legislation)	
19:00 - 21:00	**MALBEC B - Night with Science** **Lucas Helal and Carlos Frederico Martins Menck** (How to reduce the avoidable waste in research and to stay away of Predatory/deceptive journals)	

WEDNESDAY, JUNE 5th, 2019

08:00 - 09:00	**MALBEC B** **MUTAGEN SCHOOL 5** **Fernanda Rabaioli da Silva** (Applications of the system toxicology tools in genotoxicology)	**ROOM A** **MUTAGEN SERVICE 3** **Izabel Villela and Miriana Machado** (In vitro micronucleus assay: Advantages and challenges)
09:00 - 10:00	**MALBEC B - CONFERENCE 4** **Carlos Frederico Martins Menck** (How UVA light induces DNA damage and its effects on human DNA repair deficient cells)	**MALBEC C - CONFERENCE 5** **João Paulo Fernades Teixeira** (Use of human biomonitoring in toxicology and epidemiological studies)
10:00 - 10:15	**MALBEC A - Coffee break**	
10:20 - 12:00	**MALBEC B - SESSION 7** **Mechanisms that influence and regulate telomere size** **10:20 - 10:45 - Michael Fenech** Video conference (Nutrients, Genes and their interaction in the maintenance of telomere integrity) **10:45 - 11:10 - Vivian Kahl** (Telomere length measurement techniques and their application) **11:10 - 11:35 - Caroline Bull** (Chronic stress and suboptimal nutrition is associated with chromosome instability in dementia family carers) **11:35 - 12:00 - Manoor Prakash Hande** (Role of DNA repair factors in telomere integrity and genome maintenan in mammalian cells under oxidative stress)	**MALBEC C - SESSION 8** **Congenital Zika syndrome** **10:20 - 11:00 - Lavínia Schuler-Faccini** (Congenital Zika syndrome: beyond microcephaly) **11:00 - 11:30 - Lucas Rosa Fraga** (Animal models for Zika virus teratogenesis: what do they tell us?) **11:30 - 12:00 - Walter Orlando Beys da Silva** (A proteomic approach on Zika virus infection reveals a potential link to neurological disorders and brain diseases)
12:00 - 14:00	**Lunch**	

	MALBEC B - SESSION 9 **Application of genotoxicity in safety assessment of pharmaceutical impurities**	**MALBEC C - SESSION 10** **How do eating behaviors and folk medicine affect mutagenicity?**
14:00 - 15:40	**14:00 - 14:25 - Michael Urquhart** Video conference: (Managing emerging mutagenicity risks: late stage mutagenic impurity control within the atovaquone second generation synthesis)	**14:00 - 14:25 - Vanessa Moraes Andrade** (Brazil nut consumption as adjuvant in the treatment of type 2 diabetes mellitus: modulation of genomic and biochemical instability)
	14:25 - 14:50 - Miriana Machado (Genotoxicity evaluation of drug impurities: Brazilian regulatory aspects)	**14:25 - 14:50 - Siegfried Knasmuller** (Impact of overweight/obesity on DNA-stability)
	14:50 - 15:15 - Mariah Ultramari (Assessment of the genotoxic potential of pharmaceutical impurities)	**14:50 - 15:15 - Wilner Martinez** (Medicinal plant extracts: DNA damage and role in cancer chemosensitizer in vitro)
	15:15 - 15:40 - Alex Cayley (Use of in silico tools in the context of the ICH M7 guidelines)	**15:15 - 15:40 - Ilce Mara de Syllos Cólus** (How Mutagenesis can help safe use of medicinal plants?)
15:45 - 16:00	**Lunch**	
16:00 - 17:00	**MALBEC A - Poster Session** 2. Mutagenesis and human and animal health (human and animal biomonitoring, cancer, degenerative diseases, aging). 3. Genomic instability and DNA repair. 4. Modulation of mutagenicity and Nutrigenomics (antimutagenesis, anticarcinogenesis, modulation of gene expression by micronutrients).	
17:00 - 19:00	**ROOM A - MutaGen-Brasil Assembly**	
20:30 - 24:00	**MALBEC B - MutaGen 30 years dinner**	

THURSDAY, JUNE 6th, 2019

	MALBEC B - CONFERENCE 6	**MALBEC C - CONFERENCE 7**
09:00 - 10:00	**Paul White** (Quantitative analyses of genetic toxicity dose - response data - from potency determination to risk)	**Marcus Bustamante Smolka** (The DNA damage signaling network: From basic biology to cancer therapy)
10:00 - 10:15	**MALBEC A - Coffee break**	
	MALBEC B - SESSION 11 **Non-animal methods for toxicity testing: Brazilian reality and new approaches**	**MALBEC C - SESSION 12** **Biological responses to environmental factors**
10:20 - 12:00	**10:20 - 10:45 - Izabel Villela** (Alternative methods to animal use in Brazil)	**10:20 - 10:45 - Fernanda Rabaioli da Silva** (Insights into the impacts of airport environmental on birds of prey)
	10:45 - 11:10 - Márcio Lorencini (Alternative methods for cosmetics - evaluation)	**10:45 - 11:10 - Danieli Benedetti** (Evaluation of occupational health of farmers exposed to pesticides)
	11:10 - 11:35 - Rodrigo de Vecchi (3D skin model in Brazil: challenges and opportunities)	**11:10 - 11:35 - Maria Eugenia Gonsebatt** (Gestational exposure to arsenite: altered glutamate disposition and memory imparment)
	11:35 - 12:00 - Alessandra de Mello Aguiar (Micronucleus analysis by high content imaging)	**11:35 - 12:00 - Marcelo Farina** (Mechanisms mediating methylmercury - induced neurotoxicity)
12:00 - 12:30	**MALBEC B - Young Scientists Awards and Posters (by subject area) & Closing**	
13:30 - 18:00	**Cultural activity - by Membership**	

SCIENTIFIC PROGRAM

JILL ESCHER
RECORDED INTERVIEW: IMPORTANCE IN STUDYING GERM CELL MUTATIONS

DAVID DEMARINI
IDENTIFICATION OF HUMAN GERM-CELL MUTAGENS

IDENTIFICATION OF HUMAN GERM-CELL MUTAGENS

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Keywords: Germ-cell mutagens, tobacco smoke, air pollution, ionizing radiation

The existence of agents that can induce germ-cell mutations in experimental systems has been recognized since 1927 with the discovery of the ability of X-rays to induce such mutations in *Drosophila*. Various rodent-based germ-cell mutation assays have been developed, and ~45 germ-cell mutagens have been clearly identified in these rodent assays. Overall, >160 agents have some evidence that they can induce germ-cell mutations in rodents. However, no agent has been declared a germ-cell mutagen in humans. Nonetheless, evidence is emerging that ionizing radiation, air pollution, and tobacco smoke may be human germ-cell mutagens. The International Agency for Research on Cancer (IARC) is part of the World Health Organization that evaluates agents for their ability to be human carcinogens. The criteria used by IARC could be modified to evaluate an agent for its ability to be a human germ-cell mutagen. Sufficient evidence in animals would require that the agent induce germ-cell mutations in at least 2 species or that there be 2 independent positive studies in one species. In addition, in vitro, rodent, and human biomarker data would be necessary to provide sufficient mechanistic evidence in humans. Epidemiological evidence and exposure assessment would also be considered in the evaluation. Applying such criteria, the current literature indicates that there would be sufficient evidence to evaluate ionizing radiation, tobacco smoking, and air pollution as Group 1 (known) human germ-cell mutagens. Definitive molecular epidemiological evidence in the form of next-generation, genomic DNA sequence analysis, will likely provide the definitive evidence to support such evaluations. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

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LEGAL REQUIREMENTS ON GENETIC TOXICOLOGY IN BRAZIL

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Brazilian legislation prohibits mutagenic pesticides. Substances are considered mutagenic when they induce mutation in at least two tests, one to detect gene mutations and another to detect chromosomal mutations. To understand the Brazilian legislation and the mutagenic evaluation carried out by Anvisa, it is important to differentiate mutagenicity from genotoxicity. Genotoxicity assessments evaluate any type of study on cellular function which involves damage or interference during DNA replication or repair, including mutagenic effects. Mutagenicity assessments determine if the substances induce mutation in genes or chromosomes. Mutagenic effects can be permanent, leading to relevant adverse effects and hereditary changes in DNA. Therefore, evaluation of mutagenicity studies is important in the regulatory field and there are legal provisions to deny the registration of mutagenic pesticides. A weight of evidence (WoE) approach is used to determine the mutagenic potential of a substance for humans. Genotoxicity studies are also used in the regulatory assessment for understanding the adverse effects of a substance, but they do not provide sufficient evidence to ban a pesticide. Genotoxicity studies are used for the characterization of mechanisms of action, but using the WoE approach, they have a lower weight compared to the mutagenicity assays. Furthermore, when applying the WoE criteria, *in vitro* mutagenicity studies in bacteria have less weight than *in vitro* studies in mammalian cells and *in vivo* studies in mammals have the greater weight of evidence. With the WoE approach, a pesticide can be classified according to the categories of the Globally Unified Classification and Labeling of Chemical Substances (GHS). The major toxicological concern of GHS classification is germ cell mutation since its effects are inherited and difficult to detect in long term studies with animals. Therefore, it is crucial to characterize the mutagenic potential in germ cells. However, from the regulatory view, mutations in somatic cells are sufficient to conclude that a pesticide is also mutagenic for germ cells in the absence of specific studies. This is especially true when there is evidence that the substance reaches the reproductive organs and triggers adverse effects on germ cells. This framework to assess the germ cell mutagenicity of pesticides reflects the current regulatory understanding and its adoption was proposed by Anvisa in order to replace Ordinance 03/2002.

IS BRAZIL READY TO EVALUATE GERM CELL MUTAGENS AS A LEGAL REQUIREMENT?

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Evaluate the mutagenic potential of chemicals has been a legal requirement in Brazil for many years. The general feature of a standard mutagenicity test battery including a bacterial reverse gene mutation test and *in vitro* and/or *in vivo* mammalian mutagenicity test were enough for regulatory purpose. The assays to access this endpoint are available in GLP (Good Laboratory Practices) conditions in Brazil, allowing Brazilian industry to perform the studies without extra taxes. However, Brazilian legal requirements for agrochemicals are under review, and mutation in germ cells is one of the most important new suggested aspects. This inclusion is in accord with GHS regulation and brings Brazilian legislation closer to the international standards. To answer this endpoint, companies should perform specific germ cell mutation assays according to international validated guidelines under GLP conditions. These assays are completely different from the regular somatic mutation assays that are usually performed in Brazil so far. Analysing Brazilian scientific reality, there are research teams working on germ cell mutations? And, what about CROs (Contract Research Organization), any Brazilian GLP laboratory can perform these assays? How much does it cost to perform an assay outside Brazil? These are key questions to understand the impact of the legislations in Brazilian reality.

Financial Support: InnVitro Research & Development. Brazil.

GENOMIC INSTABILITY IN CHRONIC DISEASES

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The process of aging results in a host of changes at the cellular and molecular levels, which include genomic instability. Over the entire life course, these changes can be seen in unhealthy manifestations, especially in advanced age with its associated functional declines caused by the accumulation of cellular damage, together with a diminished ability to repair this damage. The maintenance of genomic stability has been considered, in several studies, as the main factor that leads to human health. Damage to human genomic DNA occurs very frequently and most of these damages are successfully repaired by mechanisms involving many biochemical factors that characterize the DNA damage response (DDR). The accumulation of oxidative damage caused by the action of free radicals (reactive oxygen species, ROS) on macromolecules such as DNA, proteins and lipids, can lead to a loss of function of these molecules. Many studies suggest that ROS participate in the pathophysiological mechanism of various human diseases, including Parkinson's, multiple sclerosis, muscular dystrophy, cataracts, retinopathies, atherosclerosis, myocardial infarction, ischemia and reperfusion syndrome, chronic obstructive pulmonary disease, diabetes mellitus type 2, hepatic cirrhosis, arthritis rheumatoid and various types of cancer. Carcinogenesis is directly related to the prolonged accumulation of injuries at different biological levels, which alter the cells both genetically and biochemically. In each of these situations there is an opportunity for intervention to prevent, delaying or stopping the gradual march of healthy cells towards malignancy. A new strategy to reduce its incidence relates to intervention programs for diet and nutrition, as well as for the development of pharmacological products that could work as chemo-preventives. Non-enzymatic antioxidants such as ascorbate, tocopherols, carotenoids and flavonoids in general, present in diets rich in fruits and vegetables, are important defences against free radicals, reducing the chances of developing degenerative pathologies. Genetic instability plays a critical role in human chronic diseases. Beyond its theoretical impact, the analysis of genetic instability may lead the way to the development of innovative therapy strategies. Constant surveillance for malignant or pre-malignant conditions should be performed.

Financial Support: CNPq, CAPES, FAPESC.

MONITORING GENETIC DAMAGE IN HUMANS OCCUPATIONALLY EXPOSED WITH THE MICRONUCLEUS BUCCAL MICRONUCLEUS CYTOME (BMCyt) ASSAY

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In various work environments it is possible to find substances which put at risk the workers health. Therefore, assays to monitor the environment as well as the effect on workers health due the occupational exposure are required. Since 1980 the Buccal Micronucleus Cytome (BMCyt) assay has been widely used to biomonitoring exposure to genetic agents such as in work environments. The BMCyt assay is a minimally invasive cytogenetic test for measuring different biomarkers such as DNA damage, chromosomal instability, cell death and the regenerative potential, in the oral epithelium. The aim of this course is to present and to describe a detailed criteria for analysis of BMCyt assay biomarkers and the usefulness of biomarkers of BMCyt assay for human biomonitoring and evaluation of cancer risk in the exposed populations.

THE USE OF SOMATIC MUTATION AND RECOMBINATION TEST (SMART) TO ASSESS NATURAL AND SYNTHETIC PRODUCTS

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The *Drosophila* wing Somatic Mutation and Recombination Test (SMART) is a bioassay that detects the loss of heterozygosity (LOH) of markers genes, as a result of gene mutations, chromosomal rearrangements and somatic recombination. Genetic changes in cells are phenotypically identified as mutant spots on the wing surface of adult flies expressing the recessive markers genes *flr*^{3} or *mwh*, responsible for changes in hair shape. It is also possible to identify genotoxins of direct and indirect action through crossings that are performed, either by acute or chronic treatment. The SMART can also be used to assess the effects of nongenotoxic chemicals, which may act as modulators when combined with genotoxins. As a test with proven reliability and applicability, several compounds of natural or synthetic origin have been evaluated.

Financial Support: CNPq, CAPES, FAPERGS and ULBRA.

IMPACTS OF AGRICULTURAL AND URBAN ACTIVITIES ON GENOTOXICITY USING *in vivo* AND *in vitro* ASSAYS

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Industrial, domestic and agricultural effluents promote the contamination of water resources due to the existence of chemical compounds that can cause genetic damage to living beings. We evaluated toxicogenic potential of surface and groundwater and analyzed the contamination by metals and emerging contaminants (ECs) to understand the relationship between their presence in the water and the use and land cover and physicochemical parameters with genetic damage. Cytotoxic and genotoxic assay *in vivo* using in blood cells of *Astyanax lacustris* and meristematic cells of *Allium cep*a were analyzed with using water samples. We determined whether ECs mixtures those are present in the water could cause effects on gene expression in zebrafish. In addition, we have assessed *in vitro* assays using culture cells to evaluate cytotoxic and genotoxic effects of metals that were presents in water (Al e Mn). Samples were collected from rivers and underground wells (MS/Brazil). The metals were analyzed using ICP-OES and EC using LC-MS/MS. The Al, Mn, Cu, Ni, Cd, Fe exceeded acceptable by Brazilian Resolution and ECs detected were caffeine, imidacloprid, carbendazim, 2-hydroxy atrazine, hexazinone, tebuthiuron, atrazine, diuron, clomazone, ametrine, malathion and bisphenol A. Toxicogenic effects of the water samples were observed in blood cells of *A. lacustris* and meristematic cells of *A. cepa*. Environmental concentrations identified in groundwater (maximum permissible values and above legislation) demonstrated cytotoxic and genotoxic effects in culture cells. We reported changes in the expression of zebrafish target genes when exposed to ECs. We demonstrated the toxicogenic potential of water samples from rivers and underground wells related land use and land cover have influence on toxicogenetic damages *in vitro* and *in vivo* assays. Areas with agricultural practices and absence of ciliary forest favor the contamination of water resources increasing the probability the genetic damage in living beings. Also, the disposal of effluents from urban areas also contributes to aggravate the problem of contamination of water resources.

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APPLICATIONS OF THE SYSTEM TOXICOLOGY TOOLS IN GENOTOXICOLOGY

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Key-words: *in silico*, bioinformatics, genetic toxicology, chemical compounds.

Advances in experimental methods in the post genomic era have provided large-scale biological data and computational tools to analysis these data sets. A novel framework and methodology for collecting and analyzing data in order to understand complex biological structures and their interactions have emerged of the system biology. System toxicology is one type of toxicity assessment that uses computational resources (i.e., methods of system biology) to organize, analyze, visualize, or predict toxicity of chemicals. These computational methods aim to complement *in vitro* and *in vivo* toxicity tests to reduce the cost and time of toxicity tests, and improve toxicity prediction. System toxicology has been associated to genetic toxicology studies to contribute in the understanding of the protein and chemicals interaction in the genetic instability context. For this, subnetworks of proteins and chemical compounds are created using mining tools STRING 11.0 and STITCH 5.0, which are databases of known and predicted protein-protein and protein-chemicals interactions with interactions including direct (physical) and indirect (functional) associations). The subnetworks prospected are merged using Cytoscape 3.7.1. Cytoscape is an open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data. Additional features are available as apps or called plugins. Apps can be applied for network analyses as: clusterization, gene ontology and centralities. As for example, authors used system toxicology tools to provide insight into the influence of pesticide exposure on telomere length maintenance in tobacco farmers; to identify as chemical compounds of coal waste sample can cause DNA damage in cells exposed; to investigate how the zinc oxide nanoparticle exposure can increase the homologous recombination and to describe the toxicological effects of the different substances in tobacco smoke on human embryonic development. Overall, these studies demonstrates that topology of networks inferred by chemical-protein interactions provides information from molecular models about how xenobiotics can affect the genetic stability.

Financial Support: CNPq, FAPERGS, UNILASALLE

OVERCOMING CHALLENGES WITH DIFFERENT CELL CULTURES IN 3D

The social-ethical pressure on the world scientific community to follow the principle of the 3R's (replace, reduce, refinement), postulated by Russell and Burch in 1959, are stimulating changes in testing of different research areas and industrial practices, especially for hazard and risk assessment of chemicals. For over decades, two-dimensional (2D) cell culture has been used as an alternative method to the animal use in understanding the mechanisms underlying cellular behavior *in vivo*. Although still considered a traditional method, monolayer culture has many limitations. Thus, with the need for development of methods with higher predictability and reliability, three-dimensional (3D) culture models emerge that mimic the cell-cell and extracellular matrix-cell interactions occurring in the human organism. However, there are some challenges to be overcome in 3D cell culture, including the tissue-tissue interface, oxygen and nutrient distributions, among other crucial factors for regulating cell behavior and function *in vivo*.

THE SALMONELLA/MICROSOME TEST

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The Brazilian Association of Environmental Mutagenesis and Genomics, MutaGen-Brasil, in an effort to harmonize and discuss the different mutagenicity methods that are required by law to the registry of pesticides, cosmetics and other chemicals in Brazil, proposed a permanent forum in MutaGen congresses: the *MutaGen Service* (Serviço MutaGen). The idea is to have private and public labs, students, scientists and anyone else interested, sitting together to discuss technical details and difficulties, result's interpretation, reports, quality control issues, etc. We also intend to plan inter-laboratorial studies, and/or other studies necessary to attend the different regulations and quality standards, with special attention to GLP and ISO 17025 guidelines.
The *Salmonella*/microsome test will be discussed in this forum.

IN VITRO MICRONUCLEUS ASSAY: ADVANTAGES AND CHALLENGES

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Micronucleus is a structure formed by acentric chromosome fragment or whole chromosome that was not included in daughter nuclei produced by mitosis. So, micronuclei can be formed after cell exposure to clastogenic and aneugenic compounds. For decades, the *in vivo* Micronucleus Test (MNT) has been globally used for chromosome mutation evaluation in academic projects and regulatory safety evaluation of drugs, agrochemical, cosmetic and medical devices. In 2010, the *in vitro* MNT guideline was firstly published by OECD and this version has been also accepted for regulatory purposes. Importantly, since 2015, the *in vitro* MNT has been recognized by the Brazilian Health Regulatory Agency (ANVISA) as a substitute method for *in vivo* Micronucleus Test and *in vivo* Chromosome Aberration Test. However, even though nine years after the *in vitro* protocol publication, acceptance criteria and data analysis still generate some concern for Brazilian industries and Contract Research Organizations. This fact is especially important this year, since it is the ANVISA's deadline for the replacement of the *in vivo* MNT by the *in vitro* version. That means, after 2019, the *in vivo* version of the assay can be only performed if the inapplicability of the *in vitro* version is properly justified. Taken together, the *in vitro* Micronucleus Test is an important tool to evaluate mutagenic potential of new substances in academic or regulatory projects and it presents several advantages when compared to the *in vivo* version. Nevertheless, besides Good Cell Culture Practices, test substance concentration range, micronuclei analysis and historical controls are key aspects to be considered in the study evaluation and acceptance.
Financial Support: InnVitro Pesquisa & Desenvolvimento – Brazil.

30 years of MutaGen - Brasil: Past, present and future

CATARINA SATIE TAKAHASHI - JOÃO A. PEGAS HENRIQUES - LUCIA REGINA RIBEIRO

During 30 years since its foundation in 1989, the Mutagenesis Society has been very active maintaining good initiatives in the national and international scenario. It will be addressed in the conference, the factors that led to the creation of the association, recovering the aspects and information that could contribute to the formation of human resources and critical mass of professionals working in the field of mutagenesis and genetic toxicology. Along three decades, the association has been working to motivate and support scientists to promote high level research in the area of mutagenesis, stimulating the publication of articles in reputed international journals. SBMCTA - Brazilian Society of Environmental Mutagenesis, Carcinogenesis and Teratogenesis, is a nonprofit civil entity that aims to develop scientific activities in the field of Mutagenesis, Carcinogenesis and Teratogenesis, as well as contributing to the training and updating of professionals in the Area. Studies in the field covered by the Association (Mutagen-Brasil, since 2013) involve research focusing on mutagenicity or genotoxicity of chemical, physical and biological agents, many of which are present in the environment, and the identification of genetic alterations that might be a consequence of environmental exposure, leading to the development of various diseases. In addition to promoting regular national meetings every two years, the Association has always been concerned about internationalization and academic exchange programs, as well as courses organized at different states of the country. In general, the purpose is to support or motivate the development of advanced techniques that can be suitable to be applied in the mutagenesis field, aiming to study different problems related to environmental mutagenesis in different organisms, including humans. In 2013, the Association Mutagen-Brasil successfully organized the International Conference on Environmental Mutagenesis (ICEM), which is held every 4 years, and the 11th ICEM took place, for the first time, in a Latin America country. The future of Mutagen-Brasil stands very promising, since young researchers working in the mutagenesis field have been stimulated to maintain the scientific activities and exchange programs in upcoming years.

ENVIRONMENTAL MUTAGENESIS: A 50-YEAR LEGACY OF PROTECTING OUR GENOME AND OUR ENVIRONMENT

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Keywords: Mutagenesis, environment, scientific societies

Just four months before astronauts were launched to the moon, a mutagenesis moonshot was launched to address the integrity of our genome relative to our environment. The impetus for this included the discovery that X-rays (Herman Muller, 1927) and chemicals (starting with Charlotte Auerbach in 1942) were germ-cell mutagens in *Drosophila* and the introduction of thousands of untested chemicals into the environment after World War II. As evidence accumulated that some of the newly introduced chemicals and drugs were mutagenic, scientists from around the world began voicing their concern that environmental mutagens may damage not only the environment but the human germline. Such voices included Joshua Lederberg (U.S., 1955), Alfred Barthelmess (Germany, 1956), Charlotte Auerbach (Scotland, 1960), Fritz Sobels (Netherlands, 1962), James Crow (U.S. 1966), and Rachel Carson (U.S., 1962) in her book "Silent Spring." In 1969 Alexander Hollaender at the Oak Ridge National Laboratory and colleagues acted on these concerns by founding the Environmental Mutagen Society, now the Environmental Mutagenesis and Genomics Society (EMGS); Fritz Sobels founded the European EMS in 1970. A succession of countries and regions also began establishing such societies, including the formation of ALAMCTA by Cristina Cortinas de Nava and colleagues in Mexico in 1980 to represent Latin America. As noted by Fred de Serres, who was one of the co-founders of the EMS, such societies were necessary because protecting populations from environmental mutagens could not be addressed by existing scientific societies, and new multi-disciplinary alliances were required to spearhead this movement. These societies gathered policy makers and scientists from government, industry, and academia who helped implement laws requiring genotoxicity testing of pesticides and drugs; created an electronic database of the mutagenesis literature; established journals; promoted basic and applied research in DNA repair and mutagenesis; and established collaborations, conferences, and training programs that expanded the science worldwide. After 50 years, the voyage continues, and vibrant environmental mutagen societies are needed to bring this daring mutagenesis mission to its intended landing site. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

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GENOMICS AND EPIGENOMICS IN PERSONALIZED NUTRITION

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In each of the stages of our lives, good nutrition is key to our health. However, what foods are part of healthy nutrition has been a controversial topic in the scientific and popular field. In order to provide health professionals and the public with a more solid basis to recommend and practice this good nutrition, different national and international institutions periodically publish nutritional guides that incorporate current scientific knowledge. However, what these guidelines do not include is the great interindividual variability in response to the food consumed. The molecular basis of this variability is complex, but part of it is associated with our genome and its multiple differences. The study of the relationship between the variations in the genome and the response to the diet is what is known as nutrigenetics or nutrigenomics and its results will be the basis for the implementation of personalized recommendations as instruments of prevention or even therapy of common chronic diseases. Nutrigenetics is a growing science and the results of the research in this area are promising thanks to advances in genetic techniques and there are already some applications that allow some limited dietary personalization. However, greater precision is needed in terms of obtaining information about the habitual diet of individuals. Other aspects that will contribute to the progress of nutrigenetics will be the integration of other technologies that define who we are and how we function, such as epigenetics, metabolomics and microbiota. In addition, nutrigenetics will require demonstration that its implementation is more effective than global recommendations and do so at the highest level of scientific evidence through randomized clinical intervention trials. Although there is still a way to go, the incorporation of this knowledge into daily practice will allow a more effective prevention of the diseases associated with aging and therefore a healthier and more vital life.

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CONFERENCE 3

JEAN-SÉBASTIEN HOFFMANN

DNA POLYMERASE THETA IN CANCER RISK AND THERAPY

DNA POLYMERASE θ IN CANCER RISK AND THERAPY

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The deficiency of DNA repair pathways is a frequent event in tumorigenesis which provides a selective growth advantage to tumor cells as this results in genetic instability and enhanced mutation rates, a driving force for tumor evolution. For example, multiple hereditary or somatic cancers are deficient in homologous recombination (HR), a repair pathway involved in the reactivation of replication forks that have been blocked and in the repair of DNA double strand breaks (DSBs). However, DNA repair–deficient cancers often become dependent on backup DNA repair pathways, which represent an "Achilles heel" that can be targeted to eliminate cancer cells. Since many years, our research lines focus on the DNA repair polymerase theta (Polθ), encoded by the *POLQ* gene, which functions in an alternative form of end-joining, referred to as microhomology-mediated end-joining (MMEJ), which competes directly with HR. Unlike HR, MMEJ is intrinsically error-prone and produces deletions and translocations. Several companies now are almost ready to propose Polθ inhibitors. I will present how we have identified *POLQ* as a powerful biomarker in different types of human cancers and find that overexpression of *POLQ* was significantly related to poor patient survival, show that Polθ overexpression correlate with increased MMEJ activity and describe the physiological contexts where the MMEJ activity of Polθ is required. I will also describe the cellular impact of pathogenic germline mutations in the POLQ gene, recently identified in bilateral breast cancer patients in southern Brazil.

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CONFERENCE 4

CARLOS FREDERICO MARTINS MENCK

HOW UVA LIGHT INDUCES DNA DAMAGE AND ITS EFFECTS ON HUMAN DNA REPAIR DEFICIENT CELLS

HOW UVA LIGHT INDUCES DNA DAMAGE AND ITS EFFECTS ON HUMAN DNA REPAIR DEFICIENT CELLS

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The UVA component of sunlight is responsible for 95% of UV reaching Earth surface, and penetrates deep on skin. UVA light causes direct DNA lesions, such as pyrimidine dimers, but indirect action effects may generate reactive oxygen species, also damage cellular components, including the genetic material. Despite many decades of investigation, the discrimination among UVA light damaging is not clear. Genetic defects in DNA damage processing mechanisms have clear harmful effects, as disclosed by the human syndrome xeroderma pigmentosum (XP), whose patients have increased skin photodamage, including tumors and photoaging, in sunlight exposed areas. Our proposal is to use cells from XP patients to amplify and, thus, better comprehend the effects of UVA light irradiation in human cells. Initial studies were performed in cells from XP-V patients, who are defective on translesion synthesis (TLS). Basically, results indicate that although pyrimidine dimers are important in the deleterious action of UVA-light, oxidative stress is also triggered several hours after irradiation and, mainly, affect the capacity of DNA repair probably due to the oxidation of proteins. The use of antioxidant gives almost full protection of the irradiated cells, including DNA replication and cell survival, although pyrimidine dimers are still induced in the genome. UVA-induced mutagenesis was also investigated using exome sequencing, to identify point mutations in the cells. Mainly C>T mutations at dipyrimidine sites were detected in UVA irradiated XP-V cells, probably due to pyrimidine dimers. However, the data disclosed also C>A mutations that may also occur due to oxidative stress, and, curiously, these mutations were also increased in non-irradiated XP-V cells, compared to TLS proficient cells. This information is highly relevant for XP patients, but may also help us to understand what occurs during skin sunlight exposure from normal human population.

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BIOMONITORING of STYRENE OCCUPATIONAL EXPOSURES

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Styrene is a commercially important chemical widely used in the manufacture of synthetic rubber, resins, polyesters, and plastics. Occupational exposure to styrene occurs in the styrene-butadiene rubber, styrene monomer and polymerisation, and reinforced plastics industries. Although the genotoxic potential of styrene is known, very limited and inconclusive information is available regarding its dose-dependent genotoxic effects in humans. The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids (MA+PGA) in urine, haemoglobin (Hb) adducts, sister-chromatid exchanges (SCE), micronuclei (MN), DNA damage (comet assay) and genotypes of polymorphic genes of some metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study. The mean air concentration of styrene in the breathing zone of workers (30.4ppm) was higher than the threshold limit value of 20ppm recommended by the ACGIH, and the biological exposure index adopted by the ACGIH for exposure to styrene prior to the next shift (MA+PGA=400mg/g cr) was exceeded, indicating that styrene exposure for this group of workers was higher than recommended. The level of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. The DNA damage was higher among styrene-exposed workers than in controls. No significant differences were observed in the MN. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers. The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses. Because the effects of these polymorphisms are relatively subtle, and some important alleles are relatively rare, a much larger study population will be necessary to evaluate their effects on biomarkers, especially when gene-gene interactions are considered.

CONFERENCE 6

PAUL WHITE

QUANTITATIVE ANALYSES OF GENETIC TOXICITY DOSE - RESPONSE DATA - FROM POTENCY DETERMINATION TO RISK

CONFERENCE 7

MARCUS BUSTAMANTE SMOLKA

THE DNA DAMAGE SIGNALING NETWORK: FROM BASIC BIOLOGY TO CANCER THERAPY

THE DNA DAMAGE SIGNALING NETWORK: FROM BASIC BIOLOGY TO CANCER THERAPY

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Kinases play central roles in the eukaryotic DNA damage response, from sensing DNA damage to coordinating DNA repair pathways. Early work in yeast and mammalian systems identified evolutionarily conserved DNA damage signaling kinases involved in cell cycle control and linked them to the “cell cycle checkpoint” concept of Weinert and Hartwell. Subsequent work revealed many of the intricacies for how these kinases establish the checkpoint and control processes beyond the cell cycle, such as apoptosis, transcription and replication origin firing. However, it remains unclear how DNA damage signaling kinases control DNA repair pathways to preserve genome integrity, representing one of the most fundamental knowledge gaps in the field. Here I will present how we are using a combination of genetic and proteomic approaches to elucidate the roles of DNA damage signaling kinases in genome maintenance and in the regulation of DNA repair machineries. Our work is uncovering fundamental mechanisms by which the kinase ATR controls the action of key homologous recombination factors from yeast to humans. Finally, I will discuss how our findings provide a rationale for the use of ATR inhibitors to selectively kill cancers that rely on homologous recombination to survive oncogene-induced replication stress.

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CARLOS RENATO MACHADO
DNA REPAIR IN TRYPANOSOMA CRUZI: WHAT WE KNOW, WHAT WE WANT TO KNOW

JOÃO A. PEGAS HENRIQUES
NEW FEATURES ON PSO2 PROTEIN FAMILY IN DNA INTERSTRAND CROSS-LINK REPAIR AND IN THE MAINTENANCE OF GENOMIC INTEGRITY IN SACCHAROMYCES CEREVISIAE

HELGA STOPPER
GENOTOXICITY IN HUMAN HEMATOPOIETIC STEM CELLS, LYMPHOBLASTOID TK6 CELLS AND PROMYELOCYTIC HL60 CELLS - INFLUENCE OF CELL DIFFERENTIATION STATE

GENOTOXICITY IN HUMAN HEMATOPOIETIC STEM CELLS, LYMPHOBLASTOID TK6 CELLS AND PROMYELOCYTIC HL60 CELLS - INFLUENCE OF CELL DIFFERENTIATION STATE

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Genotoxicity testing is usally performed with immortal cell lines or peripheral blood lymphocytes. However, the target of mutagenic effects in carcinogenesis may be stem cells. Therefore, we compared human cord blood CD34+ stem cells (HSC) and the lymphoblastoid TK6 cells regarding their response to genotoxic agents, which was quantified using the micronucleus assay. HSC were significantly less sensitive than TK6 cells for the genotoxic effects of doxorubicin, vinblastine and methyl methanesulfonate and equally sensitive for mitomycin C. However, loss of viability after mitomycin C treatment was higher in HSC than in TK6 cells. In in vivo genotoxicity testing with rodents, analyzed tissues largely consist of fully differentiated cells. To analyze the possible influence of differentiation, we used HL-60 cells, a human premyelocytic cell line that can differentiate into a granulocyte-like or a monocyte-like form in vitro. The comet assay is a gentoxicity test that does not depend on proliferation and can thus be used for both cell states. While MMS, hydrogen peroxide and potassium bromate induced DNA damage in both cell types, doxorubicin did not induce DNA damage in differentiated HL60 cells in the tested dose range. Etoposide caused significant increase in DNA damage in both cell variants, but to a larger extend in undifferentiated HL60 cells. Doxorubicin and etoposide are known topoisomerase II inhibitors, and the activity of this enzyme is needed more in actively proliferating cells than in differentiated cells. This may have to be considered when compounds with unknown mechanism of action are assessed in routine testing.

ALEXANDRE ESCARGUEIL

NEW COMPOUNDS AND STRATEGIES TO CIRCUMVENT RESISTANCE TO DNA TARGETING AGENTS IN THE CONTEXT OF CANCER

SESSION 2

RESPONSE TO DNA DAMAGE (RDD) AND GENOMIC INSTABILITY (GI)

LEONARDO KARAM TEIXEIRA

CYCLIN E1 PROMOTES REPLICATION STRESS AND CHROMOSOME INSTABILITY IN HUMAN CELLS

CYCLIN E1 PROMOTES REPLICATION STRESS AND CHROMOSOME INSTABILITY IN HUMAN CELLS

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Cell cycle progression is regulated by the Cyclin-Dependent Kinase (CDK) family of proteins, so named because their activation depends on the association with regulatory subunits known as Cyclins. Cyclin E1 normally accumulates at the G1/S boundary, where it promotes S phase entry and progression by activating CDK2. In normal cells, Cyclin E1/CDK2 activity is associated with DNA replication-related functions. On the other hand, deregulation of Cyclin E1 leads to inefficient assembly of pre-replication complexes and insufficient levels of nucleotide, causing DNA replication stress and eventually genomic instability. Cyclin E1 is frequently overexpressed in human cancers, correlating with decreased survival in breast cancer patients. However, the mechanisms by which Cyclin E1 deregulation causes genomic instability are not completely understood. Our group has demonstrated that high levels of Cyclin E1 delay S phase progression of synchronized human mammary epithelial cells, allowing cells to enter into mitosis before completion of DNA replication. In proliferating cells, Cyclin E1 overexpression leads to decreased cell proliferation, slows down S phase progression, causes DNA replication stress, and induces persistent chromosome aberrations, such as endoreduplication, chromosome pulverization, and premature separation of sister chromatids. Single-cell whole genome sequencing (WGS) experiments are being performed to evaluate structural and numerical genomic variations upon Cyclin E1 overexpression. Our work aims at understanding how oncogene-induced replication stress and genomic instability contribute to human carcinogenesis.

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TRANSCRIPTIONAL RESPONSE TO DNA REPLICATION STRESS

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In budding yeast *TOS4* is a gene involved in replication stress tolerance. During normal cell cycle progression, *TOS4* transcription is activated at the end of G1 and repressed while cells progress into S phase. However, in response to DNA replication stress, *TOS4* undergo a transcriptional reprogramming having its transcription sustained throughout the S-phase. Besides being regulated at the transcriptional level, Tos4 is also regulated at the post-translational level. During normal S-phase the activity of Tos4 is restricted by its ubiquitination and consequent degradation via proteasome. However, it's unclear if this mechanism is modulated to sustain Tos4 activity during replication stress. Using an approach combining co-immunoprecipitation followed by quantitative mass spectrometric analysis, we have identified an interaction between Tos4 and the ubiquitin protease Ubp12 during replication stress. This interaction was shown to be mediated by a protein-protein interaction FHA domain present in Tos4. Interestingly, an FHA mutant that disrupts UBP12 interaction shows a decrease in Tos4 levels during replication stress. In addition, mutation of 4 ubiquitinated residues on top of the FHA mutant partially rescues Tos4 levels. Taken together, these observations support the hypothesis that interaction with Ubp12 may be part of a mechanism to counteract the ubiquitination-dependent degradation of Tos4, thereby supporting its activity during replication stress.

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NOVEL MECHANISMS IN HOMOLOGY-DIRECTED DNA REPAIR AND GENOTOXIN TOLERANCE

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Homologous recombination (HR) is crucial for error-free repair of DNA double strand breaks and the preservation of genome integrity. Mutations in genes required for HR are associated with a range of cancer predisposition syndromes, defining a route for genomic instability-induced oncogenesis. Paradoxically, the ability of many cancers to utilize HR is essential for surviving oncogene-induced replication stress, representing an opportunity for the use of HR inhibitors in cancer treatment. Despite the therapeutic potential, the molecular mechanisms that regulate HR are not fully elucidated, limiting our ability to properly explore the concept of HR inhibition in cancer therapy. Here I will present our recent work uncovering novel mechanisms for phosphorylation-mediated regulation and surveillance of HR DNA repair. Our findings define key phosphorylation events controlling the recruitment of HR factors to sites of DNA lesions and establish the central role of the Dpb11/TOPBP1 scaffold as a “reader” of DNA damage-induced phosphorylation to coordinate DNA end resection and subsequent steps in homologous recombination-mediated repair. Taken together, this work reveals the underlying logic by which kinases and protein scaffolds monitor and regulate multiple steps in HR-mediated repair to ensure its proper execution, thereby preventing toxic recombination and the accumulation of genomic instabilities such as chromosomal rearrangements. Finally, I will discuss how the mechanistic understanding of HR regulation uncovered here provides insights for modulating HR capacity and resistance to genotoxins.

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MITOCHONDRIAL DYSFUNCTION IN CELLS WITH DNA REPAIR DEFECTS

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The mitochondrial DNA (mtDNA) is essential for mitochondrial function, as it encodes 13 polypeptides subunits of four of the 5 complexes of the oxidative phosphorylation system. Therefore, its integrity is required for cellular homeostasis and, in fact, mutations in the mtDNA cause several human syndromes and are causally linked to common human diseases such as cancer, diabetes, neurodegeneration and aging. From the known excision repair pathways, mammalian mitochondria are proficient in base excision repair, mismatch repair, double strand break repair and direct damage reversal, but not in nucleotide excision repair. Nonetheless, we have previously found that XP-C cells, mutated in the nucleotide excision repair damage recognition factor XPC, show impaired complex I-supported respiration with a shift between complexes I and II, elevated H_2O_2 production and accumulation of mtDNA damage, despite XPC not being localized in mitochondria. Here we show that this phenotype is associated with altered expression of nuclear-encoded mitochondrial proteins, in a p53-dependent manner. In XP-C cells, p53 is constitutively upregulated and treatment with the complex III inhibitor Antimycin A results in further p53 stabilization and cell death. Pre-treatment with the antioxidant N-acetylcysteine (NAC) increases glutathione levels, decreases H_2O_2 levels and p53 expression and prevents cell death. Our results suggest that lack of XPC causes mitochondrial dysfunction through an indirect mechanism mediated by p53 stabilization via increased H_2O_2 production and contributes to our understanding of the molecular mechanisms possibly involved in tumorigenesis in nucleotide excision repair deficient cells.

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PROTEIN ADP-RIBOSYLATION, GENOMIC STABILITY AND HUMAN DISEASE

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Mutations in several DNA repair genes are associated with rare hereditary diseases characterized by neurodevelopmental and/or neurodegenerative phenotypes. However, the underlying molecular mechanisms are largely unknown, precluding the identification of potential therapeutic strategies. Here we describe a novel disorder caused by biallelic mutations in the human XRCC1 gene and implicate elevated levels of protein ADP-ribosylation in the associated neuropathology.

DNA single-strand breaks (SSBs) are arguably the most abundant form of DNA damage in cells and their repair is initiated by poly-ADP-ribose polymerases (PARPs), which modify proteins surrounding the break site with poly-ADP-ribose (PAR) chains. This leads to the recruitment of XRCC1, which binds, stabilizes and stimulates the DNA end-processing enzymes that process and subsequently ligate damaged DNA termini. Single-strand break repair plays critical roles in the brain, particularly the cerebellum, as mutations in many of these factors result in rare hereditary diseases characterized by cerebellar atrophy, ataxia and oculomotor apraxia. We describe the first XRCC1-deficient patient with these clinical features and show that patient-derived cells exhibit not only reduced rates of single-strand break repair but also increased levels of DNA damage-induced protein ADP-ribosylation. Interestingly, aberrant ADP-ribose levels were also observed in cells from a patient with a related syndrome caused by mutations in the XRCC1 partner protein PNKP and in the cerebellum of untreated Xrcc1-deficient mice. Remarkably, genetic deletion of PARP1 rescued normal ADP-ribose levels both in human cells and mouse cerebellum, suggesting that delayed repair of SSBs leads to overt PARP1 signalling of these lesions. Strikingly, PARP1 loss also reduced the loss of cerebellar neurons and ataxia in these animals, identifying a molecular mechanism by which endogenous single-strand breaks trigger neuropathology. Collectively, these data establish the importance of XRCC1 protein complexes for normal neurological function and identify PARP1 as a therapeutic target in DNA strand break repair-defective disease.

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A PORTRAIT OF GERMLINE MUTATION IN BRAZILIAN AT-RISK FOR HEREDITARY BREAST CANCER

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Knowledge about the germline mutational distribution among Brazilian with hereditary breast and ovarian cancer (HBOC) is limited. We conducted a study to determine the clinical and molecular characteristics of Brazilian patients from 2009 to 2017 who underwent oncogenetic counseling and genetic testing using a panel to sequence high-risk and moderate-risk genes. Massively parallel sequencing was applied in 157 individuals (132 breast cancer-affected and 25 breast cancer unaffected individuals) selected according NCCN criteria for hereditary breast cancer. Nineteen germline variants were identified,15 pathogenic and 4 VUS (Variants of Uncertain Significance) in 27 individuals (27/157; 17% $P < 0.0001$) distributed among 7 genes. Sixty-eight percent of patients (13/19) harbor mutation in BRCA genes and 32% (6/19) in moderate risk genes. This is the first study reporting ATR deleterious germline mutation in association with hereditary breast cancer. Cancer-affected patients with moderate-risk mutation present a more aggressive phenotype, with bilateral cancer (25% vs. 13%, $P = 0.0305$), high-grade tumors (79.2% vs. 46.3%, $P = 0.0001$) and triple negative (50% vs. 22.4%, $P < 0.0001$). However, no difference in the 5 years overall survival was observed between BRCA and moderate-risk groups. This work highlights the benefits of large-scale sequencing for oncogenetic counseling and extends our understanding about the genetics of hereditary breast cancer in the multi-ethnic Brazilian population.

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DNA REPAIR INHIBITION AS A THERAPEUTIC MOLECULAR TARGET FOR THE SENSITIZATION OF DRUG-RESISTANT GLIOBLASTOMA CELLS

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Keywords: PARP-1 inhibition, glioblastoma, temozolomide, *PTEN* gene, MGMT enzyme.

Currently, in the context of molecular targets in cancer therapy, the inhibition of DNA repair pathways has been tested as a strategy for tumor sensitization. In a previous work, we demonstrated that the inhibition of BER (base excision repair) pathway was found efficient to increase cell death and DNA damage induction in T98G glioblastoma (GBM) cells, which are resistant to temozolomide (TMZ). As an alternative strategy, poly-ADP-ribose polymerase inhibitors (PARPi) have emerged as a promising tool, especially for tumors that are defective in homologous recombination repair (HRR), based on the concept of synthetic lethality. Some genes have been studied as predictive biomarkers of cellular responses to antitumoral drugs, such as MGMT (O6-methylguanine DNA methyltransferase) and *PTEN* (phosphatase and tensin homologue deleted on chromosome ten). Our purpose was to study the effects of PARPi on cellular responses to TMZ treatment in GBM cell lines presenting different genetic background, to test the hypothesis that PARP inhibition might increase TMZ cytotoxicity and cell death. We demonstrated that PARP-1 inhibition by NU1025 effectively sensitizes TMZ-resistant cells, namely T98G (*PTEN*-mutated: MGMT proficient) and LN18 (*PTEN*-wild-type; MGMT proficient); in these cell lines, the pharmacological inhibition of MGMT (with O6-BG) did not influence the responses of these cells. Interestingly, TMZ combined to NU1025 drastically reduced the cell viability of T98G and LN18 cells when collection time was extended to 20 days (recovery time) after treatment, thus revealing the potential of the drug combination in inducing cell lethality. In addition, *PTEN* silencing (siRNA) did not sensitize *PTEN*-proficient cells to PARPi, either as a single agent, or in the combined treatment with TMZ. The cellular responses to TMZ/NU1025 involved antiproliferative activity, G2/M arrest, DSBs and apoptosis induction. However, while U251MG (*PTEN*-mutated; MGMT deficient) also presented sensitization by PARPi, such effects were not observed for another cell line, U87MG (*PTEN*-mutated; MGMT deficient), suggesting that other genetic alterations possibly influence the responses to the combined treatment. Therefore, independently on the MGMT activity and *PTEN* status, TMZ combined to PARPi treatment seems to be a promising strategy to sensitizing GBM cells. **Financial support:** FAPESP – Proc. n° 2013/12033-0, 2016/17862-3 and 2013/09352-7), CNPq (Proc. Nº 309854/2017-2) and CAPES.

VERA VARGAS

BIOMARKERS OF GENOTOXICITY AS EARLY PROTECTORS OF BIODIVERSITY AND HUMAN HEALTH

GENOTOXICITY BIOMARKERS AS EARLY PROTECTORS OF BIODIVERSITY AND HUMAN HEALTH

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Studies developed in different continents have emphasized that early detection of exposure to pollutants may significantly diminish the occurrence of effects that are adverse to ecosystems and to human health. Biomarkers that are sensitive in detecting toxic substances to genetic material are important tools when adopting measures to prevent damage caused by environmental pollution. However, it is necessary to plan diagnostic strategies, associating knowledge about the areas to be studied, their geographic characteristics and land use. The early study makes it possible to define the main sources and possible dispersion routes of contaminants in the environment. It can also help in selecting the chemical and biological markers to define the toxic and genotoxic events that are to be investigated. In order to establish approach criteria, it is essential to define the environmental compartment to be investigated and the choice of safe biomarkers to subsidize effective remedial measures. In this presentation, studies using the *Salmonella*/microsome assay to screen for hazardous contaminants will be prioritized in urban and industrial areas of Rio Grande do Sul. In the atmospheric compartment, comparative data from different studies will be discussed, showing the consequences of exposure to atmospheric particles with different aerodynamic diameters. Moreover, comparative studies using tests that measure cytogenetic damage will be helpful in defining these potential relative risks. Finally, the advantages and limitations of these tools will be discussed so as to determine mutagenic effects on environmental samples, as well as their applicability in protective measures regarding biodiversity, quality of life and health of the human population.

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GENOTOXICITY OF SECONDARY METABOLITES FROM CYANOBACTERIA

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Eutrophication, generated mainly by excessive intake of phosphorous and nitrogen, reduces water quality causing the reduction of dissolved oxygen, decreased biodiversity, environmental aesthetic decay, death of fish and increase of cyanobacteria and microalgae bloom. The occurrence of these events, often in recent years around the world, comes to form dense layer on the water and can impart taste and odor due to its potential to produce metabolites such as 2-methylisoborneol (2-MIB) and geosmin (GEO), and toxicity due to production of toxins (hepatotoxins and / or neurotoxins). The need to identify the biological effects of environmental compounds, such as cyanobacteria metabolic products, and characterize their genotoxic profiles, allow drawing a more complete scenario of the environmental risk associated with these compounds. Our laboratory has been studying the genotoxic potential of causing taste and odor compounds in water, such as 2-MIB and GEO and the neurotoxin (saxitoxin) produced by *Cylindrospermopsis raciborskii* cultures. The genotoxicity of these compounds has been assessed *in vitro*, in human cell cultures, and *in vivo*, in *Drosophila melanogaster*. The possibility of associating the data obtained by genetic toxicology to molecular biology, with the evaluation of the expression of specific genes, opens a new perspective for understanding the mechanisms and DNA-level responses induced by environmental microcontaminants.

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AN INTEGRATED APPROACH FOR GENOTOXICITY ASSESSMENT OF ENVIRONMENTAL CONTAMINANTS

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In the study of genotoxicity of environmental contaminants, well-known methodologies have been used such as chromosome breaks, chromosome malsegragation, micronucleus test, comet assay and changes in gene expression. These methodologies have showed variations in the sensitivity to detect genotoxicity of chemical compounds. Studying genotoxicity of chemicals in water, an integrated approach should be done because in most cases they are present at very low concentrations. Also, in the study design, chronic or acute exposures must be taken into account. We have evaluated the genotoxicity of the residues of psychotropic pharmaceuticals in aquatic environment, using zebrafish (*Danio rerio*) as model. In our study with Nortriptin, fish were exposed at very low concentrations like found in the environment. After exposures at 0.0, 0.07, 0.19, 0.48 and 1.2 mg/L for 28 days fish were prepared for micronucleus test, comet assay and gene expression changes. The results showed no micronucleus induction nor DNA breaks by comet assay. However, changes in the profile of gene expression occurred. Microarrays using zebrafish genechip resulted in 243 genes with their expression changed. An overexpression of 77 genes and downexpression of 166 genes, which mean a genotoxic effect. We tested also Carbamazepine at 0.006 and 28.12 µg/L and Amitriptiline at 0.6 and 2,812 µg/L. Again, the results showed no micronucleus nor DNA damage, but caused changes in gene expression. In general, chemicals such as pharmaceuticals are detected in the aquatic environment at very low concentrations. Endpoints like micronucleus and comet assay have no sensitivity to detect genotoxicants at very low concentration. Then, it does not mean no genotoxicity. Using a more sensitive endpoint such as changes in genes expression genotoxicity was evidenced.

Financial Support: CNPq, CAPES

NATURAL RADIATION AND THEIR IMPACT ON GENOME INSTABILITY: AN EXPOSOME APPROACH

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Natural radiation can elicit deleterious effect through ionising particles. Among the studied radioactive elements in environment, radon gas presents a differential relevance because its inhalation is the main source of exposure to natural radioactivity worldwide; this radionuclide continues to decay inside the lungs and releasing alpha particles that can damage the DNA and rise genome instability, increasing the risk to develop lung cancer. Aside from the generally accepted potential to damage DNA, it is now evident that ionising radiation is also a potent epigenotoxic agent. Among epigenetic parameters, DNA methylation has arguably received the most attention in the context of radiation biology. In fact, more epidemiological and molecular studies are needed to analyse in an integrated way several factors that make up the exposome domains of target populations in order to better represent and evaluate the effects and risks of chronic exposure to radon in humans. In our research team, we are developing studies that evaluate changes in genome methylation, from circulating cells that serve as exposure bioindicators, in individuals exposed to natural radioactivity. Therefore, the main objective of our research is to evaluate the genome instability effects of a population chronically exposed to high levels of natural radiation. The following questions will be discussed: Are the indirect effects of ionising radiation relevant in human exposure to the point of detect significant changes in the chosen biomarkers? How other variables of human exposome, such as environmental and occupational exposures and lifestyle influence this ionising radiation indirect phenomenon?

SESSION 5

YOUNG RESEARCHER AWARD: THEMATIC AXES 1 AND 2

1. ENVIRONMENTAL MUTAGENESIS (ENVIRONMENTAL MONITORING, MUTAGENESIS OF NATURAL PRODUCTS, DRUGS, PHYSICAL AGENTS, COUNTRY LEGISLATION)
2. MUTAGENESIS AND HUMAN AND ANIMAL HEALTH (HUMAN AND ANIMAL BIOMONITORING, CANCER, DEGENERATIVE DISEASES, AGING)

BÁRBARA VERENA DIAS GALVÃO

ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF MYRCIARIA CAULIFLORA LEAF AGAINST TRYPANOSOMA CRUZI

ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF *Myrciaria cauliflora* LEAF AGAINST *Trypanosoma cruzi*

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Keywords: mutagenesis, natural products, Chagas disease.

Chagas Disease presents several therapeutic challenges, such as limited action and side effects of available drugs. These reveal the importance of the search, in plants natural products potentially active against protozoa for more effective and safer trypanocidal compounds. Parts of *Myrciaria cauliflora* (Myrtaceae) tree, known as jabuticabeira, have traditionally been used to treat respiratory and digestive disorders. Previous studies have reported the antimicrobial action of its leaves, related to the presence of flavonoids, alkaloids, saponins and tannins. Therefore, considering the importance of the experimental evolution of its biological activity, this study aimed to investigate the safety and efficacy of *M. cauliflora* leaf hydromethanolic extract against *Trypanosoma cruzi* bloodstream trypomastigote form.

The mutagenic potential was assessed by the Ames Test, in the absence and presence of metabolic activation, using *Salmonella enterica* serovar Typhimurium standard strains (TA97, TA98, TA100, TA102, TA104). HepG2 cells were assayed for cell viability determination using WST-1 assay. The trypanocidal activity was evaluated against bloodstream trypomastigote form of *T. cruzi* (Y strain, DTU II). Mutagenic effects were detected at the highest concentration tested (500 µg/plate), in the absence of exogenous metabolization in the TA100 strain, indicating base pair substitution damage, from G:C to A:T. The extract exhibited significant trypanocidal activity over a range of safe concentration for HepG2 cells (EC_{50} 2h: 4.8-11.4 µg/mL EC_{50} 24h: 4.7-10.5 µg/mL), about ten times lower than the concentration required to produce a hepatotoxic effect (EC_{50} 24h: 101 µg/mL). Mutagenic and trypanocidal potential may be related to the dose-response dependent redox imbalance of flavonoids, which in high concentrations no longer act as antioxidants and become pro-oxidative agents, damaging the DNA and altering the parasite redox balance. Furthermore, some phenolic compounds found in the extract, might have the ability to complex with parasite macromolecules, such as membrane lipids and proteins, leading to function loss. In summary, the data demonstrate that the *M. cauliflora* leaf extract presented *in vitro* trypanocidal activity in concentrations with high probability of safety for the human consumption, emerging as a potential source of active natural products against Chagas Disease etiological agent.

Financial Support: CNPq, CAPES, FAPERJ, FIOCRUZ, UERJ, UNIRIO.

RETENE, A NON-PRIORITY HYDROCARBON, IS ABLE TO GENERATE OXIDATIVE STRESS, MUTAGENIC EFFECTS, AND CELL DEATH

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Key-words: Retene, Biomass burning, *Salmonella* microsome assay, Necrosis

Retene (1-methyl-7-isopropylphenanthrene; RET) is the most abundant polycyclic aromatic hydrocarbon (PAH) released upon the burning of cellulose, although it is not considered as one of the priority PAHs and is not included for risk assessments by the US Environmental Protection Agency (US-EPA). Despite its abundance, there are only a few studies elucidating the toxic effects of RET. Therefore, the aim of this work was to evaluate whether RET plays a crucial role in the induction of oxidative stress through the generation of reactive oxygen species (ROS) by showing mutagenic activity *in vitro* and, in cell death processes (apoptosis and necrosis). Thus, human lung adenocarcinoma A549 cells were treated with different concentrations of RET as follows: 3.3 ng/mL, 10 ng/mL and 30 ng/mL for 24 and 72 hours. The oxidative stress analysis was performed using flow cytometry. In addition, cytokinesis-block micronucleus assay (CBMN) was adopted to verify the frequency of micronuclei (MN), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs). Cell death was investigated by labeling Annexin V and propidium iodide (PI). Traditional plate-incorporation *Salmonella* mutagenicity assay was performed to evaluate the RET mutagenicity using the *Salmonella* strains TA98, TA100, TA97a and TA102, with or without activation metabolic (S9 mix). Our results revealed that RET was able to hyperpolarize the mitochondrial membrane and increase the contents of mitochondria, owing to an increase in the production of mitochondrial superoxide and intracellular ROS. Moreover, RET does not induce base-pair substitution (TA100) and frameshift (TA98 and TA97a) and transition/transversion (TA102) mutations. However, treatment with RET led to a significant increase in the frequency of the formation of MN, NPBs, and NBUDs.

There was no significant difference in the viability of cells exposed to RET for 24 hours. In contrast, 72 hours of treatment with RET was adequate to decrease viability, mainly due to necrosis. Taken altogether, the results of our study provide new evidence suggesting that RET promotes oxidative stress, contributes to the processes of genomic instability and favors necrosis. Thus, we highlight the importance of including RET in routine environmental analyses in the future as a potential risk factor involved in complex diseases and carcinogenesis. Studies concerning the ability of RET to transform cells are being conducted.

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NEURONAL DIFFERENTIATION AND NEUROPROTECTIVE EFFECTS OF NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITORS DESIGNED FOR ALZHEIMER'S DISEASE THERAPY

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Alzheimer's disease (AD) is characterized by a progressive loss of episodic memory related to β-amyloid (Aβ) peptide aggregation and abnormal phosphorylation of the tau protein, leading to the loss of cholinergic function. Inhibitors of acetylcholinesterase enzyme (AChEI) are the main class of drugs used in AD therapy. Thus, the objective of this work was to evaluate the neurodifferentiation and neuroprotective effects of two hybrid molecules (TA8Amino and TAHB3, AChEI compounds) of tacrine-donepezil in SH-SY5Y cells. Firstly, cytotoxicity and hepatotoxicity assays were performed at different times (24, 48, 72, and 120h) in SH-SY5Y and HepG2 cells, respectively. Neuronal differentiation assays, protein expression, cell cycle, cell proliferation, mitochondrial changes and oxidative stress were performed on SH-SY5Y cells. Further, viability and cell death assays were performed to evaluate the neuroprotection ability of the hybrids. The compounds did not present cytotoxic effects or high hepatotoxicity and did not alter cell viability at the following concentrations: TA8Amino 0.0035 to 0.112µM and TAHB3 0.088 to 2.84µM, but both compounds were able to induce neuronal differentiation and neuritogenesis, compatible with the increase of β-III-Tubulin expression in cells treated with TA8Amino, which also induced the production of intracellular and mitochondrial ROS. While the hybrids increased SOD1 expression, they did not change the mitochondrial membrane potential and mitochondrial mass, indicating that drug-induced oxidative stress did not generate damage due to mitochondrial dysfunction in differentiated cells. Changes in PTEN $_{(Ser380\ /\ Thr382\ /\ 383)}$, AKT $_{(Ser473)}$ and COX2 protein expression indicate the involvement of the PI3K/ AKT/ COX2 pathway, which is related to neuronal differentiation. Furthermore, TA8amino and TAHB3 showed a neuroprotective effect against the neurotoxic damage induced by the Aβ peptide. In general, the results demonstrate that TA8amino e TAHB3 have advantages as potential drugs, since they are not cytotoxic at concentration levels that inhibit AChE enzyme, besides being able to induce neuronal differentiation, neuritogenesis and neuroprotection, unlike donepezil and tacrine, tested alone.

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THE INFLUENCE OF POLYMORPHISMS IN DNA DAMAGE, TELOMERE LENGTH AND GLOBAL DNA METHYLATION EVALUATED IN OPEN-CAST COAL MINING WORKERS

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Coal plants represent one of the main sources of environmental pollution due to the combustion process of this mineral and the consequent release of gases and particles capable of penetrating water sources which, in significant quantities, can lead to a potential risk to health and the environment. The susceptibility of individuals to the genotoxic effects of coal mining can be modulated by genetic variations in the xenobiotic detoxification and DNA repair processes. The aim of this study was to evaluate if xenobiotic metabolism polymorphism (GSTP1 *Ile105Val*, GSTT1, GSTM1 and CYP1A1 *Ile462val*), base excision repair polymorphisms (hOGG1 *Ser326Cys* and XRCC1 *Arg194Trp*), and non-homologous end joining repair pathway polymorphism (XRCC4 *Ile401Val*), could modify individual susceptibility to genomic instability and epigenetic alterations induced in workers by occupational exposure to coal. In this study, polymerase chain reaction (PCR – RFLP) was used to examine the polymorphic sites. The reactions, as well as the enzymes used, followed the literature. The sample population comprising 70 coal mine workers and 71 workers non-exposed to coal. The exposed workers were sampled in Candiota (Rio Grande do Sul, Brazil) open-cast coal mine, where they were involved in extraction and transport of coal to storage centers. The non-exposed group consisted of individuals from the same area. Our results demonstrated the effect of individual genotypes on different biomarkers (DI, MN, NBUD and NPB in lymphocytes, buccal MN, telomere length, and % of global DNA methylation) evaluated in the non-exposed and exposed groups.

Significant decrease in % of global DNA methylation were observed in CYP1A1 *Val/-* exposed individuals compared to CYP1A1 *Ile/Ile* individuals (P= 0.0369). Coal workers who carried the XRCC4 *Ile/Ile* genotype showed decrease NBUD frequencies (P= 0.0085), while the XRCC4 *Thr/-* genotype was associated with decrease in Buccal MN cells for the group not exposed (P= 0.0029). No influence of the other polymorphisms studied was observed. Thus, the current study reinforces the importance of considering the effect of metabolizing and repair variant genotypes on the individual susceptibility to incorporate DNA damage, as these processes act in a coordinated manner to determine the final response to coal exposure. Results of this study contribute to the understanding of DNA damage mechanisms induced by complex exposure of open-cast coal miners.

Financial Support: CNPQ, FAPERGS, ULBRA

SESSION 6

YOUNG RESEARCHER AWARD: THEMATIC AXES 3 AND 4

3. GENOMIC INSTABILITY AND DNA REPAIR

4. MODULATION OF MUTAGENICITY AND NUTRIGENOMICS (ANTIMUTAGENESIS, ANTICARCINOGENESIS, MODULATION OF GENE EXPRESSION BY MICRONUTRIENTS)

FRANCIELE BUSATTO

TOPOISOMERASE II INHIBITORS-INDUCED LESIONS - AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS

TOPOISOMERASE II INHIBITORS-INDUCED LESIONS – AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS

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Key-words: *Topoisomerase II inhibitors, NER, double-strand breaks*

Topoisomerase II inhibitors form stabilized drug-DNA-topoisomerase complexes. Processing of these complexes creates DNA double-strand breaks (DSBs). We have already shown that the Nucleotide Excision Repair (NER) pathway is involved in this process. Therefore, the aim of this study was to understand the role of CSB, a NER protein, in response to TopoII inhibitors, and the relation with DSB repair pathways. U2OS cells were transfected with siRNAs for different NER genes, treated with TopoII inhibitors and followed by 53BP1 and $_{\gamma}$H2AX foci IF analysis; the influence of NER knockdowns in the Homologous Recombination (HR) rates was done using a CRISPR based reporter assay. To evaluate the interaction of CSB and TopoII a co-IP was performed in cells treated with the same drugs. A DRIP-qPCR was performed to investigate R-Loops formation in siERCC6 and siTOP2α/β cells with and without the treatments. IF analysis showed an increase in 53BP1 and $_{\gamma}$H2AX foci after MXT treatment, but there was no difference between NER knockdowns (siERCC6, siXPC and siXPA) and the siCTRL. Interestingly, we observed an increase in HR in siXPC cells. We detected a co-IP of TopoII and CSB in response to MXT treatment. In DRIP-qPCR analysis, we found an R-Loops increase in some locus after siTOP2α/β, but not after TopoII inhibition. Our results indicate an interaction between CSB and TopoII, which could explain the implication of CSB in DSBs repair pathway in response to TopoII inhibitors induced lesions.

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Molecular Characterization of DNA Repair In Mouse Olfactory Sensory Neurons

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Key-words: DNA repair, olfactory sensory neurons, neurodegeneration.

Olfactory Sensory Neurons (OSNs) undergo neurogenesis and migration through the olfactory epithelium (OE) during maturation process; therefore, their lifespan is shorter than most neurons within the brain. Olfactory dysfunction has been reported as an early clinical symptom in many neurodegenerative diseases, which are associated with impaired DNA repair. Whether DNA repair mechanisms play a role in OSNs genomic maintenance, however, is still unknown. Our study aims to characterize DNA repair pathways in precursor and mature OSNs. For that, we analyzed data from two different transcriptomes to characterize the expression pattern for DNA repair proteins with age and stage of neuronal differentiation. In order to confirm the results obtained from the in silico analysis, we performed RT-PCR for selected DNA repair targets from newborn (4-7 days) and 3-week-old mice OE samples. The in silico analysis suggested that most DNA repair pathways are reduced in young mice, when compared to newborns. On the other hand, non-homologous end joining (NHEJ) pathway is apparently increased, which may indicate a preference for non-homologous repair pathways for repairing double strand breaks due to lack of homologous sister-chromatids in differentiated cells. However, RT-PCR did not detect significant changes in DNA repair gene expression levels between the ages. Since OSN are constantly exposed to damage by atmospheric oxygen and other toxic molecules present in the air, we hypothesized that these neurons would accumulate more DNA damage than those in the central nervous system (CNS). To test this hypothesis, we performed a long extension PCR assay comparing OE, olfactory bulb (OB) and non-olfactory neural tissue (CE) from 3-week-old mice, but we found no significant differences in the long/short fragment amplification ratio. Although our results are merely descriptive, they have never been reported before, and can be used to support new research on OSN genome maintenance and integrity.

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CHRONIC HYPERGLYCEMIA INDUCES DNA DAMAGE AND OXIDATIVE STRESS WHICH CAN BE DIMINISHED BY DIETARY RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Keywords: DNA damage; Mitochondrial alterations; Hyperglycemia; Dietary restriction; Type 2 diabetes mellitus.

Type 2 diabetes (T2D) is mainly characterized by hyperglycemia, and high glucose levels can lead to several cellular and molecular alterations related to oxidative stress and DNA damage. Considering the impact of obesity in diabetic complications, dietary restrictions (DR) has been recommended for T2D patients. Furthermore, there is evidence that DR positively affects the longevity extension and reduces age-related diseases. The aim of this study was to assess the effects of chronic hyperglycemia on molecular signaling pathways that are linked to oxidative stress, DNA repair, insulin resistance and inflammation, in addition to analyze parameters of oxidative stress at the mitochondrial level and nucleotide pool in mononuclear cells of T2D patients, evaluated before and after a protein-restricted diet (0,8g/kg/day). T2D patients with chronic hyperglycemia (without intervention) compared to healthy individuals were also evaluated. We observed that following a period of 4 weeks, the protein-restricted diet led to decreased levels of DNA damage (comet assay) in patients with T2D (n=7). Regarding the gene expression profiles evaluated for target genes (PCR array), a significant difference was not observed between “before” and “after” the DR intervention. Only a slight tendency of up-regulation was observed for *PI3K*, *PRKAA1* and *ATM* genes. For some patients there was an induction of several genes (*OGG1, MUTYH, SOD1, FOXO3, NUDT1*) with roles in DNA repair and responses to damage. In addition, it was observed that in general, T2D patients with hyperglycemia (without intervention) (n=17) presented increased levels of oxidized bases in the nucleotide pool when compared to controls (n=10). However, the intervention period was not sufficient to exert alterations in patients submitted to DR. Regarding mitochondrial analysis, significant alterations in the quantification of ROS, membrane potential and mitochondrial mass were not observed between groups of T2D patients (n=15) and healthy individuals (n=15).

Thus, chronic hyperglycemia promotes an oxidative stress condition in T2D patients, and DR during 4 weeks was found efficient in significantly reducing DNA damage levels in patients with T2D, in parallel with improvements in glycemic rates, and other clinical parameters, but changes in transcript gene expression and oxidized base levels were not observed after the intervention period.

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IN VITRO APPROACHES FOR MUTAGENICITY AND HEPATOTOXICITY INDUCED BY MARKETABLE PRE-WORKOUT SUPPLEMENTS

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Currently, the practice of physical exercises has been increasing around the world. Many of these practitioners use pre-workout nutrition through dietary supplements because of their effects on the body. However, a sequence of clinical cases of hepatic damages in the hawaiin population brings suspicious about their effect to the consumer. Thus, this project consists on investigating the mutagenic and cytotoxic potential of pre-workout OxyELITE Pro, C4 and Jack3d supplements. The *Salmonella*/Microsome assay was used to determine the mutagenic potential of the supplements investigated. *Salmonella enterica* sorovar typhimurium TA97, TA98 and TA100 strains were used for the 3 products (besides TA102 and TA104 for C4). Assays were performed using five concentrations for Oxyelite and C4 (0.0005, 0.005, 0.05, 0.5, 5 mg/plate) and six concentrations for Jack3d (0.0001, 0.0005, 0.005, 0.05, 0.5, 5 mg/plate), all diluted in DMSO 10% and analyzed with and without metabolic activation (S9 mix 4%). The assays were done in triplicate and the mutagenic effect was considered in those concentrations that had the mutagenicity index (IM = induced revertants/spontaneous revertants) higher than 2. For citotoxicity, the viability assay (WST-1) was used with the human hepatocarcinoma HepG2 cell line and an alkaline phosphatase assay was performed to check for possible hepatic disorders. The percentage of cell survival in WST and the amount of alkaline phosphatase per 10,000 cells in contact with supplements at concentrations of 0 at 5 mg/ml were observed for 24h, 48h, and 72h periods. A positive result was found for mutagenicity for OxyELITE Pro and Jack3d for strains TA98 and TA100, in the presence of metabolization, and for all three supplements for strain TA 97 under the same condition. For the WST assay, cell death occured for all three products at all concentrations after 48h incubation. In the alkaline phosphatase assay, a significant increase of phosphatase in the supernatant was detected when in contact with any of the supplements analyzed after the same period of time. Those results suggest a mutagenic and cytotoxic potential that could cause liver damage.

Financial Support: CNPq, CAPES, FAPERJ, UERJ, UNIRIO, UFRJ.

MICHAEL FENECH
VIDEO CONFERENCE - NUTRIENTS, GENES AND THEIR INTERACTION IN THE MAINTENANCE OF TELOMERE INTEGRITY

NUTRIENTS, GENES AND THEIR INTERACTION IN THE MAINTENANCE OF TELOMERE INTEGRITY

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Background: Telomeres are a TTAGGG repeat at the ends of chromosomes that, together with shelterin proteins form the telosome complex that caps ends of chromosomes. Telomeres need to be maintained at an adequate length (at least 13 TTAGGG repeats) to form the T-loop on which the shelterin proteins are assembled. Excessive shortening, strand breaks and oxidative base damage at the telomere sequence result in telosome dysfunction leading to telomere end fusions, chromosomal instability, DNA replication stress, persistent DNA damage response and senescence. Malnutrition can result in deficiency of essential substrates and cofactors required for telomere maintenance or prevention of damage to the telomere sequence by genotoxins. Methods: A systematic review was performed to determine current knowledge on the effect on telomere length in humans by dietary patterns and micronutrients. The literature on interaction of nutrition with common polymorphisms in genes involved in micronutrient metabolism and inflammation was also explored. Results: The most consistent evidence available indicates that longer telomeres are associated with (i) intake of predominantly plant-based diets such as the Mediterranean diet and the Korean prudent diet, (ii) adequate folate status, low homocysteine, and higher intake of dietary antioxidants and ω3-fatty acids, (iii) lower BMI in the normal range and weight loss in those who are obese, and (iv) low/moderate alcohol intake. Interactive effects have been reported with common polymorphisms in the following genes: MTHFR, ALDH2, FTO, IL6, TNFA, PPARγ2, TERC. There is emerging evidence that changes in expression of genes in the sub-telomere region (e.g. TERRA) may affect telomere maintenance but there are no reports yet on the effect of nutrition on DNA methylation in sub-telomeric sequences. Conclusions: There is growing evidence that telomere length is affected by nutrition and interaction with common polymorphisms in genes related to nutrient and alcohol metabolism, obesity, inflammation and telomere maintenance.

TELOMERE LENGTH MEASUREMENT TECHNIQUES AND THEIR APPLICATION

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Telomeric DNA is composed of double-stranded arrays of a (TTAGGG)n repeat unit terminating in a single-stranded 3' G-rich overhang, bound by the protein complex shelterin, which comprises the proteins TRF1, TRF2, POT1, TIN2, TPP1 and RAP1. In normal human somatic cells, telomeres shorten by approximately 200 bp in each cell division, due to limitations in the replication of linear DNA molecules. This is known as the end-replication problem and is responsible for the negative correlation that exists between human age and telomere length. Rapid telomere trimming events also contribute to stochastic telomere shortening and prevent telomeres from becoming excessively long. Such events may arise from intrinsic aspects, but also from exposure to xenobiotics. When telomeres become critically short they initiate a telomere-specific DNA damage response (DDR), which includes ATM and ATR signalling, resulting in a state of permanent cell cycle arrest known as cellular senescence. Bypass of senescence leads to catastrophic telomere shortening, or telomere crisis, which culminates in widespread genomic instability and can therefore contribute to tumorigenesis. Consequently, telomeres possess a tumour-suppressive role by limiting the number of cell divisions a cell can undertake. Over the last decade, several telomere length (TL) methods have been established for clinical and diagnostic purposes, but current techniques lack enough sensitivity to provide the distribution of TL in a cell population. This is particularly problematic as it is the proportion of short telomeres, rather than average TL that triggers cellular senescence. We have developed a novel technique called telomere fibre-FISH (TFF) that measures the lengths of single telomere molecules on DNA fibres. TFF is a direct improvement on previous techniques, as it measures the distribution of individual TL, and can be applied to fresh as well as frozen different cellular material. We will discuss the main aspects of each method, their advantages and caveats, providing new insights in TL measurement technology. Beyond the specific clinical application for the diagnosis of telomere biology disorders, TL testing is emerging as a more general prognostic marker for ageing and disease and can be applied in research field as a biomarker of chemical exposure-related effect. Therefore, the correct application of TL testing is an important tool for the clinical area and to epidemiological and basic research fields.

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Chronic stress and suboptimal nutrition is associated with chromosome instability in dementia family carers

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Telomere dysfunction and DNA damage lead to chromosomal instability and increased risk for cancers, neurodegenerative and cardiovascular diseases. Chronic psychological stress is associated with shorter telomeres, while dietary micronutrient deficiency compromises DNA replication and genome integrity. We tested the hypothesis that chronically stressed dementia family carers have elevated DNA damage, and shorter telomeres relative to non-carer controls. Nutritional and stress status was determined in carers, and age and gender matched controls (n=42 per group). Primary outcome measure was telomere length (TL). Secondary measures included perceived (PSS) and physiological stress (hair, serum, saliva), biomarkers of chromosomal and epigenome stability, psychological factors, nutritional and immune status. Carers had significantly higher perceived stress (PSS) ($p<0.0001$), and anxiety ($p=0.0002$) compared with controls. No differences were observed in TL or DNA damage, however TL was negatively correlated with age ($r=-0.5$, $p<0.0001$), homocysteine ($r=-0.23$, $p=0.03$) and alcohol intake ($r=-0.3$, $p=0.006$). Increased micronuclei (indicative of chromosome breakage/loss) were observed in carers with low serum zinc ($r=-0.36$, $p=0.02$) and high serum cortisol ($r=0.3$, $p=0.05$). IL6, was associated with lower cognitive function (MMSE) ($r=-0.23$, $p=0.04$), elevated MMA (risk factor for dementia) ($r=0.27$, $p=0.01$) and nucleoplasmic bridges (misrepair of broken chromosomes) ($r=0.25$, $p=0.03$). While the data do not support the primary hypothesis, secondary measures indicate that chronic stress, and suboptimal nutrition, may interactively impact on chromosomal stability, contributing to increased morbidity, reduced immunity and accelerated ageing observed in chronically stressed individuals.

ROLE OF DNA REPAIR FACTORS IN TELOMERE INTEGRITY AND GENOME MAINTENANCE IN MAMMALIAN CELLS UNDER OXIDATIVE STRESS

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Telomere function may have contrasting roles: inducing replicative senescence and promoting tumourigenesis. These roles may vary between cell types depending on the expression of telomerase enzyme, the level of mutations present, the efficiency/deficiency of related DNA repair pathways and oxidative stress levels in the cell milieu. The association between DNA repair factors (such as PARP-1, Ku80/70, DNA-PKcs, ATM, Brca1 and 9-1-1 complex) and telomere-genome integrity in mammalian cells has been established. Eukaryotic cells possess diverse DNA repair pathways that eliminate DNA lesions to restore genetic stability. The importance of DNA repair in genomic stability is demonstrated by the existence of a variety of human autosomal recessive disorders which are deficient in one or more DNA repair pathways. A defective nucleotide excision repair (NER) – one of the DNA repair pathways – can result in Xeroderma Pigmentosum or Cockayne Syndrome presenting with increased cancer risk and segmental progeria. The role of NER in maintaining DNA integrity under oxidative assault is studied. Oxidative stress is known to accelerate telomere attrition. Data from our studies imply that short dysfunctional telomeres and DNA repair deficiency impair the repair of oxidative DNA lesions induced by exogenous agents. Our investigations have implications in ageing and cancer therapeutics as well as in risk estimation for environmental toxicants.

LAVÍNIA SCHULER-FACCINI
CONGENITAL ZIKA SYNDROME: BEYOND MICROCEPHALY

CONGENITAL ZIKA SYNDROME: BEYOND MICROCEPHALY

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Zika Virus (ZIKV) has become a major challenge for scientists and health agencies, particularly since the identification of its involvement with microcephaly and Guillain-Barré syndrome in 2015. Firstly, in Brazil and presently in an outbreak of babies born with congenital zika syndrome infection (CZS) has hit most countries in the Americas. This newly identified human teratogen produces a recognizable phenotype of severe microcephaly secondary to brain damage, anomalies of the shape of skull and redundancy of the scalp consistent with the Fetal Brain Disruption Sequence. However, this phenotype is expanded to a spectrum of anomalies that include neurological abnormalities with normal head circumference and ocular anomalies. Presently it is estimated that 10% of prenatal exposed babies will present the CZS at birth. However much less is known about the development of the cases born with head circumference within the normal range. Although multiple experimental studies have demonstrated the link between ZIKV and neural birth defects, it is still not clear why ZIKV congenital infection produces malformations only in some patients. One study on twins with CZS shed some light on the role of genetics. When analyzing gene expression of neural progenitor cells of dizygotic twins, it was found that cells derived from affected babies displayed a differential signature in neural development genes that could explain, at least in part, the susceptibility of these cases to ZIKV infection and that would be related to the impairment of brain development. In this communication we will discuss the epidemiological impact of CZS in different countries in the Americas, the potential environmental/genetic risk modifiers, what we have learned from this epidemic, and the main unanswered questions. Moreover, the zika epidemics has brought back the topic of congenital infections as a preeminent factor for preventable congenital anomalies. Currently, ZIKV infection is a very relevant health public problem, involving multidisciplinary expertise – including epidemiologic, clinical, virological and neurochemical backgrounds to rapidly advance in the understanding of this multifaceted health problem.

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LUCAS ROSA FRAGA
ANIMAL MODELS FOR ZIKA VIRUS TERATOGENESIS: WHAT DO THEY TELL US?

WALTER ORLANDO BEYS DA SILVA
A PROTEOMIC APPROACH ON ZIKA VIRUS INFECTION REVEALS A POTENTIAL LINK TO NEUROLOGICAL DISORDERS AND BRAIN DISEASES

A PROTEOMIC APPROACH ON ZIKA VIRUS INFECTION REVEALS A POTENTIAL LINK TO NEUROLOGICAL DISORDERS AND BRAIN DISEASES

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The recent microcephaly outbreak in Brazil has been associated with Zika virus (ZIKV) infection. The current understanding of damage caused by ZIKV infection is still unclear, since it has been implicated in other neurodegenerative and developmental complications. In order to get new insights on the molecular alterations promoted by ZIKV that might be related with neurological outcomes, the differential proteome of human mesenchymal stem cells (hMSC) infected with a Brazilian strain of ZIKV was identified by shotgun proteomics (MudPIT). Our results indicate that ZIKV induces a potential reprogramming of the metabolic machinery in nucleotide metabolism, changes in the energy production via glycolysis and other metabolic pathways, and potentially inhibits autophagy, neurogenesis, and immune response by down-regulation of signaling pathways. Further characterization of the proteome found that proteins causing the greatest molecular perturbation in the system are directly related with the ubiquitin-proteasome pathway. Among those proteins, are UBA52, UBC and PPS27, recognized players in neural formation, thus demonstrating that ubiquitin-related proteins may have an important role in ZIKV infection effects, including those related with clinical outcomes.

In addition, proteins previously described in several brain pathologies, such as Alzheimer's disease, autism spectrum disorder, amyotrophic lateral sclerosis, and Parkinson's disease, among others, were found with altered expression due to ZIKV infection in hMSC. The results described here in hMSC infection proteome pointed out to the potential molecular link between ZIKV and brain diseases, other than congenital defects, triggering follow-up studies currently under progress.

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SESSION 9

APPLICATION OF GENOTOXICITY IN SAFETY ASSESSMENT OF PHARMACEUTICAL IMPURITIES

MICHAEL URQUHART

VIDEO CONFERENCE: MANAGING EMERGING MUTAGENICITY RISKS: LATE STAGE MUTAGENIC IMPURITY CONTROL WITHIN THE ATOVAQUONE SECOND GENERATION SYNTHESIS

MIRIANA MACHADO

GENOTOXICITY EVALUATION OF DRUG IMPURITIES: BRAZILIAN REGULATORY ASPECTS

GENOTOXICITY EVALUATION OF DRUG IMPURITIES: CURRENT BRAZILIAN REGULATORY ASPECTS.

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Drug impurities (DI) are formed during the chemical synthesis or drug degradation. DI includes synthesis impurities, intermediates, by-products and degradation products. Since the presence of impurities may interfere on quality, efficacy and safety of drugs, their control is of major importance. Since 1995, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has published important guidelines about risk assessment of drug impurities. In Brazil, this topic has been discussed since the 2000's by regulators and industry and it is currently regulated by RDC n. 53/2015. Published by the Brazilian Health Regulatory Agency (ANVISA) in 2015, this legislation is mainly based on ICH guidelines and establishes trheshold concentrations for reporting, identification and qualification of drug impurities. Additional documents were recently published by ANVISA as Guideline n.4/2015 and Questions & Answers guidelines (2018, 2019) that provide more information about analytical and toxicological technical aspects. Besides that, impurities evaluation is cause of concern for many Brazilian pharmaceutical companies, maily in relation to impurtities qualification. Qualification is the safety related step, which determines that depending on the impuriy concentration, drug dose and treatment scheme, the toxicological risk of some impurities have to be evaluated. In this process, impurities should be tested by *in silico*, *in vitro* and/or *in vivo* toxicological methods to be considered qualified. Genotoxicity and general toxicity are the main endpoints which have to be investigated, but other target effects should be also included in the study. Importantly, the protocols should follow validated and recognized guidelines and be carried out in Good Laboratory Practice conditions (GLP). Regarding to genotoxicity evaluation strategy, *in silico* assays can be firstly used to predict impurity potential to induce gene mutations. Depending on the results, Ames Test is performed as a confirmatory test. However, at the moment, no certified *in silico* test is available for chromosomal damage prediction. So, *in vitro* or *in vivo* methods have to be performed to evaluate this endpoint, such as the *in vitro* Micronucleus Assay. Taken together, genotoxicity is an key step on the safety evaluation of drug impurities and the study strategy have to be specifically designed for each product and following recognized guidelines and expert opinion. ***Financial Support:*** *InnVitro Pesquisa & Desenvolvimento – Brazil.*

ASSESSMENT OF THE GENOTOXIC POTENTIAL OF PHARMACEUTICAL IMPURITIES

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Nowadays the pharmaceutical industries must follow strict rules to assess the impurities in medicines. Firstly, it is necessary to develop a very sensitive analytical method to determine and quantify the impurities in the API (Active Pharmaceutical Ingredient), in the drug product, excipients and in the packaging. After identifying the compounds, they must be assessed to its genotoxic potential. The only guideline that describes how to assess the mutagenicity of impurities is the ICH M7 that allows us to use *in silico* evaluation to define the risk. This is possible using two complementary software, one statistical based and another expert rule based.

USE OF *IN SILICO* TOOLS IN THE CONTEXT OF THE ICH M7 GUIDELINES

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The current ICH M7 guidance relating to the risk assessment of impurities in drug substances with regards to their mutagenic potential, places *in silico* toxicity predictions in a pivotal role in the decision-making process. It is, therefore, of utmost importance that the predictions supplied are as accurate as possible, that the users of the software understand the strengths and limitations of the tools and that enough information is provided that a user can review individual predictions. Indeed, the guidance requires the use of two different predictive methodologies (one expert rule-based and one statistical based) and states that, “if warranted, the outcome of any computer system-based analysis can be reviewed with the use of expert knowledge” in order to make any final conclusions drawn as robust as possible. This talk will describe the two predictive methodologies for mutagenicity outlined in ICH M7, their strengths and limitations, the information provided to facilitate expert review of each prediction and finally, how they can be combined, using the detailed information provided, to reach an overall conclusion on the assessment of an impurity. The talk will be illustrated with practical examples using the expert rule-based system Derek Nexus and the statistical-based system Sarah Nexus.

VANESSA MORAES ANDRADE

BRAZIL NUT CONSUMPTION AS ADJUVANT IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS: MODULATION OF GENOMIC AND BIOCHEMICAL INSTABILITY

BRAZIL NUT CONSUMPTION AS ADJUVANT IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS: MODULATION OF GENOMIC AND BIOCHEMICAL INSTABILITY

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Type 2 diabetes mellitus (T2DM) is a metabolic disease, occurring largely due to lifestyle changes. There is a strong link between T2DM and oxidative stress, that leads to damage to lipids, proteins and DNA. Dietary interventions are essential for the treatment and prevention of T2DM-related complications. Knowing that Brazil nut (*Bertholletia excelsa, H.B.K.*) is the richest source of selenium in nature, and this mineral presents several health benefits, including improve of redox cellular status and maintenance of genomic stability, the aim of this study was to assess the effects of consumption of selenium through Brazil nut on biochemical and oxidative stress parameters, as well as genomic instability in T2DM patients. We evaluated 74 patients with T2DM, registered in the Integrated Clinics of University of Southern Santa Catarina. Participants consumed one Brazil nut a day (that provides 210 µg of selenium) for six months. Blood and exfoliated buccal cells samples were collected at the beginning and at the end of treatment. The glycemic profile (fasting glucose, insulin, glycated hemoglobin, HOMA-IR, HOMA-β and QUICKI), lipid profile (total cholesterol, HDL-, LDL- and non-HDL-cholesterol, triglycerides, and Castelli Index I), renal (creatinine and urea), hepatic (AST, ALT and GGT), oxidative stress (DCF, MDA, nitrites, total thiols, protein carbonylation, GSH, GPx and CAT), DNA damage (comet assay and micronucleus) and selenium were evaluated. The data relative to biochemical parameters presents an increased in fasting glucose levels, HDL- and LDL-cholesterol, and GGT levels. On the other hand, insulin levels and triglycerides/HDL-cholesterol ratio were decreased. Six-month Brazil nut consumption proved to be enough to significantly increase selenium and GSH levels, and GPx and CAT activity, improving the antioxidant system of patients.

In relation to oxidant production, DCF and nitrites levels were decreased. Besides, was observed an increase in total thiols, and a decrease in protein carbonyl and MDA levels, suggesting a reduction of proteins and lipids oxidized. Relative to genomic instability, the levels of basal and oxidative DNA damage in T2DM patients were significantly decreased after Brazil nut consumption, as well as the frequency of micronuclei and nuclear buds. Taken together, our results indicate that Brazil nut consumption could be an ally to module the genomic instability in T2DM patients, probably through changes in redox balance.

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IMPACT OF OVERWEIGHT/OBESITY ON DNA-STABILITY

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Health authorities are alarmed worldwide about the raise of the body mass index, *i.e.* the number of overweight/obese humans increased ca. 6-fold since 1975. It is known that excess body weight causes cancer in different organs and possibly leads also to reduced fertility and affects cognitive functions. These effects may be causally related to genetic instability. An evaluation of the literature showed that the results of human studies concerning the impact of overweight on DNA-stability are strongly controversial as a consequence of heterogeneous study designs. We conducted two studies with C57BL/6J mice under controlled conditions, in the first the animals were fed with different amounts of a Western diet (WD). Subsequently, they received either a (40%) reduced amount of the WD or a high carbohydrate low protein diet (HCLP) ad libitum. Both feeding schemes led to weight loss (*ca*. by 30%); in parallel we found reduction of DNA damage in inner organs (liver, colon, ovaries) and also a decrease of formation of oxidised purines and an increase of nucleotide excision repair (NER) in some organs. In parallel, the levels of pro-inflammatory cytokines declined while adiponectin increased. Weight loss was also associated with a clear reduction of the insulin levels. All these effects were more pronounced in the group which received the HCLP. In a later trial, we investigated DNA protective effects of dietary antioxidants (gallic acid (GA), vitamin E and epigallocatechin gallate (EGCG)) in obese (high fat diet fed) mice. We found pronounced reduction of DNA damage in many inner organs after a short intervention period with very low dose of GA (2.6 mg/kg body weight corresponding to the human uptake levels in Western countries) as well as reduced formation of oxidised DNA bases and a decrease of glucose, insulin and triglyceride levels. The activity of NF-*k*B was clearly reduced after the intervention. Also, with EGCG and vitamin E evidence for DNA protective effects was observed. Taken together, our findings show that weight loss as well as dietary components protect against obesity induced DNA damage, inflammation and improve the metabolic status. These results are relevant for the design of nutritional strategies of obese humans.

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MEDICINAL PLANT EXTRACTS: DNA DAMAGE AND ROLE IN CANCER CHEMOSENSITIZER *IN VITRO*

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Medicinal plants are commonly employing for treating several diseases. *Baccharis trimera,* popularly known as “Carqueja”, is a medicinal plant widely used in South America as anti-inflammatory, anti-spasmodic, digestive and carminative agent. Flavonoids presents in *Baccharis trimera* extracts has been correlated to beneficial effects of “Carqueja”. Besides, it has been shown that could interfere with cancer processes such as proliferation, inflammation, angiogenesis, invasion and metastasis, although the sensitizer capacity to classical chemotherapy has not been well studied yet. Therefore, we have analyzed the *in vitro* anti-proliferative effect of *B. trimera* aqueous extracts obtained from two independent collects (during summer and winter), as well as the cytotoxic and genotoxic effect on human bladder cancer cell line named T24 (either in the presence or absence of a classical chemotherapy agent) by using Resazurin test and the cytokinesis-block micronucleus (CBMN) assay. Results obtained showed an anti-proliferative activity at 0.4 mg/ml of the plant extract obtained during summer collection as well as an increase in the frequency of micronucleus after treating these cells in combination with cisplatin, a common chemotherapy drug employed in bladder cancer treatment. These results emphasize the use of extracts of *Baccharis trimera* to sensitize bladder cancer cells to the classical treatment with cisplatin as chemotherapy with the aim to minimize the side effects of classical chemotherapy.

HOW MUTAGENESIS CAN HELP SAFE USE OF MEDICINAL PLANTS

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Users of medicinal plants from around the world, keep the practice of the consumption of herbal medicines for centuries. This medicinal culture has aroused our interest in evaluating the possible toxicity of plants used by a large part of the Brazilian population to alleviate or even cure some diseases. The increase in cancer cases has reinforced the importance of studying possible products of vegetal origin, that combat its progressive development. Among the medicinal plants studied by us, *Fridericia platyphylla* (Cham.) L.G. Lohmann (FP) occurs from Mexico to Argentina, including the Brazilian cerrado. It is used by Brazilian population for the treatment of kidney stones besides disorders of the gastrointestinal tract. FP was reported as cytotoxic, anti-inflammatory and analgesic *in vitro and in vivo* studies. Our first objective was characterize the cytotoxic and antiproliferative effects of FP extract in gastric normal (GAS) and tumor (ACP02, HepG2) cell lines, searching for a selective effect among them. FP extract presented a selective cytotoxicity to tumor cells mainly attributed to necrosis induction and modulation of genes involved in apoptosis (*BCL-XL* and *BIRC5*) and cell cycle (*MET*). *Although the selectivity between the two cell types is very close, the results obtained so far highlight that even medicinal plants may present adverse effects depending on the concentrations used and the tissues investigated. In addition, cytotoxic and antiproliferative results encouraged further research into the chemotherapeutic effect of this extract and its components.* Novel flavonoids were isolated from the dichloromethane portion of the hydroalcoholic extract of the roots of *Fridericia platyphylla* and three active compounds were named brachydins, A, B and C. We evaluated these compounds for their possible toxicological effects and their influence on the motility and invasiveness of human prostate tumor cell lines (PC-3 and DU145), as well as their possible changes in protein metabolism and expression. Brachydins exerted some activities on both human prostate tumor cell lines, such as reduction of cell proliferation and induction of cell death. They has significantly decreased cell motility and invasiveness in DU145 cells. The cytotoxicity induced by the extract in both normal and tumor cells may be due to the presence of the brachydins. This data highlights the importance of evaluating medicinal plants and directing their use in therapeutics.

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SESSION 11

NON-ANIMAL METHODS FOR TOXICITY TESTING: BRAZILIAN REALITY AND NEW APPROACHES

IZABEL VILLELA

ALTERNATIVE METHODS TO ANIMAL USE IN BRAZIL

ALTERNATIVE METHODS TO ANIMAL USE IN BRAZIL

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The scientific community concern with animal testing comes from a long time. To answer the desire to see laboratory techniques become more humane for the animals concerned, William Russell and Rex Burch, published in 1959 as The Principles of Humane Experimental Technique. This publication introduced the concept of the 'Three Rs' – of Replacement, Reduction and Refinement – which in due course came to be adopted as the guiding principle for the welfare of research animals worldwide and is now required by regulatory authorities in many countries. The Three Rs are: Replacement – the use of non-animal subjects wherever possible, and research into development and validation of new non-animal research and testing models; Reduction – where replacement is not currently possible, the minimization of the number of animals used by, for example, better research design, appropriate statistical methods and use of information databases; Refinement – improvement of experimental procedures and aspects of housing and husbandry so as to minimise risks to welfare. In Brazil, the first regulatory act about the subject was "*Lei Arouca*" from 2008, which regulates animal use in teaching and research. This act recommends the use do Alternatives for Animal Testing and creates the National Counsel For Animal Experimentation Control (CONCEA), to regulate the use of animals in research and teaching. In 2014 CONCEA officially approved and recommend 17 methods as alternatives to animal use. One year later, ANVISA (National Agency for Sanitary Regulation) published resolution accepting every alternative method that CONCEA approved and determining that 5 years after CONCEA's approval these alternative methods become mandatory. To support the development of alternatives methods, a Brazilian Centre for The Validations of Alternative Methods (BraCVAM) and a Network of laboratories on alternative methods (RENAMA) were also create

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MARCIO LORENCINI
ALTERNATIVE METHODS FOR COSMETICS - EVALUATION

RODRIGO DE VECCHI
3D SKIN MODEL IN BRAZIL: CHALLENGES AND OPPORTUNITIES

ALESSANDRA DE MELLO AGUIAR
MICRONUCLEUS ANALYSIS BY HIGH CONTENT IMAGING

ALTERNATIVE TO THE ALTERNATIVE METHOD: EXPECTATIONS X REALITY FOR THE STANDARDIZATION OF THE IN VITRO MICRONUCLEUS ANALYSIS BY HIGH CONTENT IMAGING

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In vitro micronucleus test (MNvit) is a validated methodology for genotoxicity (OECD TG 487). Micronucleus (MN) are formed by acentric chromosome fragments or whole chromosomes that could not migrate to the poles during the cell division. The MNvit includes the use of cell cultures that are exposed to test substances, then allowed to grow during or after exposure to the test chemical and finally, the cells are harvested, stained and the cells that undergone one complete mitosis are evaluated for the presence of micronucleus. The more disseminated protocol for the MNvit is limited by relatively low throughput, mainly because of the laborious microscopy reading of the slides. To overcome this limitation, the aim of this study was to standardize the MNvit assay in a multi-well plate format to allow reading and image analysis in a high content imaging system to save time, save test substances and other reagents and also perform more analysis simultaneously. For this purpose, many parameters were evaluated, including V79-4 cell line seeding, the concentration of cytokinesis blocking substance cytochalasin B (cytoB), time of the treatment, fixation and staining protocols and different scripts of analysis to allow the automatic identification of cell populations, specially binucleated cells, and the micronucleus. We performed the assays with the well known aneugenic substance colchicine (C 9754 Sigma-Aldrich®) without metabolic activation with 3 h treatment before exposure to cytoB for 21h. Our results indicated that the cell preparation was critical for the test. Factors such as cell detachment, cell confluence and the presence of cell aggregates interfered with the test performance, these factors are not so critical in the traditional methodology. The staining of the nuclei and cytoplasm were required for the automatized analysis as well. To get the required number of binucleated cells for MN evaluation in 96-well plate, 149 pictures/well were used, resulting in 2-3h of reading time and 20-22Gb of data for each plate.

We also established 3 ug/mL and 21h of treatment with cytoB were the best conditions for this assay. Analysis revealed a significant increase in micronuclei frequency in binucleated (MNBN) and mononucleated (MNMONO) cells of individuals with residential proximity to open-pit coal mines compared to residents from non-mining areas. Correlation analysis demonstrated a highly significant association between PM2.5 levels, MNBN frequencies and CREST+ micronuclei induction in exposed residents. These results suggest that PM2.5 fraction generated in coal mining activities may induce whole chromosome loss (aneuploidy) preferentially, although there are also chromosome breaks. Analysis of the chemical composition of PM2.5 by PIXE demonstrated that Si, S, K and Cr concentrations varied significantly between coal mining and reference areas. Enrichment factor values (EF) showed that S, Cr and Cu were highly enriched in the coal mining areas. Compared to reference area, mining regions had also higher concentrations of extractable organic matter (EOM) related to nonpolar and polar compounds. Our results demonstrate that PM2.5 fraction represents the most important health risk for residents living near open-pit mines, underscoring the need for incorporation of ambient air standards based on PM2.5 measures in coal mining areas.

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SESSION 12

BIOLOGICAL RESPONSES TO ENVIRONMENTAL FACTORS

FERNANDA RABAIOLI DA SILVA
INSIGHTS INTO THE IMPACTS OF AIRPORT ENVIRONMENTAL ON BIRDS OF PREY

DANIELI BENEDETTI
EVALUATION OF OCCUPATIONAL HEALTH OF FARMERS EXPOSED TO PESTICIDES

MARIA EUGENIA GONSEBATT
GESTATIONAL EXPOSURE TO ARSENITE: ALTERED GLUTAMATE DISPOSITION AND MEMORY IMPARMENT

GESTATIONAL EXPOSURE TO ARSENITE: ALTERED GLUTAMATE DISPOSITION AND MEMORY IMPARMENT".

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Early life exposure to metals and metalloids have been associated with memory and learning impairments in children. In the case of arsenic, polluted water is the main cause of exposure to over 200 million people worldwide. Evidence supports the idea that populations have been exposed for generations. Inorganic arsenic (iAs) is methylated in the central nervous system by arsenic III-methyltransferase in a process that requires glutathione (GSH) and could alter the glutamate disposition. We developed a murine model to explore the expression of transporters associated with GSH and glutamate in the cortex and hippocampus of male mice that were exposed gestationally and up 90 days after birth to 20 ppm of sodium arsenic in drinking water. Results showed that animals exposed to iAs have increased expression of xCT and EAAT1 early in life associated with NMDA glutamate receptor down-regulation that continued up to 90 days when we observed increased levels of extracellular glutamate, AMPA receptor downregulation, deficient LTP induction, and lower excitability of the PP–DG pathway. iAs exposed animals exhibited deficient spatial memory using a place learning task and lower learning and memory abilities in the Morris Water Maze test. Altered glutamate disposition and AMPA and NMDA receptors expression probably due to iAs metabolism in the brain could explain mechanistically the learning and memory impairments described in the epidemiological studies performed in iAs impacted populations.

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MECHANISMS MEDIATING METHYLMERCURY-INDUCED NEUROTOXICITY

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Methylmercury is a well-known environmental toxicant ubiquitously distributed within the aquatic environments (mainly in fish and seafood). Based on experimental studies, we aimed to investigate molecular mechanisms mediating methylmercury-induced developmental neurotoxicity. Both cultured cells and *in vivo* models showed that methylmercury causes neurotoxicity mainly due to interaction with nucleophilic groups of biomolecules, leading to altered glutamatergic homeostasis, oxidative stress and neurodegeneration. Of particular importance, selenoproteins present a pivotal role in mediating methylmercury-induced neurotoxicity. These findings are presented and discussed with focus on molecular mechanisms, as well as on nutritional and environmental recommendations.

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XIV CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE MUTAGÊNESE E GENÔMICA AMBIENTAL
Mecanismos celulares e moleculares envolvidos na instabilidade genômica e desafios na manutenção da integridade do DNA
30 anos
MutaGen-Brasil
Associação Brasileira de Mutagênese e Genômica Ambiental
3 - 6 junho de 2019
Dall'Onder Grande Hotel
Bento Gonçalves - RS - Brasil

ABSTRACTS

ENVIRONMENTAL MUTAGENESIS (ENVIRONMENTAL MONITORING, MUTAGENESIS OF NATURAL PRODUCTS, DRUGS, PHYSICAL AGENTS, COUNTRY LEGISLATION)

01 TO 98

30 anos
MutaGen-Brasil
Associação Brasileira de
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INVESTIGATION OF MUTAGENICITY OF TWO DERIVATIVES OF ISONIAZID

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Tuberculosis (TB) is the second most deadly infectious disease behind only HIV/AIDS. In 2018 the World Health Organization estimated that 1.3 million people died in consequence of this disease. TB chemotherapy in Brazil is based in four different antibiotics including isoniazid (INH), it inhibs the synthesis of micolic acid, a part of *Mycobacterium tuberculosis* cell wall. With rising of antibiotic resistance in *M. tuberculosis*, due to incomplete or incorrect treatment, new options are required. In our previous research, several hydrazines derivatives of INH were investigated and two of them, PFTI and BTI, showed potential results on the hepatotoxic assay and against M. tuberculosis – even to resistant strains. *Salmonella*/microsome assay (Ames test) performed with those hydrazines showed mutagenicity for TA98. The objective of the present study was to evaluate the modulation roles of two enzymes, n-acetyltransferase (NAT) and nitroreductase (NR), on mutagenesis once INH is metabolized through phase II enzymes before phase I reactions. We performed the Ames test at 0.5, 5, 50, 500 and 5000µM, with 20 minutes of exposure and dimethyl sulfoxide (DMSO) and 4-nitroquinoline 1-oxide (4NQO) as control group. The strains used were TA98 derivative strains. TA1538 does not express recombinational repair, YG1021 overexpress NR, YG1041overexpress both enzymes, TA98NR is NR deficient and TA98-1,8DNP6 is NAT deficient. YG1021 showed an up-down oscillation on revertant colonies in PFTI and INH, presumably as result of the activation of repair system. Mutagenesis for BTI was observed only in TA1538, at 50µM. Probably, the lack of toxicity of the INH derivatives using these strains are related to acetylation of bioactive toxic intermediaries.

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EVALUATION OF THE GENOTOXIC POTENTIAL OF NEUROTOXIC INSECTICIDES THROUGH THE MICRONUCLEUS TEST IN *Tradescantia pallida*

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Key-words: Pesticides; Neonicotinoids; Phenylpyrazole; Genotoxicity

Neonicotinoids and phenylpyrazoles are classes of neurotoxic insecticides which are able to bind at different ligand sites of neural receptors, leading to the deregulation of insect neural activity and hence resulting in death. Despite the benefits conferred by the use of insecticides in the agricultural system, the misuse or indiscriminate use of these chemicals is directly associated with several toxicological effects in biota and at different trophic levels. Based on this premise, the aim of the present study was to evaluate and compare the genotoxic capacity of different concentrations of thiamethoxam (TMX), acetamiprid (ACP), imidacloprid (IMI) and fipronil (FP) through the Micronucleus Test in *Tradescantia pallida* (Trad-MCN). After acclimatization (24 h), *T. pallida* stems were treated with stablished concentrations of TMX, ACP, IMI and FP for 8 h. Then, the stems of the model organism were submitted to a recovery phase (24 h). The young inflorescences were harvested and fixed in Carnoy solution and, after 24h, were conserved in ethanol 70% until the analyzes. The obtained anthers were macerated on slides for microscopy, stained with acetic carmine dye and covered with coverslips before analysis by light microscopy. Considering the insecticides, the micronuclei (MN) frequency in plants treated at concentrations of 0.2 and 0.4 g/L for TMX, 0.2; 0.4 and 0.8 g/L for ACP, 0.1; 0.2; 0.4; 0.8 and 1.6 g/L for IMI and 0.2; 0.4; 0.8 and 1.6 g/L for FP differed statistically ($p < 0.05$, Tukey) from the MN frequency of the negative control. All chemicals evaluated revealed genotoxic activity in *T. pallida* at the highest concentrations.

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COMPARATIVE MUTAGENIC AND TOXICOLOGICAL ANALYSIS BETWEEN THE ACTIVE INGREDIENT AND FORMULATED PRODUCT OF THE INSECTICIDE IMIDACLOPRID: OTHER FORMULANTS ARE TRULY INERT?

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Keywords: Neonicotinoids; Genotoxicity; Inert ingredients; Active component.

Neonicotinoids are neurotoxic insecticides agonists to nicotinic acetylcholine receptors, with Imidacloprid (IMI) being one of their representatives. Premier® (PRM) (70%) is one of the most commercially formulated IMI-based products in Brazil. The mutagenic and recombinogenic effect were evaluated by the somatic mutation and recombination test (SMART) in *D. melanogaster*. Third-instar larvae resulting from standard (ST) and high bioactivation (HB) crosses were chronically treated (48 h) with different concentrations of IMI and PRM (1.2, 2.4, 4.8 or 9.7 x 10^{-4} mM). The results revealed high frequency of mutant spots in HB flies at all assessed concentrations of PRM and 2,4 and 4,8 x 10^{-4} mM of IMI. PRM showed a high frequency of mutant spots in the flies of ST cross. The carcinogenic effects were evaluated using the test for the detection of epithelial tumor in *D. melanogaster*. Carcinogenic effect was observed in the flies treated with 2,4; 4,8 and 9,7 x 10^{-4} mM of PRM. The results show that IMI needs to be metabolized to trigger genotoxic processes. Inert ingredients interfere with the toxicokinetics of IMI, enhancing the mutagenic effects of IMI. Because of the scarcity of data on the mutagenic effect of IMI and PRM, we propose the need for further studies in other test systems, cells, tissues or organisms, to obtain more toxicological information about these molecules and the inert ingredients present in formulated products.

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ASSESSMENT OF OXIDATIVE STRESS AND DNA DAMAGE MARKERS IN MEDICAL RESIDENTS BEFORE AND AFTER ONE YEAR OF EXPOSURE TO INHALATIONAL ANESTHETICS

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Key-words: occupational exposure, inhalational anesthetics, medical residency, oxidative stress, genotoxicity test

Worldwide, millions of operating room professionals are occupationally exposed to inhalational anesthetics, which can lead to undesirable effects. It is unknown the consequences of occupational exposure to waste anesthetic gases on young professionals, such as the medical residents. Taking into consideration the importance of human biomonitoring for assessment of possible toxic effects of occupational exposure to anesthetics, the present study monitored physicians before and after one year of exposure during their medical residency. This follow up study was previously approved by the local ethics committee and it was performed at the São Paulo State University Hospital in 23 young adult physicians who worked in operating rooms and were exposed to high concentrations of isoflurane, sevoflurane, desflurane, and nitrous oxide (anesthesia and surgery medical residents). The blood samples were evaluated before the beginning of the medical residency program (control) and at twelve months of exposure for monitoring oxidative stress (lipid oxidation - malondialdehyde by HPLC and protein carbonyl content by ELISA, and antioxidant capacity by fluorometry) and genotoxicity (DNA damage by the comet assay). There was a significant increase of lipid ($p = 0.02$) and protein ($p < 0.001$) oxidation markers, and antioxidant capacity ($p < 0.001$) compared to before the exposure. Despite the slight increase of DNA damage, it did not reach a statistical significance ($p = 0.25$). Thus, the biomonitoring during the first year of medical residency showed that high exposure to waste anesthetic gases leads to oxidative stress, but not genotoxicity. Possibly, this occupational exposure first damage lipids and proteins and then DNA. Therefore, despite the short period of exposure, the findings highlight the toxic effects observed in young exposed professionals. It is of great importance to keep following these exposed physicians at the entire period of the medical residency as well as at the beginning of their careers in order to better understand the possible toxicity of anesthetics at systemic, cellular and molecular levels.

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IN VIVO MUTAGENICITY OF THE ENVIRONMENTAL POLLUTANT 2-PHENILBENZOTRIAZOLE-9 NON CHLORINATED (NON-Cl PBTA-9), A BYPRODUCT OF THE DIPERSE VIOLET AZO DYE

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Key-words: *cytotoxicity, genotoxicity, micronucleus, water pollution*.

Literature has showed that dye compounds used in the textile industries can caused important impact on the hydrosphere. The conventional wastewater treatment can be not efficient enough for removing the hazards compounds. In addition, the water chlorination for human consumption can turn some dyes even more mutagenic. Pollution caused by azo dyes and their byproducts has been widely investigated *in vitro* systems. However, there are few studies using *in vivo* models. The 2-phenilbenzotriazole-9 non-chlorinated (non-Cl PBTA-9) has received special attention because it is derived from the Disperse Violet 93 (DV93), which is found in high quantity in the water surface under influence of textile activities. Therefore, this study aimed to investigate the cytotoxic and genotoxic effects of the acute exposure to the non-Cl PBTA-9 in Swiss adult male mice. The animals were distributed in 6 groups (n=10/each): 1) negative control: treated with filtrated tap water; 2) positive control: intraperitonealy treated with *n*-metil-*n*-nitrosurea (MNU; 50mg/kg body weight); 3) vehicule control: orally treated with 0.5% dimethylsulfoxide (DMSO); 4-6) and three groups orally exposed to non-Cl PBTA-9, at single doses of 5, 50 or 500 µg/kg/b.w. These doses were defined based on the concentrations of DV93 detected in some Brazilian rivers. Six hours after treatments, 20µL of peripheral blood was collected from the facial vein to perform the comet assay. A total of 150 nucleoids/animal were analized to measure primary DNA damage (% tail intensity). Bone marrow cells from femurs were also collected (24 hours after treatments) for cytotoxicity and micronucleus evaluation. The linear model with gamma distribution, ANOVA, Tukey-Kramer and the Poisson distribution tests were used for the statistical analyses. While an increase of primary DNA damage was detected in those animals exposed to the highest dose (500 µg/kg bw) of non-Cl PBTA-9, increased frequency of micronucleated cells was detected in all assessed doses (5, 50 and 500 µg/kg bw), when compared to the negative control ($p<0.05$). No cytotoxicity was observed. In conclusion, even at very low concentrations, non-Cl-PBTA-9, a byproduct of the DV93 azo dye, was genotoxic after acute exposure, suggesting that water contamination with these compounds can be harmful to the environment and human health.

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EVALUATION OF DNA DAMAGE IN OF BONE MARROW MESENCHYMAL STEM CELLS USING FLUOSILICIC ACID

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The higher intake of fluoride by the human population has its origin in the consumption of water. The amount of fluoride used varies by region, taking into account the amount of fluoride present in water, food and other beverages, plus some habits such as tea consumption, and cooking and dehydrating foods with coal rich in fluorides. Water fluoridation is recommended by the WHO as an appropriate method for caries prevention and is currently mandatory in Brazil. In the environment, fluoride is abundant in rocks, groundwater and soils, originating in soils similar to arsenic. Increased concentrations of these elements in soils is also a result of the use of groundwater for irrigation and / or increased capillarity of the soil. The main Brazilian aquifers have shown high concentrations of fluoride in groundwater, detected through public wells and private use. Although considered as natural exposures, these waters require monitoring by management bodies related to the use of water resources and public health. Excess fluoride in water can produce changes in tooth enamel mineralization and lead to diseases such as dental or skeletal fluorosis. There are few results presented on this subject and their conclusions are contradictory about the possible effects that fluorine can cause and its relationship with genotoxicity. The aim of present in vitro study was assess the cytotoxic and genotoxic effects of fluosilicic acid in bone marrow mesenchymal stem cells. Mesenchymal stem cells were isolated from the femur of rats and cultured under standard conditions and exposed during 3, 7 and 14 days to concentrations of fluosilicic acid (9.6, 4.8, 2.4, 1.2 and 0.6 mg/mL) to MTT assay and 3, 7 and 14 days to concentrations of fluosilicic acid (2.4, 1.2 and 0.6 mg/mL) to comet assay. Cytotoxicity was observed in 14 days of exposure, below 50%, in all concentrations of fluosilicic acid in the MTT assay and in 3 and 7 days, cells showed viability above 70%.

Alkaline comet assay results demonstrated significant increase in damage index and frequency (NC-32.00±19.90; 2.4 mg/L–234.30±16.09, $P<0.05$; 1.2 mg/L–171.80±89.21, $P<0.01$; 0.6 mg/L–129.80±35.55, $P<0.001$ and PC–218.00±48.59, $P<0.001$) for cells treated 7 days with fluosilicic acid, presenting genotoxicity. Unlike other studies, our results demonstrated genotoxicity induced by fluosilicic acid in concentrations used in drinking water. Osteogenic differentiation studies are being carried out to investigate the relationship of osteogenic potential exposed to fluosilicic acid.

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INVESTIGATION OF CITOTOXICITY, MUTAGENICITY AND ANTIMUTAGENICITY OF ARTEPILLIN C IN HUMAN GLIOMA CELLS

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Propolis is a resinous material collected by bees from buds and plant exudates. Several biological properties have been attributed to propolis, whose composition varies with its botanic origin and geographic location of source plants, resulting in numerous varieties of the product. Green propolis is collected by *Apis mellifera* from *Baccharis dracunculifolia*, and its main chemical component is artepillin C (ArtC). ArtC has strong antioxidant activity and demonstrated important antitumor effects. In order to contribute to a better characterization of ArtC, the present study investigated the cytotoxic, genotoxic and antigenotoxic effects of ArtC using different *in vitro* bioassays. Cytotoxicity was evaluated using the MTT test and chromosome instability was assessed using the cytokinesis-block micronucleus assay (CBMN). In addition, the modulator action of ArtC on lesions induced by bleomycin (BLEO) was also analyzed using the CBMN assay. The results of the MTT test show that ArtC was cytotoxic to U-87MG (62.5 and 31.2 µM), HT29 (62.5, 31.2,and 15.8 µM), and MCF-7 (62.5, 31.2, 15.8, and 7.9 µM) cell lines. We selected glioma cells, U-87MG, to evaluate ArtC genotoxicity and antigenotoxicity. The analysis of mutagenic action did not reveal any significant increase in micronucleus (MN), nucleoplasmic bridges (NPB), and nuclear buds (NBUD) frequencies after treatment of U-87MG cells with different ArtC concentrations for 4 h and 24 h. Concerning the modulator action of ArtC, the compound significantly reduced bleomycin-induced frequencies of MN during pre-treatment (ArtC 3.9, 7.8, and 62.5 µM), post-treatment (ArtC 31.2 and 62.5 µM), and co-treatment (ArtC 15.6 and 31.2 µM), indicating the protective effect of ArtC against chemically induced genetic damage. Taken together, the results of this study contribute new information for the development of pharmacological drugs based on propolis.

Keywords: Artepillin C, cytotoxicity, CBMN,U-87MG.

Financial Support: CNPq, CAPES, FAPERGS, ULBRA

CHEMICAL COMPOSITION AND GENOTOXICITY ASSESSMENT OF *Coffea arabica* WITH DIFFERENT GRAIN QUALITY AND DEGREES OF ROASTING

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Key-words: *Coffea arabica*, genotoxicity, chemical composition

During the coffee roasting process of the beans, the formation of polycyclic aromatic hydrocarbons (PAHs) may occur, which are associated with the incidence of cancer in humans due to their mutagenic and carcinogenic potentials. In previous studies, we showed that the commercial quality coffee with the highest degree of roasting was able to induce mutagenicity. Further, by the cytotoxicity assay, it can be seen that there was a dose-dependent reduction of the viability of the $HepG_2$ cancer cells under mitochondrial metabolism after exposure to the commercial and special coffee samples extract. The present study aimed to analyze the chemical constituents present in six samples of roasted and ground coffee beans (*Coffea arabica*) and evaluating their potential genotoxicity. The assays were carried out in blind tests (coffee of commercial quality 445 (light roast - Agtron 95), 659 (medium roast - Agtron 65) and 762 (dark roast - Agtron 45) and coffee of special quality: 917 (light roast - Agtron 75), 823 (medium roast - Agtron 55) and 935 (dark roast - Agtron 35). Chemical analyses were followed by gas chromatography coupled to mass spectrometry (GC-MS). Genotoxic potential was performed by Cytokinesis-block micronucleus (CBMN) assay. The coffee drinks were prepared with distilled water in an electric coffee maker. The samples were freeze-dried and the dry residues resuspended in sterile distilled water at appropriate concentrations for the assay. Chemical analysis showed that all samples contained caffeine in their composition and samples 445, 659, 762 (commercial coffee) and 823 (special coffee) also contained naphthalene. The CBMN assay showed that none of the samples induced either clastogenicity or aneugenicity in liver $HepG_2$ cancer cells. Thus, even with the presence of the compounds identified in the chemical analysis, the samples did not induce genotoxicity probably due to the presence of polyphenols commonly found in coffee.

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PHOTOPROTECTIVE EFFICACY AND SAFETY OF *n*-BUTANOL FRACTION FROM EXTRACT OF THE ANTARCTIC *Sanionia uncinata* MOSS

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Keywords: *Sanionia uncinata*, *n*-butanol fraction, photoprotection

Photoprotection is important to control skin disorders since long-term exposure of ultraviolet (UV) radiation can cause DNA damage and skin cancer. Recently we have shown that dermal formulations containing partition fraction *n*-butanol (Bf) of the hydroalcoholic extract of Antarctica moss *Sanionia uncinata* (Hedw.) Loeske, effectively protected the DNA against the induction of cyclobutane pyrimidine dimers and oxidized DNA bases on simulated sunlight by *in vitro* models. We also showed that the viability of human epidermal keratinocytes (HaCaT cells) was above 70% after 72h of treatment with Bf up to 0.04 mg/mL for monolayers and up to 1 mg/mL for 3D cell culture. The Bf did not induce phototoxicity even in association with cosmetic UV-filters. The present study investigated spectrophotometric profile, mutagenic and genotoxic activities of Bf. Cytotoxicity was carried out in HaCaT monolayers, in the absence and presence of UV-A and UV-B radiations at 20 J/cm^2 and 50 mJ/cm^2, respectively. UV-visible spectrophotometry, *Salmonella*/microsome assay, cytokinesis-block micronucleus (CBMN) assay, water-soluble tetrazolium salt (WST-1) and lactate dehydrogenase (LDH) assays were performed. The results showed that Bf has a maximum wavelength at 230 nm and weak band at 270, 330, 430, and 650 nm, similarly to the major flavonoids. No mutagenic activity was detected for TA97, TA98 (frameshift mutation), TA100 (base-pair substitution), TA102 and TA104 strains (transitions/transversions), in the absence or presence of an exogenous metabolization (S9 mix fraction). The CBMN assay showed that Bf was not able to induce clastogenicity or aneugenicity in HaCaT cells. The WST assay showed that cell viability was affected (~50-60%) by the Bf as from 0.1 mg/mL, independent of UV-A and UV-B radiation. Using LDH assay, the Bf fully protected HaCaT cells against UV-A (≥ 0.04 mg/mL) and UV-B (≥ 0.4 mg/mL) damage by LDH release. Therefore, the Bf absorbs UV-visible spectrum, does not induce mutagenicity and genotoxicity, and protects the HaCaT keratinocytes against UV-induced damage by cell membrane disruption pathway.

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INDUCTION OF CHROMOSOMAL INSTABILITY IN HUMAN MONONUCLEAR BLOOD PERIFERIC CELLS BY THE ACTION OF COMMERCIAL DEXCLORFENIRAMINE AND ACTIVE PRINCIPLE

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Key-words: chromosomal instability, antihistamine, genotoxic effect.

Dexchlorpheniramine maleate (DPN) has been used as the first choice in allergy therapy. DPN belongs to the first-generation antihistamine group that acts as a blocker of histamine H1 receptors. However, the literature does not describe whether the DPN is safe for the chromosome structure considering the concentrations reached in the therapy. The aim of this study was to evaluate whether DPN as active principle (DPN) and in the pharmaceutical form (DPN-P) were able to induce chromosomal instability in cultured human peripheral blood mononuclear cells (PBMC). Thus, chromosomal instability test was performed according OECD 487 protocol. Then, peripheral venous blood (5 mL) was collected and placed in a heparinized tube that was mixed with Histopaque® in order to obtain the PBMC. 10^6 cells/mL was adjusted for each culture plate containing 2 mL of supplemented RPMI 1640 medium at 37°C in 5% CO_2 atmosphere for 48 hours. The groups were divided in controls (negative, RPMI medium; positive, RPMI plus 10 μg/mL colchicine) and DPN or DPN-P test groups in different concentration, chosen to simulate those achieved after administration of these drugs, (0.5, 2.5, 5,10, 50 ng/mL). After 48 hours, we exposure both DPN and DPN-P for 3 h to colchicine, which disrupt the achromatic spindle causing cell cycle arrest. The cells were centrifuged and treated with hypotonic 0.0075 M KCl solution for lysis of membrane and swelling of chromosomes allowing better visibility. After, news centrifugation was performed to remove the hypotonic solution and to wash many times with methanol and acetic acid solution in order to fix on slides and to stain them with Giemsa dye. The numerical chromosomal instability was performed by counting and analyzing 300 cells in metaphase by sample in optical microscopy with 1,000X. The data were analyzed by One Way ANOVA, complemented by post-hoc Dunnett's Test, and considered as statistically significant when $p<0.05$. This study was previously approved by Ethic Committee from UNIPAMPA (n° 27045614.0.0000.5323). No breaks, ring chromosomes, horseshoes, centromeric fusions, karyopicnose, gaps or numerical changes were observed in the test samples. Therefore, we suggest that DPN in both forms used in this study and under our experimental conditions is unable to induce clastogenic effects. However, future studies should confirm and deepen this finding.

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MUTAGENICITY ASSESSMENT OF THE HIDROALCOHOLIC EXTRACT OF A FOOD FUNCTIONAL ORIGINATED FROM FRUITS AND VEGETABLES RESIDUE

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Key-words: Supplement, Flavonoids, Carotenoids, Food Safety, Mutagenicity.

The consumption of functional foods is driven by the increase of consumers who wish to improve their quality of life through food choices and incorporation of healthy habits. The presence of carotenoids and phenolic compounds, in significant amounts, indicate a good food profile and good economic value for consumption and supplementation. These bioactive compounds can be obtained in greater quantity with the total use of food. Our objective was to study toxicological effects of the hydroalcohol extract (75/25) of the flour originated from dehydrated fruits and vegetables homogenate with promising nutritional potential, Fruit and vegetable flour (FVF). A previous study evaluated the nutricional properties of FVF, for use in the production of functional food, and consumer acceptance for the FVF and their developed products, such as cereal bars and cookies. FVF was produced from the solid residue based on the entire exploitation of fruits and vegetables after the isotonic preparation, containing 50% vegetables (approximately 2% mint and rocket; 5.5% lettuce, spinach and taro; 8.5% cucumber and courgette and 13% carrot) and fruits (11% orange; 19% passion fruit and 22% watermelon). The total weight of this residue was approximately 1 kg. After processing, the residue was reduced to approximately 100g and made into flour. To determine the mutagenic potential of FVF, the Salmonella / Microsome assay was performed using the TA97, TA98, TA100, TA102 and TA104 strains of *Salmonella enterica* serovar Typhimurium. In parallel, a bacterial survival assay was performed to evaluate possible cytotoxic effects. Five concentrations (0.5, 50, 500, 5000 µg / plate), diluted in sterile water, were tested in the absence of metabolic activation using S9 mix 4% (-S9). The experiments were performed in triplicate and the results that showed mutagenicity (Mutagenicity Index = MI) >2 were considered mutagenic, and cytotoxic when survival was <70%. The hydrophilic extract of FVF presents a great variety of phytochemicals, detected by ESI-QTof-MS, mainly phenolic compounds and carotenoids. In the Ames test, mutagenicity was detected for strain TA104 (from 0.5 µg/ plate), thus having a dose-dependent behavior, and cytotoxicity were detected for strains TA98 and TA102 (from 50 µg/ plate), as FVF substances generated cell death of Salmonella enterica, characterizing the cytotoxic effect. It can be concluded that the FVF extract produced by these vegetables and fruits presented a mutagenic profile in the absence of metabolization, due to which further studies are required for the production of this functional food. **Financial support**: FAPERJ, CNPq, UNIRIO, UERJ, CAPES.

ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF *Myrciaria cauliflora* LEAF AGAINST *Trypanosoma cruzi*

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Keywords: mutagenesis, natural products, Chagas disease.

Chagas Disease presents several therapeutic challenges, such as limited action and side effects of available drugs. These reveal the importance of the search, in plants natural products potentially active against protozoa for more effective and safer trypanocidal compounds. Parts of *Myrciaria cauliflora* (Myrtaceae) tree, known as jabuticabeira, have traditionally been used to treat respiratory and digestive disorders. Previous studies have reported the antimicrobial action of its leaves, related to the presence of flavonoids, alkaloids, saponins and tannins. Therefore, considering the importance of the experimental evolution of its biological activity, this study aimed to investigate the safety and efficacy of *M. cauliflora* leaf hydromethanolic extract against *Trypanosoma cruzi* bloodstream trypomastigote form. The mutagenic potential was assessed by the Ames Test, in the absence and presence of metabolic activation, using *Salmonella enterica* serovar Typhimurium standard strains (TA97, TA98, TA100, TA102, TA104). HepG2 cells were assayed for cell viability determination using WST-1 assay. The trypanocidal activity was evaluated against bloodstream trypomastigote form of *T. cruzi* (Y strain, DTU II). Mutagenic effects were detected at the highest concentration tested (500 µg/plate), in the presence of exogenous metabolization in the TA100 strain, indicating base pair substitution damage, from G:C to A:T. The extract exhibited significant trypanocidal activity over a range of safe concentration for HepG2 cells (EC_{50} 2h: 4.8-11.4 µg/mL EC_{50} 24h: 4.7-10.5 µg/mL), about ten times lower than the concentration required to produce a hepatotoxic effect (EC_{50} 24h: 101 µg/mL).

Mutagenic and trypanocidal potential may be related to the dose-response dependent redox imbalance of flavonoids, which in high concentrations no longer act as antioxidants and become pro-oxidative agents, damaging the DNA and altering the parasite redox balance. Furthermore, some phenolic compounds found in the extract, might have the ability to complex with parasite macromolecules, such as membrane lipids and proteins, leading to function loss. In summary, the data demonstrate that the *M. cauliflora* leaf extract presented *in vitro* trypanocidal activity in concentrations with high probability of safety for the human consumption, emerging as a potential source of active natural products against Chagas Disease etiological agent.

Financial Support: CNPq, CAPES, FAPERJ, FIOCRUZ, UERJ, UNIRIO.

GENOTOXICITY OF THE BIOPESTICIDE AZADIRACHTIN IN SOMATIC AND GERM CELLS OF *Ceraeochrysa claveri*

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Keywords: Genetic damage, non-target insect, natural pesticide, comet assay, ovary and gut cells.

Azadirachtin is a biopesticide widely used as an alternative to the conventional pesticides. The compound has varied sub-lethal effects against many insect pests, including antifeedant, repellent and growth regulatory. However, the safety of the natural product is not completely proven. Herein, we used the *Ceraeochrysa claveri* as an *in vivo* model to investigate the azadirachtin potential to induce genetic damage in somatic (gut) and germ cells (ovary). *C. claveri* larvae were distributed in 4 groups: negative and positive controls, and exposed to azadirachtin (Azamax™; 1.2% active ingredient) at doses of 0.3% and 0.5%. Survival, genetic instability (comet assay – alkaline version), morphology and behavior were evaluated. Results showed increased primary DNA damage in gut and ovary cells of those insect treated with both doses of the biopesticide. Furthermore, reduced survival, changes in the larval and pupal developmental time, impairment in the animal body and in the ultrastructure of adult ovaries (thinner ovariolar sheath, complete shrinkage, alterations in cellular organization and cell death) were also detected. Taken together, these effects can potentially compromise the health of the *C. claveri*. In conclusion, azadirachtin, known as a reduced risk insecticide, is able to induce hazard effects in somatic and germ cells of *C. claveri,* an economically important non-target insect in agriculture.

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CHARACTERIZATION OF GENOTOXIC POTENTIAL OF SUNLIGHT IN SANTA MARIA, RS, BRAZIL

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Key-words: UV radiation, solar incidence, genotoxicity, DNA lesions, biological dosimetry

The ultraviolet (UV) radiation emitted by the sun differs in three different types: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm). Only UVA rays and part of UVB rays cross the stratospheric ozone layer and affect the Earth's surface. Exposure of organisms to UV radiation leads to the formation of different types of DNA lesions, especially photoproducts known as Cis-syn-cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts. In addition, UV radiation can generate oxidative damage such as 8-oxoguanine (8-oxoG). These DNA lesions form the basis of mutagenic processes which can lead to development of skin cancer. In this work, the quantification of DNA lesions induced after exposure to sunlight was correlated with data of incidence of solar UV radiation in a specific locality of southern Brazil. Measurements of solar UVB and UVA radiation were taken through the use of specific radiometers, in the municipality of Santa Maria/RS (29º S). Exposure of plasmid DNA to sunlight was performed with the DNA dosimeter system, which was exposed continuously to the sun between 10:00 a.m. to 4:00 p.m., on sunny days of the different seasons of the year. For detection and quantification of sunlight-induced DNA lesions the exposed samples were incubated with the following DNA repair enzymes at 37° C for 60 minutes: T4-endonuclease V, which cleaves CPD lesions; Formamidopyrimidine-DNA glycosylase, which cleaves oxidized purines; and Endonuclease III, which cleaves oxidized pyrimidines. After electrophoresis migration in agarose gels it was possible to observe the separation of damaged DNA from undamaged DNA. The results indicate that the formation of CPD lesions is higher than the formation of oxidative damage. In general, the frequency of formation of these lesions is directly related to the dose of solar UV radiation incident on the surface, which varies according to the seasons. Therefore, the variation of solar UV radiation that reaches the Earth's surface may result in different genotoxic impacts in exposed organisms, as well as in human health.

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GENOTOXICITY, TRYPANOCIDAL EFFICACY AND PHARMACOKINETIC PROFILE OF NOVEL AMINOQUINOLE-ATORVASTATIN HYBRID DRUGS

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Statins are drugs used to control high cholesterol levels. Atorvastatin (AVA) is a third generation statin, considered the last of synthetic pharmaceutical blockbusters. Furthermore, statins have been shown several pleiotropic effects and are potential chemotherapics to treat infectious diseases. Recently, our group described the effects of AVA on DNA damage prevention and against *Trypanosoma cruzi* infection. In this study, our aim was to evaluate the efficacy, safety and pharmacokinetic profile of four novel aminoquinolinic derivatives (4a, 4b, 4c and 4d) of AVA against *T. cruzi* using *in vitro* and *in silico* models. These compounds were synthesized by bioisosteric hybridization, combining the pyrrolic site of AVA (non-pharmacophoric region) and the aminoquinolinic region of Chloroquine or Mefloquine. *In silico* approach, using PK-CSM and LAZAR algorithms permitted to predict pharmacokinetic (ADME) and toxicity parameters. We evaluated the *in vitro* trypanocidal activity of atorvastatin against amastigotes and trypomastigotes of *T. cruzi*, from different DTU (Y strain – Tc II and Tulahuen strain – Tc IV). We evaluated the compounds cardiotoxicity using monolayer primary cultures of mouse embryonic cardiac cells and hepatotoxicity on monolayer and 3D-cultured HepG2 cells. Genotoxicity was evaluated using Ames test, and Micronucleus assay, according to international safety guidelines. The pharmacokinetic prediction suggests that besides a good ADMET profile, all of compounds were predicted as positive hepatotoxic drugs. *In vitro* antiparasitic analysis permitted to infer that all presented high trypanocidal activity, against both tryposmastigote and intracellular forms of *T. cruzi*, presenting EC_{50}'s lower than 1µM and also better selectivity than the reference drug. In bacterial model, 4a and 4b were not considered mutagenic for any strains (TA97, TA98, TA100, TA102 and TA104) of *Salmonella typhimurium*, both in absence or presence of metabolic activation. There was no evidence of toxicity in the cardiac cell model.

The compounds 4a and 4b presented time-dependent toxicity in HepG2 monolayer cultured cells but did not exerted toxic effects in 3D-cultured spheroids, opposing the *in* silico prediction. Complementarily, in non-cytotoxic concentrations, 4a and 4b did not induce micronuclei formation in monolayer cultured HepG2 cells. We can conclude that the novel AVA-aminoquinoline hybrid compounds presented *hit-to-lead* profile as antiparasitic drug prototypes.

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GENOTOXICITY ASSESSMENT OF GRAPHENE QUANTUM DOTS DESIGNED FOR NANORADIOLABELING

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Graphene is one of the crystalline forms of carbon, along with diamond, graphite, carbon nanotubes, and fullerenes, and isconsidered asa revolutionary and innovating product. The use of a graphene-based nanolabels is one of the latest and most prominent application of graphene, especially in the field of diagnosis and, recently, *in loco* radiotherapy when coupled with radioisotopes. However, its biological behavior and mutagenicity in different cell or animal models, as well as the *in vivo* functional activities, are still unrevealed. OECD recommends perform at least two *in vitro* toxicological tests before proceeding to *in vivo* assays. In this study we synthesized graphene quantum dots (GQDs) and characterized their mutagenic, cytotoxic and genotoxic potential. We proceeded the genotoxicity assessment through the standardized Ames Test protocol using TA98, TA100, TA102 and TA104 *Salmonella enterica His*$^-$ strains, in absence of metabolic conditions, from 0 to 1000 µg of GQDs. After, we measured the GQDs potential to induce mitochondrial (WST-1 assay) and cell membrane (LDH assay) damage and also micronuclei formation in mammalian cell cultures of HepG2 cell lineage, at the same exposure concentrations. The mutagenicity test has demonstrated A:T to G:C substitutions, detected by TA102 and TA104, since 1 µg of GQDs, in a dose-response curve, suggesting that GQD exhibits mutagenic activity by redox misbalance.

In eukaryotic cell models, we did not detect a severe induction of cell death by also WST and LDH assays and, at the higher concentration (1000 μg/mL) GQDs induced a slight increase, but not statistically significant, in micronucleated cells. We can conclude that our new Graphene Quantum Dots presented mutagenicity in bacterial model but were not cytotoxic nor genotoxic to mammalian cells.

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EVALUATION OF GENOTOXICITY AND MUTAGENICITY OF THE DRY TOBACCO LEAVES

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Key-words: *Nicotiana tabacum*, tobacco, genotoxicity, mutagenicity

Nicotiana tabacum is the is the most cultivated species of the tobacco in the state of Rio Grande do Sul (RS, Brazil). Tobacco growers are exposed directly to the components of the leaf during the drying process of the same. Tobacco growers are exposed directly to the leaf components during the drying process. This plant is rich in alkaloids, the main one being nicotine. Nicotine is easily absorbed by the transdermal tissue, respiratory epithelium and mucousas and it has big action in the central nervous system, due to its activity in nicotinic acetylcholine receptors. When the leaf is drying, the alkaloids undergo nitrosation and form the tobacco-specific N-nitrosamines that may interact with the DNA and are considered carcinogenic. As there are no studies in the literature on the effects that the inhalation of powdered leaves during the drying process can cause, the objective of the study was: to evaluate the concentration of nicotine and inorganic elements in dry leaf powder; cytotoxicity and genotoxicity of the aqueous extract in the Chinese hamster lung cell line (V79); and the mutagenicity of the extract in prokaryotes. The nicotine dosage was made in CG-EM Agilent® and the concentration of the inorganic elements was determined by the PIXE technique. Evaluation of the cytotoxicity was performed by MTT test, using the concentrations 0.625 mg/mL-7.5 mg/mL. Genotoxic damage was assessed through alkaline comet assay with the concentrations of 0.625 mg/mL-5.0 mg/mL. V79 cells were cultured under standard conditions and exposed for 3 hours with the different concentrations of the aqueous extract. Mutagenicity was assayed using Salmonella /microsome assay (strains TA98 and TA100). The nicotine concentration found in the tobacco leave powder was 1.56 mg/g. Were detected 13 different inorganic elements, several of them considered heavy metals, such as titanium, strontium, manganese, aluminum and iron. The results found in the MTT test showed a viability > 90% in all concentrations. The alkaline comet assay showed a significant increase in DNA damage for all concentrations when compared to the negative control ($p < 0.05$). All concentrations of the aqueous extract showed no mutagenicity to *Salmonella typhimurium* strains. Our results demonstrate DNA damage caused probably by a mixture of inorganic elements and nicotine. More studies are necessary for understand the mechanism responsible for cell damage.

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EVALUATION OF GENOTOXICICITY OF ORGANOPHOSPHORUS TRICHLORFON

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Key-words: Trichlorfon (TCF), agrochemicals, DNA damage, DNA repair enzimes.

Many natural environments are overloaded with physical, chemical and biological stressors that can affect biodiversity. Among these the organophosphates (OP) are agrochemicals widely used as pesticides because of their high toxicity. Triclorfom (TCF) is a class II OP (highly toxic) with insecticidal, acaricidal and anthelmintic properties. However, its use can affect the health of non-target organisms, including humans. The objective of this work was to verify the interaction of Trichlorfon with the genetic material through the quantification of DNA lesions. For this purpose the pCMUT (~ 1.7 kbp; C - chloramphenicol resistance; MUT - supF gene, target for mutagenesis studies) vector was used as the biomonitor. Plasmid DNA was exposed to 5 µg/L of Trichlorfon (Trichloron Pestanal 250mg, 98,4% purity, CAS-No 52-68-6, Sigma-Aldrich Brazil Ltda.) over a period of 1, 4, 8 and 24 hours, kept at room temperature and in the dark. Then, we decided to increase Trichlorofon concentration to 5, 50 and 500 µg/L to perform 24 hours incubation of plasmid DNA samples under this condition. After treatments, plasmid DNA samples were digested with the DNA repair enzymes Endonuclease III (Endo III - which recognizes oxidized pyrimidines) and Formamidopyrimidine-DNA glycosylase (Fpg - which recognizes oxidized purines). After enzymatic digestion the DNA samples were submitted to 0.8% agarose gel electrophoresis. The bands formed on the gel were analyzed and quantified by the densitometry (Image Quant 300, GE Healthcare, USA). In addition to the evaluation of oxidative damage, simple DNA breaks (SSB) were also quantified. Our results clearly indicate that TCF did not induce any type of oxidized DNA bases or SSB, regardless TCF concentration or time of exposure of DNA to this chemical compound. Therefore, this result indicates that TCF does not induce oxidative damage in DNA, as well as breaks in DNA backbone. However, it is important to recognize that there is the possibility of another type of DNA damage, not recognized by the enzymes used, being formed by TCF, which could not be detected by our technique. The following perspective of this study is to evaluate the toxicity of TCF through in vivo experiments performed with amphibians.

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ACUTE TOXICICITY AND GENOTOXIC EFFECT CAUSED BY FORMALIN IN *Danio rerio*: ASSESSMENT OF SECURE CONCENTRATIONS FOR PROFILATIC USE IN AQUACULTURE

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Key-words: formalin; formaldehyde; acute test; micronucleus, zebrafish

Formalin is one of the most commonly used fungicides in aquaculture and has a variety of applications because it is highly reactive, colorless, stable, pure in commercial form and low cost. It is widely used in treatment of diseases caused by fungi and parasites. Formalin is an aqueous solution with 37% to 40% formaldehyde is volatile and irritant and causes cancer in laboratory rats and may cause contact hypersensitivity and lung damage in humans. It is for this reason that as ideal concentrations and possible formalin residues that can be released into the aquatic environment. *Danio rerio* popularly known as zebrafish is a species of fish with internationally renowned aquarists for use in ecotoxicological trials. It has good capacity to withstand great variations of temperature, pH and water hardness and demonstrate an excellent sensitivity to high number of chemical substances. Thus, the objective of this study was to verify the toxic and genotoxic effects of the formality and to determine lethal concentrations of the chemical, to subsidize its safe use in disinfection processes in commercial fish farms. Therefore, it uses acute and chronic tests using ASTM (American Society of Testing and Materials), APHA (American Public Health Association) and ABNT (Brazilian Association of Technical Standards) methodologies. To verify the potential of the genotoxic effect we used the Micronucleus test performed on erythrocytes of the peripheral blood of zebrafish collected during the chronic test at 0h, 96h and 192h of exposure. The formaldehyde $LC_{50\text{-}96h}$ for *D. rerio* was 45.73mg.L^{-1} demonstrating higher resistance of this animal when compared to other species. This value is close to concentrations in fish disease treatment baths. Sublethal concentrations of formalin showed a positive correlation with micronuclei according to the increase in its concentration independent of the time of exposure. The incidence of micronuclei increased with concentration, and the addition of 1 mg L^{-1} formalin corresponded to an increase of 2.9% in the average number of micronuclei. We concluded that this chemical even at chronical concentrations caused mortality and genotoxic effects over time in animals. We therefore recommend further studies and other tests involving this chemical for the use of environmentally safe concentrations.

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MUTAGENICITY ASSAYS AND WATER QUALITY INDEXES: AN INTEGRATED APPROACH

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Water quality monitoring programs are designed to obtain quantitative and representative information on the physical, chemical, and biological characteristics of a water body over time and space. CETESB's Water Quality Program, established in 1974, comprises nowadays more than 400 sampling locations, and includes chemical, microbiological, hydrobiological, and toxicological analyses of surface water samples. Quality indexes are calculated, such as the Water Quality Index (IQA) with 9 parameters: fecal coliforms, pH, biological oxygen demand, total nitrogen, total phosphorous, temperature, turbidity, total residues and dissolved oxygen. The Water Quality for Public Supply (IAP) includes the IQA parameters along with THM, cyanobacteria cells, Cd, Pb, Cr, Hg, Ni and substances that affect the organoleptic quality of the water. In both indexes, each sample receives a value from 1 to 100 which allows classifying them on a scale that goes from very poor to very good quality. The present study intend to correlate the results obtained with the genotoxicity assays using the minimal effective dose (MED 1.5) and the classification obtained with the IQA and IAP indexes. The Salmonella/microsome test and induction of micronuclei in V79 cells were used to evaluate genotoxicity in 31 different sites, where waters are used as supply to water treatment plants. The comparative analyzes indicate that the results obtained in the mutagenicity tests show weak correlation with the quality indexes. About the IQA, most of the mutagenic activity was detected in samples that were classified as good for the index, and for the IAP the mutagenic activity is more equally distributed in all categories. This integrated approach is important to provide an overview into the water quality and the responses of different monitoring tools. The results reinforce the importance of this approach and indicate that to consider only the quality indexes to classify the water quality do not contemplate the presence of genotoxic activity in the aquatic environments and the inclusion of these variables in a quality index calculation could be considered as a new approach.

EVALUATION OF CELL VIABILITY AND IN VITRO GENOTOXICITY OF PIROXICAM IN VERO CELLS

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Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) that belongs to the oxicam class which is prescribed to several inflammatory diseases, such as rheumatoid arthritis, primary dysmenorrhoea and endometriosis. Its anti-inflammatory properties are well known, and it is related to its non-selective capacity for reversible inhibition of COXs (cyclooxygenase). Nonetheless, little is known about its genotoxic activity in non-neoplastic renal cells. These effects can be monitored for the prevention and control of adverse reactions and side effects. The present study investigated the cellular viability, by the MTT colorimetric assay, and the possible genotoxic effects induced in vitro by Piroxicam in African green monkey kidney cell line (VERO), using the comet method (alkaline version). The results of the cell viability test after 24 h of treatment with Piroxicam significantly reduced ($p < 0.05$) cell viability in 1.0 mM, 2.0 mM, 4.0 mM and 8.0 mM, demonstrating that the survival rate reduced in a concentration-dependent manner, and attained a decrease up to 70% in the greatest concentration, that entailed the IC 50% of 0.5mM. DNA damage index (ID) evaluated by the comet assay (0.71 ± 0.20, 0.71 ± 0.16 and 0.48 ± 0.21 for the concentrations of 0.125 mM, 0.25 mM and 0.5 mM, respectively) did not differ significantly from the ID observed in the negative control (0.41 ± 0.18), what indicates that Piroxicam did not induce DNA damage. The cellular viability data corroborate previous results from the literature performed at different experimental models, and points that viability reduces out whereas the concentration increases. However, regarding to genotoxicity, the only data from this NSAID are described in very few studies where they were evaluated through basic techniques of chromosome analysis. Finally, the investigation of the cytotoxic and genotoxic behavior of NSAIDs in renal cells is important for biomonitoring, since this drug has been widely used over the years as an anti-inflammatory treatment and whereas cytotoxic and genotoxic aspects may impact the individual's health who need to take the drug.

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EVALUATION OF THE EFFECTS OF METABOLIC ACTIVATION ON THE MUTAGENICITY OF SYNTHETIC COMPOUNDS WITH ANTITUMOURAL POTENTIAL *IN VITRO*

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Teoneladine C is a 3-alkylpyridine alkaloid obtained from the marine sponge Theonella swinhoei. It presents interesting bioactive effects, including pro-apoptotic action in human tumour cell lines. In the present work, two synthetic analogues of this compound, THP-002 and OH-002, were evaluated for their cytotoxicity, genotoxicity and mutagenicity in vitro, with and without metabolic activation (S9 fraction). Studies were performed with the human ovarian carcinoma cell line ToV-21G. MTT assays were carried out to evaluate the cell viability after treatments with the synthetic compounds in experiments with and without S9. In addition, cytokinesis-block Micronucleus assays and Comet assays were performed with concentrations of 1.2, 2.3 and 3.5 µM of OH-002 and 0.35, 0.70 and 1.0 µM of THP-002. Cells of the positive control group were treated with methyl methanesulfonate, and cells of the negative control were treated with PBS. Cells were exposed to treatments for 3 hours. The MTT assay results demonstrated that both THP-002 and OH-002 presented lower IC_{50} values in experiments with S9 in comparison to studies without S9. In addition, the Micronucleus assay results showed that the compound THP-002 was mutagenic with and without S9. However, after metabolic activation, mutagenicity was observed at lower concentrations. Complementarily, the compound OH-002 was mutagenic only after metabolic activation, demonstrating that it is an indirect mutagenic agent. Data obtained from the Comet assays revealed a probable mechanism of action of these synthetic alkaloids. In this assay, in any of the conditions employed, the compounds induced chromosomal breaks at statistically significant levels ($p > 0.05$) when compared with negative controls. Therefore, the mutagenicity observed in micronucleus assays may be correlated to chromosomal losses, and not to DNA strand breaks. We previously demonstrated that these compounds present selectivity against colon cancer cells, reinforcing the potential of these analogues to be prototypes of anticancer agents. Nevertheless, complementary studies are necessary to better elucidate the mechanism of action proposed herein and the pharmacological potential of these compounds in other biological systems.

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EVALUATION OF TOXICITY OF ATRAZINE AND 2,4 D COMPOUNDS USING *Saccharomyces cerevisiae*

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Key-words: yeast, pesticide, bioindicator

Brazil is among the countries that use pesticides the most in the world. It is also one of the largest food producers, with legislation that classifies and defines the parameters for the environmental quality of surface water, such as the Resolution of the National Environmental Council n° 357/2005. In order to comply with the legislation some analyzes take time. In this sense, microorganisms can be used in different types of tests such as toxicological, genotoxic and mutagenic, since they have advantages in relation to the cost, time and consumption of reagents. Considering that the cells can be sensitive to the time of exposure and the concentrations of the pesticides, providing an evaluation of the effects of the contaminants at the cellular level. Yeasts can be used as a bioindicator because they have a high degree of homology with human eukaryotic cells, being easy to cultivate and manipulate and are sensitive to different chemical compounds present in the environment. Thus, this study aims to evaluate the cytotoxicity of Atrazine and 2,4 D pesticides in *Saccharomyces cerevisiae*. For the mortality rate test, 0.1 g of lyophilized Fleischmann™ yeast was added in a solution containing 20 mL of ultrapure water, 2 g of sucrose and the concentrations of (0.5; 1.0; 2.0; 3.0; 4.0; 5.0) of the pesticides that remained incubated at 28 ° C for 30 and 60 minutes at 250 rpm. The control was carried out under the same conditions without addition of pesticides. After the incubation period, 10 µL of the samples were removed, and 90 µL of the methylene blue dye was added and the cells counted in Neubauer's chamber. The results showed that the yeast when exposed to Atrazine at a concentration of 0.5 µg L^{-1} in 60 min showed a 2.8% increase in the mortality rate in relation to the 30 min time for the concentration of 5.0 µg L^{-1} the increase was around 6.5% of dead cells. For the 2,4 D a similar behavior was observed, however the mortality rate was more expressive, in the concentration of 0.5 µg L^{-1} the increase in relation to the times of 30 and 60 min was of 7.5% and in 5.0 µg L^{-1} of 11.2% respectively. The 2,4 D pesticide presented a higher percentage of cytotoxicity for the yeast Fleischmann™, in relation to Atrazine. With this, the yeast studied has a potential to be used as a bioindicator.

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ANALYSIS OF FLEISCHMANN™ YEAST SENSITIVITY AGAINST PESTICIDE 2,4 D

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Key-words: *Saccharomyces cerevisiae,* metabolism, biotechnology

An important field of research in the biotechnology area is related to the use of microorganisms with specific characteristics both for obtaining products and for employment in various industrial processes. Yeasts have been extensively studied, mainly *Saccharomyces cerevisiae*, because through their metabolism can be to produce numerous compounds, because this microorganism has high capacity of biotransformation. These microorganisms also have several advantages such as being of rapid propagation, easy conservation and not being pathogenic. In this sense, yeasts can be used as a bioindicator, mainly for the detection of toxic substances present in the environment. This is because bioindicators are instruments that can measure a certain anthropic or natural factor that may impact and stress the environment or a particular area. Thus, the study aims to evaluate the sensitivity of the Fleischmann™ yeast in the presence of the 2,4 D pesticide. For the pre-inoculum, 0.1 g of the yeast was used in the YPD 2% liquid medium containing 1% (p v^{-1}) yeasts extract, 1% (w v^{-1}) of peptone and 2% (w v^{-1}) of dextrose and incubated at 28 °C for 10 hours at 160 rpm. The antimicrobial activity was performed by the solid-media disc diffusion method, the sample was suspended in saline solution according to the McFarland 0.5 scale and 100 µl of the sample were spread on the Petri dish containing 2% solid YPD medium with aid of a Drigalski handle. The 6 mm sterile filter paper disks were impregnated with 10 µL of the concentrations (0.5; 1.0, 2.0, 3.0, 4.0 and 5.0 µg L^{-1}) of the 2,4 D compound and applied to the surface of the medium and incubated at 30 °C for 48 hours and the negative control was prepared under the same conditions using ultrapure water. In this study the experiments were carried out in triplicate and the action of the pesticide in the yeast was performed through the disc diffusion test and being classified as sensitive (+) and resistant (-). Cell growth was analyzed for the presence or absence of inhibition halo. The yeast did not show inhibition halo at concentrations of 0.5 to 4.0 µg L^{-1} demonstrating resistance, however, at the concentration of 5.0 µg L^{-1}, sensitivity to 2,4-D pesticide occurred under the conditions analyzed. We can conclude that this compound had a cytotoxic effect on the Fleischmann™ yeast, since this microorganism was shown to be sensitive to a higher concentration.

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CISPLATIN SENSITIZATION BY VIOLACEIN EXTRACTTED FROM ANTARTIC *JANTINOBACTERIUM* IN HELA CELLS

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Cervical cancer is the fourth most common cancer in women around the world, and the seventh overall. Also it is the second most common cancer in women in less developed regions and the fifth cause of death by cancer in these countries. Cisplatin (CDDP) is used as a systemic treatment for advanced, persistent or recurrent cervical cancer, but its high toxicity and production of unwanted side effects (severe kidney problems, allergic reactions, decreased immunity and propensity to infections, gastrointestinal disorders, bleeding and hearing loss, among others), besides the development of resistance to CDDP, turn necessary the search of new drugs with greater therapeutic efficacy and/or fewer side effects. Violacein, a pigment produced by many bacterial strains, has demonstrated its antiproliferative activity in several derived cancer cell lines, therefore, it is important to improve its biotechnological production process. In this respect, it has been optimized the Violacein production from an Antarctic *Jantinhobacterium*, through the evaluation of different temperatures and growth bacteria medium conditions. First we determined the growth temperature for Violacein maximal production, confirming that Violacein should be produced at 20°C. Besides, we evaluated the conditions to obtain the highest yield of Violacein using different amount of TSB media (3 g/L, 15 g/L and 30 g/L) and different potential inducers such as glycerol (1 and 5 %), tryptophan (10 and 25 mM), sodium butyrate (10 mM and 25 mM), Tween 80 (1 and 5 %) and glucose (1.8 and 3.6 g/L), at 20°C and 200 rpm (initial OD of 0.05 at 660nm). Firstly, the temperature and grow medium were selected (3 g/L TSB plus glucose 3.6 g/L), which increased 10.41 times Violacein production. Subsequently, through scaling up to a 5 L bioreactor we were able to obtain a final concentration of 77.3 mg/L. Finally, Violacein anti-proliferative activity in HeLa cells was evaluated reaching an IC 50% = 0.42 µM. Moreover, Violacein in combination with 5 µM CDDP decreased the IC 50% to 0.22 µM. On the other hand, the addition of 0.15 µM of Violacein to CDDP allowed the reduction of the IC 50% to 8.34 µM (being the IC 50% of CDDP alone 17.62 µM). Violacein showed an antiproliferative effect in HeLa cells, acting as well as a sensitizer of HeLa cells to CDDP. These results are promising in the use of Violacein as anticancer or sensitizer to CDDP in cervix cancer.

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AVALIATION OF THE CYTOTOXIC AND MUTAGENIC POTENCIAL OF ANTI-PRION AROMATIC CHEMICAL COMPOUNDS

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Prion, a protein in the form of scrapie prion protein (PrPSc) develops an aspect of pathogen and causes a group of fatal neurodegenerative diseases that are called Transmissible Spongiform Encephalopathies (TSEs). At the moment, there is no effective treatment or therapy for those diseases. A previous study selected a few compounds that gave promising results in some *in vitro* and *in silico* approaches against PrPSc. Our aim was to investigate the mutagenic and cytotoxic potential of anti-prion compounds J8, J20, J35, Y13 and Y17. The Ames test was used to determine the anti-prions mutagenic potential. *Salmonella enterica* serovar Typhimurium TA97, TA98, TA100, TA102 and TA104 strains were used at five concentrations (5, 10, 20, 40 and 80 µM) diluted in DMSO. The assays were done in triplicate and the mutagenic effect was considered positive in those concentrations that had the mutagenicity index (MI = induced revertants / spontaneous revertants) higher than 2. For cytotoxicity, HepG2 cell line viability was assayed for WST according to the protocol kit (WST-1), which consists of reducing the tetrazolium salt WST-1 in formazan by cellular dehydrogenases. The integrity of the cell membrane was evaluated by measuring release of intracellular lactate dehydrogenase (LDH). The LDH activity cytotoxicity and the WST assay were carried out according to the manufacturer's instructions. Data are presented as mean ± standard deviation (SD) of triplicate measurements. In the Ames test, a positive result was found for mutagenicity for strain TA97 to J35 (from 5µM), Y13 (since 5 at 40µM) and Y17 (from 20µM); for strain TA98 to Y13 (only 40µM); for TA102 to J8 (from 40µM), J20 (5 and 10µM), Y13 (5 and 10µM) and Y17 (from 10µM); and for strain TA104 to J20 (only 10µM) and J35 (5 and 10µM). For the WST assay, the compounds did not show cytotoxicity in any period of the assay. In the LDH assay, the compounds presented membrane toxicity only at 72h of exposure. The results in bacterial model indicate that the anti-prions induced more than one type of mutation. The LDH assay indicates membrane damage in long exposure time. Arrangements of the molecules may be required to decrease the adverse effects and maintain their efficacy.

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INFLUENCE OF THE LANDSCAPE ON THE WATER PHYSICOCHEMICAL AND GENOTOXIC PROPERTIES OF A LOW ORDER STREAM AT CENTRAL BRAZIL

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Investigating the impacts of land use on water quality in low order streams is an effective tool for protecting local streams and also for improving downstream water quality. In this study, we evaluated four points (P1 to P4) along the Extrema River located in an industrial and agricultural area of Central Brazil. We performed physical (temperature, turbidity, electrical conductivity, dissolved oxygen and pH) and chemical (trace metals, ammoniacal nitrogen and orthophosphate) analysis in the water collected along these four points and correlated the landscape pattern with water physicochemical quality and genotoxic potential along the Extrema River. Physical analysis suggested that the water collected in P1 and P3 is inappropriate for human consume and chemical analysis showed that Fe and Cr content were above the limits established by law at all points. Concerning cytogenotoxic analyzes, the water collected at P1 and P2 was able to increase the *Allium cepa* root growth ($p < 0.05$), suggesting the presence of substances with mitogenic activity, but no evidence of cytotoxicity was observed in all points according to the mitotic index (MI) results and compared with a negative control (distilled water). In contrast, it was observed a higher number of chromosomal and nuclear aberrations (CNA) at P3 when compared to the other samples analyzed ($p < 0.05$), which suggest the genotoxic potential of this water sample. In concern to the permanent genetic alterations, water collected at P2-P4 was able to induce the micronucleus formation in *A. cepa* cells ($p < 0.05$) indicating a mutagenic potential. The correlation of those results with the land use revealed that the water collected at P3 was the most impacted and the contamination occurs mainly by municipal and industrial wastewater, agriculture activities and exposed soil. Different result was observed for P2 and P4, where the land use analysis related the water quality with burns. We concluded that the different uses of the soil changed the characteristics of the water, creating a vicious cycle. The *A. cepa* parameters were most related to the landscape use characteristics than to the water quality parameters per se. It highlights the importance to associate the landscape pattern with the cytogenotoxic potential of the water that runs through that terrain. To our knowledge, this is the first time that this type of approach was used.

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HEMATOTOXICITY OF *Vernonanthura polyanthes* LEAVES AQUEOUS EXTRACT AND ITS FRACTIONS

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Vernonanthura polyanthes (Spreng.) A.J. Vega & Dematt., popularly known as assa-peixe, is widely distributed and consumed in Brazil. Although this species has its verified therapeutic efficacy empirically or scientifically, few studies investigated its chemical safety. In this sense, first we performed a phytochemical screening of the *V. polyanthes* leaves and obtained an aqueous extract and three fractions (aqueous, AqF; n-butanol, n-BF; and ethyl acetate, EaF) from this. Then, we quantified total phenols, flavonoids and tannins, and investigated the antioxidant activity using the DPPH free radical scavenging method in these materials. After characterizing the work material, the cytogenotoxic potential of the *V. polyanthes* leaves aqueous extract and its fractions was investigated against human erythrocytes and lymphocytes using Trypan blue exclusion test of cell viability and CometChip. The phytochemical screening of *V. polyanthes* leaves revealed the presence of phenolic compounds, flavonoids, tannins, coumarins, terpenic compounds, and cardioactive heterosides. n-BF presented the highest phenols (~ 33%), flavonoids (~ 20%), and tannins (~ 33%) content and, consequently, the highest antioxidant activity (IC_{50} = 0.074 mg/ml). Although *V. polyanthes* leaves aqueous extract and its fractions did not cause death of erythrocytes, the cells acquired an echinocytic form. The lymphocytes demonstrated more sensibility to the treatments used in this work. *V. polyanthes* leaves aqueous extract and its fractions presented a concentration-dependent lymphotoxicity, higher than the positive control doxorubicin (DXR), being the EaF the most toxic treatment. *V. polyanthes* leaves aqueous extract and its fractions also presented lymphogenotoxicity, similar to what was observed for DXR. When *V. polyanthes* leaves aqueous extract or its fractions were co-treated with DXR, we observed that *Vp*LAE and its fractions enhanced the DXR lymphotoxicity in a concentration-dependent manner, but decreased the DXR lymphogenotoxicity. In this way, the indiscriminate use of assa-peixe by the population is not recommended, because of the toxic potential of *Vp*LAE and the possibility of *Vp*LAE to interact with DXR, an allopathic drug.

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SYNTHETIC THYOPHENES INDUCE CHROMOSOMAL DAMAGES AND CELL DEATH IN HUMAN CANCER CELL LINES

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Thiophenes are heterocyclic compounds containing a five membered ring with a sulfur as the heteroatom. This class of molecules has several biological activities, including antiproliferative effects against specific cancer cell lines. However, the selectivity and action mechanisms of these compounds are variable according to their molecular structures. The aim of the present study was to evaluate *in vitro* the cytotoxic, genotoxic and mutagenic potentials of synthetic thiophenes using cell lines derived from human solid tumors (ToV-21G (ovary carcinoma) and RKO-AS45-1 (colon carcinoma)). Different methodologies were employed to assess the cytotoxic effects of the compounds here named SB44, SB83 and SB200 (MTT assay, clonogenic assay and Annexin V staining assay) and to elucidate the chromosomal damages possibly evolved in cell death triggering (Micronucleus assay, Comet assay and γ-H2Ax assay). To assess the selectivity of the compounds, results obtained to the non-tumoral cell line WI-VA4 was used as reference. All thiophenes assessed interfered in cell survival, regardless of concentration and time of treatment. However, the compound SB200 presented an interesting Selectivity Index to the tumoral cell lines employed. In same way, all compounds evaluated caused chromosomal damages *in vitro*. The compounds SB83 and SB200 were the most promising as prototypes of antitumor agents as they demonstrated the ability to induce chromosomal mutations and lesions in the DNA of the cancer cell lines employed. The set of results obtained indicated that the compounds induced cell death by apoptosis as result of chromosomal damages. Taken together, the data suggest that the thiophenes tested herein may represent important prototypes in the development of new drugs for anticancer treatment.

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EVALUATION OF COAL GENOTOXICITY IN GASTROPODS EXPOSED IN THE COAL MINING AREA (CANDIOTA)

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Coal mining and its firing are basic sectors of the country's economy. However, the mineral coal presents a heterogeneous mixture of organic and inorganic compounds that present a high level of toxicity capable of causing serious environmental impacts. Thus, the objective of this work was to evaluate the potential genotoxic effect in hemolymph cells caused by exposure to mining and burning of opencast, using the gastropod *Helix aspersa* as a bioindicator. Twenty individuals were exposed in two places in the city of Candiota, being under direct influence of the emissions of the Thermoelectric Plant President Médice and 10 individuals were not exposed (control group) remaining in the laboratory in controlled environment. The points were chosen due to the preferential direction of the winds. After 7 days of exposure, hemolymph cells were collected for the evaluation of the genotoxicity performed through the comet assay, and pulmonary cells collected for quantification of inorganic elements through the PIXE technique. The results showed that the animals exposed from site 1 (P1; Damage index= 43.57 ± 26.9) and site 2 (P2; Damage index= 61.10 ± 29.27) near the thermoelectric showed a significant increase of genotoxic damages in relation to the control group (Damage index= 20.80 ± 14.27) ($p< 0.05$; ANOVA- Tukey). PIXE demonstrated significant increase of inorganic elements levels: Al were detected for the group P1; and Al, Si, Ca and Zn for the group P2. It is suggested that the inorganic elements present in the lung cells of the mollusks exposed to the carboniferous areas may be correlated to the damages caused to the DNA. It is believed that the highest damage levels presented by the P2 group are due to the fact that this site is located in the preferential direction of the winds. We can also observe that the *H. aspersa* can be a good indicator in the detection of bioaccumulation of inorganic particles originating from coal mining and burning.

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OXIDATIVE STRESS IN *Limnoperna fortunei* TO ZINC OXIDE NANOPARTICLES EXPOSURE

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Introduction: Nanomaterials (1 to 100 nm) exhibit high surface area and small volume, resulting in high reactivity potential and unique physical and chemical properties that differ from those of their respective bulk materials. Zinc oxide nanoparticles (ZnO-NP) have been used in wastewater treatment application, molecular biology, cosmetic, sunscreen, as an additive, food industry, paints and construction materials. Consequently, these industrial uses increases the potential for their release to the environment, causing ecotoxicological problems. In this context, the implications of ZnO-NP for human and environment health should be assessed. This study aimed to evaluate the effects of ZnO-NP on the antioxidant enzymes superoxide dismutase (Sod) and catalase (Cat) profiles in a biomonitor organism golden mussel *Limnoperna fortunei. Material and Methods:* The ZnO-NP was characterized and ZnO-NP exposure was performed at concentrations of 1, 10 and 50 µg mL^{-1} for 2, 4 and 24 h. After these periods soft bodies was processed and Sod and Cat activities measured. *Results/Discussion:* ZnO-NP size analysis demonstrated an average size of about 50 nm. In biological experiments, the results showed that ZnO-NP destabilizes the cell redox system of Golden mussels. The reactive oxygen species generation induce the antioxidants enzymes activity Sod and Cat. Antioxidant activities of Sod and Cat enzymes were significantly increased under after 24 h exposure. ZnO-NP could be generating high levels of superoxide anion radical ($O_2^{\bullet -}$), thus increasing redox imbalance in mussel cells. At 4h ZnO-NP exposure, the effective enzymatic antioxidant response starts the activity of Sod and Cat enzymes, but with no statistical difference. *Conclusions and Acknowledgments:* The exposure to ZnO-NP confirmed the sensitivity of the Golden mussel to detect the redox imbalance induced by these nanoparticles by assessment of the activities of Sod and Cat enzymes. The results confirm the ZnO-NP can cause alterations on redox metabolism of Golden mussel and this organism can be used as a potential biomonitor organism for ZnO-NP in the fresh water compartment.

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RETENE, A NON-PRIORITY HYDROCARBON, IS ABLE TO GENERATE OXIDATIVE STRESS, MUTAGENIC EFFECTS, AND CELL DEATH

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Key-words: Retene, Biomass burning, *Salmonella* microsome assay, Necrosis

Retene (1-methyl-7-isopropylphenanthrene; RET) is the most abundant polycyclic aromatic hydrocarbon (PAH) released upon the burning of cellulose, although it is not considered as one of the priority PAHs and is not included for risk assessments by the US Environmental Protection Agency (US-EPA). Despite its abundance, there are only a few studies elucidating the toxic effects of RET. Therefore, the aim of this work was to evaluate whether RET plays a crucial role in the induction of oxidative stress through the generation of reactive oxygen species (ROS) by showing mutagenic activity *in vitro* and, in cell death processes (apoptosis and necrosis). Thus, human lung adenocarcinoma A549 cells were treated with different concentrations of RET as follows: 3.3 ng/mL, 10 ng/mL and 30 ng/mL for 24 and 72 hours. The oxidative stress analysis was performed using flow cytometry. In addition, cytokinesis-block micronucleus assay (CBMN) was adopted to verify the frequency of micronuclei (MN), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs). Cell death was investigated by labeling Annexin V and propidium iodide (PI). Traditional plate-incorporation *Salmonella* mutagenicity assay was performed to evaluate the RET mutagenicity using the *Salmonella* strains TA98, TA100, TA97a and TA102, with or without activation metabolic (S9 mix). Our results revealed that RET was able to hyperpolarize the mitochondrial membrane and increase the contents of mitochondria, owing to an increase in the production of mitochondrial superoxide and intracellular ROS. Moreover, RET does not induce base-pair substitution (TA100) and frameshift (TA98 and TA97a) and transition/transversion (TA102) mutations. However, treatment with RET led to a significant increase in the frequency of the formation of MN, NPBs, and NBUDs. There was no significant difference in the viability of cells exposed to RET for 24 hours. In contrast, 72 hours of treatment with RET was adequate to decrease viability, mainly due to necrosis. Taken altogether, the results of our study provide new evidence suggesting that RET promotes oxidative stress, contributes to the processes of genomic instability and favors necrosis.

Thus, we highlight the importance of including RET in routine environmental analyses in the future as a potential risk factor involved in complex diseases and carcinogenesis. Studies concerning the ability of RET to transform cells are being conducted.

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CYTOTOXICITY OF THE RADIOPHARMACEUTICAL ^{18}F-FLUORODEOXYGLUCOSE ON HepG2/C3A CELLS

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Key-words: ^{18}FDG, nuclear medicine, radiodiagnosis, cell cycle, flow cytometry.

Developments in the field of nuclear techniques and the scientific knowledge of radiation have made it possible to use radioisotopes in industry, agriculture and medicine. The radiopharmaceutical ^{18}F-fluorodeoxyglucose (^{18}FDG) has a half-life of 109 minutes, and the radioactive decay removes electrons of the atoms, resulting in the emission of high energy photons (511keV). It is widely used in the imaging diagnosis of metastasis and several types of cancer, such as liver cancer. Because it is a source of ionizing radiation, it can interact directly with cellular components, causing damage to cells and DNA. Thus, the aim of this study was to evaluate the cytotoxic potential and possible cell cycle arrest induced by ^{18}FDG in human hepatoma cells (HepG2/C3A). Cells were treated with ^{18}FDG at equivalent doses of radiodiagnosis (0.15 μCi/mL), ten and one hundred times greater, in addition to a control group and a treatment with the cytotoxic agent methylmethanesulfonate (75 μM) for 24, 48 and 72 hours, in the MTT assay. For plasma membrane integrity and cell cycle analyzes by flow cytometry, the cells were treated for 24 hours with ^{18}FDG, in addition to a control group, and a treatment with ^{18}FDG 500 μCi as a positive control, and then labeled with propidium iodide. The experiments were performed in three biological replicates and the data were submitted to analysis of variance (ANOVA), followed by Dunnett's test. The results showed that ^{18}FDG presented cytotoxic activity at 15 μCi, in 72 hours, significantly reducing the absorbances when compared to the control. Analysis of plasma membrane integrity by flow cytometry indicated no cytotoxic effect, at the time evaluated. However, cell cycle results showed that 1.5 and 15 μCi significantly increased the Sub-G1 population of cells, possibly by increasing the frequency of apoptosis. These preliminary results suggest that the mechanisms of toxicity may be related to the increase of reactive oxygen species produced as a result of radioactive decay and as a function of the time of exposure.

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CYTOTOXIC AND GENOTOXIC EVALUATIONS OF POLYETHYLENE MICROPLASTICS IN *Danio rerio* (ZEBRAFISH)

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Key-words: plastic debris, microplastic, ecotoxicology

Uncontrolled consumption and inadequate disposal of plastics has caused a wide aquatic contamination by microplastics, becoming a huge environmental problem around world. According to the Brazilian Association of the Plastic Industry (ABIPLAST), polyethylene is one of the most used for different purposes. They are found in bags, packaging and household utensils. Microplastic debris are particles smaller than 5 mm which easily reach aquatic environment through cosmetic products or through the fragmentation of larger plastic materials. Microplastic can be ingested by animals of different trophic levels, reaching humans through the consumption of contaminated fish and seafood. In this study, we evaluate the polyethylene microparticles for genotoxic effects in *Danio rerio* (zebrafish). Polyethylene microparticles were commercially purchased from Cospheric® and measured with 60 µm. Zebrafish adults (n=7) were exposed for 96 h to polyethylene microplastics at concentrations of 12.5 $mg.L^{-1}$, 50.0 $mg.L^{-1}$ and 100.0 $mg.L^{-1}$ following the OEDC protocol n. 203. Blood samples were collected with a heparinized pipette and stored in microtubes containing 500 µL of fetal bovine serum, where 40 µL was used for the comet test and 50 µL for the micronucleus test. The comet test did not detect statistically significant DNA damages after exposures to microplastics ($p > 0.5$) when compared to the control group and Tween 80% group. Exposure at 12.5 $mg.L^{-1}$ of microplastics increased the frequency of nuclear buds ($p<0.05$) compared to the control group (Tween 80%), which means nuclear abnormalities in peripheral erythrocytes. There were not observed micronucleus induction ($p>0.5$). These results showed more cytotoxicity than genotoxicity. Further tests for cytotoxicity and genotoxicity with these polyethylene microplastics are running in other aquatic organisms to a better understanding in an ecogenotoxicological context.

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GENOTOXIC POTENTIAL OF TAQUARI RIVER SEDIMENTS IN AREAS OF WATER INTAKE FOR PUBLIC SUPPLY

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Key-words: organic extracts, mutagenesis, Ames test

The release of residues into the environment may generate changes in water sources, impairing essential uses such as water intake for supply. These agents, including the genotoxics adsorbed in the suspended particulate matter , are deposited in sediment over time. This compartment may act as a source of pollutants released during natural or anthropic disturbances. The study evaluated the presence of mutagenic compounds in organic extracts of Taquari River sediments, selecting sites for drinking water intakes. The areas sampled were: Ta063, Bom Retiro do Sul; Ta032, Taquari; Ta011, Triunfo; Ta006, General Câmara. The presence of these agents was evaluated in the Salmonella/microsome assay, microsuspension method, in the absence or presence of the liver metabolization fraction or rat liver (S9). Strains were used to measure basic molecular damage (frameshift error (TA98; TA97a; YG1041) and DNA base pair substitution (TA100; YG1042). The organic compounds (moderately polar) were extracted by ultrasound, concentrated in rotavapor (40°C) and stored at -20°C. Mutagenesis was analyzed in the non toxic linear portion of the eight dosages curves (2.5-80µg equivalent to dry mass of sediment) Direct mutagenic action (-S9) was observed at Ta032, and was more significant in strain TA100 (4244±881) and indirect action (TA97a+S9) was detected in Ta063 and Ta011 (25±12.9 and 16±6.2, respectively). At site Ta032, where higher values were found, it is used for tourism/leasure, fishing, water transport, extraction of sand/rocks and irrigated agriculture. The extracts that presented mutagenic activity in direct assays in the basic strains (TA98 snd TA100), were evaluated in the derived strains YG1041 and YG1042, selected for the presence of enzymes that raise sensibility for the detection of nitrocompounds. Mutagenesis analyzed under the same conditions and dosages as the parental strains showed non-significant responses, indicating that the activity observed was not the result of this class of compounds. The presence of toxic and genotoxic compounds at these sites on the Taquari river has been observed in various studies. Thus the continuation of the diagnosis in areas meant for the intake of drinking water will enable conclusions on the influence of the quality of the sources in the purity of water supplied to the population.

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RESEARCH ON THE GENOTOXICITY OF YERBA MATE (*Ilex paraguariensis A. St-Hil.*) FRUIT EXTRACTS

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Key-words: ecotoxicology, mutagenicity, S*almonella*/microsome assay

Vegetal extracts may be used as alternatives for pest control, thus justifying ecotoxicological studies. The immature fruits of yerba mate contain secondary metabolism substancies, outstanding among which are saponins present the highest content (12%) compared to the leaves and mature fruit. Saponins are bioactive molecules with fungicidal, antiviral and other properties. Although there is evidence of a lower relative risk of vegetal biocides, the effects on other biological systems must be delimited. In the present study the genotoxicity of extracts of the immature fruits of yerba mate was verified using the *Salmonella*/microsome assay. *Extraction:* the fruit were dried in an oven (≤40°C) and triturated. *Decoction* of the resulting powder was performed [1:10(g/ml)] in distilled water (2X) (100°C); afterwards the content was cooled, filtered and lyophilized. In the butanolic fraction the resulting powder was macerated in 70% alcohol, evaporated (40°C) and fractioned with increasing polarity solvents: cyclohexane, ethyl acetate, *n*-butanol; then the BuOH fraction was evaporated (40°C) and lyophilized. *Salmonella*/microsome assay, microsuspension method, the strains were used to measure basic molecular damage, as mutation by frameshift error (TA98) and mutation by DNA base pair substitution (TA100), in the presence or absence of a hepatic metabolization fraction of rat liver (S9). Mutagenesis was analyzed in the non-toxic linear portion of dose-response curve at the following concentrations: 0.1; 0.5; 1; 1.5; 2; 3; 4; 5; 10; 15 mg^{-1} for both extracts. The results indicated mutagenesis in strain TA98 for both extracts, in the decoction fraction in the absence of S9 (6.6±3.04 rev/mg^{-1}) and in BuOH fraction in the presence of S9 (3.8±2.12 rev/mg^{-1}). Cytotoxicity was observed for both strains, based on the 4mg and 3mg doses for the decoction fraction and 5mg and 8mg for the BuOH fraction, in +S9 (indirect mutagenic action) and -S9 (direct mutagenic action), respectively. The extraction methodologies used enabled removing different mixtures of molecules that influenced the mutagenic and cytotoxic response observed. The butanolic fraction presented less intensity of the toxic-genetic effects. New analyses are currently being performed.

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THE DRUG ORLISTAT PRESENT CYTOTOXICITY AND GENOTOXICITY IN HUMAN TUMOR CELLS IN VITRO

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Key-words: drug repurposing, antitumor activity, Xenical, antiobesity, comet assay.

Cancer is a collection of related diseases characterized by the uncontrolled growth and development of cells. Recently, with the increase in life expectancy in many countries and the nowadays lifestyle, the number of cancer cases has reached unprecedented levels, and becomes one of the main causes of death worldwide. Based on that, pharmaceutical industry has made great efforts on the search of new effective anticancer molecules. Regardless of these investments, anticancer therapy remains a remarkably challenging field, and novel fully effective options are highly desirable. One promising approach in the search of new medicines is drug repurposing, named as the discovery of new uses for old drugs, previously applied on the treatment of different diseases. Thus, the present work aimed to investigate the cytotoxic and genotoxic effect of Orlistat, produced by *Streptomyces toxytricini*, which has the property to prevent the action of lipases from the intestinal tube and, therefore, to reduce the absorption of fats, currently used in treatment of obesity, in human breast adenocarcinoma cells (MCF-7) and renal adenocarcinoma cells (786-O). To assess whether the compounds affect cell viability, the MTT tetrazolium salt reduction assay was conducted, regarding the genotoxicity, DNA damage was analyzed by comet assay, for both tests cells were treated with Orlistat concentration varying from 0.01 to 100 µg/mL. Untreated cells were maintained as negative controls, doxorubicin and methyl methanesulfonate were used as positive controls of damage in MTT and Comet assay, respectively. Our results indicated that Orlistat present cytotoxic effect against MCF-7 cells already at 1 µg/mL treatment after 48 hours, and at 100 µg/mL after 48 and 72 hours; for 786-O cell line, the drug was cytotoxic at 10 and 100 µg/mL in all times tested (24, 48 and 72 hours). In what refers to the comet assay, Orlistat induced DNA damage in both cell lines (786-O e MCF-7) at the concentration of 1 and 100 µg/mL, after 3 hours treatment. Taken together, our findings are an initial indicative that Orlistat possesses promising antitumor activity, once it presented cytotoxic and genotoxic effect against breast and kidney human cancer cell lines. Once it is an already approved drug, broadly used by population with quite low side effects, it is a great candidate for further repurposing studies on the development of a novel anticancer therapy.

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INVESTIGATION OF CYTOTOXICITY AND GENOTOXICITY OF THE ANTIDEPRESSANT VORTIOXETINE IN VITRO

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Key-words: Brintellix, DNA damage, HepG2/C3A cells, MTT, Comet assay.

Depression is a common mental disorder known by affecting a person's mind, behavior and body. It is a multifactorial disease, which affects globally more than 300 million people of all ages, identified as one of the leading causes of disability worldwide and recognized as one of the main causes of mortality in Western countries. Regarding the treatment, effective psychological and pharmacological approaches are available for depression management. Although, it is important to consider that only half of the affected people in the world receive proper treatments, possibly due to low economical resources, lack of trained healthcare staff, misdiagnosis, or even stigma-related issues of mental illnesses. Despite a range of pharmacological treatment options, there is still need of new active molecules, better in terms of efficacy and tolerability. One of the latest available drug is vortioxetine (Brintellix®), a second generation antidepressant, approved in Brazil by Anvisa in 2016. Its mechanism of action in not fully understood, but is claimed to be novel with suggested multimodal activity. It is believed that vortioxetine acts on the direct modulation of serotonergic receptor and inhibition of the serotonin transporter, therefore providing an alternative to existing antidepressant drugs. Mostly used in long-term treatments, many antidepressants have been already reported as potential genotoxic and carcinogenic agents. Thus, the present study aimed to evaluate the cytotoxic and genotoxic effect of Vortioxetine in HepG2/C3A cells (human hepatocarcinoma) *in vitro*. To assess whether the compounds affect cell viability, the MTT tetrazolium salt reduction assay was conduct, and the Comet assay was applied to identify genotoxicity, identified as a primary DNA damage. Vortiexetine was tested in different concentrations, 1.5, 3 and 6 µg/mL, chosen from the plasma concentrations of daily oral doses of 5, 10 and 20 mg used in the clinics. Our results indicated that the medicine did not reduce cell viability and neither induced DNA fragmentation in the treated groups when compared to the controls, even at the higher concentration tested. Further studies should be conduct to assess the toxicity of the antidepressant in different cell lines; as well, different methodologies are indicated to detect possible DNA damage.

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MANJERONE OIL IN CULTURED HUMAN LYMPHOCYTES: A CYTOTOXIC APPROACH

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Key-words: marjoram oil, cytotoxicity, human lymphocyte.

Marjoram is a plant with a flowering shrub that has aromatic leaves widely used as a seasoning in cooking, as well as it has been used in traditional medicine by herbal preparations as teas, plasters or even cosmetic. However, as far as we known, the literature undescribed the amount of marjoram's essential oil (MEO) is safe for humans. Thus, the aim of this study was to assess the lethal concentration of 50% (LC50) of MEO in cultured human lymphocytes (CHL). A cytotoxicity curve was performed using MEO at 1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL concentrations, in order to determine the LC50. Initially, peripheral venous blood was collected from adult voluntary over 18 years old, without use of medicines or exposure to radiations along last six months. The blood sample was mixed with Histopaque® and centrifuged at 1,500 rpm for 10 minutes in order to obtain the cells of interest. After, the lymphocytes (adjusted to ~10^6 cells/mL) were transferred to supplemented RPMI 1640 medium (fetal bovine serum, streptomycin/penicillin, gentamicin and phytohemagglutinin) and maintained at 37°C for 48 hours in a 5% CO_2 atmosphere. The samples were performed in triplicate and the protocols were previously approved by Ethic Committee of Federal University of Pampa – UNIPAMPA (n° 27045614.0.0000.5323). Cell proliferation was performed counting the number of lymphocytes through Neubauer chamber in optical microscope at 400 X magnificence. Data were expressed as mean ± standard deviation (SD). Non-linear regression analysis was used to determine the LC50. The LC50 for lymphocytes was 238.50 µg/mL. Other studies should be conducted to elucidate this phenomenon, such as genotoxic studies to evaluate possible damage to the DNA of the cells.

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OCCUPATIONAL EXPOSURE TO ENDOTOXIN: FORKLIFT DRIVERS

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Many studies have already associated endotoxin exposure, especially in workplaces, to airways disease, yet threshold limit values have not been set, nor a standardized procedure for the analysis of endotoxin levels. Inhaled endotoxins may cause bronchial asthma and may increase the allergic response in hypersensitive people. Endotoxins are biologically active lipopolysaccharides (LPSs) produced by the most external layer of cellular walls of gram (−) bacteria. They are commonly found at workplaces where large amounts of bioaerosols are generated, like in waste sorting plants, where microorganisms growing on organic residue are present at high concentration in the air. Endotoxins are relatively heat stable so they are released into the air during cell growth and after cells' death when the integrity of the cell wall is ruptured. Up to the present, endotoxin values are measured with non-uniform methods and therefore values are of limited benefit for assessment and limits' establishment. In this study, eleven filters belonging to the filtration system from forklifts operating in one waste sorting industry located in the Lisbon region and one control filter were analyzed. The 11 filters were used in forklifts between 794 and 22,240 working h. All the assessed filters were composed by activated charcoal and belong to category 2 (pores typically from 3.0 µm or greater dimension) according to protection requirements (EN 15695) ensuring protection against dust inside the cabinet. In these settings, criteria for filter replacement in the air conditioning system are dependent on visual observation by the maintenance service to accomplish preventive maintenance program. Endotoxins levels were analysed following the European Standard (2003) EN 14031 and varied between 3,6 EU/cm^2 and > 625 EU/cm^2. Considering that standard filters have an area of about 1226 cm^2, the total charge of endotoxins in the studied filters ranges from 4382 EU to 766 250 EU which raises questions about the security and for how long is safe to keep the filters in use in this and other particular indoor settings.

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EVALUATION OF THE CYTOTOXIC AND GENOTOXIC EFFECTS OF 2,4-D HERBICIDE IN HUMAN HEPATOCARCINOMA TUMOR CELLS (HepG2/C3A)

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Key-Words: environmental monitoring, ecotoxicology, agrochemical, MTT assay, Comet assay.

Among the agrochemicals most commonly used today, also responsible for damages to the environment, stands out the herbicide 2,4-D. 2,4-Dichlorophenoxyacetic acid is one of the herbicides widely used in the state of Paraná, Brazil, in the cultivation of soybeans, corn, wheat and sugar cane, among others. The herbicide is a growth regulator that has an analogous effect to the hormone auxin, belonging to the family of phenolic compounds. It is used to show selectivity to plants with narrow leaves, having a greater phytotoxicity for broadleaf species, being used in no-tillage, mainly in mixture with other herbicides such as glyphosate, in the desiccation of weeds before sowing of several crops. In the last decades several ecotoxicological tests have been carried out in in vitro tests to evaluate the effects of chemical agents derived from processes, industrial and agricultural. These tests allow to estimate the damages that chemical agents can cause on the organisms. Thus, the objective of this work was to evaluate the genotoxic and cytotoxic effects of the 2,4-D herbicide on human hepatocarcinoma (HepG2 / C3A) cells by the Comet and MTT tests. The ability of DNA damage was verified by the Comet test, which consists of the incorporation of cells in agarose and lysates, followed by electrophoresis. Cells with fragmented DNA form a comet after electrophoresis and their size depends directly on the damage. Cytotoxicity was quantified by the MTT assay, which consists of the ability of the mitochondrial and cytosolic dehydrogenases, active in viable cells, to convert the yellowish MTT salt into formazan, purple crystals. Thus conversion is directly proportional to cell viability. The doses used were ADI (Acceptable Daily Intake) 0.01 µg/mL and 10, 100 µg/mL. The results submitted to statistical tests showed no significant changes in cell viability and DNA stability. Despite this, further studies should be developed, varying the concentrations, the cell lines and the types of tests, in order to clarify the safety of use of this herbicide.

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INVESTIGATION OF CYTOTOXICITY AND GENOTOXICITY OF HERBICIDE GLYPHOSATE IN TUMOR CELLS OF HUMAN HEPATOCARCINOMA (HepG2/C3A)

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Key-Words: environmental monitoring, ecotoxicology, agrochemical, MTT assay, Comet assay.

In the last years the ratio between planted area/kg of pesticide employed had a significant increase. The use of these chemical agents in an irregular way is responsible for several environmental imbalances. Studies show an increase in the number of cases of chronic intoxication, mainly in groups of rural workers, caused by herbicides. Chronic intoxication can generate DNA damage as well as cause cytotoxicity to any cell type. One of the herbicides most used in Brazil and associated with cases of intoxication is glyphosate. (N-(phosphonomethyl) glycine), Glyphosate or Roundup is a non-selective organophosphorus herbicide of systemic action desiccant of cultures, by the inhibition of the enol-piruvilshiquimato-phosphate-synthase enzyme (EPSPs), responsible for the production of proteins, vitamins (K and E), hormones, alkaloids and other products associated with plant development. Its application causes paralysis of the growth, necrosis and later death of the vegetable. However, some studies argue that its toxicity is low for human organisms, and other studies prove the herbicide's relationship to cases of autism. In the face of speculation as to its safe use, the present study aimed to evaluate the cytotoxicity and the ability to promote direct DNA damage in human hepatocarcinoma (HepG2/C3A) cells. Cytotoxicity was quantified by the MTT method, which consists of the ability of mitochondrial and cytosolic dehydrogenases, active in viable cells, to convert the yellowish MTT salt into formazan, purple crystals. Thus conversion is directly proportional to cell viability. The ability of DNA damage was verified by the Comet test, which consists of the incorporation of cells in agarose and lysates, followed by electrophoresis. Cells with fragmented DNA form a comet after electrophoresis and their size depends directly on the damage. The doses used were ADI (Acceptable Daily Intake) 0.05 µg/mL and 50, 500 µg/mL. Those resulting from statistical tests did not demonstrate significant changes in cell viability and DNA stability. Despite this, further studies should be developed, varying the concentrations, the cell lines and the types of tests, in order to clarify the safety of use of this herbicide.

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HYPOLIPIDEMIC EFFECTS OF *Campomanesia xanthocarpa* (MART.) O. BERG. AND ITS MODULATION ON OXIDATIVE STRESS AND GENOMIC INSTABILITY

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Key-words: *Campomanesia xanthocarpa*, DNA damage, dyslipidemia, oxidative stress, tyloxapol.

Hypercholesterolemia is associated with overweight and obesity which have been increasing in many countries including Brazil. This condition increases the cardiovascular risks to atherosclerosis and stroke as well as the genomic instability making the discovery of new approach therapies needed. In this sense, *Campomanesia xanthocarpa* (Mart.) O. Berg (Myrtaceae) popularly known as Guabiroba is a plant spread in Latin America, more specifically in Brazil, Argentina, Paraguay, and Uruguay. This plant is empirically used to decreasing cholesterol, losing weight, treating cystitis and uretritis and also as a depurative, antidiarrheal, anti-inflamatory, and antirheumatic. However, studies to prove its activities on lipid metabolism and its genotoxic/antigenotoxic effects have not received sufficient attention. The aim of this study was to evaluate hypolipidemic effects and to assess protective actions against oxidative stress and DNA damages of a *C. xanthocarpa* aqueous leaf extract (CxAE). The tyloxapol- induced hyperlipidemia model was used to evaluate the hypolipidemic properties of CxAE and its genotoxic/antigenotoxic effects. Wistar rats were treated with CxAE 250 and 500 mg/kg by gavage for 7 consecutive days before tyloxapol administration. Biochemical analyses and oxidative stress levels measuring thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), and catalase (CAT) enzyme activities, and glutathione S-transferase (GST) activity were performed. DNA damages were assessed using alkaline comet assay in several tissues and micronucleus test in bone marrow. The data were analyzed using the one-way analysis of variance (ANOVA) followed by Tukey's test or Newman-Keuls Multiple Comparison test with statistical significance at $p<0.05$.

CxAE decreased cholesterol and triglyceride levels in serum and DNA damage in liver and kidney tissues of tyloxapol- treated rats. There was no effect on the micronucleus frequency in bone marrow. The extract increased CAT activity and decreased GST activity in kidney tissue. In conclusion, CxAE showed hypolipidemic effects, reinforcing its value in folk medicine by lowering cholesterol, it improved oxidative stress parameters, and protected DNA against damage induced by tyloxapol-induced hyperlipidemia, providing chemopreventive properties associated with its hypolipidemic effect.

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INFLUENCE OF BRAZILIAN RED PROPOLIS ON THE MUTAGENICITY INDUCED BY DOXORUBICIN IN MICE

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Key-words: Red propolis, micronucleus test, modulating effect, doxorubicin.

The Brazilian red propolis, produced in the state of Alagoas, is a product synthesized by bees of *Apis melífera* species, from the exudates collect of *Dalbergia ecastophyllum* (L) Taub. This plant species is found mainly in estuaries, mangroves and in restinga vegetation. Rich in flavonoids, tannins, xanthones and terpenoids, this propolis has antibacterial, antiparasitic, antifungal, anti-inflammatory, antioxidant and anti-tumor activity. Considering the biological properties of this natural product, the present study aimed to evaluate the modulating effect of red propolis hydroalcoholic extract (RPHE) on doxorubicin (DXR)-induced mutagenicity in Swiss mice using micronucleus test. In addition, the serum creatinine and urea levels were analyzed in order to evaluate the nephrotoxicity of the treatments. The animals were treated with three different doses (12.5, 25 and 50 mg/kg of body mass [b.m.]) of RPHE combined with the DXR (10 mg/kg b.m.). The negative (water), solvent (dimethylsulfoxide 5%), RPHE (50 mg/kg b.m.), DXR (10 mg/kg b.m.) and solvent plus DXR control groups were included. The animals treated with RPHE and DXR showed frequencies of micronucleated polychromatic erythrocytes that did not significantly differ from those treated with only the mutagen. The ratio between the polychromatic erythrocytes and the total erythrocytes showed the absence of cytotoxicity of the treatments, when compared with the negative control group. The analysis of biochemical parameters demonstrated that creatinine and urea levels were not significantly different from those observed in untreated animals. Therefore, under the experimental conditions used, RPHE was not able to influence the induction of chromosomal damage by DXR. In addition, the treatments were neither cytotoxic nor nephrotoxic.

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GENOTOXICITY ASSESSMENT OF PHTHALATES IN *Rhamdia quelen* AFTER SUBCHRONIC EXPOSURE

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Key-words: Comet assay, DBP, DiPeP, Neotropical fish

Phthalates are considered as emerging contaminants in aquatic environments. Di-iso-pentyl (DiPeP) is a phthalate widely used in Brazil and its production is related to the process of sugarcane production. Another phthalate is di-n-butyl phthalate (DBP), which is widely used worldwide and is classified as a substance of regulatory concern due to its toxic potential. Ecotoxicological studies with trophic and sub chronic bioassays are rare in the literature. Our study proposes the evaluation of possible toxic effects of DBP and DiPeP by utilizing non-usual biomarkers for these substances. *Rhamdia quelen* specimens were divided into 8 groups (n=15): negative control, DBP (5 ng/g, 25ng/g and 125 ng/g), DiPeP (5 ng/g, 25 ng/g and 125 ng/g) and positive control. The doses of phthalates were injected in gelatinous capsules according to the weight of the animal. These capsules were inserted in blocks of commercial food with colorless gelatin solidified and offered to the animals. This procedure was performed 15 times at 48 h intervals, totaling 30 days of exposure. Forty-eight hours after the administration of the last dose, the animals were euthanized and samples of posterior kidney were collected for the alkaline comet assay procedures and quantification of biochemical biomarkers involved in the detoxification process (SOD, CAT, GPx, and GST activities;GSH concentration). The comet assay of posterior kidney did not show any differences between treatments and controls. Nevertheless, all doses of DiPeP showed reduction in the biochemistry biomarkers GPx (p<0,001) and GSH (p<0,05 in 5 and 25ng/g and p<0,01 in 125ng/g treatment) just the highest dose of DBP showed reduction in GSH concentration (p<0,05). The SOD activity reduced at the two highest DBP(p<0,01) doses and at the highest DiPeP dose (p<0,01). The intermediate dose of DiPeP showed a reduction in activity of CAT (p<0,05).The decrease in the antioxidant system activity can reduce the cellular capacity to destroy the free radicals and in this way, make the macromolecules susceptible to oxidative damage. Thus, the absence of DNA damage in the groups exposed to phthalates may be due to the time of exposure that was not sufficient for genotoxicity to be observed. Our study is one of the first reports utilizing environmental relevance doses of phthalates and sub chronic exposure in a freshwater fish species.

Suporte financeiro: CNPq

TOXICOLOGICAL ANALYSIS OF PHTHALATES ISOLATED AND MIXTURES USING THE FISH EMBRYO ACUTE TOXICITY TEST WITH EXTENDED TIME

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Key-words: Di-n-butyl phthalate, Di-iso-pentyl phthalate, Teratogenesis.

Phthalates are additives used in several commercially available plastic products, to increase their flexibility. Di-n-butyl phthalate (DBP) has well-described mechanisms of toxicity in rats, however little is known about its effects in fish. Di-iso-pentyl phthalate (DiPeP) is widely used in Brazil because its production is correlated with the production of ethanol fuel, for this reason it has been reported in environmental samples and in biological samples of Brazilian women. It is important to consider that the environment receives a mixture of different phthalates, whose combined action can enhance their toxic effects. Thus, the aim of this study was to evaluate the toxicity of DBP, DiPeP and the mixture between them, using the Fish Embryo Acute Toxicity (FET) Test in *Danio rerio* embryos, with the extended exposure time. The methods followed protocol 236/2013 of the Organization for Economic Co-operation and Development (OECD), with adaptation in the exposure time from 96 to 168 hours. The solutions were prepared with dilution water and the treatments were: negative control; solvent control (1% methanol v/v); groups exposed to DBP: 0.0012; 0.0035; 0.007; 0.015; 0.031; 0.062 and 0.125 mg/L; groups exposed to DiPeP: 0.0012; 0.0035; 0.007; 0.015; 0.031; 0.062 and 0.125 mg/L; groups exposed to their mixture (DBP + DiPeP): 0.0012; 0.0035; 0.007; 0.015 and 0.031 mg/L and a positive control (4 mg/L of 3,4 dichloroaniline). The test solutions were renewed after 72 hours of exposure. Mortality and malformations were analyzed every 24 hours for 168 hours. Mortality was observed from 0.031 mg/L DBP and 0.125 mg/L DiPeP. The mixture did not cause significant mortality in the *Danio rerio* embryos. Several changes in embryo development in *Danio rerio* were observed after exposure to phthalates such as yolk sac and pericardial edema, non-absorption of the yolk sac, spinal deformity, dwarfism, hemorrhage, bradycardia and delay in development of the swim bladder. In addition, equilibrium disturbances of larvae were observed from 96 hours post fertilization. DBP has been shown to be more toxic than DiPeP causing mortality at lower concentrations. The mixture did not cause an increase in mortality. However, the malformations observed appeared early in the lower concentrations during the embryonic development.

Financial Support: CNPq, CAPES

EVALUATION OF THE CITOPROTETOR EFFECT OF HOMEOPATHIC COMPOUND CANOVA® IN AFRICAN GREEN MONKEY KIDNEY LINE EXPOSED TO SODIUM DIPYRONE DRUG

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Keywords: cytoprotection, sodium dipyrone, canova

Dipyrone has been highlighted in the literature as one of the most commonly used drugs in the practice of self-medication mainly because of its potent analgesic and antipyretic effect. Despite its wide use, our research group has shown that dipyrone has genotoxic and cytotoxic effects. In this way, they are important the studies with drugs that provide protection or that minimize the possible damages caused by this drug. The homeopathic compound Canova® (CA) seems to be a good candidate for this purpose, since it reduces the side effects of drugs associated with it. Therefore, the present study aimed to evaluate the possible cytoprotective effect of CA on African Green Monkey Kidney line (VERO) exposed to the drug dipyrone sodium using the alkaline version of the comet assay, cell death with fluorescent dyes and fluorescence immunocytochemistry. The concentration of dipyrone used in the present study was defined according to Gomes (2016). In relation to the CA concentrations used in this study, these were defined according to the work of Seligmann et al. (2003). Our results demonstrated by the comet assay that, after three hours of exposure, in the dipyrone single concentration 5 mM induced a significant increase ($p < 0.01$) in the damage index (DI) of the DNA in VERO line. However, when these cells were co-treated with 5mM dipyrone plus CA 4, 8 and 16% (v / v), a significant ($p < 0.01$) reduction in ID was observed in the DNA of these cells at the 3 concentrations. In relation to the cell death assay it was observed that dipyrone induced a significant increase ($p < 0.01$) in the number of apoptotic cells in both 24 and 48 h. However, when this drug was associated with CA, a significant reduction ($p < 0.01$) was observed in this effect in the three CA concentrations. Regarding the results of immunocytochemistry, a significant increase ($p < 0.01$) in the expression of caspases 8, 9 and cytochrome C was demonstrated when cells were exposed to dipyrone; on the other hand, co-treatment significantly reduced this effect only in relation to Caspase 8 and cytochrome C.Thus, our results demonstrated that CA had antigenotoxic, anticytotoxic effects and an effect on the reduction of expression of Caspase 8 and cytochrome C in VERO cells exposed to sodium dipyrone.

Financial Support: CAPES

MORPHINE WITHDRAWAL INDUCED DNA DAMAGE AND THE EFFECT OF *Passiflora incarnata* L. (PASSIFLORACEAE) EXTRACT

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Drug addiction is a complex disorder, which is influenced by genetic and environmental factors, suggesting that substance use disorders could be associated with DNA damage. *Passiflora incarnata* L. (Passifloraceae) has been traditionally used for anxiety and nervousness. Its depressive effect in nervous system attracted its use for the treatment of drug addiction. Based on this, there are reports of its use to treat morphine dependence in India. The aim of this study was to investigate the effect of a standard commercial extract of *P. incarnata* on the DNA damage of morphine-dependent mice. Mice (n = 8-10/group) were morphine injected (ip) 3 times/day for 3 days at increasing doses in order to become dependent. On 4th day, the animals orally received the *P. incarnata* extract (PI 50, PI 100, PI 200 mg/kg). After 24h, mice were euthanized, and the blood was collected from the vena cava and transferred to tubes containing heparin as anticoagulant. Comet assay was performed in whole blood immediately after collection. GelRed-stained slides were examined under a fluorescence microscope, 500x magnification. In each sample, images of 100 randomly selected cells (50 cells in duplicate) and the percentage of DNA in the tail (% Tail DNA) was analyzed. The experiments were carried out in accordance with current guidelines for the care of laboratory animals and were approved by the University Ethics Committee (nº 30520). All morphine dependent mice presented a significant increase of DNA damage in relations to naive animals (that did not receive morphine), however PI 50 mg/kg treated mice showed a significant decrease in DNA damage (ANOVA/Bonferroni, $p<0.05$). The PI chemical analysis indicated the presence of flavonoids as major compounds, which could act as antioxidants and protect the DNA from oxidative damage. This treatment should be investigated as a potential new therapy in the morphine withdrawal syndrome.

EVALUATION OF MUTAGENIC ACTIVITY OF EXTRACTS OF TREATED WATER FROM THE LOWER TAQUARI RIVER REGION

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Key-words: Drinking water; Mutagenicity of organic compounds; *Salmonella*/microsome assay

Surface water contamination has contributed to reducing its quality, directly influencing the water used for public supply. This is one of the ways in which human can be exposed to pollutants present in water resources, even after conventional treatment. Thus, it is valid to consider that the quality of treated water depends on the raw water, since many contaminants can be carried to the final treatment. The lower portion of Taquari River has been studied because it is influenced by compounds originating in these activities, leading to great concern about the drinking water supplied to the population of cities in this region. Thus, our objective was to analyze the genotoxic potential of samples of water to be used for public supply, as a possible indication of hazard to human health. Mutagenic effects were evaluated by the *Salmonella*/microsome assay using strains that measure frameshift error (TA98) and base pair substitution of the DNA (TA100) in the absence of S9 mix. The mutagenesis found showed higher values detected by TA100 at most of the sites studied in neutral and acid pH. There was an increase in the positive responses in the samples treated compared to the raw ones indicating that the sub-products of disinfection were detected by the strains tested with higher values in the acid samples but also present in neutral extracts. Higher mutagenesis was also found in a raw sample than in that treated at site Ta011 in acid pH (346±75,5 rev/L), possibly as a result of the complexity of interactions of the mutagenic compounds present in surface water. Analyses associated with the human metabolic fraction (S9) are being performed to help detect the effects of metabolites generated by the hepatic system. Literature suggests that mutagenesis without S9 in acid extracts of drinking water indicate the presence of halogenated sub-products of disinfection. However, the results found in the neutral fraction indicate a warning of the presence of other contaminants. Hence, the positive results of the treated water from these sites suggests the interference of pollutants generated by anthropogenic activities close to the regions of study, besides creating new challenges for the scientific community to define methodologies of treatment and analysis of newly emerging stressors present in the waters at very low concentrations, making them difficult to detect.

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EFFECT OF HAZARDOUS CONTAMINANTS IN THE SURFACE WATER OF TAQUARI RIVER AT SITES USED FOR PUBLIC SUPPLY.

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Key-words: Runoff of compounds; Mutagenesis; *Salmonella/*microsome assay;

Degraded water sources alter sediments and reduce the quality of water impairing its uses such as for supply. The purity of drinking water depends on its quality at the sources, since many contaminants may be carried to the final treatment. Joint projects are being developed by FEPAM/PPG Ecologia, UFRGS in the region of the Lower Taquari sub-basins. The purpose of the present study is to evaluate the mutagenic potential of raw water samples, prioritizing sites that are to be used for supply. In the current phase, the following sites were analyzed: Ta063, Ta032, Ta011, Ta006, Ta referring to the river and the distance in Km from the mouth. The samples were submitted to extraction for organic compounds in $XAD_{4,}$ resins, natural and acid pH, with elution in methanol/dichloromethane (moderately polar/nonpolar compounds) and methanol/ethyl acetate (polar compounds), respectively, and the eluates were concentrated in rotavapor. Mutagenesis and cytotoxicity were evaluated in the *Salmonella*/microsomal assay in strains that detect frameshift error (TA98) and base pair substitution (TA100) of the DNA in the absence (-S9) and presence (+S9) of metabolic activation (human hepatic microsomal fraction). The dose-response curve for mutagenesis was analyzed in the SALANAL program. The significant results were predominant in the presence of S9, TA100 strain. At the Ta063 site, the neutral sample presented 647.1±81.8 revertants per liter (rev/L) and the acid one 1065.6±27.2. In direct assays the neutral sample presented an induction of less than 274.0±74 rev/L. The results at a downstream site (Ta011) were 617.6±65 (neutral) and 566±66 (acid) rev/L, also for TA100+S9. No sample was cytotoxic. These results support the concern about the quality of water from this source. Site Ta063, located in the Arroio Sampaio/Estrela sub-basin was initially considered an area of reference for this stretch in the basin. Its main uses, besides water intake, are tourism, fishing and others. Site Ta11 presents the influence of cultivation, and is less than 1 km upstream from a site contaminated by wood preservatives. The mutagenesis found in these areas was the result of a complex mixture of substances and indicates the presence of mutagenic organic compounds in the water that will be treated for supply.

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IN VIVO EVALUATION OF PROLACTIN'S ANTIMUTAGENIC POTENTIAL AGAINST DAMAGE CAUSED BY METHYLMERCURY

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Key-words: Methyl Mercury, Prolactin, Mutagenic

In the aquatic environment, the metals come from natural sources and through anthropic and industrial sources. Metals such as mercury, because they are not biodegradable, accumulate in organisms by bioaccumulation and biomagnification, and it can reach high concentrations upon reaching men. Human contamination by mercury present in water and food is extremely important, since this metal corresponds to one of the most widespread and deleterious organ-specific contaminants. In Brazil, studies show that several species of carnivorous fish in the Amazon have high levels of methyl mercury (MeHg), which increases the concern with the possible riverside communities' health problems, since fish is the main type of feeding of these communities. Prolactin (PRL) is a protein hormone that acts in more than 300 biological activities. Several studies have demonstrated protective functions of PRL, including in vitro cytoprotective action against cytotoxic and mutagenic effects of MeHg. The present study aimed to investigate in vivo the cytoprotective potential of PRL against the mutagenic action of MeHg in mammals, using the micronucleus test in bone marrow cells of mice (Mus musculus) to verify this action. From the CEUA / UEPA approval (protocol 16/2017), 56 healthy animals, young adults of both sexes were obtained from the vivarium of the Evandro Chagas Institute and kept in the State University of Pará laboratory. The animals were divided into six treatment groups, a negative control group, a group receiving MeHg (30 µg / kg / day), two groups receiving PRL (25 and 250 µg / kg / 12h) and two groups receiving the MeHg and PRL conjugate treatment concentrations already mentioned. After 45 days of treatment the animals underwent euthanasia to prepare the slides for the test. Micronucleus (MN) frequency was evaluated in 2000 polychromatic erythrocytes per animal. Statistical analysis of the data revealed a significant increase in the number of micronucleated erythrocytes in the MeHg treated group, about seven times greater than the number of micronuclei in the control group; characterizing a mutagenic action of this metal; when MeHg was administered with PRL, the number of micronuclei significantly reduced, and there was no statistical difference with the control group, demonstrating an important protective action of this hormone against the mutagenic effect of MeHg.

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ANTIPROLIFERATIVE EFFECT OF *Vernonanthura polyanthes* LEAVES AQUEOUS EXTRACTS

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Keywords: Assa-peixe; *Allium cepa*; *Artemia salina;* antitumor; cytogenotoxicity.

Vernonanthura polyanthes (Asteraceae), popularly known as assa-peixe, has been widely used in traditional medicine to treat a variety of diseases, including bronchitis, pneumonia, hemoptysis, persistent cough, internal abscesses, gastric and kidney stone pain. The purpose of this study was to evaluate the toxicity, cytotoxicity and genotoxicity of *V. polyanthes* leaves aqueous extracts using the *Artemia salina* and *Allium cepa* assays. For the *A. salina* assay, three groups of 10 larvae were exposed to *V. polyanthes* leaves aqueous extract at the concentrations of 5, 10, 20, 40 and 80 mg/ml and, after 24 h, the survival rates (%) were recorded. The negative control was the saline water and the positive control was the solution of potassium dichromate. For the *A.* cepa assay, the bulbs were exposed to *V. polyanthes* leaves aqueous extract at the 10, 20 and 40 mg/ml and then submitted to microscopic analysis using Giemsa stain. Water was used as a negative control and sodium azide as a positive control. As result it was identified a toxicity and cytotoxicity of *V. polyanthes* dependent on the extract concentration. The *Artemia salina* assay suggests that concentrations greater than 24 mg/ml are toxic for shrimp and kill 50% of naupllis; while the *A. cepa* assay suggests the *V. polyanthes* leaves aqueous extract are toxic in all concentrations tested and cytotoxic only at the 40 mg/ml concentration. This cytotoxicity may be assigned to the flavonoids found in the species *V. polyanthes*, since different pharmacologic effects, such as antiproliferative and anticarcinogenic action, are attributed to these compounds. No genotoxic activity was observed in *V. polyantes* aqueous extract tested. Our results suggest that *V. polyanthes* could be a good natural source of antitumor compounds and may be has potentially used in human medicine; however more detailed studies need to be performed to confirm its potential.

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URBAN OCCUPATION INCREASES WATER TOXICITY IN AN IMPORTANT RIVER IN CENTRAL BRAZIL

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Keywords: *Allium cepa*; cytogenotoxicity; indicators, Meia Ponte River; phytoplankton

Meia Ponte River supplies water for 2 million people and a large part of the economy depends on it for various purposes, such as: water supply, irrigation of crops, supply of animal drinking water, leisure and for disposal of domestic and industrial sewage. Here we evaluate the environmental quality and toxicity of surface water along the course of this river. Physicochemical analyses of water from the Meia Ponte River in the source and urban perimeter of Goiânia were higher than the limits of Brazil environmental regulations for fresh water. The biological community was heterogeneous along the river. Samples taken closer to Goiânia showed classes of phytoplacton found more common in polluted environments. Bioindicators analyses were performed using the *Allium cepa* model in five collection points along the course of the river. The results showed no reduction in the length of roots, suggesting the absence of toxicity in the Meia Ponte River water. Also, it was observed in four of the five collection points, higher mitotic index (MI) than the negative control, which suggests that the samples of water were not cytotoxic in most collect points. Different from cytotoxic analysis, the majority from collected water points (4 from 5 points), presented a significant increase in chromosomal aberrations, which revealing the genotoxic potential of the water from Meia Ponte River. This genotoxic levels represent a risk for aquatic biota and humans, once the genotoxic agents in water samples might cause the loss of DNA integrity, inducing damages and DNA breaks and consequently can lead to increase of tumor rate, cellular and tissue damage and also bioaccumulation. Considering that the Meia Ponte river supplies water for millions people, the biomonitoring is necessary, to diagnose contamination aiming at the recovery of water quality. This work is important to alert the authorities about the need for water quality improvement of the Meia Ponte River.

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ROSMARINUS OFFICINALIS L. EXTRACT INDUCE GENOMIC INSTABILITY *IN VITRO*

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Key-words: cell culture, chromosome aberrations, rosemary

Rosmarinus officinalis has been used since ancient times in traditional medicine and as food additive to enhance the aroma and flavor. Recently, various studies have described the use of rosemary extracts or formulations to prevent or cure several health disorders. However, the rosemary uses and its genetic effects, especially in normal conditions is still unknown. Thus, the main objective of this study was to evaluate the impact of rosemary on healthy human lymphocyte chromosomes. This study was previously approved by Ethic Committee from Federal University of Pampa – UNIPAMPA (n° 27045614.0.0000.5323). The *Rosmarinus officinalis* extract was made using leaves mildly macerated and 70% ethanol (v/v). After 7 days, extracted mixture was filtered and the solvent was eliminated with a rotary evaporator at 40 ºC. Cytogenetic analyses by using the chromosomal abnormality assay was performed in lymphocytes exposed to 1, 10, 25, 50 μg/mL of rosemary extract, according to acceptability criteria (cytotoxicity and solubility) for 3 h according OECD 487 (2016) protocol. About 300 well-spread metaphases were scored for the presence of chromosomal aberrations, numeric and structural. The mitotic index was computed for each treatment as the number of dividing cells percent. The data were analyzed by One Way ANOVA**,** complemented by post-hoc Tukey test, and considered as statistically significant when $p<0.05$. Our results illustrate rosemary induced structural alteration and decreased the mitotic index significantly at all concentrations assayed compared with negative control. At the highest concentration, no metaphase was found, signaling blockage of cell division. These findings indicate interference in the regular process of cell division. Previously, carnosic acid (one of the major compounds in rosemary) decreased cyclin A levels and cell cycle arrest at the end of S phase or at the beginning of the G2/M phase. These effects may be associated to decreased CDK2 activity and cdc2, which are essential for the progression from S to G2/M phase. In summary, our results showed that *Rosmarinus officinalis* extract interference in the regular process of cell division and increase in chromosome aberrations in healthy human lymphocytes.

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ANALYSIS OF GENOME OXIDATIVE DAMAGE IN INHABITANTS OF A MEDIUM-HIGH BACKGROUND RADIATION AREA

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Keywords: 8-oxo-guanosine, ionizing radiation, exposome

In the state of Rio Grande do Norte, Brazil, the Lajes Pintadas (LP) city has high concentrations of radon (Rn), a noble gas derived from the uranium decay chain, has a half-life of 4 days and is concentrated in closed places. When inhaled, the alpha particles emitted by this gas and its decay products interact with the lung cells, can cause DNA damage and generate oxidative oxygen species from the radiolysis of the water present in cells. Ionizing radiation is a known mutagenic and carcinogenic agent. However, more epidemiological and molecular studies are needed to analyze in an integrated way several variables that make up the exposome of target populations in order to better represent and evaluate the effects and risks of chronic exposure to environmental ionizing radiation in humans. Therefore, the aim of this work is to analyze the levels of genome oxidative damage in inhabitants of Lajes Pintadas/RN, considered a medium-high background radiation area. Thus, it was performed a quantification of 8-hydroxy-2'-deoxyguanosine (8-oxoG) with the DNA/RNA Oxidative Damage ELISA Kit. For this test, it was collected urine samples from 82 adults from LP and 46 from people who live in a normal background radiation area as a control group; also, they all were interviewed with a questionnaire to obtain socio-demographic status, health history and environmental and occupational exposures information. The 8-oxoG median (and interquartile range) of the control and LP groups were, respectively, 129.33 (91.04) and 136.11 (155.08) ng/mL. The difference of 8-oxoG concentration between the groups was not significant (p-value = 0.188) and this variable was not correlated with radon levels (Rho = -0.171, p-value = 0.403). When testing 8-oxoG as dependent variable in a multiple linear regression, it was obtained a significant model with buccal cell's nuclear buds frequency (B = -0.234, p-value = 0.045), buccal binucleated cells frequency (B = -0.287, p-value = 0.019) and platelets concentration (B = 0.255, p-value = 0.032). These variables explained 25% of 8-oxoG variance [R^2 = 0.25, F (4, 59) = 4.911, p-value = 0.002]. It can be concluded that high Rn levels did not significantly influenced the genome oxidative metabolism of the subjects analyzed in this study. Only variables representing the internal exposome was capable to explain part of 8-oxoG levels. More studies are necessary to investigate which other factors are mainly responsible for genome oxidative damages.

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EVALUATION OF CITOTOXICITY AND DNA DAMAGE OF GRAPHENE OXIDE IN H9c2 CELLS

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Keywords: nanotoxicology; cardiomyoblasts; viability tests; genotoxicity.

Graphene is a two-dimensional (2D) monolayer of carbon atoms, tightly packed, forming a honey comb crystal lattice, with physical, chemical, and mechanical properties greatly used for energy storage, electrochemical devices, and in nanomedicine. Recently, GO has gained interest as scaffold material for electrical conduction in the therapeutic of heart failure and myocardial infarction, but little is known about the safety of its use. Therefore, the aim of the present study was to evaluate the cytotoxicity and DNA damage of nano-graphene oxide (nano-GO) in the rat cardiomyoblast cell line H9c2. The cell viability was evaluated with the MTT reduction, neutral red uptake, fluorescein diacetate (FDA)/propidium iodide (PI) and in the trypan blue exclusion assays. Genotoxicity was evaluated in comet assay and low molecular weight DNA experiment. Nano-GO was previously characterized with different protocols, showing that the material, which was obtained from a commercial source, was a single layer, with a good-sized distribution and easily suspended in water. MTT reduction and neutral red viability methods presented an increase in absorbance, accompanied by the increase in nano-GO concentration. However, a significant reduction of cell viability with 20, 40, 60, 80, and 100 µg/mL nano-GO was observed in FDA/PI and trypan blue tests after 24 h incubation. DNA breaks in comet assay were observed at 40, 60, 80, and 100 µg/mL. This DNA damage was accompanied by a significant increase in LMW DNA only at 40 µg/mL. In conclusion, we observed an interference of nano-GO in absorbance of MTT reduction and neutral red uptake assays but it was possible to observe a cardiotoxic effect with FDA/PI and trypan blue assays. Furthermore, nano-GO might interact with DNA, indicating the importance of the further evaluation of the safety of nanomaterials.

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GENOTOXIC EFFECT OF METHANOLIC EXTRACT OF *Neonothopanus gardneri* IN MURINE MODEL FOR BREAST CANCER: TOXICOLOGICAL AND CYTOGENETIC MONITORING

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Key-words: 7,12-dimethylbenzanthracene; ductal mammary carcinoma; comet assay.

Neonothopanus gardneri stands out for its food, nutraceutical, antimicrobial and antiviral activities. The aim of the study was to isolate and characterize some of the chemical compounds from the methanolic extract obtained from *N. gardneri*, and to evaluate the genotoxic effects in murine model for breast cancer induced by 7,12-dimethylbenzanthracene. The methanolic extract was obtained and its phytochemical characterization and isolation of chemical compounds characterized by chromatography on Sephadex LH-20 and magnetic resonance. Virgin and non-pregnant Swiss albino mice (6-7-week old) weighing between 20 and 50 g were used for the induction of breast cancer with 7,12- dimethylbenzanthracene (6 mg.Kg-1 v.o), for eleven weeks. Invasive ductal mammary carcinoma was characterized by histopathological analysis and by Ki67 labeling. The extract was tested at 10 mg.Kg-1 v.o and cyclophosphamide at 25 mg.kg-1, for three weeks of therapy. Toxicological monitoring (behavioral [open field and rota rod test], organ weight, hematological and biochemical analysis) was done during cancer induction and therapy with the extract. We identified sugars, tannins, catechins, depsitions and proteins in the extract, as well as the substituted amides 7,8-dihydroxy-13-oxo-heneicosa-9,11-dienamide and 7,8-hydroxy-13-oxo-octadeca-9,11-dienamide, as major constituents. Only the group treated with 7,12-dimethylbenzanthracene showed renal lesions, change in organ weight and behavioral. On the other hand, the extract showed genotoxic effects, due to mechanisms associated with DNA damage, possibly due to oxidative effects, as well as by induction of apoptosis, which can be attributed to its chemical compounds, which should be tested for antitumor pharmaceutical formulations.

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CYTOTOXICITY EVALUATION OF THE ETHANOLIC EXTRACT FROM THE BARK OF *STERCULIA STRIATA* A. ST. HIL. & NAUDIN IN LINEAGE RAW264.7 MACROPHAGE CULTURE.

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Key-words: Cell viability; MTT technique; medicinal plants.

Sterculia striata A. St. Hil. & Naudin, also known as chichá or arachachá, is a species that belongs to the Malvaceae family with a wide distribution from Amazonas to Piauí, besides Mato Grosso, Minas Gerais and Rio Grande do Sul. Its seeds are widely used in human food, since it has in its chemical composition substances with antioxidant potential. In folk medicine, *S. striata* leaves have been used topically with warm butter or olive oil for the treatment of boils. However, despite the applications in different human activities, there are still no reports in the literature about its toxicity. Therefore, the present work aimed to evaluate the cytotoxic potential of the ethanolic extract from the bark of *Sterculia Stiata* A. St. Hil. & Naudin *in vitro* by the MTT assay. First, RAW 264.7 macrophages were cultured in a 96-well plate, at a concentration of $1x10^4$ cells per well, under standard culture conditions. Subsequently, they were treated with concentrations of the ethanolic extract of the *S. striata* bark ranging from 3 to 750 µg/ml, as well as the negative (DMEM) and positive (Triton X-100 3%) controls for a period of 48h. After the treatment period, 20 µL of the MTT [3- (4,5-dimethylthiazolone-2-yl) -2,5-diphenyl tetrazon bromide] dye was added to the wells at 5 mg/mL concentration. Then, the plates were centrifuged, the supernatant was discarded and 100 µL of DMSO was added for the dissolution of the formazan crystals. Finally, the plate was submitted to spectrophotometer reading. Statistical analysis was performed with T *Student* test and analysis of variance (ANOVA) with *p* values lower than 0.05. Our results demonstrated that all the concentrations used herein were able to reduce the viability of the RAW 264.7 macrophages significantly and in a dose-dependent manner, in comparison to the negative control. Particularly the concentrations of 500 and 750 µg/ml presented cell death higher than 40%. These findings corroborate with the results previously obtained by our group through the *Artemia salina* bioassay. In view of these results, it can be concluded that the ethanolic extract from the bark of *Sterculia striata* A. St. Hil. & Naudin is cytotoxic *in vitro*, at least in the concentrations and test used.

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CYTOTOXICITY OF THE ETHANOLIC EXTRACT FROM THE FLOWER OF *PITYROCARPA MONILIFORMIS* (BENTH) *IN VITRO.*

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Key-words: MTT test; medicinal plants, Cell viability.

The species *Pityrocarpa moniliformis* (Benth), popularly known as “angico de bezerro”, is a fast growing species belonging to the family Fabaceae, being found in the Brazilian northeast. It is used in folk medicine for the treatment of respiratory and gastrointestinal diseases and also demonstrates antimicrobial and antioxidant activity. The MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) has been widely used in the evaluation of cytotoxicity, proliferation and cellular activation *in vitro*. This assay is based on the ability of mitochondrial dehydrogenase enzymes from the living cell to convert the aqueous solution of the MTT substrate to a formazan salt. The amount of this salt produced is proportional to the number of viable cells. The objective of this work was to evaluate the cytotoxic potential of the ethanolic extract from the flower of *P. moniliformis* (Benth) in culture of macrophages RAW 264.7 through the MTT assay. First, cells were cultured in a 96-well plate, at a concentration of $1x10^4$ cells per well, under standard culture conditions. Subsequently, they were treated with concentrations of 3, 81, 243, 500, 750 and 1000µg/mL of the ethanolic extract from the flower of *P. moniliformis* (Benth), as well as the negative (DMEM) and positive (Triton X-100 3%) controls for a period of 48h. After the treatment period, 20µL of the MTT [3-(4,5-dimethylthiazolone-2-yl)-2,5-diphenyl tetrazon bromide] dye was added to the wells at 5mg/mL concentration. Then, the plates were centrifuged, the supernatant was discarded and 100µL of DMSO was added for the dissolution of the formazan crystals. Finally, the plate was submitted to spectrophotometer reading. Statistical analysis was performed with T *Student's* test and analysis of variance (ANOVA) with *p* values lower than 0.05. The results demonstrated that the ethanolic extract from the flower of *P. moniliformis* (Benth) significantly reduced the viability of the treated cells from the concentration of 243µg/ml in relation to the negative control and in a dose dependent manner. At the concentration of 1000µg/mL the cell death was even higher than 50%. In contrast, at the lowest concentrations used, 3 and 81µg/mL there was no reduction in cell viability. Thus, in view of the obtained results, it can be concluded that the ethanolic extract from the flower of *Pityrocarpa moniliformis* B. is cytotoxic at high concentrations, at least in the assay used.

EVALUATION OF MUTAGENIC ACTIVITY OF PLATINUM COMPLEXES AND ITS EFFECTS ON THE EXPRESSION OF REPAIR AND APOPTOSIS GENES IN *Drosophila melanogaster*

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Key-words: carboplatin, cisplatin, mutation, oxaliplatin, somatic recombination

Cisplatin, carboplatin, and oxaliplatin are some of the most often used alkylating chemotherapeutic agents. In view of the paucity of data on the genotoxicity of oxaliplatin, this study compares the mutagenic activity of cisplatin (0.006, 0.012, 0.025, 0.05 mM), carboplatin (0.1, 0.2, 0,5, 1.0 mM), and oxaliplatin (0.1, 0.2, 0,5, 1.0 mM) using the somatic mutation and recombination test (SMART) in somatic cells of *Drosophila melanogaster*. Standard and high-bioactivation crosses were used, which present basal and high levels of cytochrome P450 (CYP450) metabolization enzymes, respectively. In addition, changes in the expression of the *mus308*, *spn-A*, *mei-9*, *spel1*, *bsk* and *p53* genes were evaluated using real-time PCR. All concentrations of cisplatin and carboplatin induced lesions in genetic material in both crosses, while oxaliplatin was mutagenic only to high bioactivation flies treated with 0.1, 0.2, and 1 mM of the compound. No significant differences were observed between genotoxicity values of cisplatin and carboplatin. However, CYP450 enzymes may have affected the mutagenic action of oxaliplatin. Quantification of genetic damage based on origin of lesions showed that carboplatin induced mainly mutation events, while cisplatin triggered mostly mutation and recombination events when low and high doses were used. Most events induced by oxaliplatin were generated by somatic recombination. Important differences were observed in genotoxic potential of platinum chemotherapeutic compounds, possibly due to the origin and type of the lesions induced in DNA and the repair mechanisms involved. Regarding the expression of the repair and apoptosis-related genes, cisplatin and oxaliplatin were found to have varied responses, inducing and reducing gene expression of the different genes, whereas carboplatin in the two concentrations used caused reduction in the expression of all the genes.

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MUTAGENICITY FROM AIRBORNE FINE PARTICLES (PM2.5) AND EARLY GENOTOXICITY IN LYMPOCYTES OF CHILDREN IN URBAN AND INDUSTRIAL AREA OF THE SOUTHERN BRAZIL

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Key-words: *Salmonella*/microsome, comet assay, air pollution

Investigation of environmental quality using biomarkers of genotoxicity contributes to the identification of areas with potential health risks to exposed populations. This study aimed to analyze comparative genotoxicity data of DNA damages in lymphocytes of children and airborne inhalable particles (PM2.5) from Rio Grande city (RS), southern Brazil. PM2.5 were collected with high volume samplers placed at two different areas in the city, one at an urban-industrial area (Site 1) and the other at an urban-residential area (Site 2). The samples were collected once a week (24h) during winter (August/09, July/10, August/10) and Autumn (April/10, May/10). Filters were pooled by month and submitted to organic extraction by sonication. The mutagenicity of organic extracts was assessed by *Salmonella*/microsome assay, microsuspension method, using strain TA98 with and without mammalian liver metabolization (S9 mix). Children between 5 and 12 years old living and studying in these two sites were evaluated for primary DNA damage in peripheral blood lymphocytes using comet assay during the same periods (study approved by the Ethics Committee at Hospital de Clínicas de Porto Alegre - project nº 07-042). The concentrations of fine inhalable particles were similar among the sites sampled and ranged from 1.28 to 35.05 µg/m³. The highest particles concentrations occurred in the winter when two samples (Site 1: 32.67; Site 2: 35.05 µg/m³;) surpassed the PM2.5 limit of 25 µg/m³ recommended by the World Health Organization. All PM2.5 organic extract samples were positive for mutagenicity, ranging from 0.99 to 17.08 rev/m³ (Site 1) and from 1.77 to 9.70 rev/m³ (Site 2). Generally, mutagenicity was higher without metabolization, indicating the predominance of direct action substances. The primary damage marker in the DNA (tail intensity) in children living in the two areas showed significantly increased in Site 2 group (8.05%; n=41) compared to Site 1 group (5.91%; n=37). The tail intensity mean for all studied subjects was 7.03%. A study that evaluated children at the same age from an area of reference for PM2.5 in the same state at southern Brazil described similar index for children (7.3%). The mutagenicity of PM2.5 samples indicates that the value established for air quality is insufficient to avoid environmental damages. The results of comet assay suggest the need for advance in the parameters that could to estimate genotoxic effects of environmental exposure children.

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CYTOTOXICITY OF MAGNOLOL IN HUMAN HEPATOMA (HepG2/C3A) AND HUMAN BREAST ADENOCARCINOMA (MCF-7) CELLS

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Key-words: *Magnolia officinalis*, neolignoid, antitumor activity, mitochondrial activity.

Magnolol is a phenolic compound belonging to the neolignans class, and is mainly found in the bark of *Magnolia officinalis* (Magnoliaceae), a plant traditionally used in Chinese, Korean and Japanese medicine for the treatment of depression, diabetes, cancer, among others diseases. Some studies suggest that Magnolol has antioxidant and anti-inflammatory properties, as well as antitumor activity, acting on the expression of different genes involved in the survival of human tumor cells, proliferation and cell death. Thus, due to the pharmacological properties presented by Magnolol and since the literature shows that this compound exhibits different antiproliferative effects on numerous human tumor cell lines, this work aimed to evaluate the cytotoxic effect of Magnolol in human hepatoma (HepG2/C3A) and human breast adenocarcinoma (MCF-7) cells, by means of the colorimetric assay for measuring the activity of enzymes that reduce MTT. The cells ($0.4x10^4$ HepG2/C3A or MCF-7 per well) were seeded in 96-well culture dishes. The groups evaluated were: Control (DMEM culture medium supplemented with 10% fetal bovine serum); cytotoxic agent Doxorubicin (Doxo, 18µM) and Magnolol at concentrations of 10, 20, 30, 40 and 50 µM for 24 and 48 hours exposure. The absorbance reading was performed on a microplate reader at 550nm in three independent experiments and the results of the absorbance were submitted to analysis of variance (ANOVA), followed by the Dunnett's test ($\alpha=0.05$, $p<0.05$, n=3). The results demonstrated that human tumor cells presented different behaviors after exposure to different concentrations and times of Magnolol, since a significant reduction in mitochondrial activity was observed for the concentration of 50 µM (24 hours) and 30, 40 and 50 µM (48 hours) in human hepatoma HepG2/C3A cells, whereas for MCF-7 breast adenocarcinoma cells, cytotoxicity occurred for the concentration of 40 µM (24 hours) and for all the concentrations evaluated (10, 20, 30, 40 and 50 µM) in 48 hours were cytotoxic, when compared to the Control group. The reduction of mitochondrial activity may be related to the effect of the compound on cell metabolism, interfering with mitochondrial membrane potential, cytochrome C release and cell cycle regulatory mechanisms. Thus, the results of the present study indicate that further research should be performed to evaluate a possible antitumor activity of this compound.

Financial Support: CNPq, CAPES, Fundação Araucária.

CYTOTOXIC AND GENOTOXIC ACTIVITY OF HYDROXYBUPROPION HYDROCHLORIDE METABOLITE IN HUMAN TUMOR CELLS HepG2/C3A

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Key-words: antitumor activity, drug repurposing, antidepressant, BUP, comet assay.

Depression is considered a serious mental disorder according to the World Health Organization, affecting more than 300 million people. Several drugs can be used to reverse the depressive symptoms, generating a well-being and attenuating its symptoms, which include insomnia, anxiety, lack of appetite, deep sadness and, in more severe cases, leading to self-harm and suicide. Bupropion Hydrochloride (BUP) is a medicine used in the treatment of depression and for smoking cessation. This drug inhibits the reuptake of dopamine and norepinephrine and, when metabolized in the cytochrome P450 located in hepatocytes, it is converted into three secondary metabolites, namely Hydroxybupropion Hydrochloride (HH), Erythro-hydroxybupropion and Threo-hydroxybupropion. HH is the main metabolite found in plasma, being pharmacologically more active than its precursor, BUP. In order to analyze the cytotoxic and genotoxic effect of HH on human hepatoma cell line HepG2/C3A, the MTT assay was performed, which evaluated mitochondrial activity and cell viability, as well as DNA fragmentation, by the comet assay. Cells ($1x10^4$ cells/well in 96-well plates for the MTT assay, 24 and 48 hour exposure times, and $2x10^5$ cells/well in 6-well plates for the comet assay for 3 hours of exposure) were seeded, evaluating different concentrations of HH. Three independent experiments were performed and the results were submitted to analysis of variance (ANOVA), followed by the Dunnett's test ($p<0.05$, n=3). In the assessment of cytotoxicity by the MTT assay, it was observed that HH was cytotoxic at concentrations of 25, 50, 100 and 250 µM at both exposure times (24 and 48 hours), significantly reducing mitochondrial activity when compared to Control. For the comet assay (3 hours of exposure), damage index (DI) analysis showed that HH was genotoxic at concentrations of 25, 50 and 100 µM. The results of the present study indicated that Hydroxybupropion Hydrochloride has cytotoxic and genotoxic activity for HepG2/C3A cells, since it interfered in the mitochondrial activity of these cells possibly due to their action on cellular and mitochondrial dehydrogenases enzymes, and their pro-oxidant potential, inducing the formation of reactive oxygen species that can cause DNA fragmentation, which qualifies this substance as a potential agent for the development of new therapies against cancer.

Financial Support: CNPq, CAPES, Fundação Araucária.

ADMINISTRATION OF LEMON BALM ALCOHOLIC EXTRACTS DURING ACTIVE METABOLISM AND ITS INTERFERENCE IN NUCLEOLAR ACTIVITY (PRELIMINARY STUDY).

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Key-words: *C. citratus*, *L. alba*, *M. officinalis*, AgNOR impregnation techinique

Biological rhythms control regulates fundamental aspects in cell growth and survival, presenting a relationship to cancer development and progression. Cell malignancy is also associated with increased protein synthesis, a phenomenon dependent of ribosomes synthesis, governed by the cellular nucleolus. *Cymbopogon citratus* (D. C.) Stapf, *Lippia alba* (Mill.) N. E. Brown and *Melissa officinalis* L. are lemon balm species commonly used in folk medicine, which have been studied in antitumor therapies, because selective cytotoxicity activity. The present study aimed to evaluate the interference in the nucleolar activity of *C. citratus, L. alba* and *M. officinalis* alcoholic extracts, associated to photoperiod of administration, in health and tumor animal models. Swiss male adult mice received ethanolic (EE) or methanolic extracts (ME) of *C. citratus* (CC), *L. alba* (LA) or *M. officinalis* (MO), via gavage, for 14 days, in light phase (LP) or in dark phase (DP) of the day. Swabs of blood (healthy groups) and tumor (groups inoculated with 10^3 Ehrlich's Ascitic Tumor cells on the 7^{th} day of treatment); were submitted to impregnation with silver nitrate ions (AgNOR) to evaluate the position and integrity of the nucleolus. Partial results indicated more than 72% of the blood cells analyzed for the CC and MO extracts had evident nucleolus in a central position, coinciding with the data from the health control group; however, LA extracts it was possible to observe nucleolar fragmentation: EELA (50%) and EMLA (30%), being dispersed in the nucleus. There was no difference between the extract's administration in LP or DP, indicating that the photoperiod did not interfere in the nucleolar activity in healthy groups. However, results for tumor groups showed all the methanolic extracts reduced the nucleolar activity (high level of nucleoli fragmentation) combined to the reduction of the tumor growth, when administered in the DP, indicating that the extraction vehicle as well as the moment of the administration had differential effect on the tumor groups. As nucleolar activity is a factor related to tumor growth and aggressiveness, it can be concluded that the methanolic extracts administration in the dark phase of the day, contributed to the control of tumor growth; however *L. alba* extracts interfered in the nucleoli assembly in health groups, because dispersed nucleolar fragments are incapable of rendering nucleolar reorganization and triggering the cessation of the cell cycle.

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Cymbopogon citratus ALCOHOLIC EXTRACTS EXHIBIT ANTITUMOR, PROTECTIVE AND SELECTIVE ACTIVITIES, IN VIVO

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Key-words: Lemongrass, Micronucleus assay, Comet Assay, Antitumor activity.

Cymbopogon citratus (CC) is a popularly used lemongrass because it presents several therapeutic and great biological potentials. The aims of this study were to investigate the mutagenic and the genotoxic activities of ethanolic (EE) and methanolic (ME) extracts of CC in Swiss mice: healthy (H) or inoculated with Ehrlich Ascites Tumor (EAT) (CEUA/USC: 8706290315; 8918021216). The oral tolerance test defined extracts concentrations (100 mg of extracts/ kg of body weight), then, the following groups were treated, by gavage, for 14 days: (H) healthy mice, received distilled and autoclaved water; (EECC) EECC administration; (MECC) MECC administration; (EAT) received distilled and autoclaved water, mice were inoculated with Ehrlich Ascites Tumor; (EECC+EAT) EECC administration + EAT inoculation; (MECC+EAT) MECC administration + EAT inoculation. On the 7th day, 10^3 Ehrlich Ascitic Tumor cells were inoculated into the EAT groups, intraperitoneally. Peritoneal cells counting indicated tumor progression inhibition of approximately 50% in EECC+EAT and 70% in MECC+EAT. The micronuclei frequency (peripheral blood and bone marrow) EECC and MECC was similar to H, however, EECC+EAT and MECC+EAT, a reduction around 50% was observed when compared to EAT, suggesting antimutagenic activity against the mutation's accumulation during tumorigenesis. Comet assay was performed on blood and tumor cells. Extracts were genotoxic for leukocytes, showing scores similar to TAE, both in healthy and in EAT animals. In tumor cells, EECC+TAE (466.0 ± 138.2) and MECC+TAE (390.0 ± 146.2) presented higher scores than TAE (152.0 ± 37.1), due to higher frequency of class 3 damage observed on treated groups, suggesting the targeting of the tumor cells to apoptosis. The administration of CC alcoholic extracts, besides improving the physiological status of animals by probable antioxidant processes and antitumor activities, also present antimutagenicity in bone marrow and peripheral blood, both in a healthy system and in the face of tumor interaction, and a probable genotoxic activity in blood and tumor cells. Comparisons between the groups inoculated with the Ehrlich tumor and the healthy groups suggest that the extracts also exhibit protective and selective activity in relation to non-repairable damages (micronucleus tests) and reparable, fine damages (comet test). These results highlight the importance and necessity of studies on the properties and potentials of plants used in folk medicine.

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SETTING THE BASIS FOR THE MICRONUCLEUS ASSAY FOR THE TROPICAL MARINE AMPHIPOD *PARHYALE HAWAIENSIS*

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Key-words: genotoxicity, *Parhyale hawaiensis*, Crustacea, environmental monitoring

Micronucleus and other abnormalities assay have been widely used to monitor early effects of genotoxic compounds in the environment. This technique is simple, sensitive and reliable. The benthic detritivore amphipod *Parhyale hawaiensis* is widely distribute in tropical areas and easy to cultivate. The objective of this work was to optimize the micronucleus and other abnormalities assay for *P. hawaiensis*. Zinc was used as genotoxicant as it is considered a strong clastogenic agent in many biological systems. Organisms cultivated in the laboratory were exposed to Zn (as $ZnSO_4.7H_2O$) at the concentrations of 0.0 (control), 0.5, 1.0, 2.0 and 4.0mg/L prepared in salt water for 48hrs. Three independent experiments were performed, the first one with 4 organisms (2 males; 2 females) and the others with 6 organisms (3 males; 3 females). Hemolymph was collected with a fine capilar and slides prepared with Giemsa 10% and acridine orange 0.1%, for comparison. Several experiments were performed to define the best condition for slide preparation. The best one was to incubate hemolymph in seawater for 15min at humid chamber, to wash the slides and to fix them in 1:10 formalin in seawater. The frequency of micronucleus, cells with segmented and bubble nucleus and the sum of abnormalities were evaluated in 6000 cells for each condition. Two-way ANOVA was used to detect differences between concentrations of zinc and dyes (independent variables) and nuclear abnormalities (dependent variable). The viability of the hemocytes was evaluated with the tripan blue exclusion method in 2 organisms from control and from 4.0mg/L. The hemocytes viability decreased after exposure to zinc, but it remained above 85%. There was a significant increase of micronucleus, cells with bubble nucleus and total abnormalities in the concentrations of 1.0, 2.0 and 4.0mg/L in relation to the control and of 2.0 and 4.0 in relation to 0.5mg/L. The frequency of micronucleus found for the highest concentration was similar to that found for aquatic invertebrates exposed to toxic compounds (*P. hawaiensis*: 8.0±1.6‰ for acridine orange, 11.4±4.1‰ for Giemsa; mollusks: 11.1±4.2‰; daphnia: 11.0‰). No significant differences were found between dyes, thus both dyes can be used, but Giemsa has the advantage that slides can be stored after staining. The technique was successfully optimized for *P. hawaiensis* and can be used to monitor genotoxic compounds as well used to evaluate organisms collected from the field.

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ASSESSMENT OF MUTAGENIC AND RECOMBINOGENIC POTENTIAL OF BthTX-I PLA_2 ISOLATED FROM *Bothrops jararacussu* VENON

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Key-words: Somatic mutation and recombination test, *Drosophila melanogaster*, phospholipase A_2, miotoxin

Cancer is the major public health issue in several countries and has also been suggested as being the second most common death cause both globally and in Brazil. New therapeutic strategies against cancer have been developed, such as the use of ophidian toxins extracted from snakes. Phospholipase A_2 BthTX-I (Bothropstoxin-I) is an isolated myotoxin from *Bothrops jararacussu* venom and has already been associated with antitumor efficacy treatment, causing cytotoxicity through apoptosis or necrosis, cell cycle interruption between G_0 and G_1 phases, and induced tumor size reduction in rats. The objective of the present study was to evaluate a prospective DNA damage caused by BthTX-I myotoxin, from *B. jararacussu*, *in vivo*, through the Somatic Mutation and Recombination Test (SMART) in wing cells of *Drosophila melanogaster*. Two types of crosses were performed, namely: standard cross (ST) and high bioactivation (HB) cross. The ST cross had mwh males crossed with flr^3 females, and the HB cross with mwh males being crossed with ORR,flr^3 females, characterized by high constitutive levels of cytochrome P450 enzymes. Third instar larvae (72 $\pm$ 4h), from both crosses, were submitted to a chronic treatment (approximately 48h) with different concentrations of BthTX-I: 6.72, 13.44, 26.88, 53.75, 107.5, 215, or 430 µg/mL. Ultrapure water and Urethane (10 mM) were used as negative and positive controls, respectively. All treatments were performed in duplicate. There was no significant change in the survival rate of the individuals at any concentration from both the ST and HB crosses. The highest amount of mutant spots was observed for the ST cross at the highest concentration (430 µg/mL) of BthTX-I, being essentially recombinogenic. As for the HB cross, when metabolized, the BthTX-I acted early in the development, inducing an increase in the number of mutant spots at intermediate concentrations (53.75 and 107.5 µg/mL), being the 53.75 µg/mL highly mutagenic and 107.5 µg/mL recombinogenic. The highest tested concentrations were not mutagenic-associated, which may indicate cytotoxicity at high concentrations. Further studies should be performed to elucidate the mechanism of action of BthTX-I PLA_2 and its possible use in therapeutic strategies against cancer.

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GENE EXPRESSION BIOMARKERS IN PATHWAYS INVOLVING CELL DEATH, OXIDATIVE AND ENDOPLASMIC RETICULUM STRESS INDUCED BY PIPERLONGUMINE IN HepG2/C3A CELLS

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Keywords: oxidative stress, genotoxicity, cell signaling

Understanding the molecular mechanisms of action of candidate chemotherapeutic drugs is essential for the development of new pharmaceutical agents with greater effectiveness and reduced adverse effects. Piperlongumine (PLN) is an alkaloid with antiproliferative properties, but information on its mechanisms of action is scarce. This study correlated the antiproliferative effects of PLN using morphological and molecular analyses in a human hepatocellular carcinoma cell line, HepG2/C3A. This study assessed the cytotoxicity of PLN in MTT assay; interference in cell cycle, membrane integrity, induction of apoptosis and reactive oxygen species (ROS) by flow cytometry; genotoxicity by Comet assay; and mRNA expression by RT-PCR. Cytotoxic concentrations of this drug were ≥20 µM. PLN reduced cell proliferation as detected by the arrest of cells in G2/M and emergence of monoastral standard spindles. Furthermore, induction of cell death by apoptosis was observed. Apoptosis appeared to have been induced by increased expression of pro-apoptotic mRNAs *BAK1* (3.1-fold) and *BBC3* (2.4-fold) and by increased caspase 9 and 3/7 activity. PLN causes cellular injury by inducing ROS and DNA damage. We also observed changes in mRNA expression of cell cycle control genes *CDKN1A* (4.9-fold) and *CCNA2* (-2-fold). DNA damage signaling also activated *MDM2* expression (3.0-fold). This gene is also associated with the emergence of monoastral spindles in mitosis. Genes associated with degradation of ROS also exhibited increased mRNA expression (*GSR,* 2.0-fold; *SOD1,* 2.1-fold). Additionally, PLN induced endoplasmic reticulum stress, with elevated expression of *ERN1* (4.5-fold) and *HSPA14* (2.2-fold) mRNAs. Genes involved in metabolism of xenobiotics showed increased expression of mRNAs for *CYP1A2* (2.2-fold) and *CYP3A4* (3.4-fold) induced by PLN. Therefore, we observed that many avenues of damage induced by treatment with PLN contributed to antiproliferative effects, revealing gene expression biomarker mRNAs and the formation of monoastral spindles.

Financial Support: CNPq, CAPES, FINEP and Fundação Araucária.

CORRELATIONS OF mRNA EXPRESSION WITH APOPTOSIS AND DNA DAMAGE IN p53 WILD-TYPE AND MUTANT CELLS TREATED WITH HOMOHARRINGTONINE AND HARRINGTONINE

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Keywords: phytochemical; cell death; TNF; genotoxicity; cell cycle; HepG2/C3A.

The search for compounds derived from natural sources, such as phytochemicals, has grown because these compounds may represent a new approach for the treatment and prevention of cancer. Therefore, we evaluated the mode of action of the alkaloids harringtonine (HT) and homoharringtonine (HHT) in p53 wild-type (HepG2/C3A) and mutant (HuH-7.5) human hepatocyte cell lines and performed cell viability tests, flow cytometry for analysis of apoptosis, cell cycle status, membrane integrity, and genotoxicity, and assessed the relative expression of mRNAs involved in apoptosis, cell cycle regulation, xenobiotic metabolism, endoplasmic reticulum stress, and proliferation control. HT and HHT induced significant increases in the induction of initial apoptosis in both cell lines in addition to cell cycle alterations, with significant increases in G2/M cells in each line when treated with HHT and significant increases in the number of S cells in the C3A line after exposure to HT. Relative gene expression measurements revealed increased expression of apoptosis-inducing genes such as *TNF* and *BBC3* and decreased expression of *BCL2* and *BAK*, indicating that the compounds induce cell death by apoptosis. Expression of genes involved in metabolism was altered after treatment with the compounds, with *CYP2E1*, *CYP2C19,* and *CYP3A4* expression being elevated, the latter only in the HuH-7.5 lineage. Endoplasmic reticulum stress genes *ERN1* and *EIF2AK3* exhibited significant increases in their expression after treatment with HT and HHT, which may be linked to the principal action of such compounds being related to the inhibition of cellular protein expression. Endoplasmic reticulum stress may also cause increased expression of other genes, such as *TRAF2*, which was elevated following both treatments, as well as induction of tumor necrosis factors correlated with the observed increase in *TNF* gene. Thus, we suggest that HT and HHT induce endoplasmic reticulum stress by altering protein levels, thus causing cell death through the intrinsic and extrinsic pathways attributed to the increased expression of *BBC3* and *TNF,* respectively.

Financial Support: CNPq, CAPES, FINEP and Fundação Araucária.

PRELIMINARY CYTOGENOTOXIC INVESTIGATION OF THE ETHANOLIC EXTRACT FROM THE LEAF OF *STERCULIA STRIATA* A.ST. HIL. & NAUDIN

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Key-words: Mitotic index, Chromosome breaks, *Allium cepa*.

Among the cytogenetic tests used to evaluate the cytotoxic and genotoxic potential of medicinal plants, the *Allium cepa* test system stands out. This assay is widely used for the evaluation of chromosomal damage and changes in the mitotic cycle due to its low cost, simplicity and reproducibility. *Sterculia striata* A.St. Hil. & Naudin, is popularly known as Chichá or monkey chestnut and belongs to the family Malvaceae, being present from North to South of Brazil. Popularly, its leaves have been used for topical use in the treatment of boils and their seeds used for feeding, showing signs of antioxidant activity. This work aimed to investigate the cytotoxicity and genotoxicity of the ethanolic extract from the leaf of *Sterculia striata* A. St. Hil. & Naudin, through the *Allium cepa* bioassay. For this, the concentrations used ranged from 500 to 1000 µg/ml. Initially, the bulbs were grown in distilled water for 48h at 25°C and constant aeration. Subsequently, for each extract concentration, five bulbs were used and treated for 24h. After this period, the roots were collected, fixed in Carnoy solution (3:1-ethyl alcohol and acetic acid) for 24h, washed in distilled water and hydrolyzed in 1N HCL for 5 min to prepare the slides. These were stained with 1% acetic orcein and analyzed under an optical microscope at an increase of 400X. All experiments were compared to the negative control, which consisted of distilled water. Statistical analysis was performed using the variance test (ANOVA), followed by Tukey's multiple comparisons test with *p* values lower than 0.05. As parameter of cytotoxicity the mitotic index (MI) was used, which is given by the ratio of the number of cells divided by the total number of cells, multiplied by 100. The results demonstrated a significant increase in MI values for all tested concentrations of the ethanolic extract from the leaf of *Sterculia striata* A. St. Hil. Naudin, compared to the negative control. For the mutagenicity parameter, a significant increase in the number of chromosomal changes in treated cells was also observed, especially for the 750 and 1000 µg/ml concentrations. The main alterations were chromosomal delays and released chromosomes in anaphase. In view of the preliminary results obtained, it can be concluded that the ethanolic extract from the leaf of *Sterculia striata* A. St. Hil. Naudin has a proliferative effect and is mutagenic, at least at the concentrations and test used.

DAMAGES INDUCED BY HERBICIDE ATRAZINE AND ITS PHOTODEGRADATED PRODUCTS IN ERYTHROCYTES OF *Astyanax altiparanae*

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Key-words: environmental monitoring, ecotoxicology, fish, heterogeneous photocatalysis, Micronucleus Test.

Atrazine (ATZ) is widely used throughout the world to limit weed growth by increasing the productivity of agricultural crops. However, the indiscriminate use, accidental spillage and discharge of effluent containing this herbicide represents a threat to ecosystems such as aquatic biota, especially because of its difficult degradation. For this reason, researches that aim to degrade this pesticide have been developed. The Advanced Oxidative Processes (AOPs) are technologies that use hydroxyl radicals as a basis for the degradation of persistent organic pollutants and disinfection of pollutants. Among the types of AOPs employees for the degradation of atrazine it highlights the photocatalysis, which uses a combination of light, as UV, and a catalyst semiconductor to degrade pollutants by means of oxidation-reduction reactions. The objective of this study was to investigate the mutagenicity, by the Micronucleus Test, of the herbicide ATZ and products of the treatments performed by heterogeneous photocatalysis in erythrocytes of *Astyanax altiparanae* exposed for 24 and 96 hours. In addition, detecting the presence of ATZ and its degradation products by means of mass spectrometry in water samples collected from their aquariums. For the photocatalysis the catalysts were used: zinc oxide, titanium dioxide and zinc oxide impregnated with titanium dioxide, doped with iron. The results obtained demonstrate that no significant differences were observed for the 24 hours exposure time, however, after 96 hours all the groups induced to the formation of nuclear changes of the type vacuolized. No group induced the formation of micronuclei. ATZ was detected only in the atrazine group in the two sample times, desethyl mercapturic atrazine was detected in all groups in the time of 24 hours while desalkyl mercapturic atrazine after 24 and 96 hours. Hydroxyatriazine and deisopropyl hydroxyiatrazine were found only in the treatments by photocatalysis in the two sampling times. Terbuthylazine-2-hydroxy was observed only in zinc oxide photocatalysis at both times. Although the treatments by photocatalysis are efficient in the degradation of ATZ and accelerate this process, the obtained results show that even as the atrazine, the metabolites from the degradation process have as damaging effect as the precursor molecule. These data reinforce the importance of the use of AOPs, however, demonstrate the need to optimize these treatments for complete mineralization of the pesticide.

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NUCLEAR AND HISTOLOGICAL CHANGES IN *Astyanax altiparanae* EXPOSED TO EFFLUENT FROM THE ANODIZING OF ALUMINUM PROCESS

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Key-words: environmental monitoring, ecotoxicology, liquid effluent, fish, micronucleus test.

Aluminum is a metal widely used, since domestic utensils until the civil construction. The anodized aluminum is a procedure provided to the metal to form a thick layer of aluminum oxide on the surface, in order to confer greater resistance to the material. This process generates an effluent with acid pH and high concentration of sulfate. Alternatives for the treatment of this type of effluent involve the use of the technique of chemical precipitation. Can be used for both salts of barium chloride ($BaCl_2$), calcium chloride ($CaCl_2$) and commercial calcareous with aluminum hydroxide ($Cc/Al(OH)_3$). The objective of this study was to evaluate the toxicity in *Astyanax altiparanae* (*lambari*), of the raw effluent (E1 and E2) and treated by chemical precipitation, by the micronucleus test in erythrocytes and histology of the gills, after 24 hours of exposure. The microcosm consisted in 18 aquariums, being 3 repetitions for each group: E1, $BaCl_2$, $CaCl_2$, E2 and $Cc/Al(OH)_3$. For the study was performed to collect the blood of the tail vein and the second branchial arch left, of each animal. According to the results, the effluent E2 induced damage in erythrocytes cells after 24 hours. The most frequent was the induction of vacuolated nuclei. For the gill histopathology, considering the index of Bernet, which evaluates the importance of the lesion, all effluents, except $Cc/Al(OH)_3$, induced significant changes in the gills. The lesions observed with greater frequency were epithelial desquamation, hypertrophy of epithelial cells and loss of cell adhesion. These changes are initial responses to a stressor agent, possibly are indicative of changes in the level of cellular membranes, therefore, aluminum alters the permeability of the membrane. Already the treated effluent with $Cc/Al(OH)_3$ may have been more efficient, because, besides of the sulphate can also remove metal cations and anions. This study reinforces the importance of biological tests for the evaluation and validation of the physicochemical techniques already used, effluent treatment, as well as the development and application of biological parameters to be used prior to the launch of these in water bodies.

Financial Support: CNPq, CAPES.

GENOTOXICITY, CITOTOXICITY AND CONCENTRATIONS OF METALS IN BLOOD AND URINE OF WORKERS EXPOSED TO WELD EMISSIONS

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Introduction: At present, exposure to smoke emissions or welding aerosols in the work environment is frequently observed at an industrial level. These are produced by the evaporation and solidification of metals that are released from the foundry, resulting in aerosols of metal oxides (oxides of aluminum, cadmium, chromium, copper, iron, lead, manganese, nickel, titanium, vanadium) and gases such as ozone, nitrogen dioxide and carbon monoxide that are generated by the decomposition of electrode coatings and the action of ultraviolet rays. According to the International Agency for Research on Cancer (IARC) workers in shoe factories, metallurgy, paint and welding exposed to organic solvents and heavy metals, may be at increased risk of developing cancer of the trachea, lung, liver and colon. Objective: The aim of this study was to evaluate the potential cytotoxic and genotoxic effects of welding fumes on peripheral blood lymphocytes and buccal epithelial cells in occupationally exposed workers. Materials and methods**:** in this study were included 98 exposed workers and 100 non-exposed control individuals. Genotoxicity and cytotoxicity was determined by cytome of lymphocytes and oral mucosa analysis and the metals concentration present in blood and urine was performed through the PIXE method. Analysis of differences in the biomarkers was performed using the non-parametric Mann–Whitney U test. Statistical differences between the groups were analysed using the nonparametric two-tailed Kruskal–Wallis test with Dunn's correction for multiple comparisons to perform a non-parametric analysis of variances. Results: Significant differences were observed between the two groups in terms of biomarkers by cytokinesis-block micronucleus cytome (CBMN-Cyt) assay; frequency of micronucleus (MN), nucleoplasmic bridge (NPB), nuclear bud (NBUD) and necrotic cells (NECR) and levels of biomarkers for micronucleus, binucleated cells (BN), karyorrhexis (KRX), karyolysis (KRL) and condensed chromatin (CC) by the buccal micronucleus cytome (BM-Cyt) assay.

Also, there was a significant concentration of metals in blood of the exposed group such as magnesium, aluminum, chromium, manganese, iron and nickel. Regarding the urine metals concentrations analyzed significantly higher concentrations of chromium, iron and copper were observed in the exposed group compared to the control group. These results are the first data in Colombia on the cytotoxic and genotoxic effects induced by continuous exposure to welding fumes and thus showed the usefulness of biomarkers of the comet, CBMN-Cyt and BM-Cyt assays for human biomonitoring and evaluation of cancer risk in the exposed populations. Conclusions: These cytotoxic and genotoxic effects in the workers exposed to welding fume could be consequence of the oxidative damage produced by the exposure and inhalation of metals present in the welding fumes.
These results are the first data in Colombia on the cytotoxic and genotoxic effects induced by continuous exposure to welding fumes and thus showed the usefulness of biomarkers of CBMN-Cyt and BM-Cyt assays for human biomonitoring and this information generate guidelines in order to promote industrial and hygiene safety programs whose purpose is to generate awareness of the existence of risks and care in the worker.

Keywords: Welding, emissions, metals, DNA oxidative damage.

THE ANTIPROLIFERATIVE EFFECTS OF 1,3-DIMETOXY-5- (4-METHOXYSTIRYL) BENZENE (*cis*-TMS) AGAINST THE LUNG ADENOCARCINOMA CELL LINE A549

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Lung cancer is considered one of the leading causes of death worldwide, with a survival rate ranging from 15 to 20% after five years. The high chemoresistant phenotypes and extensive side effects of conventional chemotherapy treatment, such as cardio and nephrotoxicity, requires new effective therapies for the treatment of this disease. The search for natural products or their synthetic derivatives becomes relevant for the development of new drugs in the treatment of cancer. Experimental evidence shows that 1,3-dimethoxy-5- (4-methoxystyryl) benzene (*cis*-TMS) has better bioavailability as well as more pronounced cytotoxic effects than its structural precursor, resveratrol. The aim of this work was to study evaluate the antiproliferative effects of *cis*-TMS in the pulmonary adenocarcinoma cell line A549. The experiments were conducted using 24 hs of treatment with *cis*-TMS in the concentrations of 0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 µM. Cytotoxicity was determined using XTT and clonogenic survival assays. Wound healing assay estimated the cellular migration and apoptotic cell death was determined by cytomorphological analysis. The results showed that *cis*-TMS was cytotoxic to the A549 line at the concentration of 2.5 µM ($p<0,05$), as demonstrated by XTT colorimetric assay; but when assessed for its long-term cytotoxicity by the clonogenic survival assay, it was cytotoxic in concentrations up to 0.625 µM ($p<0,0001$). *Cis*-TMS significantly inhibited cellular migration and induced apoptotic cell death after 24 h treatment with 1.25 and 2.5 µM ($p<0,001$). Altogether, these data demonstrate the cytotoxic and antiproliferative effects of *cis*-TMS against the lung adenocarcinoma cell line A549 at very low concentrations; however, further studies will elucidate the molecular pathways involved in this process.

Key-words: Antiproliferative activity, *cis*-TMS, cytotoxicity, lung adenocarcinoma, stilbene.

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INVESTIGATION OF THE ANTITUMORAL ACTIVITY OF IDARRUBICIN COMBINATION WITH MEBENDAZOL IN VITRO

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Idarubicin (IDA) is an antineoplastic topoisomerase II inhibitor that has been used in the treatment of several types of neoplasias, which may be chemoresistant to this drug. Mebendazole (MBZ) is an anthelmintic whose association with antineoplastics has been investigated, and it acts through the depolymerization of tubulin and subsequent de-structuring of microtubule function, thus, it may play an important role in the inhibition of the expression of genes related to chemoresistance and contribute to potentiate the treatment. This study aimed to evaluate the antitumor activity of the molecule idarubicin in combination with mebendazole in the metastatic human gastric adenocarcinoma AGP01. Thus, the present study evaluated the cytotoxic potential of IDA in combination with MBZ through the MTT assay to determine the IC50 of the drugs isolated and combined in the AGP01 cell line. Cell cycle analysis and cell death index were measured by flow cytometry to evaluate cytostatic effects and cell death rates. The 50% inhibitory concentration (IC50) was 242 nM for IDA and 300 nM for MBZ, with the highest cytotoxic activity conferred on the association of the substances with the IC50 of 123.8 nM for IDA and 153.5 nM for MBZ. In addition, both substances were able to block the S-cell cycle for isolated IDA and combination of MBZ + IDA ($p < 0.001$), and in the G2 / M phase for isolated MBZ ($p < 0.001$), in addition to inducing death ($p < 0.001$) isolated and in combination at the two concentrations tested (IC 50 and half). The MBZ and IDA drugs have excellent antineoplastic activity against the gastric cancer cell line AGP01. These results may represent a new strategy for the treatment of this type of tumor, since the association of IDA with MBZ reinforces the induction of cytotoxicity, cell death and cell cycle blockage in gastric cancer cells. Therefore, their combination may contribute to a more effective treatment, since the combination of these two drugs is a promising therapeutic strategy, mainly for the possibility of reducing the dosage necessary to obtain beneficial effects to the patient and contribute to a possible reduction of adverse effects caused by idarubicin.

Financial Support: CAPES

VALUATION OF THE ANTIGENOTOXIC AND ANTICITOTOXIC EFFECT OF THE BIOPRODUCT CANOVA® METHOD IN HUMAN LYMPHOCYTES EXPOSED TO ANTI-MALARICIAN ARTESUNATO

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Malaria is one of the most serious infectious-contagious diseases in the world, with an extensive geographic distribution in tropical zones, where temperature and humidity favor the development of vectors. Generally, its treatment is based on the administration of drugs like artemether and artesunate. However, studies have shown that artesunate may cause genotoxic and cytotoxic damage, so treatments that minimize these effects are strongly needed. The CANOVA® Method (CA) is a Brazilian immunomodulator of homeopathic formulation indicated under clinical conditions at which the immune system is compromised. The present study aimed to evaluate in vitro the possible cytoprotective effects of CA on peripheral human lymphocytes exposed to artesunate. Comet assays were performed for the evaluation of genotoxicity / antigenotoxicity and the detection of apoptosis by multiple dyes. The results of the comet assay demonstrated that the CA has the ability to significantly reduce ($p < 0.05$) DNA damage index (ID) induced by ART (artesunate) in all combinations tested, the ART ID was 2 (2 µg / mL) + CA 4%, ART (2 µg / mL) + CA 8 (95% confidence interval [CI]), whereas the IDs of ART + CA combinations were approximately 1.03, 1.14 and 0.66 for ART % and ART (2µg / mL) + CA 16%, respectively. There was also a significant difference ($p < 0.05$) between ART ID (2.98) and negative control ID (1.04). In cell cultures tested only with CA, no significant variations in lymphocyte ID were observed when compared to negative control ID. The evaluation of the anticytotoxic potential of CA was obtained after 24h and 48h of treatment, presenting a decrease of both apoptotic and necrotic cells in the combined treatments of ART + CA when compared to the treatment of ART alone, however, it was significant ($p < 0, 05$) only in the combination of ART + CA16% in relation to ART alone treatment. Thus, CA can collaborate in the treatment of malaria, minimizing the genotoxic and cytotoxic effects caused by artesunate, since all CA concentrations significantly reduced the damage and induction of apoptosis caused by ART administration, that characterizes an antigenotoxic action and anti-toxicity in vitro of this compound in relation to artesunate.

Financial Support: CNPq

ANTICARCINOGENIC POTENTIAL OF SILIMARIN IN SOMATIC CELLS OF *DROSOPHILA MELANOGASTER*

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Keywords: protective effect, silimarin, epithelial tumors

Silymarin is a chemical compound extracted from the seeds of *Silybum marianum* (L) *Gearth*, is a mixture of three structural isomers: silybin, silidianine and silicristin, the former being the most active component. In addition to its hepatoprotective effects against the most severe necrosis, studies report a satisfactory antioxidant and anticarcinogenic power. In view of this, the objective of the present study was to verify the carcinogenic and / or anticarcinogenic effect of silymarin by means of a test for the detection of epithelial tumors (*warts*) in *Drosophila melanogaster*. We used 72 hour larvae resulting from crossing *mwh x wts/tm*^{3} were treated with three different concentrations of silymarin (12.5,25 and 50 mg / mL). The DXR (0.4 mM) was adopted as positive control, and for the negative control, reverse osmosis water was used. To assess whether the compound in question has anticarcinogenic characteristics, silimaria was combined with DXR. As a result, the frequencies found in the isolated concentrations of silymarin (12.5,25 and 50 mg / ml) in relation to the negative control did not present significant differences in tumor frequency ($p < 0.05$). Therefore, the compound in question had no carcinogenic potential. However, the results of the compound associated with DXR show a significant reduction of the tumor action, that is, the contact with the inducer of epithelial tumors, silymarin was considered to be potentially anticarcinogenic. Therefore, in the present experimental conditions, silymarin presented anticarcinogenic effect, since it was able to modulate the chemotherapeutic action reducing statistically the frequency of tumors generated in *D. melanogaster.*

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GENOTOXIC RISK OF TREATMENT WITH ^{131}I AND GENETIC FACTORS OF SUSCEPTIBILITY TO THYROID CANCER

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In view of the fact that the number of thyroid cancers is increasing in Bolivia and therefore the treatment using 131I, in this study we determined the magnitude of the genotoxic damage after treatment in patients with thyroid cancer using the test of Micronuclei in binucleated cells and the comet assay (P = 0.000, Wilcoxon Test). Finding statistically significant data of damage in patients with both biomarkers. Furthermore, in contrast to 5% to 10% of the population that presents with nodules, thyroid cancer is the cause of less than 1.6% of all cancers. Therefore, it is a priority to find tracking methods capable of identifying individuals at risk of cancer among nodule carriers. Some clinical and epidemiological characteristics, among other factors, are important in this identification. Molecular biology techniques allow the identification of genetic polymorphisms in this study of the rs2145418s and rs4658973 polymorphisms that make up the basis of thyroid cancer susceptibility. In the analyzed population, SNP rs4658973 showed an association with thyroid cancer, while SNP 2145418 showed no association. The results obtained in the genotypes with the variant allele G of the marker rs4658973 showed a protection to thyroid cancer with an odds ratio of 0.52. Regarding the modulating effect of environmental genetic factors, in the SNP rs4658973 a reduction of the risk associated with the tobacco habit could be observed as protection to thyroid cancer with an OR = 5.44 (CI: 1.62 - 18.26)The findings confirm the usefulness of the micronucleus assays in binucleated cells and the comet for the performance of human biomonitoring studies to evaluate genotoxic damage, as well as the importance of determining the polymorphisms of genes related to thyroid cancer as biomarkers of susceptibility. Key words: Genotoxic risk, thyroid cancer, genetic polymorphisms, Genetic susceptibility.

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MUTAGENIC AND GENOTOXIC EFFECTS OF DIFFERENT DEXCLORPHENYRAMINE MALEATE PREPARATIONS IN CULTURED PBMC

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Key-words: mutagenicity, genotoxicity, antihistamine, dexchlorpheniramine.

Compounds that competitively block histamine at H1 receptors have been used clinically years. Dexchlorpheniramine (DPN) belong to the alkylamine class of antihistaminic drugs that often used to treat allergic symptoms. The aim of this study was to evaluate the mutagenic and genotoxic effects of DPN in peripheral blood mononuclear cells (PBMC), as pure active (DPN-A) and as commercial form (DPN-C). Initially, 5 mL of venous blood was collected from healthy adult voluntary, over 18 years old. The blood was separated with Histopaque® to obtain the PBMC. 10^6 cells/mL were incubated in supplemented RPMI 1640 medium at 37°C in 5% CO_2 atmosphere, without DPN (negative control, just RPMI; and positive control, with RPMI plus demecolcine 10 μg/mL), or with DPN (DPN-A and DPN-C) in different concentrations for 24 hours, as following: 0.5, 2.5, 5,10, 50 ng/mL. All protocols were approved by the Ethics Committee of UNIPAMPA (n° 27045614.0.0000.5323). After that, both micronucleus and comet tests were performed. For the micronucleus test, an aliquot of each culture was placed over slide and stained. The micronuclei were counted under optical microscope at 1,000 X. For the comet essay, an aliquot of each culture was homogenized with low melting point agarose and dispensed on a slide pre-coated with normal melting point agarose. After drying, the slides were undergoing to the lysis solution for 24 h. After, the electrophoresis was carried out to obtain the DNA migration. In sequence, firstly the slides were immersed in a neutralizing solution, and secondly in a fixative solution. The slides were stained with silver nitrate and the comets counted under an optical microscope at 400 X, considering the following scores: zero, 1, 2, 3, and 4 damage levels, where zero represent absence of DNA damage; and 4, the maximum damage. Data were analyzed by One Way ANOVA, complemented by post-hoc Dunnett's Test, and considered as statistically significant when $p<0.05$. The mean negative damage indices of the control and samples were kept at the house of 5 points, while the positive control reached values close to 30 points, showing that the test compound did not cause lesions. For the micronuclei test, the same profile of the comet test was found, but with indices of 2.5 for negative control and samples and 12.5 for positive control. In conclusion, our data showed that DPN was unable to induce both mutagenic or genotoxic effects in human PBMC in the experimental conditions performed.

Financial Support: FINEP, FAPERGS and CNPq

EVALUATION OF POLYMORPHISMS IN GENES RESPONSIBLE FOR XENOBIOTIC METABOLIZING ENZYMES IN PATIENTS WITH CLEFT LIP AND PALATE IN THE STATE OF PARÁ.

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Key-words: Cleft lip and palate, Genetics, Polymorphism, Agrotoxic

Cleft lip or palate is one of the most frequent congenital defects and several studies relate this malformation to multifactorial causes. Among the various environmental causes are maternal smoking habits, as well as the use of pesticides. The response of the human embryo to teratogenic agents is well known. However, it is known that different organisms metabolize in a different way the same chemical component, this is due to intrinsic genetic characteristics related to different enzymatic functions. Such differences can be investigated from the analysis of polymorphisms in genes related to the metabolism of these xenobiotics, which may thus be related to the etiogenesis of palatine lip fissures. The objective of our study was to analyze polymorphisms in seven genes**,** *PON1* (rs662), *PON1* (rs854560), *MTHFD1, CYP2E1, EPHX1, ABCB1, AHR*, where a correlative analysis with environmental factors such as exposure to pesticides was performed in order to to evaluate whether or not there is influence of different polymorphic variants and such environmental interactions in the etiogenesis of cleft lip palates. The total number of samples analyzed was 166 individuals, 83 of whom were patients with fissures, with a mean age of 7 years (SD 5 years) and 83 mothers. In our samples, the male gender was 64% of the total affected. a record for the collection of epidemiological data was developed for the study; the biological material collected for analysis was blood. In relation to the polymorphism of the enzyme PON1 (rs662), when genotype frequencies were analyzed, the AA genotype was more frequent in the affected population when compared to the African and Asian populations. This genotype is associated with clefts of the Labiopalatina type (p = 0.009)Another important relation was the findings related to the PON1 polymorphism (rs854560), when analyzed the genotype frequency, the genotype AA, was more frequent in both the mother and the affected population in relation to the global population. In addition, this genotype had a higher prevalence in patients with cleft palate (p = 0.039). Rescuing the relevance of our study when evaluated its intersection with pesticide.In TT genotype, it was more frequent in the affected population when compared to the African and Asian populations. Proving that our target may actually have been affected by exposure to the related environmental factor (pesticide). In relation to polymorphism of the EPXH1 gene, most of the group of mothers exposed to pesticides were homozygous AA (p = 0.019), suggesting a relationship between the use of pesticides and this genotype in the etiology of cleft lip and palate. Among the group of mothers exposed in the MTHFD1 gene, most of them were homozygous AA (p = 0.019), suggesting a relationship between the use of pesticides and this genotype in the etiology of cleft lip and palate.

In relation to the ABCB1 gene, the frequency of the TT genotype was significantly higher for the population of affected and mothers when compared to the African population.
In the analysis of the AHR gene, the maternal population had a higher frequency of the GG genotype when compared to the African and Asian populations, and a lower frequency of this same genotype when compared to the Caucasian population. The distribution of the genus and types of fissure was similar to the data already reported in previous studies in individuals with cleft lip and palate of different populations. Among the mothers who participated in the study there were reports of exposure to at least one environmental (agrotoxic) factors related to increased risk of cleft lip and palate.

Financial Support: CAPES and FAPESPA

ANALYSIS OF THE CYTOTOXICITY OF HOSPITAL LAUNDRY EFFLUENTS, WITH AND WITHOUT PHYSICAL-CHEMICAL TREATMENT, IN *Allium cepa* L.

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Key-words: environmental monitoring, ecotoxicology, water pollution, advanced oxidation processes, liquid waste.

Water bodies suffer constant impacts due to increasing anthropogenic actions that are caused by industrialisation and urbanisation. Among the several sectors that generate liquid effluents in urban centres, hospitals have been the focus of several studies due to their polluting potential. In this context, hospital laundries are responsible for a significant part of the amount of effluent that is generated in hospitals. Advanced oxidation processes are technologies that involve the generation and use of strong oxidizing agents, such as ozone and hydrogen peroxide (H_2O_2), in the presence or absence of ultraviolet light (UV), for the generation of highly reactive free radicals capable of mineralizing organic substances present in conventional effluents. Considering the social importance, as well as the preservation of the environment and protection of water resources, the objective of the present study was to evaluate the cytotoxic potential of different laundry effluents from the Regional University Hospital of Maringá, Paraná, Brazil, on *Allium cepa* L. meristematic root cells. The effluents were characterised by the actions of rinsing, wetting, prewashing, washing and softening, together with the wastewater (the effluent generated at the end of the washing process), the wastewater that was treated by physicochemical processes (PC - coagulation/flocculation/filtration) and the wastewater that was treated by advanced oxidation processes (PC + UV, PC + H_2O_2 and PC + UV/H_2O_2). The mitotic indexes were calculated by counting 5,000 cells per group and the statistical analyses were performed by the Chi-square test ($\alpha = 0.05$). Although the analysed effluents did not present a statistically significant cytotoxic potential, the results showed that the PC + UV/H_2O_2 treatment reduced the critical parameters, such as the biochemical and chemical oxygen demands, to tolerable levels that are required by the legislation for the disposal or the reuse of effluents. Upon considering that each washing process may result in different effluent compositions, with different concentrations of toxic substances, it becomes indispensable to implant appropriate treatment methods for these effluents, allowing for the rational reuse of water and in a compliance with the legislation standards for an effluent discharge, while at the same time, reducing the environmental impacts.

Financial Support: CNPq, CAPES.

EVALUATION OF THE CYTOTOXIC AND GENOTOXIC ACTIVITIES OF THE FLAVONOID TAXIFOLIN IN HUMAN KIDNEY TUMOR CELLS (786-O)

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Key-words: dihydroquercetin, flavanonol, natural compound, MTT assay, comet assay.

The beneficial activities that flavonoids demonstrate have been of great interest for the development of new forms of therapy and prevention of several diseases. Taxifolin is a polyphenolic flavonoid belonging to the family of flavanones, it can be found in several fruits, such as strawberries, and possesses antioxidant, antibacterial, cytoprotective, genoprotective, cardioprotective and cholesterol-lowering properties. For this reason, the objective of this work was to evaluate the cytotoxic potential of Taxifolin by the MTT assay and the genotoxic activity by the comet assay in human kidney tumor cells (786-O). Cells were treated with Taxifolin, in addition to a control group, and a treatment with the cytotoxic agent doxorubicin (18µM) for 24, 48 and 72 hours for the MTT assay. In the comet assay, cells were seeded in culture flasks, and treated with Taxifolin, in addition to a control group, and a treatment with the genotoxic agent methylmethanesulfonate (75µM), for 3 hours, prior to the alkaline electrophoresis. The experiments were performed in three biological replicates and the data were submitted to analysis of variance (ANOVA), followed by the Dunnett's test for the MTT assay, and the Tukey's test for the comet assay. The results showed that Taxifolin did not present cytotoxic activity, regardless of the exposure time, for any of the concentrations evaluated (50-500 µM), as it did not reduce significantly the absorbances when compared to the control. These data suggest that the compound did not interfere in the activity of cellular and mitochondrial dehydrogenase enzymes. For the comet assay, the flavonoid showed no genotoxic activity at concentrations of 300 and 400 µM, however it was significantly genotoxic at the concentration of 500 µM, possibly due to the pro-oxidant activity performed by flavonoid when found in very high concentrations. This pro-oxidant effect may be related to the generation of reactive oxygen species, which may have been responsible for the primary DNA-damage, as observed in the comet assay.

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WATER CYTOTOXICITY OF THE IRRIGATED RICE FARMING FROM THE IVAÍ RIVER VALLEY - PR, IN *Allium cepa* L.

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Key-words: ecotoxicology, environmental monitoring, *Oryza sativa* L., aquatic ecosystems, water management.

In the State of Paraná, Brazil, irrigated rice (*Oryza sativa* L.) farming areas are located close to dams or floodplains, especially in the Ivaí River valley, which brings to the farmers constant environmental problems regarding the exploration of water source areas or environmental protection. In addition, the large volume of water used for flood irrigation has major impacts on the quality of aquatic ecosystems, since the irrigated rice production system is characterized by the widespread use of pesticides that may present cytotoxic, genotoxic, mutagenic and carcinogenic properties. For this reason, the objective of this work was to investigate the cytotoxic potential of water samples collected from an irrigated rice farming in the municipality of Tapira – PR, during the 2018 harvest, as well as the water of the Ivaí River, which supplies and drains the crop, on meristematic root cells of *Allium cepa* L. The experimental design consisted of five groups, with five bulbs each, and received different treatments: negative control (filtered water); water samples from three different places, in the beginning, in the middle and at the end of the rice farming; and water from the Ivaí River, which receives the water drained from the farming. The roots of the bulbs were collected at three different times: control of the bulb (Co-0h); treatment with water samples for 24 hours (Tr-24h); and recovery in filtered water for 24 hours (Re-24h), for reversal of possible damages. The mitotic index was calculated by counting 5,000 cells per group and the statistical analysis was performed by the Chi-square test (α=0.05). The analysis of the results indicated cytotoxic activity to the water sampled in the middle of the farming, with a significant reduction of the cell division for Tr-24h (40%) and Re-24h (52%) treatments, when compared to the control of the bulb. This may have occurred because the irrigated system can leach the pesticides used in rice cultivation to the lower parts of the farming, which coincided with this collection point. The water sampled at the end of the farming also indicated cytotoxicity (Tr-24h, reducing the cell proliferation by 58%), but recovery from damage was observed after Re-24h treatment (66% of cell proliferation, statistically similar to the control). The evaluation of these damages and the use of bioindicators are of great importance, since short-term results can be obtained before the occurrence of severe and deleterious effects, allowing the adoption of preventive impact strategies before more environmental damage occurs.

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CYTOTOXIC AND MUTAGENIC ACTIVITIES OF ALUMINUM ANODIZATION INDUSTRIAL EFFLUENTS ON *Allium cepa* L.

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Key-words: environmental monitoring, ecotoxicology, liquid effluent, barium chloride, ettringite.

The aluminum anodizing industry generates liquid effluents containing several metals, sulfuric acid, and other contaminants, which are often disposed in the public sewage system or in the environment without even receiving proper treatment. Barium chloride and ettringite are used in the treatment of effluents from the anodizing process to remove excess sulfate. Thus, the objective of this study was to evaluate the cytotoxic and mutagenic potential of the raw effluents treated with ettringite and barium chloride, produced by the aluminum anodization process of an industry from Maringá – PR, by the *Allium cepa* L. test system. The experiment was distributed in five groups, with five bulbs each: raw effluent, raw effluent treated with ettringite; raw effluent treated with barium chloride, and negative control (treated with filtered water all the time). The roots of the bulbs were collected in three sample periods: control of the bulb, treatment with effluents for 24 hours, and recovery in filtered water for a further 24 hours, for reversal of possible damage. The negative control remained throughout the sample period in filtered water. The mitotic index was calculated by counting 5,000 cells per group and the statistical analysis performed by the Chi-square test (α=0.05). The analysis of the results did not show cytotoxic activity for the raw effluent or the raw effluent treated with ettringite, however, the raw effluent treated with barium chloride was cytotoxic, with complete inhibition of cell division even after the recovery time in the water. In addition, several chromosomal alterations were observed in the meristematic root cells, such as chromatin condensation abnormalities and mitotic spindle dysfunction, possibly induced by the barium chloride. Both treatments were efficient for removing sulfate from the effluent, but it was demonstrated that this industrial effluent must not be directly discharged in the environment after barium chloride treatment due to its cytotoxic and mutagenic effect. The *Allium cepa* L. bioassay demonstrated sensitivity in identifying the toxic potential of this type of effluent, reinforcing the important role of toxicological genetics and plant biomonitoring as effective tools for environmental monitoring.

Financial Support: CNPq, CAPES.

ACUTE AND GENETIC TOXICITY OF *HOVENIA DULCIS* THUNB. (RHAMNACEAE) LEAVES IN *EUSENIA FETIDA.*

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Key-words: invasive species, medicinal plant, micronucleus test, earthworm

Hovenia dulcis Thunberg (Rhamnaceae), popularly known as the Japanese raisin tree, is a species native from Asia and introduced in different continents. Parts of this plant (seeds, fruits, roots and leaves) has been widely used as functional foods and natural health products and its metabolites may have an important role in these benefits. However, the knowledge about the effects *H. dulcis* on biological mechanisms is poor. Thus, the objective was to investigate the toxic effect of the *H. dulcis* leaves in *Eusenia fetida* by contact filter paper test. For this, the infusion preparation was undertaken with fresh and dry leaves from plants of *H. dulcis*. These were placed in boiling water for 10 min, strained and placed to cool. The follow treatments were used: 5, 15 and 30 mg/ml of leaves. The *E. fetida* is a currently used invertebrate species for toxicological assessment. Adult earthworms (weighing between 350 and 500 mg) with well-developed clitella were used to the contact filter paper test (OECD, 1984). A piece of filter paper was placed in a Petri dish and treated with the infusions and one earthworm was placed on it. Ten individuals were exposed per group. The dish was incubated in the dark at 20±1 °C for 48 hs. After, the mortality and earthworm weight were recorded. An earthworm was considered dead if it failed to respond to a gentle mechanical touch on the front end. After, coelomic fluid of the five earthworm containing coelomocytes was obtained from the coelomic cavity by the extrusion method and an aliquot of 10 µL of coelomic fluid from each earthworm at each infusion concentration was smeared on a glass slide, using two slides for each concentration. When the fluid dried, the coelomocytes were fixed with a fixative solution and stained by Giemsa. A total of 2,000 coelomocytes were scored to determine the micronuclei frequency. When the mortality percentage is higher than 10%, the sample is considered toxic. So for fresh leaves infusion, there was no toxicity. But for dry leaves infusion at concentrations of 15 mg/ml and 30 mg/ml were toxic. Also, it was observed that a greater weight loss in individuals exposed to higher concentrations as well as a micronucleus frequency increased when compared to negative control (tap water). In conclusion, higher concentration of dry leaves infusion of *H. dulcis* caused acute and genotoxic toxicity to earthworm exposed via contact filter paper test.

Financial Support: CNPq, FAPERGS, UNILASALLE.

ASSESSMENT OF THE GENOTOXIC POTENTIAL OF NIOBIUM OXIDE NANOPARTICLES

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Key words: nanoparticles, genotoxicity, Comet assay.

The term Nanoscience refers to the study of objects and devices that have their physical dimensions with only a few tens of nanometers, known worldwide as nanoparticle (NP). Niobium (Nb) is a metallic element, has low resistance to oxidation and has the property of superconductivity at very low temperatures. Materials containing Nb are used in high technology industries, especially in aerospace, with superalloys of metal and electronics. Considering the lack of information on the biological effects of niobium oxide (NbO) NPs, this study assessed the genotoxic effects of NbO NPs using the comet assay (*Single-Cell Gel Assay*), which detects DNA damage in individual cells. The NbO NPs used for the test were synthesized in the Laboratory of Nanostructured Materials of UNIPAMPA. For the test, Chinese hamster ovary (CHO) cells were cultured in flasks containing DMEM medium with 10% fetal bovine serum and 1% penicillin/streptomycin in an incubator with 5% CO_2 at 37 °C. The cells were transferred to cell culture dishes, and after 24 hours the following concentrations of NbO NPs were assessed: 6.5; 13.15; 26.31; 52.62; 105; 210.5 and 421 µg/mL, and the positive and negative control (distilled water) during a period of 4 hours. The alkaline version of the comet assay (pH>13) detects double-and single-strand DNA breaks, alkali-labile sites, DNA/DNA and DNA/protein associations and incomplete DNA repair by excision after single-strand breaks. Results were analyzed using the image analysis software *Comet Assay IV* (*Perceptive Instruments,* UK) based on tail intensity (% of DNA in the tail) to assess DNA damage. Data were compared using the one-way analysis of variance (ANOVA) and the Dunnett test at $p<0.05$. The results demonstrated that NbO NPs concentrations of 52.62; 105; 210.5 and 421µg/mL increased significantly the DNA damage frequency in CHO cells in comparison with the negative control group. Negative control showed 5.9% of DNA in the tail, while concentrations that were genotoxic (52.62, 105.0, 210.5 and 421 µg / mL) showed, respectively, the following percentages: 13.8%; 16.2%; 18.3% and 13.9% of DNA in the tail (tail intensity). Due to wide spectrum of applications of NbO NPs, the next investigations should be focused on the evaluation of the mutagenic potential of this nanomaterial.

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EVALUATION OF THE CYTOTOXIC EFFECT OF THE MEBENDAZOL AND VANDETANIBE IN VITRO ASSOCIATION

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The search for new alternatives for the treatment of gastric cancer represents an important advance in the reduction of mortality rates. Among the possible strategies is the use of the combination of single target drugs to affect different pathways of tumor development. In this context, Vandetanib has potential since it has been observed the importance of the family of Vascular Endothelial Growth Factors (VEGF) in the process of GC carcinogenesis, as this drug acts to inhibit this pathway. Mebendazole, which has been studied in anticancer treatment, is a viable alternative since it acts on cellular microtubules. Thus, the present study aimed to evaluate the antineoplastic potential of the combination of Vandetanib and Mebendazole, through the in vitro MTT assays and cell migration in a human metastatic gastric adenocarcinoma (AGP01) lineage. Vandetanib has been shown to reduce metabolic activity and cell proliferation in a dose-dependent manner with the IC50 (Inhibitory Concentration) value of 600nM for the AGP01 line after 72 hours of exposure. Under the same conditions, mebendazole has also been shown to reduce cell activity and proliferation in a dose-dependent manner with the IC50 value of 300 nM. When cells were treated with the combined drugs, inhibition of cell proliferation was significantly increased and the IC50 was reduced by 50.5%, thus presenting IC50 values of 300 nM for vandetanib and 150 nM for mebendazole. Regarding cell migration, the results demonstrated that treatment with vandetanib alone, at the concentration of half of the IC50, inhibited the migration of the cells from the time of 6 hours ($p < 0.05$). When the treatment was performed using the drug combination, the migration was inhibited from the 6 hour time with the IC50 ($p < 0.001$) and 12 hours with half the IC50 ($p < 0.01$). Both drugs had good antineoplastic effects and although the combination did not potentiate the inhibitory effect under migration ability, the concentrations required to kill 50% of the cells decreased considerably, demonstrating that the combination may be an effective treatment strategy, that produced antineoplastic effects similar to those observed in the isolated treatments, but using a lower concentration of each drug, which could represent a decrease in the toxicity.

Financial Support: CNPq

OXIDATIVE STRESS INDUCED BY THE LEISHMANIC ALCALODE JULOCROTINA IN HUMAN LYMPHOCYTES

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The enormous pharmaceutical potential in the Amazon region attracts attention of the scientific community. Among the many Amazon natural substances carrying therapeutic potential we have Julocrotina, a glutarimide alkaloid isolated from *Croton pullei var. glabrior* Lanj., extensively found in the Amazon Forest. Several plants of the genus Croton have been shown to have anti-inflammatory and leishmanicidal effects, besides antitumor properties. Studies suggest that Julocrotina is cytotoxic to intracellular parasites, increasing the effectiveness of the microbicidal response of nitric oxide (NO) and reactive oxygen metabolites produced by macrophages. Although there are several natural products used to contain the action of leishmaniasis, it is worth noting that the use of such products is not yet effective in eradicating any form of the disease. In the last years, it has been proposed that ROS participate in the mechanism of action of azole antifungals. However, this antifungal mechanism is not fully elucidated and the reactive species of oxygen by azoles, especially fluconazole still needs to be further investigated. The objective of this study was to evaluate the effects of different concentrations of the alkaloid glutarimide julocrotina isolated from the *C. pullei* species on the production of reactive oxygen species (ROS) in culture of human lymphocytic cells from human peripheral blood. To perform the test, blood samples were obtained by venipuncture from three healthy non-smokers aged 20 to 30 years, two females and one male, with no exposure event to mutagenic agents. For the Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay, generation of intracellular ROS was evaluated using the fluorescent probe dichlorofluorescein diacetate (DCFH-DA) in human lymphocytes and exposed to 316 µM and 632 µM Julocrotina. Samples were then analyzed by spectrophotometry with an emission wavelength of 528 nm and an excitation wavelength of 485 nm and H_2O_2 (2 mM) was the positive control. In our result, we observed that there was a significant increase ($p < 0.05$) in ROS rate at all treatment concentrations tested for Julocrotina compared to the negative control. Thus, our data suggest that the induction of ROS could be one of the probable mechanisms by which Julocrotina induces the leishmanicidal effects, which reinforces the need to take the appropriate care before its use as leishmanicide phytotericide.

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MUTAGENICITY AND TOXICITY OF REACTIVE DYE RED 239, AND TEXTILES EFFLUENTS TREATED BY ELECTRON BEAM IRRADIATION

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The textile industry has an expressive scenario in the world economy, with an annual billing more than US$ 330 billion. This sector uses an important quantity of water during the production, while generating effluents usually with high color and toxicity. Textile effluent is a mixture of dyestuff, surfactants, dispersants, acids, alkalis, bleaching agents, humectants, among other compounds, and many of them are highly soluble and relatively recalcitrant. The Advanced Oxidative Processes are used to improve the treatability of this type of effluent, complementing the biological treatment. Electron beam irradiation (EBI) has been proposed as a technology for the reduction of toxicity, coloration and other parameters in textile effluents. The objective of this study was to evaluate the mutagenicity and toxicity of a Reactive Dye Red 239 and a textile effluent containing the same dye, before and after electron beam treatment. The dye and effluent were submitted to EBI treatment, doses of 2.5 kGy and 5.0 kGy. For the *Salmonella*/microsome *mutagenicity* test, three bacterial strains were used: TA98, TA100 and YG1041, with concentrations between 1 mL and 20 mL. Solid phase extraction (SPE) of the samples was performed with Oasis HLB cartridges, elution with methanol. The ecotoxicological assay was carried out with *Daphnia similis* crustaceans. The organisms were exposed to samples during 48 hours, and the effect observed was immobility after this time, the results expressed by EC50 (median effective concentration). Mutagenicity was not detected neither with the pure dye nor with raw and EBI treated effluents. Toxicity was observed at doses 10 and 20mL to TA100 *Salmonella* strain. About toxicity assays, for a Reactive Dye Red 239, to *D. similis* EC 50% = 69.0 ± 0.66, after EBI no toxicity reduction was observed. For the textile effluent, EC50 values (%) ranged from raw: 2.93 ± 0.13; 2.5 kGy: 9.27 ± 0.41 and 5 kGy: 20.90 ± 1.48. The EBI treatment was effective for toxicity reduction, with efficiency higher than 60% (2.5 kGy) and 80% (5.0 kGy). Electron beam irradiation is a promising technology for reducing toxicity and other parameters in textile effluents, at relatively low doses. The monitoring of contaminants, such as dyes and textile effluents, can support action plans to assist in the maintenance and preservation of the water bodies that receive these effluents.

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CLASTOGENIC AND ANEUGENIC EFFECTS OF ARTESUNATE ARE FOLLOWED BY CHANGES IN MARKERS OF OXIDATIVE-NITROSATIVE STRESS AND APOPTOSIS IN HUMAN LYMPHOCYTES

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Keywords: artesunate, lymphocytes, toxicity

Artesunate (ARS) is a semi-synthetic derivative of artemisinin, used as an outstanding antimalarial drug, which also displays antitumor, anti-inflammatory and immunosuppressive effects. In spite of the numerous reports showing the antitumor activity of ARS, the particular mechanisms associated with its cytotoxicity and genotoxicity in non-neoplastic human cells remains unclear. The present work aimed to assess the specific chromosome damages and the changes in markers of oxidative-nitrosative stress and apoptosis triggered by ARS exposure in human peripheral blood lymphocytes. Cultures were incubated in the presence of ARS and the number of binucleated cells was determined. To discriminate between micronucleus (MN) containing a whole chromosome or an acentric chromosome, the micronucleus test was employed in combination with the fluorescence in situ hybridization (FISH) assay. Alterations in the levels of superoxide anion (O_2^-) and nitric oxide (NO) were measured by the nitroblue tetrazolium (NBT) and Griess assay, respectively. Changes in the expression of the apoptotic markers were assessed by immunocytochemistry. We found that ARS induced significantly both centromere-positive (C+ MN) and centromere negative (C– MN) micronuclei. These cahanges were accompanied by an increase in both cellular levels of O_2^- and total NO production, and a remarkable enhancement in the expression of the apoptotic markers cytochrome C and caspases 8 and 9. Together these findings reveal that ARS displays both clastogenic and aneugenic effects which are followed by changes in the oxidative-nitrosative and apoptotic status of human lymphocytes. I emphasize that I am interested in participating in the Young Researcher Award.

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CYTOXICITY AND GENOTOXICITY SCREENING OF THE ETHANOLIC EXTRACT FROM THE LEAF OF *Anadenanthera colubrina* (VELL.) BRENAN.

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Key-words: Medicinal plants; *Allium cepa;* Chromosome Aberrations

Medicinal plants are commonly used by the population for the treatment of several diseases, however, the vast majority of them have not been sufficiently studied with regard to their cytogenotoxic and mutagenic potential. *Anadenanthera colubrina*, popularly known as Angico or Angico preto, belongs to the family Leguminosae, subfamily Mimosaceae and has wide distribution in Brazil, from the states of the north and northeast to the south of the State of Paraná. In folk medicine it is used in the treatment of cough, bronchitis and affections of the respiratory tract, it also has depurative, astringent, anti-flu, antirheumatic, healing and anti-inflammatory properties. The present work aimed to evaluate the cytotoxic and genotoxic potential of the ethanolic extract from the leaf of *Anadenanthera colubrina* (Vell) Brenan through the bioassay *Allium cepa*. The concentrations defined to perform the test were 9 µg/ml, 27 µg/ml and 81 µg/ml. Initially, the bulbs were grown in distilled water for 48h at 25°C and constant aeration. Subsequently, for each extract concentration and controls, five bulbs were used and treated for 24h. After this period, the roots were collected, fixed in Carnoy solution (3:1-ethyl alcohol and acetic acid) for 24h, washed in distilled water and hydrolyzed in 1N HCL for 5 min to prepare the slides. These were stained with 1% acetic orcein and analyzed under an optical microscope at an increase of 400X. All experiments were compared to the negative control, which consisted of distilled water. Statistical analysis was performed using the variance test (ANOVA), followed by Tukey's multiple comparisons test with *p* values lower than 0.05. The results obtained for the cytotoxicity parameter demonstrated a significant increase in the mitotic index (MI) when compared to the negative control (MI=26,8%), mainly for the concentrations of 27µg/ml (MI=147,2%) and 81µg/ml (MI=173%). For the genotoxicity parameter, the results also showed a significant increase in the number of chromosomal aberrations (CA) in a dose dependent manner, negative control (CA=2), 27µg/ml (CA=52,8) and 81µg/ml (CA=105,6). The main chromosomal changes observed were chromosomal bridges in anaphases and telophase delays. Thus, the results obtained here indicate the proliferative and genotoxic effect of the ethanolic

A REVIEW OF GENOTOXICITY STUDIES ON METALLIC AND METALLIC OXIDE NANOPARTICLES IN NEOTROPICAL FRESHWATER FISH SPECIES.

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Keywords: características físico-químicas, estresse oxidativo, mecanismos antioxidantes, região Neotropical, contaminantes emergentes.

In this study, we selected bioassays involving the evaluation of genotoxicity of metal and metal-oxide nanoparticles (NPs) in freshwater fish as test organisms, mainly Neotropical fish. The vast majority of studies published so far use the species recommended by international guidelines (OECD, USEPA), such as the species *Danio rerio* and *Poecilia reticulata*. However, the use of key species that are considered representative of ecosystems and that play a relevant ecological role in them would ensure better protection of aquatic communities as a whole. Thus, due to the great variety of freshwater fish species present in the Neotropical region, the objective of this review is to evaluate what is currently known about the toxicity of metallic and oxide-metal nanoparticles in fish and fish cells, since the use of these species is essential for more realistic genotoxicity testing. Regarding the mechanisms of toxicity described for these NPs, there are indications that oxidative stress may be involved in most of the effects to the DNA molecule observed in the studies. However, some studies point out that a mechanism of direct action of certain nanoparticles in the DNA molecule may also occur. In addition to the size of the NPs, surface reactivity and the state of dispersion of the nanomaterials also play an important role in the induction of genotoxicity and in the bio-distribution of these NPs. Although this study provides information on the genotoxic potential of some nanomaterials for Neotropical fish species, data is still very limited, especially when we take into account the different types of nanomaterials available and their possible modifications in the aquatic environment. In order to fill in the gaps with respect to genotoxicity data, we believe that additional studies should be carried out involving organisms of the Neotropical species on different trophic levels. There should also be an increase in the use of in vitro alternatives, in an attempt to elucidate the mechanisms of toxicity of these NPs.

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Development of three-dimensional (3D) spheroid cultures of the continuous rainbow trout gonad cell line RTG-2 for detecting genotoxicants.

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Keywords: fish cell line, in vitro model, aquatic toxicology, comet assay, germ cell line.

The health of aquatic organisms can be compromised by the exposure to toxic substances present in these environments. Fish are excellent test organisms for aquatic toxicology studies; however, ethical and legal issues have led to the development for *in vitro* alternatives to reduce the number of animals, including fish, in research in the academic and industrial sectors. *In vitro* fish cells have been proposed as alternatives to ecotoxicological studies with fish (*in vivo*). Cells can be cultured in different 2D and 3D cell culture systems, the latter being more similar to architecture and physiological conditions of organs and tissues *in vivo*. The gonadal cell line of rainbow trout (RTG-2) was the first lineage established from cold-blooded vertebrates and because it is a germ cell line, it has great application in the area of Genetic Toxicology, being one of the most widespread strains currently available. In this study we introduce a three-dimensional (3D) *in vitro* experimental system based on spheroidal aggregate cultures (spheroids) of the continuous rainbow trout gonad cell line RTG-2 and provide a first description of their structural and functional properties including growth, viability, metabolic activity, ultrastructure and cytochrome P450 1A (CYP1A) activity. In addition, we also have described the sensitivity observed in this 3D system as an alternative *in vitro* model for detection of cytotoxicity and genotoxicity. Our results show that RTG-2 cells in 3D spheroids (including those cells in the interior) were viable and metabolically active. The available evidence suggests that RTG-2 spheroids (3D) may have enhanced cell functions and may be considered a superior *in vitro* model for assessing gonadal effects and acute genotoxicity compared to conventional cell monolayer cultures (2D). In this way, we hope to establish consistent *in vitro* alternatives for studies with fish that are sensitive for the detection of chemicals potentially harmful to the environment and to future generations.

Financial Support: CNPq, CAPES

BIOMONITORING OF THE CYTOGENOTOXIC POTENTIAL OF THE PARANHANA RIVER, RS, USING THE *Allium cepa* L. BIOASSAY

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Keywords: genotoxicity, cytotoxicity, *Allium cepa*, water pollution.

The Paranhana River is an important tributary of the Sinos River, located in Southern Brazil, and is used for public supply and recreational purposes. However, this water resource is under anthropogenic impacts which can affect the aquatic biota and human health. Therefore, the present study aimed to assess the cytogenotoxic potential of water samples collected at two sites of the Paranhana River using the *Allium cepa* L. bioassay. Water samples were collected every three months in 2016 at two sites of the river – source and mouth, located in the municipalities of Três Coroas and Taquara, RS, respectively. Seeds of *A. cepa* were previously germinated in Petri dishes containing distilled water and then exposed to water samples for 24 h. A control group was maintained in distilled water during the same period. After exposure, roots were fixed in Carnoy solution and store in ethanol 70% at 4 °C. In order to prepare the slides, roots were hydrolyzed with HCl 1N and stained with acetic orcein 1%. A total of 10 slides were prepared and analyzed for each group. For the cytotoxicity evaluation, the number of cells under division was recorded (analysis of 1000 cells). For genotoxicity, the frequency of micronucleus in 1000 interphase cells and frequency of chromosomal aberrations in 100 anaphase-telophase cells were assessed. Statistical analysis was performed using the Kruskal-Wallis test, followed by the Dunn's multiple comparisons test, when appropriated. A significant reduction in the mitotic index was found in roots exposed to water samples collected at the source and mouth of the Paranhana River in comparison to control roots ($p<0.001$). Additionally, results observed for the mitotic index in the mouth of the river significantly differed from the source ($p<0.001$) in all sampling periods, except in the winter. For genotoxicity endpoints, no significant differences were observed among the groups. In addition, no significant seasonal variation was found among the sampling sites. Therefore, both sites of the Paranhana River are under impacts – the source, probably affected by the water transposition from the Caí River; whereas the intense urbanization could influence the water quality at the mouth.

Financial Support: FAPERGS.

TOXIC EFFECT OF SACCHARINE SWEETENER IN CULTURED HUMAN LYMPHOCYTES

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Key-words: saccharine, lymphocytes, cytotoxicity.

The high consumption of sweeteners in general population is attributed to influence from commercial information that are not founded by scientific bases. This phenomenon cooperates to the consume of several products in the day-day in order to replace the white sugar. Saccharin was the first sweetener discovered, perhaps for this it has be well known and widely used. As main organoleptic characteristic, saccharine is 200-fold as sweet as sucrose. However, recent studies have shown controversy over its use as a beneficial product in health prospects to individuals, warning concern on the its carcinogenic potential. The objective of this study was to evaluate the cellular safety of the use of sweetener saccharine based on lymphoproliferation, a test that has direct relation with our immune system. For this test, we used concentrations of 1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL and 500 µg/mL. Lymphocyte cultures were prepared from human peripheral venous blood that was mixed with Histopaque (Sigma-Aldrich®) and centrifuged at 1,500 rpm for 10 min. After, it was separate the phases of interests and immediately transferred to RPMI 1640 medium supplemented with fetal bovine serum, streptomycin/penicillin, gentamicin and phytohemagglutinin. Cells were maintained at 37°C for 72 hours in 5% CO_2 atmosphere. The assay was performed in triplicate and receive previously approval by Ethic Committee of Federal University of Pampa – UNIPAMPA (n° 27045614.0.0000.5323). We assessed the total of lymphocyte cells counting them in a Neubauer chamber. Data were expressed as mean ± standard deviation (SD). Non-linear regression analysis was used to determine LC50. The calculated LC50 for lymphocytes was 321.2 µg/mL. Our data suggest that in spite of an apparent innocuousness, this lymphotoxicity found may be harmful to the functioning of the immune system and should be studied in more detail to understand the mechanics involved in the process.

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SYNTHETIC β-CARBOLINE ALKALOIDS INDUCE CHROMOSOMAL MUTATIONS IN HUMAN CANCER CELLS

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On a daily basis, the genetic material of live organisms is exposed to harmful agents that are present, for example, in the air, food and water. These agents can cause mutations that may cause severe degenerative diseases, including cancer. Most of the drugs used to treat cancer are unspecific and cause damage to normal cells and several collateral effects in patients. Thus, the search for new and more selective anticancer compounds is an important challenge. β-carboline alkaloids present diverse pharmacological activities, and studies with these molecules have produced promising results in the development of new anticancer drug candidates. In the present work, the genotoxicity and the mutagenicity of two synthetic β-carboline alkaloids, termed NQBio-001 and NQBio-006, which were designed as anticancer candidates, were evaluated employing the human ovary carcinoma cell line ToV-21G. The cytokinesis-block micronucleus assay and the comet assay were performed with different concentrations of the synthetic alkaloids. The compound NQBio-001 presented positive results in the comet and micronucleus assays; it induced chromosomal breaks that resulted in heritable genetic alterations. The compound NQBio-006 induced a significant increase in the frequency of binucleated cells with micronuclei when compared with a negative control. However, the scores were not different from a negative control in comet assay studies with this compound. Thus, based on the results observed with NQBio-006, we infer that this compound induced the micronuclei via chromosome mis-segregation and not by chromosomal breaks. The results obtained and presented herein demonstrate that the evaluated compounds can induce tumour cell death triggered by chromosomal mutations. However, additional studies are necessary to better understand the mechanisms of action and the type of cell death induced in tumour cells. Nonetheless, the compounds NQBio-001 and NQBio-006 are interesting prototypes for the development of new anticancer drugs.

Financial Support: FAPEMIG and CNPq

BIOLOGICAL EFFECTS OF *Petiveria alliacea* L. ETHANOLIC EXTRACT IN BACTERIAL CELLS AND PLASMID DNA

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Keywords: *Escherichia coli,* cytotoxicity, genotoxicity, mutagenicity, medicinal plant.

Petiveria alliacea L. is native from the Amazon region and belongs to the Phytolaccaceae family. It is used in folk medicine and some biological active compounds, as well as therapeutic activities, have already been identified. However, its toxicological effects are not fine elucidated yet. Therefore, the present work evaluated the cytotoxicity and genotoxicity of the *P. alliac*ea leaves ethanolic extract in *Escherichia coli* and plasmid DNA. Leaves collected in Niterói, RJ, were dried at 50 °C for 24 hours, crushed and macerated with absolute ethanol and the extract obtained was evaporated under reduced pressure. To evaluate the cytotoxic potential, mid-log phase cultures of *E. coli* wild type AB1157 were incubated with different concentrations of the plant extract (5, 10, 15, 20, 25 and 30 mg/mL) for 60 minutes. Genotoxicity was assayed *in vitro* by agarose gel electrophoresis of plasmid DNA (pUC 9.1). Genotoxic action was measured *in vivo* by plasmid pUC 9.1 transformation assay and also by *E. coli* DNA agarose gel electrophoresis. The mutagenic potential was analyzed by the rifampicin resistance induction assay. The crude *P. alliacea* extract was not mutagenic but also not able to change the transformation potential of pUC 9.1 plasmid DNA. However, the ethanolic extract showed to be citotoxic in concentrations higher than 15 mg/mL and after 40 minutes of incubation. The electrophoresis approach indicated that the extract were able to induce strand breaks in *E. coli* genomic DNA, but did not induce breaks in plasmid pUC 9.1. A delay in the plasmid migration pattern was observed, as a function of plant extract increasing concentration. Obtained data suggest that, *in vitro*, *P. alliacea* extract is able to promote some kind of interaction with the DNA, as there are changes in the migration pattern of the plasmids in the agarose gel. However, this interaction does not result in breaks in the molecule, nor interfere with the transforming capacity. As the extract is able to decrease cell viability and promote bacterial DNA breaks, it is possible that one or more components of the plant extract could be metabolized by bacteria, causing breaks in the genomic DNA and culminating in the cell death. The mutagenic potential should be better evaluated and further studies are need to validate the therapeutic use of preparations obtained from *P. alliacea*, to achieve safe doses, to evaluate side effects, as well as synergistic effects during treatment.

Financial Support: CAPES, CNPq, FAPERJ, UERJ e PGBV.

EVALUATION OF TOXICITY / CYTOOTOXICITY AND MUTAGENICITY OF BROMELINE BIOATIVE COMPOUND IN NON-CLINICAL STUDIES

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Key words: Bromelain, protease, toxicogenetic

Toxicogenic studies with medicinal plants have been of great importance due to the interest in proving the non-toxicity of phytochemicals, as well as the discovery of other pharmacological activities, such as antitumor activity. Among the natural complexes obtained from plants, bromelain, a protease derived from pineapple (Ananas comosus) has been studied for several therapeutic applications, among them the anti-inflammatory, cytotoxic and antitumor activity. Thus, the objective of the study was to evaluate the toxic, cytotoxic and mutagenic effects of bromelain in different test systems. The bioassays used were: Mortality test in Artemia salina with exposure time of 24 and 48 hours at concentrations of 31.25 to 1000 ug / mL and Allium cepa test in 48 hours at concentrations of 50, 100 and 200 µg / mL . In both tests were used: distilled water - negative control (CN) and potassium dichromate and cyclophosphamide - positive control (PC). In the A. salina test, all concentrations showed toxicity in comparison to the negative control, with mortality values varying from 40% to 95%, except for the lowest concentration of 31.25 µg / mL exposed for 24h. In relation to the positive control (CP), concentrations of 62.5 to 1000 µg / mL bromelain were not statistically different from this control for 24h, however, for the 48h time, the concentrations of 125 and 1000 µg / mL differed from CP, being less toxic. The lethal dose (LD50) of bromelain was 505.2 and 303.0 µg / mL at the time of 24 and 48h, respectively, which justified the selection and use of the concentrations for the bioassay of Allium cepa. Cytotoxicity results measured by mitotic index (MI) showed significant cytotoxic effects for the two highest concentrations of bromelain (100 and 200 µg / mL) with IM values of 20.7% and 25.9% compared to the negative control (56.24%). Regarding mutagenicity, only the concentration of 200 µg / mL showed significant mutagenic effects due to the observed high chromosomal delays of 8.2%. Therefore, bromelain showed toxicity, antiproliferative capacity and genotoxicity in eukaryotic cells, interesting effects for additional studies of antitumor activity.

MUTAGENESIS AND HUMAN AND ANIMAL HEALTH (HUMAN AND ANIMAL BIOMONITORING, CANCER, DEGENERATIVE DISEASES, AGING)

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IN VITRO EXPOSURE OF CD4$^+$ AND CD8$^+$ HUMAN CELLS TO DEXCLORFENIRAMINE: AN IMMUNOTOXICOLOGICAL STUDY

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Key-words: Dexchlorpheniramine, lymphocytes T, cell viability.

Dexchlorpheniramine (DPN) is a dextrorotatory isomer of chlorpheniramine, belonging to the pharmacological class of histamine H1 receptor antagonists. This drug acts in the prevention and relief of allergic manifestations and hypersensitivity reaction. These clinical settings may be triggered when an organism is exposed to an allergen. This phenomenon will be mediated by immune cells, where the lymphocytes play a decisive role in the adaptive immune responses. Thus, the aim of this study was to evaluate the potential DPN immunotoxicity on cultured human peripheral blood mononuclear cells (PBMC) using commercial (capsules) and pure (powder - Sigma®) DPN in different concentrations (0.5 to 50 ng/mL – that ranging those in bloodstream of individuals undergoing DPN therapy), in particularly, evaluating if DPN has influence on CD4$^+$ and CD8$^+$ T lymphocytes. The cultures (RPMI medium supplemented with bovine serum, streptomycin, penicillin, gentamycin and phytohemagglutinin, as extensive described by literature) were maintained at 37°C during 48 hours in 5% CO_2 atmosphere. It was evaluated the cell viability of PBMC by trypan blue exclusion method, counting and distinguish 300 cells per sample in Neubauer chamber using optical microscopic in 400 X of magnification. It was also assessed the absolute number of CD3$^+$ (lymphocytes), CD4$^+$ and CD8$^+$ T lymphocytes by flow cytometry (BD® FcasCalibur). Protocols were approved previously by the Ethics Committee of Federal University of Pampa – UNIPAMPA (n° 27045614.0.0000.5323). The data were analyzed by One Way ANOVA, complemented by post-hoc Dunnett's Test, and considered as statistically significant when $p<0.05$. Our data showed that all tested DPN concentrations were unable to induce decreasing in cell viability when compared to negative control (98% of cell viability). The absolute number of CD3$^+$, as well as the both CD4$^+$ and CD8$^+$ lymphocytes were similar to the negative control. Thus, our data suggest that DPN is unable to induce immunotoxicity in the CD4$^+$ and CD8$^+$ lymphocytes, at least in the range concentration and experimental conditions performed in this study.

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BUCCAL MICRONUCLEUS ASSAY FOR MONITORING THE DNA DAMAGE IN CAREGIVERS OF ELDERLY ALZHEIMER

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When people face stressful situation, the organism starts physiological reactions to keep the body safe. However, if that situation extends for a long time the cells can suffer damage. Currently many people have a stressful routine, especially people who have a large daywork as caregivers of patients affected with neurodegenerative diseases. Therefore, the objective of this study was to evaluate the genotoxic stress through the quantification of micronuclei and apoptotic cell in oral mucosa. In this work, caregivers over 18 years are being selecting for the analyzes, which could be relatives or professionals. Until now, 42 samples of caregivers were obtained through scraping of oral mucosa of the individuals and preserved at 1:1 solution of methanol and acetic acid for a day. Control samples were obtained from 10 undergraduate and graduate students from UFSM. Then, the samples were centrifuged 1000 rpm for 15 min, the supernatant was discarded, and added 3:1 methanol and acetic acid solution. This process was repeated 3 times until get the final solution that was used to prepare the slides. Slides were then heated by 60ºC and added 2 drops of the solution containing the cells. Cells were stained with the dye Geimsa 5% for 7 minutes. These slides were analyzed at the optical microscope with 100X of magnification and 1000 cells were scored identifying the micronucleus, apoptotic and the normal cells. The comparison of the frequency of micronucleus and apoptotic cells between the caregivers and the control group was done by Student's t-test. The results indicate that the caregivers of patients affected with neurodegenerative diseases showed many cells with micronucleus, as well as apoptotic cells. Therefore, we can conclude that they are probably suffering with more genomic instability than the expected. This may happen because of the stressful routine allied to excessive consume of stimulants substances or sedentarism. It’s important to highlight that there are many individual variations and other factors that must be further considered in this work.

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GREEN TOBACCO SICKNESS IN FARMERS IS ASSOCIATED WITH GENOTOXIC IMPAIRMENT

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Key-words: Tobacco Farmers; Genotoxicity; Green Tobacco Sickness (GTS).

Brazil is the second largest tobacco producer worldwide, being the Southern region the one that contributes most to the country's productivity. Tobacco farming promotes contact with nicotine present on the leaf surface, mainly during the harvest period. Nicotine is absorbed by dermal contact and metabolized majority to cotinine, which can lead to the Green Tobacco Sickness (GTS), an occupational illness. Thus, the aim of this study was to evaluate the incidence of GTS symptoms in farmers of the municipality of Santa Cruz do Sul during the harvest period; the oxidative balance and presence of DNA damage and its relation with age and exposure time. The study involved 40 males, 20 of the exposed group (farmers who participate in the tobacco harvest) and 20 of the control group (people who did not work in agriculture), paired by age (42.0 $\pm$ 14.0 years old). Individuals completed a questionnaire and had blood samples collected. The cotinine dosage in plasma samples was performed by HPLC–UV. A significant increase in serum cotinine level was observed in the farmers group when compared to the control group, confirming exposure. The main GTS signs and symptoms described by farmworkers were nausea (55%), abdominal cramps (50%), headache (50%), vomiting (15%), and dizziness (5%). The mean exposure time was 29 years. The oxidative balance was determined using Trolox Equivalent Antioxidant Capacity (TEAC) and serum lipid-peroxidation was measured by Thiobarbituric Acid Reactive Substances (TBARS). The results showed a significant increase only of TBARS levels for farmers in relation to the control group. Damage to DNA was evaluated by the alkaline Comet assay, and 100 cells were analyzed per individual, classifying them into class 0 to 4 damages.

Comet assay results showed a significant increase in both damage index and frequency for exposed group in relation to controls. An inverse significant correlation was found between DNA damage and age for both groups, as well as for farmers regarding DNA damage and exposure time. It is suggested that elderly men are more prone to suffer cell death, which could explain the decreased damage. This study highlights the genotoxic risks of exposure to nicotine, which induces oxidative stress. GTS prevention must be based on methods to reduce dermal absorption of nicotine, such as the use of personal protective equipment (PPE), as it is not linked only to clinical symptoms of acute poising, but also with genotoxic impairment.

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OXIDATIVE DNA DAMAGE, SYSTEMIC AND MOLECULAR INFLAMMATORY MARKERS IN PHYSICIANS OCCUPATIONALLY EXPOSED TO ANESTHETICS

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Key-words: occupational exposure, anesthetic gases, comet assay, inflammation

Operating room professionals are scarcely aware of their occupational exposure to the inhalational anesthetics. The occupational exposure to waste anesthetic gases (WAGs) may lead to oxidative stress, which can damage DNA (genotoxicity) and lead to inflammatory process. Considering that inflammation and genotoxicity are linked events, and that limited literature is available on possible genetic and inflammatory effects of WAGs in exposed professionals, this study evaluated oxidative DNA damage, systemic inflammatory cytokines and related gene expression. After Institutional Review Board approval, we evaluated 60 physicians who worked in a Brazilian university hospital, according to the exposure (anesthesiologists exposed to the most modern inhalational anesthetics; exposed group, n = 30) or lack of exposure (physicians with no exposure to the anesthetics; control group, n = 30). The groups were matched by age, sex and lifestyle. All participants signed the Informed Consent Form and answered a detailed questionnaire. Fasted blood samples were collected from both groups and were freshly prepared. Lymphocytes were isolated and the comet assay was applied by using the FPG enzyme for detecting oxidized purines. Serum pro-inflammatory interleukins IL-6, IL-8 and IL-17 were assessed by flow cytometry. In addition, the genes *IL-6*, *IL-8* and *IL-17* were detected by quantitative real-time PCR. As expected, demographic data did not differ between groups ($p > 0.05$). The results showed that there were no statistically significant differences between groups in relation to oxidative DNA damage ($p = 0.61$) and IL-8 ($p = 0.10$), since both IL-6 and IL-17 values were below the detection limits and, therefore, they were not analyzed. In addition, no statistically significant differences were found in relation to expression of *IL-6* ($p = 0.06$) and *IL-17* ($p = 0.60$) between groups. However, the exposed group showed increase of *IL-8* expression when compared to the control group ($p = 0.04$). Thus, our findings showed that exposure to the most modern WAGs did not induce oxidative DNA damage, systemic and molecular inflammation markers, with the exception of the pro-inflammatory *IL-8*, which is related to inflammation in the respiratory tract. Hence, we suggest that *IL-8* might be a biomarker of exposure to the WAGs, but further studies are needed to confirm our data.

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EVALUATION OF THE LEVELS OF GENE AND PROTEIN EXPRESSION IN WORKERS EXPOSED TO CRYSTAL SILICA

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Introduction: Silicosis is a lung inflammatory disease caused by long time exposure to crystalline silica (CS). This study aimed to investigate potential early peripheral biomarkers of inflammation through protein and gene expression in workers occupationally exposed to CS. Methods: The non-occupationally exposed workers at CS (NEW) group (n=22) and CS exposed workers (CSEW) were divided into two groups: workers diagnosed with silicosis (CSEW I, n=26) and workers without silicosis (CSEW II, n=28). Serum reactive C-protein (RCP) was measured by immunoturbidimetry. Lymphocytes and monocytes protein expression of L-selectin, CD18 and CD54 by flow cytometry and gene expression of CXCL2 and IL-8 by RT-PCR were performed. CEP: 1.868.122. Statistical analyses: IBM SPSS (version 22). Significance was accepted at $p \leq 0.05$. Results/Discussion: Lymphocytes protein expression of L-selectin was significantly decreased in the exposed groups (CSEW I: 25.73 ± 1.51 %; CSEW II: 40.56 ± 1.73 %) compared to the NEW group (48.51 ± 2.18 %) ($p<0.05$) and was significantly different between CSEW I and CSEW II groups ($p<0.001$). No significant difference was observed between the CD18 and CD54 groups. Significant higher values of RCP in CSEW I (16.36 ± 5.08) than in CSEW II (1.91 ± 0.54) and NEW (2.40 ± 0.97) ($p<0.05$), indicating an increase of inflammatory activity. Otherwise, CESW groups presented significant lower gene expression of CXCL2 and IL-8 (CSEW I: 0.15 ± 0.03 and 0.53 ± 0.07, respectively; CSEW II: 0.27 ± 0.06 and 0.52 ± 0.07, respectively) when compared to the NEW group (1.88 ± 0.33 and 1.03 ± 0.03, respectively) ($p<0.001$). Also, were observed that the more T cells were activated, the lower were the gene expression of these inflammatory markers (L-selectin *vs.* IL-8 r= -0.293 $p<0.05$; L-selectin *vs.* CXCL2 r= -0.420 $p<0.001$). As the exposure to CS causes an excessive inflammatory response, increasing adhesion molecules levels in plasma, we can suggest that the downregulation of IL-8 and CXCL2 chemokines is a negative feedback mechanism for organism protection against inflammation. Conclusions: Our findings help us to increase our understanding of the complex mechanism of silicosis pathogenesis and to identify new early biomarkers of precocious diagnosis. These results may be considered for designing new studies with more details in the future.

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EVALUATION OF NITROUS OXIDE ANESTHESIA ON MICRONUTRIENTS, HOMOCYSTEINE AND OXIDIZED DNA BASES IN SURGICAL PATIENTS

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Key-words: nitrous oxide, vitamins, DNA damage, elective surgical procedures

General anesthesia is commonly applied in millions of patients around the world. Although the anesthetic gas nitrous oxide (N_2O) has been used for several decades in anesthesia practice, little is known about its possible side effects in healthy surgical patients. Prolonged or repeated exposure to N_2O was shown to inactivate vitamin B12 (cobalamin) and methionine synthetase, which is required for folate and DNA synthesis, besides increase homocysteine that can be toxic. However, no study has evaluated so far whether this anesthetic can alter folate, vitamin B12, homocysteine and oxidative DNA damage during and after one day of anesthesia maintained for a short period of time. After ethical approval and registry at the Brazilian Clinical Trials, the study was performed in 20 healthy individuals of both sexes, aged between 18 and 50 years, who underwent septoplasty and received 60% of N_2O associated with the halogenated anesthetic desflurane. Fasted coded blood samples were collected before the patient received anesthesia (control), at 90 minutes after the beginning of anesthesia and on the morning of the day after anesthesia. The homocysteine was evaluated by high performance liquid chromatography (HPLC), while vitamin B12 and folate were evaluated by chemiluminescence, and oxidized DNA bases were detected by the comet assay. All the experiments were run in duplicate and under dim light. The results showed that the use of N_2O with the halogenated did not statistically alter homocysteine, vitamin B12 and folate values nor induce oxidized bases during and after surgical procedure. Therefore, we suggest that the use of N_2O in healthy subjects who undergo minimally invasive surgery with a short duration (1.5 h) is safe since neither leads to changes in folate cycle nor induces oxidative DNA damage.

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ARSENIC IS ASSOCIATED WITH HYPERMETHYLATION OF CpG SITES RELATED TO GENES FROM INSULIN SIGNALING PATHWAYS

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Obesity´s prevalence is high in Brazil and in the world. Substances known as obesogens, such as Arsenic (As), can act as endocrine disruptors, altering gene expression, which can be regulated by epigenetic mechanisms, such as DNA methylation in the CpG sites. Anthropogenic activities increase the exposure to metals such as As and the major sources are drinking water and contaminated food. Despite knowledge about the toxic potential of this compound, it is not yet established how it contributes to the etiology and progression of chronic diseases. The median lethal dose of As is 0.07 g/kg. There are reports in the literature of a positive correlation of arsenic with BMI. Large-scale genomic studies allow thousands of regions to be evaluated simultaneously and can provide a global approach for clinical studies. The objective of the present study was to identify the DNA methylation pattern of obese women and eutrophic women and to correlate with the serum concentration of As. Materials and methods: this transversal study enrolled obese (Ob) (BMI ≥ 30 kg/m2) and eutrophic women (Eu) (18.5 kg/m^2 ≥ BMI ≤ 24.9 kg/m^2). Both groups were evaluated and consent with anthropometric data and peripheral blood collection. DNA were extracted from peripheral blood mononuclear cells (PBMCs) and used to hybridize 450k beadchip, wich has 450,000 CpG methylation sites available to be analysed methylation analysis. Serum was used to As determination using ICP-MS. Bioinformatics analysis were based on the Champ package pipeline and the Functional Enrichment were performed with DAVID and KEGG. Statistical analysis were done with SPSS software. The seric concentration of As were different between groups (Ob:13.49 ± 1.93 µg/L p=0.01, Eu: 12.72 ± 0.55 ug/L, *p=0.01*). Linear Regression analysis revealed 96.946 (Benjamini-Hochberg, *p<0,05*) CpG sites related to As seric concentration. Enrichment analysis revealed the top 70 genes with higher amount of CpGs related to As. Most of the CpG sites were hypermethylated and the Functional Enrichment showed 4 main pathways involved with the genes from the dataset, most of them related with insulin signaling pathways. Conclusion: Obese women seemed to be more exposed to As and this may be associated with the disruption of pathways. This results suggest that arsenic may have effects on DNA methylation and further studies may confirm its metabolic impact.

Keywords: Obesity, Arsenic, Insulin, DNA methylation.
Financial Support: CNPq, CAPES

BIOLOGY SYSTEM TO UNDERSTAND THE COAL EXPOSURE AND THE INFLUENCE ON TELOMERE LENGHT

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keywords: coal, telomere length, PAH, inorganic elements, system biology

The coal is a complex mixture of organic chemicals, containing notedly, several polycyclic aromatic hydrocarbon (PAH) and, minor amounts, inorganic compounds as oxides compounds and different inorganic elements. Exposure to coal and its genotoxic and mutagenic effects has been studied with different approaches and also in different organisms. In a recent study, our group detected a significantly shorten telomere length (TL) in individuals occupationally exposed to coal. However, the molecular mechanism of chemical constituents of coal action on telomere length is not fully understood. Our study evaluated the interaction between the chemical constituents of coal as PAHs (Naphthalene, Acenaphthene, Phenanthrene, Anthracene, Fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene) and inorganic elements (Na, Mg, Al, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Zn, Sr) associated to proteins involved in TL control. Subnetworks of proteins and chemical compounds were created using the data mining tools STRING 10.0 and STITCH 5.0 and merged with Cytoscape 3.4.0. Cluster analysis and bioprocesses assessment were performed using the Complex Molecular Detection (MCODE) and the Biological Network Gene Ontology (BiNGO) tools, respectively. The centralities analysis was performed using CentiScaPe. Our analysis showed that one cluster was associated with TL proteins that act in bioprocesses such as i) regulation of mitotic cell cycle, ii) cell-cycle checkpoint, iii) interphase of mitotic cell cycle and iv) DNA repair. In addition, chemical constituents of coal up regulate proteins associated to oxidative response, as AKT and TP53, molecules that could modulate telomerase reverse transcriptase (hTERT) activity and, consequently, the telomere length. This study to provide preliminar insight regarding the influence of chemical constituents of coal exposure on TL maintenance.

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EVALUATION OF CYTOTOXICITY AND GENOTOXICITY *Aloysia gratissima IN VITRO*.

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The plants are widely used popularly for treatment of various diseases, however, although they can be beneficial to health, can cause toxicity. The *Aloysia gratissima* is a plant commonly used in the treatment of colds, flus and gastrointestinal problems, but their toxic effects are still unknown. We studied the cytotoxic and genotoxic effects of leaves aqueous crude extract of *A. gratissima* in vitro using human neuroblastoma cells (SH-SY5Y). Cytotoxicity was assessed using the test of cell viability (MTT) and genotoxicity assay using Comet assay. Cytotoxicity was observed for the concentrations higher than 0.90 mg/mL (viability < 20%). DNA damage was significantly elevated in the concentrations tested (0.112-1.800 mg/mL; $P<0.05$ - ANOVA) when compared to negative control. Phytochemical screening was performed with *A. gratissima* leaves to investigate the presence of flavonoids, coumarins, anthraquinones, saponins, tannins and alkaloids. The results obtained for the phytochemical screening were positive for volatile and flavonoid oils and negative for other classes of compounds. As for the total phenolic compounds content of the extract, 174.44 ± 0.11 mg/g EAG (gallic acid equivalent) was obtained. When evaluated, the total flavonoid compounds content was 3.46 ± 0.02 mg/g EQ (quercetin equivalent).The inorganic elements were quantified by PIXE technique, and were identified Magnesium (Mg), Silicon (Si), Phosphorus (P), Sulfur (S), Potassium (K), Calcium (Ca), Manganese (Mn), and Zinc (Zn). Based on our data, crude aqueous extract of *A. gratissima* under the conditions of this study present a cytotoxic and genotoxic effect, probably related to flavonoids, which suggests further studies for safety in its use.

Financial Support: Lutheran University of Brazil – ULBRA, FAPERGS, CNPQ, CAPES.

CYTOTOXIC AND GENOTOXIC EVALUATION OF *SIDA PLANICAULIS* EXTRACTS USING SH-SY5Y CELLS

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Sida planicaulis heterotypic S. *carpinifolia* is known as guanxuma. Its infestation is widespread in Rio Grande do Sul (RS; Brazil) and it causes important intoxication in livestock with a negative impact on the country's economy. Since the 1990s, the relationship between guanxuma and increased mortality in cattle has been documented. Studies have shown that such an effect is related to the Swainsonine compound (SW) present in the plant, a toxin that causes *locoism* and induces addiction in the animal. SW promotes important cytotoxic effects causing physiological complications, mainly in the Central Nervous System (CNS) that lead the animal to death. Reproductive dysfunction, embryonic and fetal lethality, congenital defects, alterations in the endocrine and immune systems have been reported in the literature. It is important to understand the damaging mechanisms that involve the effect of *S. planicaulis* in the CNS, in this study, we evaluated the cytotoxic and genotoxic effect of the extracts from leaves collected during winter and summer using SH-SY5Y (human neuroblatoma cells). Phytochemical constitution was performed by colorimetric quantitative and qualitative analyzes and inorganic elements by PIXE technique using ethanolic extract. Cytotoxicity was evaluated using MTT colorimetric assay, and genotoxicity using alkaline comet assay. Modified comet assay was used to evaluation of oxidized purine and pyrimidine bases (Endo III and FPG). Phytochemical screening showed mainly alkaloids and flavonoids (winter<summer). *S. planicaulis* demonstrated a weak antioxidant potential, mainly for winter extract. MTT results demonstrated cytotoxicity higher than 70% forall concentrations 0.007-4.000 mg/µL. Genotoxicity was demonstrated for winter samples in different concentrations (1.75-7.00 mg/µL) and summer samples only for one concentration (7 mg/µL). Modified comet assay demonstrated both oxidized purines and pyrimidines at all concentrations. The PIXE method showed the higher concentration of sodium, phosphorus, sulfur, chlorine, potassium, calcium, chromium, iron, nickel and zinc in the winter sample than summer sample, except potassium. Extracts of *S. planicaulis* presented cytotoxicity and genotoxicity in neuroblastoma cells under the conditions of this study, mainly extracts from winter. The oxidative damage observed is probably related with phenolic compounds (including SW) and inorganic elements.

Financial Support: CNPq, CAPES, FAPERGS, ULBRA.

BIOMONITORING OF FARMERS OCCUPATIONALLY EXPOSED TO PESTICIDES FROM MARINGÁ - PR, BY THE BUCCAL MICRONUCLEUS CYTOME ASSAY

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Key-words: biomonitoring, agrochemicals, ecotoxicology, health of rural workers, individual protection equipment.

The agricultural sector stood out in the history and development of the Brazilian economy. However, with the growing market, there has been a need to increase production, resulting in the transformation of Brazil into the largest consumer of pesticides in the world since 2008. Pesticides are often used indiscriminately by farmers, leading to risks to the environment and to the health of rural workers. Thus, the objective of this study was to evaluate the micronucleus frequency, nuclear abnormalities, cell death, and the frequency of basal cells by the buccal micronucleus cytome assay (BMCyt), in individual exposed or not to pesticides, from Maringá - PR. Fifty male volunteers participated for buccal mucosa collection (27 subjects not exposed to pesticides and 23 exposed), and provided epidemiological information by a questionnaire adapted from the International Commission for Protection Against Environmental Mutations and Carcinogens. The population not exposed to pesticides was between 21 and 68 years old, and the population exposed was between 20 and 70 years old. The weekly working day of most farmers was 40 hours, and only 52.5% used personal protective equipment. A statistically significant difference was observed between the groups in the micronucleus test ($p<0.001$), and the farmers presented an increased number of basal cells ($p<0.05$), as well as cytogenetic alterations, represented by condensed chromatin ($p<0.01$) and karyolitic cells ($p<0.001$). A significant increment of differentiated cells was observed in the non-exposed individuals ($p<0.001$) when compared to the exposed ones. In the comparison of the nuclear morphologies with the epidemiological data, it was observed that the individuals that presented a higher frequency of condensed chromatin cells were the ones that made the preparation of the pesticides ($p<0.05$) and the transport of the pesticides to the sprayer machines ($p<0.05$), possibly resulting from the inadequate use of personal protective equipment. Therefore, individuals exposed to agrochemicals are more prone to DNA damage. These results suggest that the development of public health policies are mandatory, including the periodic biomonitoring of farmers from the Maringá rural area, as well as their awareness of the adequate use of protective equipment.

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BUCCAL MICRONUCLEUS CYTOME ASSAY: INTER-LABORATORY PROFILE OF MICRONUCLEUS FREQUENCIES AND NUCLEAR ABNORMALITIES IN DIFFERENT BRAZILIAN POPULATIONS

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Since the 1980's decade, the Buccal Micronucleus Cytome (BMCyt) assay has been used to measure genetic effects due to environmental and occupational exposures, impact of nutrition status, lifestyle factors and associated to different diseases. Besides the BMCyt could evaluate different parameters as DNA damage, chromosomal instability, cell death and the regenerative potential. Moreover, Brazil is a country of great extensions, which, together with the fact that it has received immigrants from various parts of the world, helps us to understand the regionalism observed in the Brazilian population. Therefore, the aim of present study was determine the range of reference values for BMCyt endpoints in Brazilian populations from different regions. Each one of 10 laboratories sought to collect 90 healthy non-smokers subjects (45 men and 45 women; grouped in three categories: 16 to 30 years old; 31 to 45 years old; and 45 to 60 years old), and 30 smokers (15 men and 15 women; 31 to 45 years old), from their local region. Our results are quite close to those described to health population. No differences between male and female individuals were observed, not including the binucleated cells frequencies, that was higher in female ($P<0.05$). Comparing individuals from different age groups, reduction in normal differentiated and binucleated cells frequencies in individuals aged 31 to 45 years and 45 to 60 years in relation to 16 to 30 years old individuals were observed ($P<0.05$), as well, in karyorrhectic, pyknotic and karyolysis cells frequencies only in individuals aged 31 to 45 years when compared to 16 to 30 years old individuals ($P<0.05$). Smokers individuals present a significative reduction in normals basal and differentiated cells frequencies, in relation to healthy individual, on the other hand, an increase in cell death (condensed chromatin, karyorrhectic and pyknotic cells) and DNA damage (binucleated cells, MN, Bud + Broken Eggs) parameters were observed ($P<0.05$).

Financial Support: CNPq, CAPES, FAPERGS, MutaGen-Brasil

MUTAGENIC AND CITOTOXIC EFFECTS OF HEXAVALENT CHROMIUM IN TADPOLES OF *Lithobates catesbeianus* (SHAW, 1802) (ANURA, RANIDAE)

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Keywords: Hexavalent chromium, bullfrog, Micronucleus test, Flow cytometry.

Urban and industrial growth is one of the main factors responsible for increasing the quantity and complexity of waste that is released into the aquatic environment, which causes serious ecological and toxicological problems. The hexavalent chromium compounds are strong oxidizing agents and present in aqueous solution through the species: chromates $[CrO_4]^{2-}$, dichromates $[Cr_2O_7]^{2-}$, chromic acid $[H_2CrO_4]$ e acid chromate $[HCrO_4]^-$. Potassium dichromate $[K_2Cr_2O_7]$ is a compound with several applications in the industry, such as cement components, chrome objects, fabric dyeing and leather tanning. Different aquatic organisms such as bivalve molluscs, fish and amphibians have been used to investigate genotoxicity in water through bioassays. The bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura, Ranidae), is important for raniculture and used in universities for research. The objective of this work was to evaluate the mutagenic and cytotoxic effects of hexavalent chromium in *L. catesbeianus* tadpoles, through the micronucleus test and flow cytometry, respectively. Tadpoles (n = 55) were exposed to three nominal concentrations of potassium dichromate (4, 12, and 36 mg L^{-1}) and 5 mg L^{-1} of cyclophosphamide as a positive control, for 24 h. A negative control was also added to the experiment. Micronucleus analysis was performed by a single observer, under an optical microscope with a count of 1000 erythrocytes per individual. The data were submitted to analysis of variance and the level of significance considered was 5%. The results showed that, in general, there was a statistically significant difference in micronucleus frequency between the negative control and all treated groups ($p < 0.05$) with a dose-dependent effect. In addition, the positive control did not differ from the treatments with chromium. Chromium treatments also promoted cell retention in the sub-G1 phase and decrease of cells in the S and G2/M phases, indicating inhibition of the cell cycle. The results found, therefore, confirm the toxic effects of hexavalent chromium and *L. catesbeianus* as an experimental model suitable for monitoring the pollution of aquatic ecosystems.

EVALUATION OF GENOTOXICITY AND MUTAGENICITY IN TAXI DRIVERS FROM RIO GRANDE DO SUL, BRAZIL

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Keywords: occupationally exposed, comet assay, micronucleus assay (MN), oxidative damage

Introduction: Some workers, such as taxi drivers are occupationally exposed to many xenobiotics, which could be harmful to their health. Among these xenobiotics, we highlight air pollution, which may lead to several kinds of DNA damage. Genotoxicity and mutagenicity assays are important to detect and evaluate the effects caused by these pollutants. This study aimed to evaluate the genotoxicity and mutagenicity in taxi drivers occupationally exposed to environmental pollution. Methods: This study included 34 taxi drivers (TD) exposed to air pollution and 22 subjects non-exposed to xenobiotics (NE). Blood samples were collected to determine DNA damage by comet assay analysis. Urinary concentrations of 8-hydroxy-2-deoxyguanosine (8-OHdG) were determined using EIA Kit 8-OHdG (Cayman, MI, USA). Additionally, cells from oral mucosa were collected for the micronucleus assay (MN). Ethics Committee approval (No. 21728/11). The results were expressed as mean ± standard error mean. Comparisons between groups were achieved by ANOVA-one way/Bonferroni. Results/Discussion: It was possible to observe a significant increase in DNA damage index (DI) for TD group in relation to the NE group ($p<0.001$). In the TD group, MN frequency presented no significant difference when compared with the NE group ($p>0.05$). Based on these results, we suggest that occupational exposure to environmental pollutants induces DNA damage, since there was an increased genotoxicity evidenced by comet assay. Additionally, 8-OHdG levels were positively correlated with DNA DI and MN. The excretion of urinary 8-OHdG reflects both the oxidation of DNA and cellular excision repair. It is important to consider that other factors may be involved in increasing DNA damage, such as lifestyle (eating, drug use, alcoholism, smoking and physical inactivity. Conclusion: Our results indicate that the occupational exposure to environmental pollutants is linked to genotoxicity and oxidative damage.

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INFLUENCE OF *PON1* GENE IN THE ACTIVITY OF SERUM CHOLINESTERASE AND CHROMOSOMAL INSTABILITY IN SOYBEAN FARMERS EXPOSED TO PESTICIDES IN THE STATE OF Rio Grande do Sul (Brazil)

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Brazil is the second largest exporter and producer of soybeans in the world, with emphasis on the South and Center-West regions. Planting requires the application of a broad mix of pesticides, mainly organophosphates, and fertilizers, considered essential to preserve the plant from insect and pest attack. Therefore, Brazil is also considered one of the largest consumers of pesticides in the world, especially during the cultivation of soybeans, which represents 63% of total consumption. The exposure of workers to the chemical agents present in the composition of pesticides is related to toxicity and genotoxicity in humans, and the activity of butyrylcholinesterase (BChE) is the only test required by the legislation. There is a great variability of cellular responses in farmers exposed to pesticides, which is due in general to the variation in the metabolism of the individuals. The goal of the present study was to evaluate the activity of BChE and the frequency of micronuclei in buccal cells in soybeans farmers chronically exposed to pesticides, as well as verify the modulation of these by the metabolism gene *PON1*. The study included 134 workers exposed to pesticides and 83 non-exposed, all male and non-smokers. The mean age (± standard deviation) was 47.5 years (± 12.5) in the exposed group and 41.7 years (± 15.1) in the non-exposed group. Soybean farmers exposed to pesticides showed a significant increase in micronuclei, binucleated cells and nuclear bud ($P < 0.001$; t-Student test), when compared to non-exposed individuals. Regarding the serum levels of BChE in this study population, no significant differences were observed between the groups. The metabolizing gene *PON1* showed no influence on DNA damage index and likewise on BChE activity. Mutagenicity, cytokinetics defects and gene amplification were demonstrated for soybean farmers. BChE has not been shown to be a good biomarker of exposure for farmers in cases of chronic exposure. These results are preliminary, since the sample tends to be expanded in order to evaluate other polymorphisms of metabolism and repair.

Key-words: Pesticides; Soybean farmers; Genotoxicity
Financial support: FAPERGS, CAPES, CNPq

EVALUATION OF DNA DAMAGE IN GAS SATION ATTENDANTS OCUPATIONALLY EXPOSED TO BENZENE

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Introduction: Gas station attendants are occupationally exposed to benzene, wich can be harmful to their health. Benzene is an example of xenobiotic that can lead to multiple types of DNA damage. The aim of this study is to evaluate the DNA damage caused in gas station attendants occupationally exposed to benzene and other xenobiotics. Methods: 20 gas station attendants (GSA group) with minimum exposure time of 6 months and 20 subjects without known occupational exposures (NE group) were selected. Smokers were excluded. This study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul/RS (No. 80 20322/11). All volunteers provided their written informed consent. It was collected 2 mL of blood using vacutainer tubes with heparin. For the comet assay, it was used 30 µL of total blood. Buccal cells were collected for the micronucleus assay (MN). To evaluate genotoxicity, alkaline comet assay was made by electrophoresis using Singh et al method (1988). Mutagenicity was evaluated by micronucleous assay. Analysis of the data was performed with SPSS Statistics software. ANOVA–one way/Bonferroni was the statistical test used and correlation tests were performed by Spearman's test. Values of $p \leq 0.05$ were considered significant. Results/Discussion: Exposure time of GSA group was 11.03±1.3 years. DNA damage index evaluated by comet assay was 81.9±7.5 for GSA group and 49.3±7.5 for NE group. Significant differences were observed between groups ($p < 0.05$). MN frequency was of 49.3±7.5 for GSA group and 0.7±0.2 for NE group. Correlation between DNA damage index and exposure time was observed in GSA group ($r=0.37$; $p < 0.01$). It was also found between MN frequency and exposure time ($r=0.52$; $p<0.001$). Data confirmed genotoxicity is caused by benzene, wich suggest that chronic exposure contribute to the development of genotoxic and mutagenic effects. Significant differences were found between GSA and NE group and comet assay is capable of evaluating DNA reversible damage. Conclusion: Biomarkers used were able to evaluate the harmful effects caused by xenobiotics exposure in the work place.

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MELATONIN HEPATOPROTECTIVE EFFECT IN THE EXPERIMENTAL MODEL OF NON-ALCOHOLIC ESTEATE-HEPATITIS

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Melatonin (MLT) has been widely cited in different experimental models as a potent antioxidant. The term non-alcoholic fatty liver disease (NAFLD) comprises a broad spectrum of conditions associated with accumulation of lipids in the liver, ranging from steatosis to non-alcoholic steatohepatitis (NASH), and may progress to more severe forms such as fibrosis, cirrhosis and hepatocellular carcinoma. NASH is defined by the presence of inflammatory infiltrate, presenting a hepatocellular lesion (balonization). The NASH experimental model is performed with methionine and choline deficient diet (MCD). The objective of this study was to evaluate the effect of MLT on the hepatic tissue in mice with NASH, induced by MCD diet. Thirty-two male mice of the C57BL6 lineage were divided into four groups: Control (CO), CO+MLT, NASH, NASH+MLT. The animals of the NASH and NASH + MLT groups received MCD diet for 4 weeks. MLT (20 mg/kg) was administered intraperitoneally (i.p) from day 15 of the of experiment after induction of NASH until the end of the experiment. After cardiac puncture blood was collected for comet assay, hepatic tissue for histological analysis (HE and Picrossiruis), and to evaluate oxidative stress. In the evaluation of lipoperoxidation by thiobarbituric acid reactive substances (TBARS), a significant increase of the NASH group was observed when compared to the control groups and a significant reduction in the NASH + MLT group. The enzyme catalase (CAT) had a significant increase of the NASH group in relation to the control groups and a significant decrease in the NASH + MLT group. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes present a reduction in the NASH group in relation to the control groups and a significant increase in the NASH + MLT group. In the histological analysis of the liver of animals with NASH, a hepatic parenchyma, inflammatory infiltrate and presence of fibrosis are observed. In the comet assay the NASH group presented a higher frequency of DNA demage when compared to the control groups, and the NASH + MLT group presented a significant reduction in relation to the NASH group. The MCD-induced NASH generated cellular and tissue damage in the same exposed animals, observed by the increase in lipoperoxidation, altered activity of antioxidant enzymes and changes in histological evaluation. Melatonin was able to attenuate the damages oxidative stress and genotoxicity caused in this experimental model.

Financial Support: CNPq, FAPERGS, CAPES, FIPE-HCPA.

ASSOCIATION OF INTERLEUKIN-10 GENES POLYMORPHISMS AND LEPROSY.

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Keywords: Leprosy; Mycobacterium leprae; Genotyping; Immune Response.

Cytokines play an important role in the host immune response against *Mycobacterium leprae*. Polymorphisms of cytokine genes have been implicated as a host factor influencing susceptibility to infectious diseases. This study aimed to evaluate the relationship between the human polymorphisms -1082, -819, -592 of the IL-10 gene with leprosy. In this study, individuals from the municipalities of Canaã dos Carajás, Rondon do Pará and Curionópolis in the State of Pará were studied. A total of 105 individuals were included in the study, of which 28 were paucibacillary (BP) treated, 25 multibacillary (MB) treated, and 55 contacts of whom lived with the ex-patients (intradomiciliares) throughout the course of the disease and did not get sick. Blood samples were collected for DNA extraction and for analysis of the polymorphisms IL-10 -1082, -819, -592. Blood samples were collected for DNA extraction and analysis of the polymorphisms of IL-10 -1082, -819, -592, which were typed by the polymerase chain reaction (PCR) technique using the kit, according to manufacturer's instructions. The GCC/GCC genotype was associated with disease predisposition. The “A” allele at position -1082 with leprosy protection, with a greater predominance of ACC/ATA (31.3%) and GCC/ATA genotypes (37, 5%) (p = 0.03) and the “A” allele in the -1082 position (76.85%) (p = 0.043) in the contact groups, while GCC/GCC was strongly found in the MB group (22.2%) (p = 0.05). The results indicate the participation of IL-10 polymorphism in leprosy susceptibility. These results may contribute to the identification of human haplotypes that can modulate the immune response to leprosy.

Financial support: Ministry of Health of the Federal Government

EVALUATION OF THE GENOTOXIC AND MORPHOLOGICAL EFFECTS OF CHRONIC EXPOSITION TO METHYLMERCURY IN THE *Physalaemus ephippifer* LARVAE (STEINDACHNER, 1864) (ANURA: LEPTODACTYLIDAE)

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Key words: genotoxicity, amphibians, comet assay, methylmercury

A decline in amphibian populations has been reported worldwide in the last decades, much of this decline is due to the destruction and fragmentation of habitats and trade in wild animals, among other factors. However, this may also be related to contamination by heavy metals such as mercury. Mercury is present in the environment from natural sources, however, the action of man can increase the occurrence of this metal in ecosystems. Amphibians are excellent biomarkers and can also be used in experimental studies to evaluate the isolated effect of certain contaminants. In this context, the objective of this study is to evaluate the effect of methylmercury in the larval phase of *Physalaemus ephippifer*, a species native to Amazonia, using the comet assay to identify genotoxic damages and histological analysis to identify morphological damages in animals exposed to subchronic concentrations of MeHg. Tadpoles of Physalaemus ephippifer were subjected to concentrations of 0.0004, 0.0007, 0.004, and 0.007 µg / mL of MeHg from hatching for 28 days. As a positive control, oxygen peroxide (H_2O_2) was used. After exposure, the animals were euthanized and processed to perform the comet assay and morphological analysis. The DNA damage index of the treatments with MeHg and H_2O_2 showed to be significantly higher than the negative control, evidencing the damage due to the genotoxic agents used. The mean of the positive control was significantly higher than the negative control and similar concentrations of 0.0007, 0.004 and 0.007 µg / mL MeHg used in the experiment. The highest DNA damage index was 0.007. In the morphological analyses there were no significant differences between treatments. The present study is an important step towards understanding the possible effects of chronic methylmercury contamination on Amazonian wildlife.

Financial Support: CNPq and UFPA.

ASSOCIATION OF TAQ I AND APA I POLYMORPHISMS IN VITAMIN D RECEPTOR (VDR) GENE WITH LEPROSY

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Keywords: Leprosy; *Mycobacterium leprae*; Vitamin D receptor; Immune Response

The evolution of leprosy depends on the immunological and genetic aspects of the host, being an active form of vitamin D essential for the regulation of the immune system. Studies have implicated the variation of single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) gene with susceptibility to various diseases, including tuberculosis and leprosy. The objective was to verify the relationship between the ApaI SNPs (rs7975232) and TaqI (rs731236) of the VDR gene and leprosy. The study was carried out with individuals from the municipalities of Rondon do Pará, Goianésia, Curionopolis, Parauapebas and Canaã, in the state of Pará, Brazil. We included 246 individuals, 61 multibacillary (MB), 35 paucibacillary (PB) and 150 contacts. Blood collection for DNA extraction and analysis of the polymorphism ApaI (rs7975232) and TaqI (rs731236) of the VDR gene, submitted to the polymerase chain reaction (PCR) technique and typed using the ABI 3130 Genetic Analyzer (Applied Biosystems®). For the TaqI SNP, no difference was observed in the distribution of genotypes between the groups, with the A/ A genotype being the most found in the study population. For the ApaI SNP, an association of the A / A genotype with the multibacillary form of leprosy and the C / A genotype with the disease protection was observed in the present study. An association of the C/C A/A haplotype with the multibacillary form and the C/A A/A haplotype with disease protection was observed. The G/A genotype of TaqI was associated with a better cellular response and better protection profile of the BCG vaccine. There was no statistically significant association between the TaqI and ApaI polymorphisms of the VDR gene and positivity or negativity for anti-PGL-I.

Financial support: Ministry of Health of the Federal Government, CAPES, PIBIC IEC/CNPq

MATERNAL PARTICULATE AIR POLLUTION EXPOSURE AND DNA DAMAGE IN UMBILICAL CORD BLOOD

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Air pollution is a significant urban-related environmental risk factor and one of the major contributors to the global burden of disease. Although this is an universal issue, particular attention must be paid to susceptible populations, such as children or elderly, and to critical windows of susceptibility, such as *in utero* development. So far, different studies have shown that particulate matter exposure in pregnancy is associated to different adverse health outcomes, but also to molecular alteration, namely alterations in DNA methylation, biochemical parameters and genetic damage. With data obtained in the frame of the NEOGENE project, we investigate here the influence of maternal residential exposure to particulate air pollution on levels of DNA damage analysed in umbilical cord blood. Air quality of 65 homes of pregnant women living in Porto Metropolitan Area (Portugal), specifically, PM_{10}, $PM_{2.5}$ and UFP were concurrently measured indoors and outdoors (DustTrak DRX Aerosol Monitor 8533, TSI; P-Trak UFP counter 8525, TSI). Information regarding the pregnant subjects was collected via a detailed questionnaire, after approval of the Ethical Committee of Centro Hospitalar de São João. DNA damage was assessed by alkaline and FPG-modified comet assay in umbilical cord blood samples. The significance of other relevant exposures (such as tobacco smoke) and other demographic, socioeconomic and lifestyle variables in the analysed outcomes was also explored. Data obtained will contribute to better understand the underlying mechanisms by which early life exposure to particulate matter may induce adverse health effects later in life. Furthermore, it could provide scientific evidence to improve environmental policies and public health measures, focused on major sources of air pollution, in order to reduce the burden of disease attributable to particulate matter air emissions.

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MECHANISMS OF ZIKV TERATOGENESIS: *in vivo* APPROACH USING CHICKEN EMBRYOS AS EXPERIMENTAL MODEL

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From the recognition and establishment of the Zika virus (ZIKV) as a human teratogen, several researches have been conducted to understand the cellular and molecular mechanisms involved in the harmful effects caused by ZIKV infection in the developing embryo. However, several questions about ZIKV teratogenesis remain unanswered. Basic science experiments are essential to understand and to identify mechanisms of teratogenicity as well as strategies to prevent the occurrence of negative outcomes caused by ZIKV. Therefore, this work aims to characterize the cellular and molecular mechanisms of ZIKV teratogenesis from the establishment of an experimental model using chicken embryos. For that, an *in vivo* model of congenital ZIKV syndrome was established in chicken embryos, a model recognized and widely used in development and teratogenic studies. Firstly, an initial screening with the effects of different amounts of ZIKV on chicken embryo was performed, when phenotypic and morphological aspects were analyzed. A control group with no virus infection (cell culture media only) was used to comparison. In addition, determination of the viral load present in each embryo was also performed using quantitative Real Time PCR. Tests were performed to determine the viral curve and to analyze the mortality of the embryos. The infected embryos were compared to controls in the evaluation of morphological changes. We found that ZIKV infected embryos presented a higher prevalence of defects such as reduced developing encephalon, face anomalies (e.g. micrognathia) mesencephalon when compared to control embryos. Also, we found that some ZIKV infected embryos were understaged and shorter than expected for the developmental stage. Once the phenotypes found in chicken embryos exposed to ZIKV recapitulate some found in humans we can use this model to investigate the pathways that, when disrupted, lead to embryonic damages observed in children. Therefore, as perspective, we will analyze cellular and molecular changes caused by ZIKV on the developing embryos. This work has potential to contribute to the test of substances that can minimize the observed malformations, projecting possible preventive and therapeutic targets to be evaluated in other animal models. Finally, the establishment of the use of this experimental model may be useful in the study of other infectious agents whose embryotoxic potential is not yet described.

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TERATOGEN INFORMATION SYSTEM IN LATIN AMERICA: INCREASE OF CALLS ON THE TERATOGEN AND PROBABLE MUTAGEN VALPROIC ACID.

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Valproic acid (VPA) is a well-known teratogen in humans, with teratogenic risk being estimated on 10% of exposed pregnant women. Neurobehavioral teratogenesis is of special concern after VPA exposure in pregnancy, including autism. the mutagen effects of valproic acid are not as well specified, and so is of great concern the increase in its use among child-bearing age women. One of the probable mutagen mechanisms of valproic acid is its action as a histone deacetylase inhibitor. SIAT (*Sistema de Informações sobre Agentes Teratogênicos*) is a free of charge Teratogen Information Service in Brazil directed mostly to pregnant women or planning pregnancy. SIAT was implemented in 1990 at Hospital de Clínicas de Porto Alegre and its 27 first years of work are registered in a database. Our aim is to to evaluate calls received by SIAT on valproic acid comparing two periods of time: 1990 to 2006 (Period 1) and 2007 to 2017 (Period 2). Data were retrospectively. Comparisons were made using the chi-squared test. SIAT received a total of 12,486 calls on Period 1, being 24% of them about medications with action on the central nervous system (CNS). In Period 2 a total of 7,979 calls were registered with a significant increase on CNS medications questions (47%) ($p<0.01$). VPA motivated 63 calls in Period 1 (2.6% out of 2,282 CNS medications) and 113 in Period 2 (4.0% out of 2,784) ($p<0.01$). In conclusion, there was an increase in calls referring CNS acting medications in recent years, and particularly VPA was increasingly prescribed for women in reproductive ages. Its teratogenic risk and possible mutagenic potential should be pondered before prescribing this drug.

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THE USE OF LYMPHOCYTE CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY TO MONITORING PESTICIDE-EXPOSED FARMWORKERS FROM CASIMIRO DE ABREU/ RJ

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Introduction: Brazil has been the main consumer of pesticides since 2008, and intensive and indiscriminate use has placed the environment and human health at risk, especially the population of rural workers. The literature shows that pesticides can cause genetic damage and an increase of the micronucleated cell rate is describe. The municipality of Casimiro de Abreu, located in the coastal plain of the State of Rio de Janeiro, has intense agricultural activity. The present study aims to evaluate the frequency of micronuclei in rural workers of Casimiro de Abreu and to compare the results to those observed in workers of the city who are not exposed to pesticides. Methods: This is a cross-sectional study with individuals living in the municipality of Casimiro de Abreu. The study involved 100 individuals of both sexes, living in the rural area and 100 control subjects with the same characteristics as farmers, except exposure to pesticides. (INCA - CAAE: 64799217.3.0000.5274). Exposure assessment is performed using the micronucleus assay with cytokinesis block. Whole blood samples (500uL) of each volunteer are incubated for 72 hours (RPMI 1640 medium, 20% fetal bovine serum, 3% phytohemagglutinin) at 37 °C with 5% CO_2. Cytokinesis is blocked with cytochalasin B (4.5 ug/mL), added 44 hours after the culture has been started. At the end of the culture the samples undergo hypotonic treatment (0.075 M KCl) and fixation (methanol/acetic acid 5:1 and 3:1), when finally stained (Giemsa 5% solution) and analyzed under an optical microscope. The cytotoxic potential is determined by the nuclear division index (NDI) and the genotoxic potential by frequency micronuclei in binucleate lymphocytes. Results/Discussion: To date, 50 rural workers and 100 control subjects not exposed to agrochemicals have been recruited. The NDI for farmworkers was 1.16 ± 0.1 (M ± SD, N = 35) and for 82 individuals not exposed to agrochemicals was 1.14 ± 0.1.Regarding the genotoxic potential, the results are available for 8 volunteers (4 exposed and 4 controls). Partial results show that the frequency of binucleate cells containing micronuclei was 5.75 ± 2.4 in those exposed versus 4.38 ± 2.5 in the controls. When evaluating the total micronuclei, the exposed had 6.13 ± 3.1 and the control group 4.63 ± 3.0. Conclusion: The results are still partial, but the study intends to identify genetic damages that may contribute to the development of cancer, aiding in decision making, as early as possible.
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BIOMARKERS OF GENOTOXICITY AND OXIDATIVE STRESS IN CHILDREN EXPOSED TO METALS AND PESTICIDES IN A RURAL AREA

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Introduction: Rural children are exposed to environmental chemicals, including pesticides and metals. We evaluated the possible association between the environmental exposure of rural children to cholinesterase inhibitor insecticides and metals and DNA and oxidative damages. Methods: 55 children (5-16 years) from the rural area of Agudo, RS, Brazil, participated of this study. Blood and buccal cells samples were collected in two different moments: low and high exposure periods to pesticides. Butyrylcholinesterase (BuChE) serum activity was determined spectrophotometrically as biomarker of exposure to cholinesterase inhibitors. Blood metals (As, Cr, Ni, and Pb) were measured by ICP-MS. DNA damage was evaluated through comet assay in blood and micronucleus (MN) test in buccal cells. Activities of antioxidant enzymes, glutathione S-transferase (GST) and glutathione peroxidase (GPx), were determined by spectrophotometry. Data were analyzed with the software IBM SPSS (version 22); $p<0.05$ was considered significant. The Research Ethics Committee of UFRGS approved this study (CAAE: 51920115.2.0000.5347). Results/Discussion: BuChE activity was significantly inhibited in the high exposure period (6,830.8 ± 2702 U/L) in comparison to the low exposure period (8,357.4 ± 1785 U/L) ($p<0.01$). Cr and Pb were significantly increased in the low exposure period, As and Ni were significantly increased in the high exposure period ($p<0.01$). Although no significantly different, % of DNA in tail and the frequency of MN were higher in the high exposure period than in the low exposure period. Activities of the enzymes GST and GPx were significantly increased in the high exposure period ($p<0.01$), suggesting an effort of the organism against oxidative damage caused by environmental chemicals. In fact, a significant association was found between % of DNA in tail and GPx in the high exposure period ($r= 0.390$; $p<0.01$). In the low exposure period, As was correlated with GPx ($r= 0.397$; $p<0.01$). Cr ($r= 0.334$) and Ni ($r= 0.338$), two known carcinogens, were correlated with the frequency of MN in the low exposure period ($p<0.05$). Positive correlations were found between some metals, and between high levels of metals and lower activity of BuChE, reinforcing the hypothesis of co-exposure to multiple chemicals. Conclusion: Children from rural communities are environmentally exposed to several xenobiotics, including insecticides and metals, being susceptible to suffer deleterious health effects.

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ANTIMONATE N-METHYLCUCAMINE AND ANTIMONIUM INDUCE DECREASING PROLIFERATION IN CULTURED HUMAN LEUKOCYTES

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Key-words: Leishmaniosis, leukotoxicity, genotoxic effect

N-methylglucamine antimoniate (Sb-GAM) is the drug of choice to treat leishmaniasis according to the World Health Organization. The literature describes this drug's toxic effects, especially due to the presence of antimony (Sb) in its molecule. However, as we known, the literature has a lack on the genotoxic potential of Sb-GAM. The aim of this study was to evaluate if Sb-GAM and Sb^V may exert effect on the cell proliferation in cultured human leukocytes at the range concentration of 5, 10, 20, 40 and 50 µg/mL. The two last ones represent the plasma peak concentration of the Sb-GAM for humans after administration per injection. To perform the culture, peripheral venous blood (5 mL) was collected in heparinized tube and centrifuged at 1,800 rpm for 10 min. The leukocytes suspension (LS) was adjusted in order to obtain 10^6 cells/mL. 1 mL of this LS was added in plate containing 9 mL of supplemented RPMI 1630 medium (streptomycin, penicillin, phytohemagglutinin, and fetal bovine serum) at 37°C in 5% CO_2 atmosphere for 72 hours. The groups were divided in control groups (negative, just RPMI medium; and positive, with RPMI plus 4 µM H_2O_2); and tested groups (Sb-GAM and Sb^V). To assess the proliferation, an aliquot of 100 µL from different cultured groups was mixed with 100 µL Türk solution in order to count the leukocyte number in Neubauer chamber using optical microscope at 400 X magnificence. The data were analyzed by One Way ANOVA, complemented by post-hoc Dunnett's Test, and considered as statistically significant when $p<0.05$. Cell proliferation is a standard genotoxicological test for xenobionts. Our data showed that both Sb-GAM and Sb^V was capable to decrease the leukocyte proliferation in all tested concentration (5-50 µg/mL), when compared to negative control ($p<0,001$). Additionally, Sb-GAM and Sb^V did not differ each other both in their respective effects and tested concentrations. However, further studies are needed to clarify the mechanism of this phenomenon observed.

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DIPHENYL DITELLURIDE SENSITIZES TEMOZOLOMIDA RESISTANT GLIOBLASTOMA MULTIFORME CELLS

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Despite advances in the oncology field, malignant gliomas are characterized by low incidence but high mortality rates, both of which are likely to increase in coming years, especially in developing countries. Despite the initial responsiveness to standard treatment with the chemotherapeutic alkylating temozolomide (TMZ), few advances have been made in the prognosis of patients in the last 10 years. This is due to the fact that these tumors are rarely amenable to surgical resection, have a high rate of recurrence and chemotherapeutic resistance. In this scenario, the discovery of new substances that can act with additive or synergistic effect and increase the sensitization of tumor cells to the treatment, becomes a therapeutic strategy in the field of oncology. Diphenyl ditelluride (DPDT), solid and hydrophobic, is an organotellurium compound used in various organic synthesis reactions, studied due to antioxidant, chemoprotective, cytotoxic and potential antitumor activities *in vitro*. The aim of this study was to evaluate the cytotoxic effects of DPDT and the widely used chemotherapeutic agent TMZ, in isolated and associated regimens, after acute and chronic exposure of non-resistant (M059J) and resistant glioma cells (GBM). In the analysed results, cumulative population doubling showed to be significantly lower in the cell number for M059J and GBM treated with TMZ, during the 120 h of drug exposure, after drug withdrawal and these cells still decreased their number indicating the long-term effect of TMZ. These results confirmed the resistance of GBM cell line, because of the higher IC_{50} value found in viability assay, 50µM for M059J and 100µM for GBM. Furthermore, it was possible to determine that DPDT clearly has a dose-dependent cytotoxic effect on the M059J and GBM lines, in a lower concentration range than that used with TMZ. Evidenced by the decline in cell viability, DPDT sensitized the cells to TMZ treatment. It is important to point out that this sensitization occurred in low and approximate IC_{50} values after both, 24 h (2.5 µM for M059J and 2.3 µM for GBM) and 120 h (and 2.5 and 2.0 nM, respectively) of treatment, being the effects of the DPDT independent of the resistance profile to TMZ. Therefore, observing the effects of DPDT on M059J and GBM cells, there was a potentiation of the effects of TMZ, with a decline in cell viability to around only 20% survival. The data here presented suggest using DPDT and its association to TMZ in the treatment of gliomas, aiming to reduce the doses of TMZ used in the clinic and to allow a real decrease of the side effects to the patients.

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DIALLYL DISULFIDE/SORAFENIB ASSOCIATION INDUCES SYNERGISTIC CELL DEATH, APOPTOSIS AND PREVENTS CELL MIGRATION IN HEPG2 HUMAN CELLS

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Key-words: diet bioactive compounds; nutrigenomics; antiproliferative

Hepatocellular carcinoma is one of the most common types of cancers in adults, and sorafenib is the drug of choice in the advanced stages of the disease. However, many patients exhibit tumor progression during treatment, which drives the search for new therapeutic options. The occurrence of the disease may be reduced and/or its development controlled by synergistic mechanisms of drugs and dietary bioactive compounds. Diallyl disulfide, a compound found in garlic and other plants of the genus *Allium*, exerts anti-tumor activity *in vitro*, including in hepatocarcinoma. Here we present results from diallyl disulfide (1 - 800 µM) and sorafenib (2 - 32 µM) and its association. Cytotoxic activity was assessed by resazurin (24, 48 and 72 hours), apoptosis by Annexin V-FITC/PI (72 hours), genotoxicity by comet assay (24 hours) and cell migration by wound healing assay. Treatment with diallyl disulfide reduced cell viability in 48 (100-800 µM) and 72 hours (200 and 400 µM). Diallyl disulfide alone induced apoptosis at 200 µM, genotoxicity (50, 100 and 200 µM) and prevented cell migration at all concentrations tested. While treatment with sorafenib alone (8-32 µM) reduced cell viability at 24, 48 and 72 hours and 8 µM induced apoptosis and prevented cell migration. The associated treatment of the bioactive compound (50, 100 and 200 µM) with sorafenib (8 µM) had a synergistic effect on the decrease of cell viability, induction of apoptosis and cytostatic effect. In conclusion, the association between diallyl disulfide and sorafenib induces cell death, prevents cell migration and induces apoptosis by DNA damage in HepG2 cells. Moreover, this association represents a promising new strategy to treat human hepatocarcinoma.

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EVALUATION OF ANTIGENOXIC AND ANTIMUTAGENIC EFFECTS OF PRE-TREATMENT WITH MELATONIN IN A MODEL OF MELANOMA SKIN CANCER IN MICE

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Key-words: Melanoma; B16F10 cells; Melatonin; DNA damage; antioxidant.

Melanoma is an aggressive skin cancer, originating of the melanocytes, and can be disseminated by the primary tumor, with a risk of metastasis to lung, liver and cortex, causing mutagenic effects on DNA. The melatonin is an endogenous hormone, identified as a free radical scavenger, with an ability to protect the DNA, and has antiproliferative effects in melanoma cells. Thus, the aim of the present study was to evaluate the effects of B16F10 melanoma cells and the effects of melatonin supplementation on genotoxic parameters in mice submitted to animal model of melanoma cancer. In this study thirty-two male C57Bl/6 mice, were divided in four groups: PBS + vehicle (n=6), melanoma + vehicle (n=10), PBS + melatonin (n=6) and melanoma + melatonin (n=10). The melanoma groups received a B16F10 cell injection and the melatonin was administered during 60 days. After treatment, measurement of tumor and metastasis were evaluated. The DNA damage was determined in peripheral blood, lung, liver and cortex using the comet assay and bone marrow for micronucleus test. Results demonstrated there was no founded metastasis in lung, liver and cortex, this result may be attributed to the type of analysis performed because we only evaluated metastasis by visual observation. The B16F10 cells were effective to induce DNA damage in all tissues analyzed and melatonin supplementation decreased these damages in blood, liver and cortex it is because this hormone exerts strong anti-tumor activity via several mechanisms, including anti-proliferative, pro-apoptotic effects and antioxidative properties. However, was not found the same results in the lung, the hypothesis is because the melatonin can be induced apoptosis in cancer cell, effect not evaluated by the comet assay. In conclusion, this study has evidence B16F10 cells is effective to induce genotoxicity and mutagenicity and melatonin can reduce the damages.

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SILENCING OF *APE1* IN GASTRIC CANCER CELL LINE TREATED WITH HYDROGEN PEROXIDE AND *HELICOBACTER PYLORI* EXTRACT INDUCES G2/M ARREST AND APOPTOSIS

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Helicobacter pylori (*H. pylori*) infection is the major risk factor associated with the development of the gastric cancer. The chronic inflammation promoted by this bacterium in the host gastric mucosa results in reactive oxygen species (ROS), promoting oxidative stress and DNA damage. APE1 endonuclease, an essential enzyme with a role in the Base Excision Repair (BER) pathway, and DNA damage response (DDR) genes have important role in the recognition and repair of DNA damage, and may be regulated by miRNAs. Thus, we aimed to investigate whether the silencing of *APE1* by shRNA can interfere with survival of AGS gastric cancer line after treatments with hydrogen peroxide (H_2O_2) and/or *H. pylori* extract (HPe) and its relation with the expression of DDR genes (*ATM*, *ATR* and *H2AX*) and miRNAs. In AGS cell line expressing *APE1* was observed an increase in the proliferation rate and intracellular ROS level in the combined treatment of H_2O_2 + HPe, while higher levels of DNA damage (γH2AX foci) were observed only after treatment with H_2O_2. In addition, treatments with H_2O_2 or HPe significantly increased the percentage of initial apoptosis, whereas treatments with HPe and the combination of H_2O_2 + HPe led to a reduction of cells in the G0/G1 phase. The H_2O_2 treatment caused high expression of the *APE1* gene, miR-15a and miR-21, and repression of the *ATM*, *ATR*, and *H2AX*. Moreover, treatment with HPe increased the expression of the *APE1* and *ATR*, miR-15a and miR-421, while the combined treatment (H_2O_2 + HPe) increased the miR-24 expression. In silenced AGS line (AGS shAPE1) we observed that the treatments with H_2O_2, HPe or the combined treatment (H_2O_2 + HPe) led to a greater increase in cell proliferation, in the level of intracellular ROS and higher levels of DNA damage (γH2AX foci) in relation to AGS line- *APE1* not silenced. Moreover, the treatment combined increased the percentage of late apoptosis, accompanied by reduction of cells in the S phase and G2/M blockade, and reduction of the expression of miR-421. In addition, treatment with H_2O_2 also caused *ATM* repression, whereas the treatment with eHP promoted *ATR* induction. In conclusion, our results show that H_2O_2 and/or HPe treatments increase the occurrence of DNA damage in AGS cell line, as well as the expression of genes and miRNAs involved in DDR and DNA repair, while AGS shAPE1 may led the cell to late apoptosis and blockade of cell cycle, can be a promising target for the control of gastric cancer progression.

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CARCINOGENIC EFFECT OF THE PHENOBARBITAL ASSESSED BY THE EPITHELIAL TUMOR CLONES DETECTION TEST (*WARTS*) IN *Drosophila melanogaster*

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Keywords: *Drosophila melanogaster*. Phenobarbital. Carcinogen.

Phenobarbital is an anticonvulsive barbiturate widely used in veterinary medicine. It is frequently used in the treatment of epilepsy, one of the main neurological pathologies that affects dogs and cats. Several studies have associated phenobarbital's use with hepatic cancer. In this context, the present study was aimed at assessing phenobarbital's carcinogenic effects using the clone detection of epithelial tumors test in *Drosophila melanogaster*. A preliminary toxicity assessment assay was done at five different phenobarbital concentrations. Toxicity was determined based on the number of third instar larvae that did not reach adulthood. Concentrations of 10.75, 21.5, and 43.1 mM phenobarbital were selected for the test. The third instar larvae of *D. melanogaster* also underwent treatment with the selected phenobarbital concentrations that were associated (co-treated) with 0.4 mM doxorubicin. Doxorubicin at 0.4 mM was also used as a positive control, and ethanol (5%) was the negative control. Adult flies were analyzed, and tumor incidence numbered. The results showed a significant increase in tumor frequency with both the 10.75 and 21.5 mM phenobarbital concentrations when compared with the negative control. In addition, there was a statistically significant difference when the different phenobarbital concentrations were associated with doxorubicin and compared to the positive control, suggesting a modulatory effect by phenobarbital on doxorubicin's actions. Several studies have revealed that phenobarbital is metabolized by CYP450 enzymes, which convert xenobiotics into carcinogenic compounds; this enzyme complex also induces oxidative DNA damage that eventually leads to cell death. Under the present experimental conditions, we conclude that phenobarbital showed carcinogenic effects that demonstrate tumor induction and CYP450-induced doxorubicin modulatory effects, however, further studies are needed to better understand the mechanisms underlying the activities of phenobarbital.

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EPIDEMIOLOGIC AND *IN SILICO* DATA SHOWS *XRCC4* GENE VARIANT AS A RISK FACTOR TO CHROMOSOMAL REARRANGEMENTS INVOLVED IN LEUKEMOGENESIS IN INFANTS

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Acute leukemias are the most common and severe group of neoplasms during childhood. Infant acute leukemia (i-AL; up to 24 months old) is characterized by a high frequency of *KMT2A* rearrangements (*KMT2A*-r, previously known as *MLL*-r) both in infant acute lymphoblastic leukemia (i-ALL) and infant acute myeloid leukemia (i-AML). *KMT2A* is an important regulator gene of hematopoietic tissue differentiation during embryogenesis and several evidences point to the origin of rearrangements during fetal life. Previous investigations highlighted maternal exposure to xenobiotic compounds during pregnancy as a risk factor for i-AL and *KMT2A*-r, specially inhibitors of topoisomerase II like flavones and isoflavones of diet, benzoquinones of tobacco smoke and drugs such as quinolones, sodium dipyrone and estradiol. The inhibition of topoisomerase II could lead to double strand breaks (DSBs) on DNA and its incorrect repair may be responsible for chromosomal translocations. Here, we analyzed the risk for i-AL and the non-homologous end joining's (NHEJ) *XRCC4* intron 3 indel (30 bp) polymorphism rs28360071. Using data from the Brazilian Collaborative Study Group of Infant Acute Leukemia (2000-2013) the genotypes were determined by PCR from samples of 592 individuals (up to 24 months old): 292 cases (i-ALL =173; i-AML = 119) and 300 controls. Calculations of allelic/genotypic frequencies and Hardy-Weinberg equilibrium were determined using GenePop 4.2; p-values and odds ratios (OR) were calculated using SPSS Statistics 22.0. Bone marrow expression of *XRCC4* was performed by qPCR. *XRCC4* cDNA and polymorphic region of rs28360071 sequencing were performed on Applied Biosystems 3500 Genetic Analyzer. We found an increase of risk for i-ALL in patients harboring *KMT2A*-r (codominant model IIxID: OR = 2,23, CI: 1.17-4,25, p = 0.014). There was a higher expression of *XRCC4* among patients with ALL than among patients with AML, but this was not related to the genotypes of rs28360071. Human Splicing Finder software pointed to the activation of a cryptic splicing site on intron 3 due to the deletion allele. However, sequencing and melting curve of the amplification fragment of *XRCC4* cDNA didn't show any abnormalities. Our results suggest that *XRCC4* intron 3 indel variant may have a role on *KMT2A* rearrangement generation and leukemogenesis in i-ALL patients.

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LACK OF CARCINOGENICITY OF DIPYRONE IN SOMATIC CELLS OF *Drosophila melanogaster*

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Keywords: analgesic, cancer, epithelial tumour test, *wts*.

Dipyrone (DIP) belongs to the class of non-steroidal anti-inflammatory drugs, and is used as analgesic, antipyretic, anti-inflammatory and spasmolytic. Its mechanism of action is the inhibition of the synthesis of prostaglandins, prostacyclins and thromboxanes, and the reversible and irreversible inhibition of the cyclooxygenase (COX) enzyme in its known isoforms. Exaggerated consumption may result in adverse reactions, including agranulocytosis, aplastic anemia, and other reactions. Some studies have shown that DIP has shown toxic and mutagenic effects in different test organisms. The large-scale consumption and easy acquisition of DIP has aroused interest in several research centers that seek to elucidate the possible effects of the drugs on cancer formation. However, there is as yet no research to prove the effect of this drug on tumor formation. The aim of the current study was to evaluate the carcinogenic effect of dipyrone through the Epithelial Tumour Test (ETT) in *Drosophila melanogaster*. Third instar larvae from *wts/*TM3 virgin females mated with *mwh/mwh* males were treated with different concentrations of DIP (0.5, 1.0 and 2.0 mg/mL). Water ultrapure and doxorubicin (DXR 0.4 mM) were used, respectively, as negative control and positive control. The results showed no significant increase in tumours compared with the negative control. In conclusion, under these experimental conditions, DIP had no carcinogenic effects in *D. melanogaster*.

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EVALUATION OF THE CARCINOGENIC EFFECT OF PARACETAMOL, ASSAYED THROUGH THE EPITHELIAL TUMOUR TEST (ETT) IN *Drosophila melanogaster*

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Keywords: analgesic, cancer, doxorubicin, *wts*.

Paracetamol (PAR) is a drug belonging to the class of non-steroidal anti-inflammatory drugs widely consumed in Brazil, being used as analgesic and antipyretic. It has a low anti-inflammatory action and a proven clinical efficacy in the temporary relief of mild to moderate pain and a decrease in fever, being effective in infants, children and adults. In addition, PAR does not present some of the side effects of other non-steroidal anti-inflammatory drugs, such as gastric ulceration and blockage of platelet aggregation. However, the adverse effect of overdose of this drug results in fatal hepatic injury. Some studies have shown that PAR has toxic and mutagenic effects in different test organisms. The large-scale consumption and easy acquisition of PAR has aroused interest in several research centers that seek to elucidate the possible effects of the drugs on cancer formation. However, there is as yet no research to prove the effect of this drug on tumor formation. The aim of the current study was to evaluate the carcinogenic effect of PAR through the Epithelial Tumour Test (ETT) in *Drosophila melanogaster*. Third instar larvae from *wts/*TM3 virgin females mated with *mwh/mwh* males were treated with different concentrations of PAR (0.5, 1.0 and 2.0 mg/mL). Water ultrapure and doxorubicin (DXR 0.4 mM) were used, respectively, as negative control and positive control. The results showed no significant increase in tumours compared with the negative control. In conclusion, under these experimental conditions, PAR had no carcinogenic effects in *D. melanogaster*.

Financial Support: UNIPAM

LACK OF MUTAGENICITY AND CARCINOGENICITY OF VITAMIN D3 SUPPLEMENTATION IN SOMATIC CELLS OF *Drosophila melanogaster*

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Keywords: cholecalciferol, epithelial tumor test, somatic mutation and recombination test

Vitamin D deficiency is associated with several chronic diseases, such as obesity, diabetes, rheumatic arthritis, Parkinson's, Alzheimer's, osteomalacia, osteoporosis, and cancer. Vitamin D supplementation has increased and also been associated to antineoplastic properties, with diverse effects on cancer development and progression. The metabolism and functionality of vitamin D are changed in different types of cancer though. Such changes allow a greater resistance to antitumorigenic effects of the vitamin D and contribute to the development and progression of cancer. The aim of the current study was to evaluate the mutagenic and carcinogenic potential of cholecalciferol, commonly called as Vitamin D3 (VD3), a fat-soluble secosteroid compound. Mutagenic effects were evaluated using the somatic mutation and recombination test (SMART) on wing cells of *Drosophila melanogaster*. Third-instar larvae from standard (ST) and high bioactivation (HB) crosses were treated with different concentrations of VD3 (6.25, 12.5, 25, 50, 100 mM). Water (Reverse Osmosis), 1% Tween 80 and doxorubicin (DXR 0.4 mM) were used, respectively, as negative control, solvent control and positive control. The results showed that VD3 did not induce mutation at any of the concentrations used in both crosses. The carcinogenic effect of VD3 was assayed through the Epithelial Tumour Test (ETT) in *D. melanogaster*. Third-instar larvae from the cross between naïve *wts/*TM3 females and *mwh/mwh* males, were treated with the same concentrations used in the SMART. Water (Reverse Osmosis), 1% Tween 80 and doxorubicin (DXR 0.4 mM) were used, respectively, as negative, solvent and positive controls. The results showed no significant increase in tumor, when compared to the solvent control. Therefore, our results showed that under experimental conditions, VD3 supplementation had no mutagenic and carcinogenic effects in *D. melanogaster*.

Financial support: FAPEMIG; CAPES; CNPq, UFU; UNIPAM.

GROWTH INHIBITION OF HUMAN TUMOR CELLS LINES BY AN AQUEOUS EXTRACT OF *Annona muricata* PULP

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Key-words: *Annona muricata*, cytotoxicity, anticancer activity

Annona muricata Linnaeus, popularly known as *graviola*, is a tree found in tropical and low altitudes, belonging to the family Annonaceae. Pharmacological effects of its fruit (soursop) have been widely investigated. We have shown that the aqueous extract of soursop pulp does not induce mutagenicity and shows antimutagenic potential avoiding damage to DNA caused by oxidative stress. Besides, the aqueous extract has a significant phenolic content with low capacity to scavenger 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. We suggested that this effect can be due to the presence of compounds with low antioxidant potential such as *p*-coumaric acid. The present study investigated the cytotoxic potential of the aqueous extract of *A. muricata* pulp on cancer cell lines: hepatocarcinoma ($HepG_2$), colorectal adenocarcinoma ($CaCo_2$) and breast cancer (MCF-7). Cell death was evaluated by water-soluble tetrazolium salt (WST-1) and lactate dehydrogenase (LDH) assays. Dose-dependent inhibition of cell growth by aqueous extract on $HepG_2$, $CaCo_2$ and MCF-7 cells either by alteration of mitochondrial activity or by membrane rupture were detected, with greater toxicological sensitivity on mitochondrial activity of the MCF-7 line. In the WST-1 assay, no cytotoxic activity was observed in $HepG_2$ cells, but it was observed in the MCF-7 and $CaCo_2$, reaching 57.8 and 48.7% of survival reduction, respectively, after 72h exposure. These results suggest that the aqueous extract present bioactive compounds with selective cytotoxic properties when under mitochondrial metabolism. However, cytotoxic induction via membrane rupture was observed by the LDH assay in all lines tested. Greater sensitivity to cell death was observed in MCF-7 line, followed by $CaCo_2$ and $HepG_2$ lines, reaching 90.7, 84.8 and 37.5% of survival reduction, after 72h exposure. The cytotoxic induction may be due to the presence of acetogenins or flavonoids which are present in the *A. muricata* pulp. It is concluded that the extract has cytotoxic potential on tumor cells lines, and seems to have selective action only on mitochondrial activity.

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ANTITUMORAL EFFECTS OF THE FLAVONOID BRACHYDYN A IN DU-145 HUMAN PROSTATE CANCER CELLS CULTURED AS 3-DIMENSIONAL-SPHEROIDS *in vitro*

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Prostate cancer (PCa) in the metastatic stage is treated with hormonal therapy followed by chemotherapy. However, the main chemotherapeutic agents used in metastatic PCa (i.e., taxanes) treatment have been showing tumor resistance. Therefore, the research for novel chemotherapeutic agents that act inhibiting PCa progression has been stimulated. Bioactive substances of plants, known as phytochemicals, has been demonstrated cytotoxic and antiproliferative action in PCa cells *in vitro*. Brachydin A (BrA), a glycosylated flavonoid from *A. brachypoda*, showed cytotoxic effects in PCa cells *in vitro* cultured in monolayers (2D). Nevertheless, due to the capacity of tumor microenvironment replication, a three-dimensional (3D) *in vitro* tumor cell line approach can provide a more accurate response about the potential use of BrA in metastatic PCa. We aimed to investigate the antiproliferative, cytotoxic and genotoxic effects of BrA in prostate DU-145 (brain metastatic isolated) tumors cells cultured as 3D-tumor-spheroids *in vitro*. Initially, the structure (morphology) and growth (volume) were evaluated at different time points (72, 120, and 168h) with BrA concentrations from 10 to 100 µM. Besides, cytotoxic potential of BrA (10-100 µM) was analyzed using resazurin and acid phosphatase assays by 24, 48 and 72h. The comet assay investigated the genotoxic effects of BrA (40-100 µM) after 4h. The experiment design also included negative, solvent (DMSO; 1%) and positive (docetaxel; 50 µM) controls. Results showed that BrA at concentrations of 40 to 100 µM modulates the structure, growth, and volume of DU-145 3D-spheroids only after treatment-time by 72h. Cell viability results showed that BrA induced cytotoxicity in concentrations higher than 60 µM and after 48h treatment-time in both assays performed. Interestingly, BrA showed more notable spheroid desegregation and cell viability decreasing rate than docetaxel (taxane chemical). Furthermore, BrA causes genotoxicity at the two higher concentrations (80 and 100 µM). In conclusion, our results demonstrated that BrA promotes disintegration, changes in volume/grown, besides showing cytotoxic and genotoxic effects in metastatic PCa DU-145 cells cultured in 3D-spheroids. At this moment, BrA data reveals a promising candidate to antitumoral agent (as chemotherapy or adjuvant) in metastatic PCa treatment.

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EVALUATION OF THE ASSOCIATION BETWEEN SULFORAPHANE AND VITAMIN D ON CYTOTOXICITY IN HUMAN PROSTATE CANCER CELLS

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Key-words: nutrigenomics; *in vitro;* antiproliferative; calcitriol.

Prostate cancer is the third leading cause of cancer death in men worldwide. Despite the available treatments and the effective results in most cases, there is no efficient treatment for the advanced or metastatic stages of this disease. The identification of new compounds with promising antitumor activity could increase the options of therapeutic approaches for these patients. So, many dietary bioactive compounds have been investigated for their anticancer properties, including sulforaphane and vitamin D. These compounds have been shown to be anticancer in *in vitro* and *in vivo* studies, however, when used in clinical therapy, their effects have been limited due to their bioavailability. Thus, sulforaphane and vitamin D were combined in this study to evaluate the possible cytotoxic activities of this combination in human prostate cancer cells, DU145 (ATCC® HTB-81™) and PC-3 (ATCC® CRL-1435™) obtained from the American Type Culture Collection, at achievable plasma concentrations. Cell viability was analyzed by MTT and neutral red with exposure of cell lines for 72 hours with sulforaphane (1, 2, 4, 8 and 16 µM) and vitamin D (2, 4, 8, 16 and 32 nM). The effects of the combination of compounds were investigated using protocols of simultaneous, pre- and post-treatment. The results demonstrated that vitamin D was cytotoxic in DU145 (32 nM) and PC-3 (4 - 32 nM) cells, whereas sulforaphane reduced the cell viability of the two cell lines in concentrations greater than or equal to 2 µM in MTT assay. Analyzing cell viability by the neutral red assay, vitamin D was cytotoxic in both cell lines (32 nM), as well as sulforaphane, which reduced cell viability of DU145 (8 - 16 µM) and PC-3 (4 - 16 µM). The results of the association of sulforaphane and vitamin D were not different from the results reported by sulforaphane alone, so the combination of the compounds did not exhibit a better cytotoxic effect than sulforaphane when used alone. Although no synergistic or antagonistic effects of the combination of sulforaphane and vitamin D have been observed, these results are important for the determination of safe concentrations of these compounds to be given as supplements, and may help in inferring possible pharmaceutical interactions.

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THE ROLE OF DOUBLE STRAND BREAK REPAIR, TRANSLESION SYNTHESIS AND INTERSTRAND CROSSLINKS IN COLORECTAL CANCER

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Key-words:colorectal cancer, DNA damage response, prognostic biomarkers, Translesion Synthesis, Double Strand Break.

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the world. The current management is basically dependent on the TNM staging system based on histopathological criteria. However, many patients present a different prognosis of their classification group and there may be other influencing factors such as DNA repair. Deficiencies in the Mismatch repair pathway (MMR) lead to Microssatelite Instability (MSI). CRC with MSI shows several distinct clinicopathological features and may have some influence in response to chemotherapy and serve as a predictive factor to 5-FU response in CRC patients. Thus, the study of DNA repair becomes a promising area to identify new biomarkers in CRC. Aim: To evaluate the prognostic value of molecular modulation of double strand break (DSBR) - XRCC2 and XRCC5 - core components; DNA damage tolerance/translesion synthesis (DDT/TLS) - POLH, POLK and POLQ – and, finally, of interstrand crosslink repair (ICLR) -DCLRE1A – pathways in sporadic colorectal cancer (CRC). Methods: Tumour specimens and matched health mucosal tissues from 47 CRC patients who underwent surgery were assessed for gene expression of XRCC2, XRCC5, POLH, POLK, POLQ and DCLRE1A by qRT-PCR; protein expression of Polk, Ku80, p53, ki67 and mismatch repair MLH1 and MSH2 components were assessed by immunohistochemistry (IHC); CpG island promoter methylation of XRCC5, POLH, POLK, POLQ and DCLRE1A was performed. Uni and multivariate analyses were employed to determine associations with clinicopathological features and prognostic value of molecular data. Results: Neoplastic tissues presented induction of POLK ($p < 0.001$) and DCLRE1A ($p < 0.001$) and low expression of POLH ($p < 0.001$) and POLQ ($p < 0.001$) in comparison to healthy paired mucosa. Low expression of POLH was associated to mucinous histology and T1-T2 tumours ($p=0.038$); low tumour expression of POLK was associated to distant metastases ($p=0.042$). POLK promoter methylation was associated to early stages CRC ($p=0.011$) and POLH promoter methylation to high grade tumours ($p=0.023$). CRC harboring POLK promoter methylation presented better disease free survival (DFS) ($p=0.005$).

Conclusion: This study mainly demonstrated that low expression or unmethylated POLH and POLK were related to worse biological behavior tumors. However, POLK methylated was associated with better DFS. POLK and POLH are probable potential prognostic biomarkers in CRC.

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ABSENCE OF CARCINOGENIC EFFECT OF THE CELECOXIB, ASSESSED BY *WARTS* TEST IN *DROSOPHILA MELANOGASTER*

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Key- words: Anti-inflammatory drug; Anticarcinogenic; *Drosophila*; Tumor; *Warts.*

Celecoxib is a selective nonsteroidal anti-inflammatory drug for inhibition of cyclooxygenase – 2 (COX-2). Some studies involving celecoxib have been performed to evaluate its action on carcinogenesis, due to this enzyme being expressed in some tumors. Thus, this study aimed to assess the carcinogenic effect of the celecoxib through *warts* test to detect clones of epithelial tumors in somatic cells of *Drosophila melanogaster*. For this purpose, 72-hour larvae resulting from the crossing of *wts / TM3, Sb1* virgin females and *mwh / mwh* males were treated with three different isolated concentrations of the celecoxib (0.375, 0.75, 1.5 mg/mL). A positive control (DXR 0,4 mM) and a negative control (reverse osmosis water) were also included in the study. After undergoing metamorphosis, 200 flies for each concentration were analyzed to detect the presence or absence of epithelial tumors in the eye, head, wing, body, legs and halters. Only males and females presenting genotypes (*wts +/+ mwh)*, phenotypically characterized as trichomes hair, were analyzed. The results showed that isolated concentrations of the celecoxib did not increase ($p > 0.05$) the frequency of tumors when compared to the negative control. In conclusion, under the present experimental circumstances, celecoxib did not induce the occurrence of epithelial tumor in *Drosophila melanogaster*, therefore, it did not show carcinogenic effect.

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METHYLATION PROFILE AND EXPRESSION LEVELS OF *HOX* GENES ARE ALTERETED IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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Esophageal cancer (EC) is the seventh most-incident and the sixth leading cause of death by cancer worldwide. Esophageal squamous cell carcinoma (ESCC) is an aggressive cancer and represents more than 90% of EC cases. However, the molecular mechanisms involved in initiation, promotion and progression of ESCC are poorly understood. In this scenario, studies that aim to improve both the early diagnosis and the prognostic of patients with ESCC are necessary. Deregulation of *HOX* gene expression has already been reported in some tumors. However, in ESCC there are few studies evaluating the impact of gene deregulation of this family. *HOX* genes family encodes transcription factors that have key roles in establishing the identity of cells and tissues in early stages of development. In this study, our goal is to evaluate the methylation profile and expression levels of *HOXA7*, *HOXC9*, *HOXC10*, *HOXC13*, *HOXD8* and *HOXD13* (identified in our microarray data) in 40 ESCC and paired non-malignant surrounding mucosa, and 10 esophageal biopsies from healthy volunteers and to compare it with clinical-pathological data. Initially, real-time PCR was used in order to analyze the expression profile of *HOXA7*, *HOXC9*, *HOXC10*, *HOXC13*, *HOXD8* and *HOXD13* in paired samples of individuals diagnosed with ESCC and 10 esophageal biopsies from healthy volunteers. We found that tumor and surrounding samples of *HOXA7*, *HOXC9*, *HOXC13* and *HOXD8* were upregulated compared to healthy samples. In addition, *HOXC9*, *HOXC10*, *HOXC13, HOXD8* and *HOXD13* were upregulated comparing tumor with surrounding samples. Using Illumina 450k we evaluated the methylation profile of *HOXA7*, *HOXC9*, *HOXC10*, *HOXD8* and *HOXD13* and we found that *HOXD8* and *HOXD13* were differentially methylated on healthy and surrounding samples compared to tumor samples. Wanderer *in silico* platform showed that the tumor samples of *HOXA2*, *HOXA7*, *HOXC10*, *HOXD8* and *HOXD13* were hypomethylated compared to healthy samples. Together, these data suggest that gene expression can be regulated by DNA methylation and may be an early event in esophageal carcinogenesis.

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A SILVER COMPLEX WITH BIOLOGICALLY ACTIVE LIGAND: MUTAGENICITY AND CYTOTOXICITY STUDIES

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Key-words: Silver metal complexes, Ames test, cytotoxicity.

Cancer is the consequence of DNA coding errors that arise either directly from mutagenic events or indirectly from cell proliferation especially if sustained. Chemicals that act via direct interaction with DNA can induce cancer because they cause mutations which can be carried forward in dividing cells. Chemicals that act via non-genotoxic mechanisms must be dosed to maintain a proliferative environment so that the steps toward neoplasia have time to occur. Therefore, the objective of this research was to evaluate the mutagenic and cytotoxic effects of Ag complex with sulfonamide-derivative bioactive ligand named Ag-SFD, due its potential as a lead compound for drug development. The mutagenicity was evaluated by Ames test, a widely used assay that detects mutations at the gene level through strains genetically modified of the *Salmonella typhimurium* bacteria. This assay was performed using TA98, TA100, TA97a and TA102 strains of *S. typhimurium*, in the absence (-S9) and presence (+S9) of metabolic activation system in five concentrations, varying from 1.25 to 10 µg/plate. Cell viability was analyzed through the resazurin assay in a tumor cell line (HepG2 - human hepatocellular carcinoma). The results obtained showed that Ag-SFD did not induce any increase in the number of revertant colonies relative to the negative control, indicating the absence of mutagenic activity. Ag-SFD show concentration-dependent results and exhibited IC50 of 42.4 ± 1.6 µg/mL in HepG2 cells after 24 h, suggesting new potential chemotherapeutic agent. The absence of mutagenic effect by this metal complex against *S. typhimurium* bacterial strains in the Ames test is highly relevant and is a positive step towards ensuring its safe use in medicine. However, further investigations exploiting mutagenesis and cytotoxicity mechanisms should be conducted.

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ASSESSMENT OF MUTAGENICITY OF NOVEL COPPER (II) COMPLEXES CONTAINING ISONIAZID-BASED LIGANDS

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Previous studies have shown that Cu(II) complexes, formed from the interaction of Cu(II) ions with biologically active ligands, demonstrated great activity against *Mycobacterium tuberculosis*, the main agent of tuberculosis. Considering the preclinical requirements, in this study we determined the cytotoxic and mutagenic potential of novel Cu(II) complexes with isoniazid (INH) ligands: $CuCl_2(INH)_2.H_2O$ (**I1**); $Cu(NCS)_2(INH)_2.5H_2O$ (**I2**) and $Cu(NCO)_2(INH)_2.4H_2O$ (**I3**). The cellular viability was evaluated by resazurin assay in a normal cell line (GM-07492 - human lung fibroblasts). Mutagenic activity was evaluated by the *Salmonella*/microsome assay (Ames test), using the *Salmonella typhimurium* tester strains TA98 and TA97a (detect frameshift mutations), TA100 (detect base-pair-substitution mutations) and TA102 (normally used to detect mutagens that cause oxidative damage and base-pair-substitution mutations), with (+S9) and without (−S9) metabolization, by the preincubation method. The micronucleus assay was performed in HepG2 cells to investigate chromosome mutations. The complexes demonstrated low cytotoxicity with IC_{50} of 257 µg/mL for **I1**, 232.2 µg/mL for **I2** and 335.4 µg/mL for **I3**. The Ames test showed, after metabolic activation (+S9), mutagenicity indexes next to 2.0 in the experiments with the TA100 strain for **I1**, TA97a for **I2** and these both strains for **I3**. Similarly, the evaluated complexes were able to induce chromosomal damages in HepG2 cells with a statistically significant increase of nuclear buds (NBUDs), nucleoplasmic bridges (NPBs) or micronuclei (MNs) when compared to the negative control. These results contribute to a better understanding of the genetic toxicological events that the complexes can cause, in addition to providing data for the continuity of preclinical and clinical research to clarify the mechanisms and conditions that mediate their long-term biological effects.

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HUMAN NEUROBLASTOMA CELLS (SHSY-5Y) IN THE CYTOTOXIC AND GENOTOXIC EVALUATION OF NICOTINE AND ITS COTINE METABOLITE

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Nicotine is the main cause of cigarette addiction and most adverse health effects. This alkaloid is easy absorption through the skin, and the systemic toxicity can occur through its manipulation. Its main metabolite is cotinine and studies suggest that it's should be examined for its possible involvement in damage to nicotine-induced DNA. Due to neurotoxicity studies using nicotine still present inconsistencies, it is essential to evaluate the effect of cotinine on nicotine, Human neuroblastoma cell line is commonly used in studies related to neurotoxicity, oxidative stress and neurodegenerative diseases. The present in vitro study assessed the effects of nicotine and cotinine on the viability and DNA damage in SH-SY5Y neuroblastoma cells, as well as genotoxicity related to oxidative stress mechanisms. An alkaline comet assay modified by repair endonucleases (FPG, OGG1 and Endo III) was used to detect oxidized nucleobases. SH-SY5Y neuronal cells were cultured under standard conditions and exposed during 3 h to different concentrations nicotine (2.0 µL/mL, 1.0 µL/mL, 0.5 µL/mL, 0.25 µL/mL, 0.125 µL/mL) and cotinine (2.0 mg/mL, 1.0 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL). Cytotoxicity was observed at higher doses of nicotine and cotinine in the MTT assay; and for trypan blue assay, cells showed viability above 80% for both compounds. Alkaline comet assay results demonstrated significant increase in damage index and frequency for cells treated with nicotine and cotinine, presenting genotoxicity. The results of the enzyme-modified comet assay suggest an oxidatively damaged DNA induced by nicotine. In conclusion, our study indicated that nicotine and cotinine induced cytotoxicity to exposed cells and DNA damage, mainly by acting on mitochondrial activity. The mechanisms of action of genotoxicity suggested here are associated with the induction of oxidative stress. The results of the modified comet assay using enzymes demonstrated oxidized purine bases suggesting an oxidizing nature in DNA damage in cells treated with nicotine. The similar DNA damage effects observed for these two pyridine alkaloids (nicotine and cotinine) may be due to the similarity of their structures.

Keywords: Cotinine; cytotoxicity; genotoxicity; nicotine; SH-SY5Y cell.

Financial support: CNPq; CAPES; FAPERGS, ULBRA.

EVALUATION OF THE CARCINOGENIC EFFECT OF MEDROXYPROGESTERONE BY MEANS OF THE *WARTS* TEST IN *Drosophila melanogaster* CELLS

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Key- words: Carcinogenic; *Drosophila*; Progesterone; tumor; *Warts*.

Medroxyprogesterone acetate (MDA) is a synthetic analog of progesterone used in veterinary medicine as contraceptive for female dogs . However, the literature reports that MDA has many adverse effects, among them cystic endometrial hyperplasia and mammary neoplasia due to excessive or incorrect use of the drug. Therefore, the objective of this study was to evaluate the carcinogenic potential of MDA, through the warts test in somatic cells of *Drosophila melanogaster*. For this, the third stage larvae were treated with different concentrations of MDA (0.25, 0.5 and 1.0 mg/mL), were also negative control (5% ethanol) and a positive control using the chemotherapeutic doxorubicin (DXR 0.4 mM). The analysis of the long trichomes individuals, carriers of the gene under study (wts), revealed that the greatest concentration of MDA showed increased frequency of tumors when it was compared with the negative control ($p < 0.05$). In view of the above, it is possible that 1.0 mg/mL of MDA has been elevated to *Drosophila*, capable of triggering an activation of proliferative pathways. Because steroid hormones are liposoluble, bind to intracellular receptors and activate genes, thus can increase the synthesis of messenger RNA and protein synthesis and alter the cell cycle. Therefore MDA, in the present experimental conditions, promoted the carcinogenic effect in high concentration.

Financial Support: PIBIC, UNIPAM.

CYTOTOXIC EFFECT OF CHLOROFORM EXTRACT OF ANTARTIC SEAWEED *Desmarestia anceps* IN HCT 116 CANCER CELL LINE

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Key-words: colorectal cancer, apoptosis, Antartic seaweed

Colerectal cancer is the third most common tumor worldwide. Recently, natural products from seaweed evidencing apoptotic activity have attracted attention given its properties as alternative and complementary preventive or therapeutic anticancer agents, besides being sources of new drugs with anticancer activity. The aim of this study was to evaluate the cytotoxicity and mechanisms of cell death induced after treatment with chloroform extract of Antartic seaweed *Desmarestia anceps*. The cytotoxicity of the chloroform extract against the colorectal carcinoma HCT 116 cell line was evaluated by MTT assay after 24, 48 and 72 hours to extract exposition. DMSO 0,5% was added to medium as a negative control. The cell viability rate (%) was calculated as follows: (OD of the treated group/OD control group) × 100. Death induction pathway and effect on the cell cycle of the HCT 116 cell line by flow cytometry after exposure to the extract at IC_{50} and double concentration rate were also analyzed. The results of all analysis represent the averages of 3 independent experiments performed in triplicate. The chloroform extract of *D. anceps* presented cytotoxic activity against HCT 116 cell line in a dose dependent manner. IC_{50} varied according incubation time, 37.77 ± 1.57 $\mu g.mL^{-1}$ for 24h, 25.99 ± 1.41 $\mu g.mL^{-1}$ for 48h and 30.48 ±1.48 $\mu g.mL^{-1}$ for 72h. The extract also caused a reduction in the number of cells in the G1 phase of the cell cycle, an increase in the M-cell population and multinucleated cells, suggesting that tumor cells can escape from mitosis without cell division. The mechanism of induced cell death after exposure of the HCT 116 tumor cells to the chloroform extract for 24 hours was related to the apoptotic process. The inhibition of apoptosis in colorectal cancer cells enhances tumor growth, promotes neoplastic progression, and confers resistance to cytotoxic anticancer agents. Thus, bioactive compounds that induce apoptosis in cancer cells can be used as agents for cancer chemoprevention and/or chemotherapy.

These data suggest that Antartic brown seaweed *D. anceps* is candidate as potential sources of molecules with antitumor activity. This was the first study to report the anticancer activity of extract of *D. anceps* brown seaweed, as well as the mechanism of death and effect caused in the cell cycle of the HCT 116 cell line, contributing to future studies for adjuvant treatment of colorectal cancer.

Financial Support: CAPES, University of Caxias do Sul (UCS), RedeAlgas, PROANTAR

CANCER RISK IN AGRICULTURAL POPULATIONS EXPOSED TO PESTICIDES IN THE STATE OF RIO GRANDE DO SUL – BRAZIL.

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Key words: neoplasias, pesticides, micronuclei, relative risk.

Pesticides are a chemical family which have brought many benefits to mankind, especially in the agricultural area. However are growing studies associated to the exposure of these substances to the prevalence of certain diseases, among them cancer. Thus, the aim of the present study was estimate the risk of the development neoplasias and of the micronuclei cells frequency (FR) in individuals exposed to pesticides as well as in populations living in agricultural areas, especially of soy and tobacco in the state of Rio Grande do Sul, Brazil. Therefore was performed data analysis in a population database published in the years 2000 to 2016 by the Mortality Information System (DATASUS – SIM - Brazil), which were reviewed, classified and calculated the Relative Risk (RR), according to the primary location of the neoplasias. Thus, it was possible to observe a RR of 1.4-5.0 for the development of different neoplasms in populations living in agricultural regions, involved in the soybean and tobacco production when comparing with resident populations in regions without agricultural or industrial activitys. Our results show that resident populations of the soybean producing regions show a RR up to 2 times higher for the development of pancreas, kidney, bladder, multiple myeloma and prostate cancers, up to 3 times higher for leukemias and up to 5 times higher for laryngeal cancer. In populations of the tobacco producing regions were observed increased risk up to 2 times higher for the development of prostate, colorectal, pancreas, bronchi and lungs, bladder, brain and esophagus cancers, 3 times higher for leukemias and 5 times for laryngeal cancer. In addition, from data from different studies with farmers conducted in our laboratory it is observed a RR 3-fold higher to the RF of MN cells in soybean and tobacco farmers (exposed to pesticides) compared to non- exposed individuals. Therefore, in addition to the recognized correlation between increased MN frequency and increased cancer development, epidemiological and experimental studies are necessarity to complement and support mechanisms by which mutagenic events and the onset of neoplasms are prominent in agricultural populations

Financial Support: FAPERGS, CAPES, ULBRA.

ANTIOXIDATIVE DEFENSE OF ASCORBIC ACID AND RETINOL PALMITATE AGAINST OMEPRAZOLE-INDUCED OXIDATIVE STRESS AND TOXICOGENETIC EFFECTS

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Keywords: antioxidants; omeprazole; oxidative stress; toxicogenic effects.

Omeprazole (OM), a prototype proton pump inhibitor, oxidizes thiol groups and induces DNA damage. This study aimed to evaluate the oxidative effects of OM and its interactions with ascorbic acid (AA) and retinol palmitate (RP) in proficient and deficient *Saccharomyces cerevisiae* strains, as well as levels of cytogenetic damage in Sarcoma 180 (S180) cells. OM was tested at concentrations of 10, 20 and 40 µg/mL. As positive control, we used hydrogen peroxide (10 mM) and cyclophosphamide (20 mg/mL), while saline (0.9% NaCl solution) was used as negative control. In *S. cerevisiae* strains, OM induced oxidative effects (28.4 ± 2.24, $p<0.001$). However, OM co-treatment with AA (50 µM) and RP (100 IU) showed a prominent modulation (12.2 ± 1.46, $p<0.05$) of oxidative damage in *S. cerevisiae* strains. In S180 assay, OM did not increase micronucleus formation and chromosomal bridges, but induced an increase in shoots. Also, we evidenced an increase in karyolysis and karyorrhexis in S180 after OM treatment, which was modulated by the co-treatment with AA and RP. In conclusion, much precaution is recommended with OM and antioxidant co-treatment.

Financial Support: CNPq, CAPES, PPSUS

ANALYSIS OF THE CARCINOGENIC AND/OR ANTICARCINOGENIC EFFECT OF *Aloe vera* IN SOMATIC CELLS OF *Drosophila melanogaster*

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Aloe vera is a plant widely used in traditional medicine, because of its healing, laxative, antihyperglycemic, antiviral, antibacterial, antifungal, anti-inflammatory, antioxidant and anticarcinogenic properties. In view of this fact, the present study aimed to evaluate the anticarcinogenic or carcinogenic activity of the aqueous extract of *Aloe vera* in somatic cells of *Drosophila melanogaster*. The tested concentrations were determined from the toxicity test. The extract of *Aloe vera* was prepared in aqueous media, aiming at maintaining the properties of the *Aloe vera* components. The test for the detection of epithelial tumor clones (ETC Test) in *D. melanogaster* was performed using larvae *wts+/mwh+*, descendants from the crossing of *wts*/TM3 females with *mwh/mwh* males, which were chronically submitted to a negative control (reverse osmosis water), a positive control (doxorubicin - 0.4 mM) and three different concentrations of *Aloe vera* aqueous extract (10g/L, 20g/L and 40g/L). The results revealed a significant increase in the tumoral frequency of the three *Aloe vera* extract concentrations when compared to the negative control. It was also evidenced that such effect is dose dependent (the higher the concentration of the *Aloe vera* extract, the greater the frequency of tumors was observed). It is concluded that, under current experimental conditions, the extract of *Aloe vera* significantly increased the formation of tumors in *D. melanogaster* in the three used concentrations, therefore exerting carcinogenic activity.

Keywords: *Vitis vinifera*, *Drosophila melanogaster*, Carcinogenic Effect.

IN VITRO STUDIES ON THE CYTOTOXICITY, CELL DEATH INDUCTION AND MIGRATION INHIBITION EFFECTS OF THE SYNTHETIZED COMPOUND LQFM11

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Key-words: LQFM11; cytotoxicity; reactive species; lactate dehydrogenase; cellular migration; apoptosis induction

The compound LQFM11 was synthetized through molecule hybridization of two inhibitors of myocardial phosphodiesterase III, cilostazol and milrinone, drugs that increase the heart's contractility. To measure the possible cytotoxic activity of these compounds, MTT, neutral red (NR), LDH and quantification of intracellular reactive species (RE) assays were conducted. The concentrations assessed were 10-400μM for 24, 48 and/or 72 hours of treatment on HuH7 cell line (human hepatocellular carcinoma). Possible induction of apoptosis or necrosis, wound-healing time (400μM-24hours) and the clonogenic rate (100, 200 and 400μM) were assessed. No statistically relevant alterations were detected in colony formation by clonogenic assay after 7 days of treatment. No changes were observed after 24, 48 and 72 hours after MTT assay, but LQFM11 was able to decrease cell viability after 24 hours of treatment on the NR assay. Both experiments are indicative of cell viability, but they demonstrate different paths through which the compound is metabolized: MTT assess mainly mithocondrial metabolism and NR is related to integrity of lisossomes. LQFM11 treatment elevated LDH levels indicating the disruption of the membrane integrity. The compound also induced an increase intracellular of reactive species. Treatments with 400μM increased the population of apoptotic cells by comparison with non-treated group. Another interesting finding was the decreasing in cellular mobility after 24 hours of treatment with 200μM on the wound-healing assay, indicating that the compound could inhibit tumor migration. Therefore, take together the results of the decreasing cell viability, apoptosis induction and migration inhibition it is possible to affirm that LQFM11 may be effective in the treatment of liver cancer. Further studies must be conducted to determine which proteins could be involved on the deregulation of the tumoral cells by LQFM11.

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EVALUATION OF CYTOTOXICITY, GENOTOXICITY AND ABILITY OF INHIBITION OF CELL MIGRATION OF LQFM20 IN HUH7 CELLS *IN VITRO*

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Key-words: wound-healing assay, neutral red, comet assay

Cilostazol and milrinone are phosphodiesterase 3 inhibitors, potent vasodilator agents used to control cardiovascular disease. However, the administrations of these medications can lead to side effects such as headache, tachycardia, palpitations, among other related pathologies. Therefore, it is necessary to investigate new compounds that minimize these effects. Through molecular hybridization of cilostazol and milrinone was developed the prototype LQFM20 at the Laboratory of Medical Pharmaceutical Chemistry (LQFM) of the Faculty of Pharmacy of the Federal University of Goiás, Brazil. The current study investigated the cytotoxicity, genotoxicity, interference in the production of reactive species and the process of cell migration of this compound in HuH7 cells *in vitro*. To evaluate the cytotoxicity, the MTT, Neutral Red (VN) and LDH assays with serial concentrations between 50 and 450 µM were performed. From the results of these tests, some concentrations were chosen for intracellular reactive species (CM-H_2DFCDA probe), cell death (apoptosis and necrosis) and genotoxicity (comet test) assays. The results were statistically analyzed by the Shapiro-Wilk normality test and by analysis of variance (ANOVA) followed by Tukey's post-test. The compound LQFM20 did not lead to 50% decreasing in cell viability in MTT, NR and LDH assays. Evaluations of intracellular reactive species, cell death and genotoxicity did not indicate statistically significant results. However, the migration activity of the HuH7 cell was inhibited after 12 and 24 hours of LQFM20 treatment at concentrations of 150µM and 400µM, suggesting that the compound has chemotherapic profile to be used as coadjuvant in the treatment of hepatocarcinoma. Further research is necessary to understand the mechanisms of action of LQFM20 in non-tumor and other tumor cell lines.

Financial Support: CNPq grant 444213/2014-7 and CAPES/PROAP permitem

CARCINOGENIC EFFECT OF PROGESTERONE-BASED BOVINE CYCLE REGULATOR IN SOMATIC CELLS OF DROSOPHILA MELANOGASTER

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Keywords: carcinogenic, progesterone, epithelial tumors

The use of drugs to reproduce the effect of natural progestins has been widely used to promote synchronization of estrus during artificial insemination in bovine females, bringing many advantages, mainly in relation to genetic gains and cost reduction. However, the use of female sex steroid hormones is described as a crucial factor for the development of tumors and the onset of neoplasms. Considering what has been stated, the objective of the present study was to evaluate the carcinogenic effect of a progesterone-based drug for the synchronization of the heat in cattle through the warts test in *Drosophila melanogaster.* To evaluate carcinogenic potential, the drug was tested at four different concentrations (30 µM, 60µM, 120µMe 240µM), as the positive control was used the DXR (0.4mM) and the concentrations tested were statistically compared to the negative control (1 % Tween-80). The results obtained evidenced an increase in the frequency of tumors in the heterozygous descendants of *D. melanogaster*, treated with the progesterone-based medicine, at concentrations of 60, 120 and 240µM. Only the concentration of 30 µM did not show a significant statistical difference *(P <0.05*) in relation to the negative control. Increased tumor frequency occurred as drug concentrations were increased, suggesting a carcinogenic effect on somatic cells of *D. melanogaster*. In this sense, it was concluded that the drug presented carcinogenic potential at the concentrations tested, raising the frequency and the total number of tumors in somatic cells of *D. melanogaster*.

Financial Support: PIBIC, FEPAM

IN SILICO PREDICTIONS AND *IN VITRO* TESTS: CYTOTOXIC AND GENOTOXICOLOGICAL STUDIES OF ACYCLOVIR

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Key-words: Acyclovir. Citotoxicity. Genotoxicity. Mutagenicity.

Acyclovir is widely used drug in therapy for prevention and treatment of Herpes virus infections. However, the literature still lacks genotoxicological data proving the safety of its use. The aim of this study was to evaluate the safety of acyclovir through toxicological *in silico* and *in vitro* tests. *In silico* tests used the following programs: Osiris Property Explorer®, ADME-Tox®, ACD/Labs team®, Lazy Structure Activity Relationships®, ADMET Structure-Activity Relationship Server® (admetSAR, and pkCSM). *In vitro* tests assessed cytotoxicity (Trypan blue exclusion test), DNA damage (alkaline comet assay) and mutagenic (micronucleus test). The leucocyte cultures were performed using 5 mL of venous blood from a volunteer (over 18 years, self-declared healthy, no drug user and no exposure to radiations along 6 months). 0.5 mL of adjusted Leukocytes suspension (10^6 cells/mL) were transferred to 10 mL supplemented RPMI 1630 culture medium (fetal bovine serum, phytohemagglutinin, and streptomycin/penicillin). The cell cultures were incubated at 37°C in 5% CO_2 atmosphere for 72 hours. For the tests, seven groups were used as follow: negative control (RPMI medium), positive control (4 mM H_2O_2), and acyclovir concentrations, as following: 1 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, and 20 µg/mL (plasma peak for humans). The tests were performed in triplicate and previously approved by Ethic Committee of Federal University of Pampa – UNIPAMPA (n° 27045614.0.0000.5323). The data were analyzed by One Way ANOVA, complemented by post-hoc Dunnett's Test (when propitious), and considered as statistically significant when $p<0.05$. *In silico* analysis suggests the acyclovir has potential mutagenic risks (admetSAR and pkCSM). The *in vitro* tests point out the four highest acyclovir concentrations showed cytotoxic effect, however, the acyclovir, at concentrations and experimental conditions assayed was unable to show DNA damage and mutagenic effect. About the comparative between the *in silico* and *in vitro tests* in this study, it was possible to observe that the first one has predictive value but, effectively, their results must be reiterated by testing on biological matrices, at least until mathematical software models have developed enough to dissolve these differences.

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GENE FUSIONS AND WBC COUNT IN ACUTE LYMPHOCYTIC LEUKEMIA: CLUES FOR PROGNOSIS AND A POSSIBLE TARGET PATHWAY?

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The world faces a high incidence of pediatric cancer (0 to 18 years) caused by ALL, considered the most often and accounts for 75% of total infant cancer cases. Despite the therapeutic advances, up to a quarter relapse due to the frequent genetic alterations, which 50% of them are chromosomal translocations. Therefore, the studies have sought to identify the genetic alterations that contribute to its leukemogenesis, as well as the medical outcome and potential new prognostic tools that can lead to a suitable processing, or in the identification of new therapeutic targets. In this context, some technologies have been potentially useful, such as the Polymerase Chain Reaction (PCR). This work aims to detect and analyze the main gene fusions (TCF3-PBX1, MLL-AF4, BCR-ABL1, TEL-AML1 and SIL-TAL) in pediatric patients with Acute Lymphoid Leukemia (ALL) in the State of Pará. We collected 155 peripheral blood samples or bone marrow from ALL patients attended at the Ophir Loyola Hospital. Afterwards, lymphocyte isolation, RNA extraction, RT-PCR, and direct sequencing of all samples were performed. The results were analyzed and associated with clinical data. Descriptive analysis and chi-square test ($p \leq 0.05$) were performed. We detected the existence of 86 (55%) of ALL patients with gene fusions. Patients with TCF3-PBX1 were associated with more cases of leukocytosis ($p = 0.05$). BCR-ABL1 increased with age ($p = 0.0001$) and showed to be more deathly ($p = 0.0001$). TEL-AML1 fusion was more frequent in younger children and leukopenic patients ($p = 0.003$). Finally, the MLL-AF4 fusion was exclusive to infants ($p = 0.0001$) and was observed in patients at high risk ($p = 0.01$). We concluded that the PCR technique is a fast and efficient test to detect gene fusions which suggest been useful to classify individuals at distinct risk groups. Moreover, further investigations should be conducted on the gene pathways that are disrupted by gene fusions represented by the leucometric alterations that imply on the course of the therapy.

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EVALUATION OF CITOTOXICITY AND GENOTOXICITY OF COMMERCIAL GLYCOLIC EXTRACT OF *ALOE VERA* (L.) F. BURM ON DIFFERENTS CELL LINES EUKARYOTIC

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Key-words: viability; DNA damage; cancer; babosa

Since the beginning of humanity, plants with medicinal purposes are used for treatment, healing and prevention of several diseases. A plant commonly used by the population, as cosmetics and medicinal purposes, is *Aloe vera*, known popularly as babosa. Many studies has shown some biological effects caused by Aloe's compounds as antinflammatory, antioxidant and antineoplastic properties. According to WHO, one of the main causes of death in the world is cancer, however the conventional treatment, still remains very expensive and carries many side effects. Nowadays, the main goal of cancer treatment is to find selective chemotherapeutic agents, whose act in neoplastic cells without causing injurious effects in non-tumour cells. Therefore, this work has the purpose to evaluate the citotoxicity and genotoxicity of *Aloe vera*'s commercial glycolic extract, as well as its potential antineoplastic effect, comparing neoplastic cell lines (MDA-MB-231 and MCF-7) with a non-neoplastic cell line (Vero). Therefore, it was used the proliferation cell assay WST-1 and trypan blue exclusion assay to evaluate cell viability, while the DNA damage were evaluated through comet assay. Two different exposition times (2h and 24h) and six concentrations (50%, 25%, 12.5%, 6.25%, 3.12% and 1.56%) of extract were tested. The results showed that it has significant difference ($p<0,05$) between neoplastic and non-neoplastic cell lines on both time interval studied, indicating greater possibility of antineoplastic effect on 24h exposition period.

Financial Support: UERJ, CAPES, FAPERJ

IN VITRO CYTOTOXICITY OF BRAZILIAN RED PROPOLIS AND CHANGES IN MORPHOLOGY OF 3D CANCER SPHEROID CULTURE OF HEP-2 CELLS

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Propolis is a resinous product made by honey bees that has been used for centuries in folk medicine. It has a wide variety of biological effects, including antimicrobial, antioxidant and anticancer activities. Common cultures of 2D cells are not able to replicate the complexity and variety of clinical tumors as tumors *in vivo* grow in a three-dimensional (3D) configuration with specific organization and structuring. The aim of this study was to analyze the chemistry and tumor cytotoxicity of Brazilian red propolis samples, collected in Maceió (Alagoas state), as well, to evaluate changes in 3D culture of Hep-2 (human laryngeal epidermoid carcinoma) cancer cells. The ethanolic extract was prepared with 10 g of red propolis and 100 mL of ethanol 70%, for 24 hours under agitation. The extract was filtered and the solvent evaporated at room temperature and then ground to a fine powder. The ethanolic extract was analyzed by ESI-MS/MS for their chemical characterization. The cytotoxicity of propolis extract in tumor Hep-2 cell lines was determined by MTT assay. Hep-2 cancer cells were incubated in culture media containing different concentrations of propolis extract, ranging from 5 µg/mL to 175 µg/mL, for 72 hours. Morphological changes of the cells were observed and photographed under a light microscope. Our results showed that the hydroalcoholic extract presented identical main chemical compounds, including high amounts of flavonoids and phenolic acids as formononetin, liquiritigenin, medicarpin and biochanin A. At the same time, red propolis extracts exhibited cytotoxic effects *in vitro* for Hep-2 cancer cells (IC50 = 51.34 ± 6.56 µg/mL), inhibiting their cell growth in higher concentration. Red propolis treatment changed considerably the 3D cellular morphology. The examination of cancer cells treated revealed altered morphological structure dependent of the concentration of sample used while the 3D cell culture in the negative control group that receive hydroalcoholic solution showed a regular appearance. In conclusion, the Brazilian red propolis showed similar bioactive compounds and their biological properties affects the viability of Hep-2 cells. Further viability test in 3D culture is needed to better understand their effects on larynx cancer treatment, once two-dimensional 2D culture model lack realistic complexity. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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GENOTOXIC AND CYTOTOXIC EFFECT OF A SYNTHETIC CHALCONE (GCCG29) IN HUMAN LYMPHOCYTES

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Key-words: human lymphocytes, genotoxicity, chalcone

The chalcones are compounds uncovered on many plants and classified as open-chain flavonoids. Its structure is very explored due to the possibility of chemical transformations and synthetic production of different analogous with diverse biological activities. However, just few studies have investigated the effects of chalcones in normal cells. Thus, the aim of this study was the evaluation of the genotoxic and cytotoxic effect of the (E) -N- {4- [3- (4-NITROFENYL) ACRYLLYL] PHENYL} CETAMIDE (GCCG29), a synthetic chalcone, on human lymphocytes. The cytotoxicity evaluation was performed using the MTT assay - lymphocytes were seeded in a 96 well plate, cultivated for 24 hours and treated with GCCG29 Chalcone in the range of 1.5625 to 100 µM in triplicate for 72 hours. As a positive control we used doxorubicin and no drug/treatment was used as negative control. Absorbance results were obtained after reading in a spectrophotometer (562nm), and the values were used to calculate the half maximal inhibitory concentration (IC_{50}). For the evaluation of genotoxicity, the alkaline comet assay was performed in which the cells were treated with chalcone GCCG29 for 3 hours at the concentrations of 2, 4 and 46.18 µM; positive and negative controls were the same as the MTT test. Slides containing the cells were analyzed under a microscope, the damage index (DI) of each group was calculated based on the rate of nucleus degradation. The IC_{50} founded was 46.18 µM and no different damage index was observed when compared to the controls. These data show a cytotoxic effect only in high concentration comparing to others well-knowns cytotoxic agents, and non-genotoxic property of this chalcone on lymphocytes. This is an excellent result, since the main objective of testing this drug on lymphocytes was not to cause damage or death in this cell type, as well as in other healthy cells. On the other hand, this chalcone is expected to have a greater harmful action on tumor cells, as we have already observed in an another study (IC_{50} 2 µM on HCT116) of our group. So GCCG29 seems to have a good perspective of use when thinking about tumor chemotherapy. Although it is fundamental that complementary evaluations of its effectiveness and selectivity be done analyzing other parameters and models.

ANTIPROLIFERATIVE EFFECTS OF DIPHENYL DITELLURIDE AND CELL CYCLE ARREST IN COLORECTAL CANCER - HCT116 CELLS

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Cancer is the one of most concerns in public health and has one of the highest mortality rates in worldwide. Diphenyl ditelluride (DPDT) is an organotellurium (OT) compound with biological effects, such as potential antioxidant, antigenotoxic and antimutagenic at low concentrations (≤1 µM). Otherwise, DPDT showed cytotoxic effects inducing oxidative damage, DNA strand breaks in mammalian V79 cells (1 – 10 µM) and cell cycle arrest in several cell lines. Thus, this work aims to investigate the antiproliferative and cell cycle arrest potential of DPDT in human colorectal cancer cells (HCT116 *wt,* HCT116 $p21^{-/-}$, HCT116 $p53^{-/-}$ and HCT116 $BAX^{-/-}$) and human fibroblast cells (MRC5) to elucidate further potential applications of DPDT. Results showed a decrease in cell viability after DPDT exposure for 24 h in all cell lines, as evaluated by MTT (1 – 20 µM) and clonogenic assays (1 – 5 µM). The sensitivity was clearly higher for the HCT116 *wt* cell lines when compared to the MRC5, evaluated by MTT, given values of 5.17 ± 1.07; 1.14 ± 1.19; 5.38 ± 2.36; 5.98 ± 1.01 and 5.77 ± 2.75 µM (IC_{50} ± SD) for MRC5, HCT116 *wt,* HCT116 $p21^{-/-}$, HCT116 $p53^{-/-}$ and HCT116 $BAX^{-/-}$ respectively. In the clonogenic assay, for the same cell lines, in 24 h exposure, the values 3.56 ± 0.03; 2.05 ± 0.19; 4.22 ± 0.544; 2.15 ± 0.12; 2.13 ± 0.10 µM (IC_{50} ± SD) were found. The cell cycle analysis by flow cytometry in 24 h of DPDT exposure (1, 5 and 10 µM) detected G2/M phase arrest, for MRC5 and HCT116 *wt,* showing that DPDT accts with same mechanism of action. Taken together, HCT116 colon cancer cells were more sensitive to DPDT antiproliferative effects than MRC5 cells, but show the same cell cycle arrest profile in both cell lines. The action of DPDT apparently occurs in the S phase, taking place to cell cycle arrest in G2/M, leading to the checkpoint activation. The HCT116 $p21^{-/-}$ showed a more pronounced viability when compared to HCT 116 *wt* and this difference should be explored, investigating a possible checkpoint mechanism via p21 pathway. The employment of genotoxic and checkpoint activators agents that leads to DNA damage response are targets in cancer research for their specificity or immunotherapeutic activity. Thus, the results of this work open up possibilities for future studies on further investigation of possible mechanisms of molecular action of DTDF in colorectal cancer cells and other cancer models.

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ANGIOTENSIN-(1-7) POTENTIATES DOXORUBICIN AND MITOXANTRONE ANTI-PROLIFERATIVE AND ANTI-MIGRATORY EFFECTS IN BREAST CANCER CELL LINE

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Keywords: Breast cancer; Angiotensin (1-7); doxorubicin; mitoxantrone; Mas receptor.

Chemotherapy is the preferential treatment for breast cancer (BC), which frequently includes doxorubicin (DOX) and mitoxantrone (MTX). Oncological effectiveness of DOX and MTX is limited by risk of cardiotoxicity. Pharmacological modulation of Renin-Angiotensin System (RAS) has been employed as a cardioprotective approach. Beyond cardiovascular system, RAS exerts pleiotropic effects, including in cancer cells. Angiotensin II (AngII) and its receptor AT1 (AT1R) are implicated in stimulation of proliferation and survival, angiogenesis and migratory capacity. Another RAS axis, which includes Angiotensin-(1-7), (Ang-(1-7)), and its receptor Mas (MasR), counterbalances AngII/AT1R axis actions, leading to antitumoral features. Thus, we aimed to evaluate the potential antitumoral effects of Ang-(1-7)/MasR axis modulation in BC associated to DOX and MTX chemotherapy. MCF-7 cells were treated simultaneously with DOX or MTX and a MasR agonist, Ang(1-7), or antagonist, A-779. MCF-7 cells viability was assessed by MTT and Trypan blue exclusion assays; migration was evaluated by Transwell assay; DNA damage was assessed by comet assay and apoptosis induction was detected by flow cytometry. Ang-(1-7) peptide co-treatment enhanced DOX and MTX cytotoxic and anti-migratory properties. This effect was abolished by A-779 suggesting that the decrease of viability/proliferation in MCF-7 cells treated with Ang-(1-7) and DOX or MTX is mediated by MasR. Ang-(1-7) co-treatment do not change the amount of DNA strand breaks induced by DOX or MTX in breast cancer cells. As expected, individual DOX or MTX treatment induced higher levels of DNA strand breaks in comparison to control after 24h treatment. Our data suggest that Ang-(1-7)/MasR axis modulation in BC is a potential new therapeutic target to increase antineoplastic effects of DOX and MTX.

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CHEMOPROTECTIVE EFFECT OF DIPHENYL DITELLURIDE ON DOXORUBICIN INDUCED TOXICITY IN MAMMALIAN CELLS

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Doxorubicin (DOX) is an anthracycline chemotherapeutic agent which main action is Topoisomerase II inhibition. Anthracycline-induced cardiotoxicity is believed to be related to the generation of reactive oxygen species (ROS) by at least two mechanisms: enzymatic reduction of the quinone with subsequent redox cycling and/or formation of an iron-anthracycline complex capable of intramolecular reduction and redox cycling. Some chemotherapeutic approaches have proposed the use of antioxidants to minimized cytotoxicity and the damage induced in normal tissues by antitumor agents that produce free radicals. Diphenyl ditelluride (DPDT) is a compound with antioxidant and antigenotoxic potential. However, the beneficial properties occur in a limited concentration range due to a bimodal nature of this agent. The aim of this study was to evaluate the effect of low DPDT concentrations on DOX-induced toxicity and genotoxicity in Chinese hamster fibroblasts (V79), as well as in human fibroblasts proficient (MRC5) and deficient in NER (XPD). For this purpose, the cell lines MRC5, V79 and XPD were treated with doxorubicin in the presence or absence of DPDT pre-treatment. Measurement of cell viability was performed using MTT assay. The DNA damage induced by DOX was studied in the comet assay and modified comet assay including incubation with the enzymes formamidopyrimidine DNA glycosylase (Fpg), that is specific for oxidized purines, and endonuclease III (Endo III) that recognizes mainly oxidized pyrimidines. The intracellular ROS levels were visualized by fluorescence microscope following incubation with 2'-7'-dichloro-dihydrofluorescein diacetate. DOX at concentration of 0.6 µg/mL induced genotoxicity, increase in the Fpg- and Endo III sensitive sites and elevated intracellular ROS levels after 3h treatment. The effect of a range of DPDT concentrations (10 nM, 50 nM and 100 nM) on DOX induced cytotoxicity and genotoxicity at the same conditions was evaluated. The concentration of 10 nM DPDT decreased DOX-induced genotoxicity and ROS formation. Concentrations above 100 nM DPDT enhanced the cytotoxic effects of DOX. Our results showed that low DPDT concentrations exhibit chemoprotective effect on DOX-induced DNA damage without decreasing of its cytotoxicity in mammalian cells. This finding suggests that DPDT can be useful for preventing the Anthracycline-induced genotoxic damage in normal tissues.

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CORRELATION OF CLINICAL AND PATHOLOGICAL DATA WITH PROTEIN EXPRESSION OF CYCLINE D1 IN PATIENTS WITH PENILE CANCER

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Penile cancer is a frequent neoplasm in Brazil, while the state Maranhão has the highest incidence worldwide. In this context, it is extremely important to investigate the events related to this cancer, especially regarding to the search of molecular markers that may improve the accuracy of *prognosis* and therapeutics. In this scenario, Cyclin D1 participates in the molecular pathogenesis as a regulatory protein, which may represent potential oncogenesis with expression reported in different types of carcinomas. Therefore, the aim of this study was to analyze the protein expression of Cyclin D1 in patients with penile cancer treated at two health services in the state of Maranhão, and correlate these data with clinical-pathological characteristics. This research was carried out on 62 patients with anatomopathological diagnosis of squamous cell carcinoma of the penis, attended at the President Dutra University Hospital and Aldenora Belo Hospital, located in the city of São Luís, MA, from 2013 to 2017. A survey of the clinical epidemiological data by medical records was obtained, and the analysis of Cyclin D1 expression was performed using the immunohistochemistry technique. Our results show the highest incidence in men between 40 and 49 (19.3%) and 60 to 69 years old (26%), most of which were farmers (53%) and married (58%) from São Luís and surroundings (37%), and with a low level of schooling (86%). Regarding the association of the protein expression of Cyclin D1 with the clinical-pathological features, the largest frequency of Cyclin D1 cases was observed for patients with usual histological subtype (27%), tumors larger than 2 cm (69%), histological grade 2 and 3 (66%) and presence of phimosis in 51%, and presence of HPV (50%), although there was no statistical difference. The data obtained in this research contributed to the better understanding of this neoplasia, in a region of high incidence. Most of the clinical-pathological characteristics of the patients in this study were similar to those described in the literature. The elevated expression of Cyclin D1 was found in patients with the worst prognosis and risk factors, such as HPV and phimosis. These data reveal the role of this biomarker in this neoplasia as a potential marker that may aid in the prognosis of this neoplasia.

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GENOTOXICOLOGICAL SAFETY ASSESSMENT OF A ZINC COMPLEX WITH BIOLOGICALLY ACTIVE LIGAND

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Key-words: Metal complexes, Zinc, Ames test, cytotoxicity

Zinc (Zn) is an important trace element in human bone, and Zn species play diverse roles in biological functions, such as DNA synthesis, enzyme activity, nucleic acid metabolism, biomineralization, and hormonal activity. Previous studies showed that Zn complexes, formed from the interaction of Zn(II) ions with biologically active ligands, have shown excellent antimicrobial activity. Considering its potential as a lead compound for drug development, the aim of the present study was to investigate the mutagenic activity of Zn(II) complex with sulfonamide-derivative bioactive ligand named Zn-SFD, by the Ames test, besides the cytocompatibility in a normal cell line (GM-07492 - human lung fibroblasts) by Resazurin assay. The Ames test was performed using TA98, TA100, TA97a and TA102 strains of *Salmonella typhimurium*, as sensitive indicators of DNA damage, in the absence (-S9) and presence (+S9) of metabolic activation system in five concentrations, varying from 0.125 to 100 µg/plate. The results showed that the Zn-SFD has no mutagenic potential by the Ames test since they did not induce a significant increase in the number of revertant colonies, compared to the negative control, in none of the concentrations and strains used, in the experiments with and without metabolic activation. Moreover, on cytotoxicity, it showed statistically significant differences when compared to the negative control at the highest concentrations, with IC50 of 459.8 µg/mL. The absence of mutagenicity and the low cytotoxicity are extremely relevant because it provides reliable data to support future clinical researches. However, further toxicological tests are needed to ensure its safe use.

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SALIVARY DNA DAMAGE IN CAREGIVERS WITH STRESS AND DEPRESSION SYMPTOMS

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Stress has been associated with an increased risk of developing mental illnesses, including depression, and may also arise from physically, emotionally and psychologically exhausting work conditions. The caregivers are professional or relatives highly vulnerable to stress and mood disorders, particularly whose work with chronic neurodegenerative diseases. The aim of this study is to evaluate the salivary DNA damage in caregivers of elderly people with neurodegenerative diseases and its association with stress and depression symptoms. This study is part of the work "Evaluation of caffeine consumption and its association with stress and depression in caregivers of elderly people with Alzheimer's Disease", approved by the Research Ethics Committee prot. nº. 84597318.5.0000.5306. Volunteers are 38 women and 7 men (n=35; age 53,22 ± 16,62 years), residents in Santa Maria, RS, which are professional caregivers or relatives of people with neurodegenerative diseases. They answered psychological distress inventories following collection of saliva samples for DNA damage analysis. All analyses were performed by blind researchers. The data were analyzed by Kolmogorov-Smirnov test, correlation test between the variables, Mann- Whitney or Fischer's exact test (SPSS software; $p < 0.05$). The population was dichotomized by stress symptoms. Depressive symptoms were found in 65.71% (n=23) of the volunteers. The stress assessment indicated symptoms in 54.28% (n=19) of the population, and all of them displayed depressive symptoms. There was no correlation between the variables stress, micronuclei and apoptosis. When we analyzed only the stressed participants who presented depressive symptoms, there was no correlation with micronuclei and apoptosis. Data analysis indicated homogeneity between populations for micronuclei and apoptosis of cells of the buccal mucosa tissue. Many studies have demonstrated the effect of stress on DNA integrity, because it leads to oxidative changes, which may affect DNA replication and transcription. In our sample, stress and depression symptoms were not correlated with DNA damage in saliva.

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ZIKA VIRUS INFECTION OF HUMAN MESENCHYMAL STEM CELLS RESULTS IN SEVERE DISTURBANCE IN THE UBIQUITIN-PROTEASOME PATHWAY

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Introduction: The Zika virus (ZIKV) is a mosquito-borne flavivirus that causes neurodiseases, such as microcephaly and Guillain-Barré syndrome in infected individuals. The current molecular understanding of the deleterious effects and its extensions promoted by ZIKV infection remains unclear. Moreover, ZIKV has been implicated in other neurodegenerative and developmental outcomes. In order to get new insights related with mechanisms implicated in ZIKV infection and pathogenesis, we further analyzed a proteome dataset of human Mesenchymal Stem Cells (hMSC) that related ZIKV infection to brain diseases. Methods: hMSC differential proteome of ZIKV infection was analyzed applying a system biology approach. The list of identified proteins were submitted to Centiscape 2.2 application in Cytoscape software to calculate the degree related with the predicted regulatory relevance of each node of system. Results and discussion: Our results indicate that ZIKV induces a potential reprogramming of the metabolic machinery in nucleotide metabolism, changes in the energy production via glycolysis and other metabolic pathways, and potentially inhibits autophagy, neurogenesis, and immune response by downregulation of signaling pathways. In addition, proteins previously described in several brain pathologies, such as Alzheimer's disease, autism spectrum disorder, amyotrophic lateral sclerosis, and Parkinson's disease, were found with altered expression due to ZIKV infection in hMSC. In addition, we detected that proteins causing the greatest molecular perturbation in the system are directly related with the ubiquitin-proteasome pathway according to Network Degree Centrality analysis. Among these proteins, are UBA52, UBC and PPS27, recognized as important in neural formation, thus demonstrating that ubiquitin-related proteins may have an important role in ZIKV infection effects, including those related with clinical outcomes. Conclusion: Our system biology approach points out to a major disturbance of ubiquitin-proteasome pathway as effect of ZIKV infection in hMSC.

Financial Support: CNPq, CAPES

NEURONAL DIFFERENTIATION AND NEUROPROTECTIVE EFFECTS OF NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITORS DESIGNED FOR ALZHEIMER'S DISEASE THERAPY

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Alzheimer's disease (AD) is characterized by a progressive loss of episodic memory related to β-amyloid (Aβ) peptide aggregation and abnormal phosphorylation of the tau protein, leading to the loss of cholinergic function. Inhibitors of acetylcholinesterase enzyme (AChEI) are the main class of drugs used in AD therapy. Thus, the objective of this work was to evaluate the neurodifferentiation and neuroprotective effects of two hybrid molecules (TA8Amino and TAHB3, AChEI compounds) of tacrine-donepezil in SH-SY5Y cells. Firstly, cytotoxicity and hepatotoxicity assays were performed at different times (24, 48, 72, and 120h) in SH-SY5Y and HepG2 cells, respectively. Neuronal differentiation assays, protein expression, cell cycle, cell proliferation, mitochondrial changes and oxidative stress were performed on SH-SY5Y cells. Further, viability and cell death assays were performed to evaluate the neuroprotection ability of the hybrids. The compounds did not present cytotoxic effects or high hepatotoxicity and did not alter cell viability at the following concentrations: TA8Amino 0.0035 to 0.112µM and TAHB3 0.088 to 2.84µM, but both compounds were able to induce neuronal differentiation and neuritogenesis, compatible with the increase of β-III-Tubulin expression in cells treated with TA8Amino, which also induced the production of intracellular and mitochondrial ROS. While the hybrids increased SOD1 expression, they did not change the mitochondrial membrane potential and mitochondrial mass, indicating that drug-induced oxidative stress did not generate damage due to mitochondrial dysfunction in differentiated cells. Changes in PTEN $_{(Ser380\ /\ Thr382\ /\ 383)}$, AKT $_{(Ser473)}$ and COX2 protein expression indicate the involvement of the PI3K/ AKT/ COX2 pathway, which is related to neuronal differentiation. Furthermore, TA8amino and TAHB3 showed a neuroprotective effect against the neurotoxic damage induced by the Aβ peptide. In general, the results demonstrate that TA8amino e TAHB3 have advantages as potential drugs, since they are not cytotoxic at concentration levels that inhibit AChE enzyme, besides being able to induce neuronal differentiation, neuritogenesis and neuroprotection, unlike donepezil and tacrine, tested alone.

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ZIKA VÍRUS ACTIVITY ON NERVOUS SYSTEM CELLS DNA

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Key words: ZIKV, comet assay, genotoxicity, astrocyte.

The Zika Virus (ZIKV) belongs to the Flavivirus from Flaviviridae family. ZIKV is transmitted by the *Aedes aegypti* mosquito, which is phylogenetically related to other mosquitoes that transmit diseases such as japanese encephalitis, west nile fever, dengue fever, yellow fever and tick-borne encephalitis. The first reported human virus infection occurred in 1952 at Uganda and the Federative Republic of Tanzania. Between 2013 and 2014, there was an outbreak of people infected with the Zika virus in French Polynesia. Thus, ZIKV transmission has been associated with increased development of an autoimmune neurological disease known as Guillain-Barré Syndrome (GBS). At the beginning of 2015, the most reported cases of ZIKV infection were identified in Brazil northeastern. Few months later, the cases of infection spread to more than 14 Brazilian states, followed by an increasing in babies borned with microcephaly. The difficulty of developing methods to control the transmission of this pathogen is due to that even today, although the main transmission vector is through the *Aedes aegypti* mosquito, there are still other forms allowing the disease propagation. Thus, studies to enhance the understanding of the physiological and molecular mechanisms of Zika virus action becomes relevant. The present study aimed to evaluate the ZIKV genotoxicity with the comet assay in ASTC15, ASTCF2 and NSC cells compared to their respective controls. Besides, the ascorbic acid protective effect on the DNA of ASTC15 and ASTCF2 cells was also assayed. The results showed that the cells infected with ZIKV presented a significant difference ($p<0.05$ – ANOVA) in the DNA damage when compared to control group. So, we can conclude that the virus is genotoxic for all three cell lines evaluated. In addition, ascorbic acid showed a protector effect to ASTCF2 infected cells, but not concerning to ASTC15 lineage.

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MELATONIN SUPPLEMENTATION FOR DIFFERENT TIME PERIODS UNTIL AGING MODULATES GENOTOXIC PARAMETERS IN MICE

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Key-words: Melatonin; Aging; Genomic instability; DNA repair.

Aging is a multifactorial phenomenon, correlates with decreased physiological and cellular functions, the ability for stress response and increased incidence of various degenerative diseases, characteristic of this phase. Studies about melatonin (N-acetyl-5-methoxytryptamine), a potent natural antioxidant secreted by pineal gland of all mammals, including humans, is gaining attention. Melatonin is the main pineal hormone, synthesized from tryptophan predominantly at night. With aging, nocturnal melatonin production decreases in various animal species, including humans, thus questioning the role of melatonin in aging. Based on these findings, the aim of this study was to evaluate the effects of melatonin chronic consumption on genotoxic and mutagenic parameters of old Swiss mice. For this, 240 male Swiss mice with 3-month-old were divided into eight groups: groups that started melatonin consumption (2mg/L) at 3, 6, 12 and 18 months of life and continued consuming until they were 21 months old, groups G1, G3, G4, and G6 respectively; animals that started melatonin consumption at 3 or 12 months until natural death, groups G2 and G5 respectively; and animals that received only water with 0.04% ethanol (ethanol was used because the melatonin is dissolved in ethanol at the final concentration of 0.04%) until 21 months or natural death, G7 and G8 respectively. After 21 months, animals that were not destined to remain receiving supplementation until natural death were subjected to behavioral test. After the behavioral test animals were submitted to euthanasia for the dissection of the structures for later genotoxic and biochemical analyzes. Our results in the tail suspension behavioral test, melatonin presented antidepressant effect, reducing the immobility of the animals. In the survival curve, the melatonin prolonged the animals life span. Relative to genomic instability, melatonin, at tested dose, independently of initiation age, was effective in reducing DNA damage caused by aging, presenting antigenotoxic and antimutagenic activities. The group receiving melatonin for 18 months, had high levels of the enzymes responsible for the DNA repair system (APE1 and OGG1).In conclusion, our results showed that melatonin presents an efficient antioxidant mechanism aiding in modulating genetic and physiological alterations due to aging.

Financial support: CNPq, FAPESC, Capes, UNESC.

GENOMIC INSTABILITY AND DNA REPAIR

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FUNCTIONAL CHARACTERIZATION OF TOS4 AND UBP12 INTERACTION DURING REPLICATION STRESS

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Keywords: *Tos4 protein, ubiquitination, replication stress*

TOS4 (*Target of SBF 4*) is a *Saccharomyces cerevisiae* cell cycle-dependent gene. During cell cycle progression, *TOS4* transcription is activated at the end of G1 and inhibited while cells progress into S-phase. At the end of S phase, Tos4 is ubiquitinated and driven to degradation by the Ubiquitin- Proteasome Complex. Although *TOS4* function is still unclear, it was demonstrated that its transcription is sustained during S-phase in response to replication stress. Tos4 is characterized by the presence of a protein interacting Forkhead Associated (FHA) domain. Interestingly, we found that Tos4 mutant cells lacking functional FHA domain (FHA mut) showed increased sensitivity to hydroxyurea. To better understand the role of Tos4 during replication stress, we performed an immunoprecipitation assay followed by mass spectrometry analyses and found that Tos4 interacts with Ubp12 (Ubiquitin-specific Protease 12). Ubp12 interaction was confirmed by co-immunoprecipitation and demonstrated to be dependent on Tos4 FHA domain. By performing a cycloheximide chase assay in S-phase progressing cells, we showed that the FHA mut form of Tos4 has a short half-life compared to the wild type protein. To support this idea, we also demonstrated that in the presence of a proteasome inibihitor (MG-132), the FHA mut has a long half-life compared with the FHA mut that was not treated with MG-132. Based on previous evidences that Tos4 is ubiquitinated at four Lysine's residues (K22, K34, K37 and K197), we also performed a cycloheximide chase assay in cells containing point mutations at these four residues (K4A). Interestingly, this assay demonstrated that the double mutant FHA mut-K4A rescued the stability of Tos4 protein when compared to the single mutant FHA mut Tos4. Together, our results suggest that Ubp12 interaction may be important to counteract Tos4 degradation, contributing to sustain its activity during S phase in conditions of replication stress.

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EVALUATION OF CHEMOPREVENTIVE EFFECTS OF VITAMIN D IN AN ANIMAL MODEL OF COLORECTAL CANCER

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Key-words: Colorectal cancer. 1,2 - DimetylHydrazine. Vitamin D. Chemoprevention. DNA damage.

Colorectal carcinogenesis is a complex process which can last many years, having as classical substrate the sequence adenoma-carcinoma. However, aberrant crypt foci has substituted microadenomas as early biomarkers in the colorectal tumorigenesis. Aiming to breake the cascade, and particularly the occurrence of aberrant crypt foci, many chemopreventive actions have been tested intending to block the carcinogenesis progression. The antioxidant group has been highlighted by neutralizing the free radicals action. The aim of this study was to evaluate the chemopreventive effects of vitamin D in an animal model of colorectal Carcinogenesis induced by 1,2-dimetilhidrazine (DMH). It was utilized Balb-c adult mouse in an experiment with 12 weeks of duration. The animals were divided in four groups : EDTA group, DMH group, Vitamin D + EDTA and Vitamin D + DMH. The animals received Vitamin D (1500 UI a week) during 12 weeks, and DMH induction was made during the 4th and 5th week of treatment with a dose of 40 mg/kg twice a week. In the end of 12th week blood samples from the retroocular venous plexus was taken and after that the animals were sacrificed on 12 week of the experiment. Then the animals were submitted at laparotomy to remove the medium-distal colon and small fragment of the liver. The analysis were: Comet Assay and Aberrant Crypt Foci detection. The results have shown that the intervention demonstrate antigenotoxic properties in both blood and liver, reducing even the DNA oxidative damage. In the aberrant crypt foci evaluation, the intervention demonstrated anticarcinogenic effect in the colon of the animals exposed to DMH when comparing to positive control. In conclusion, the results have shown the chemopreventive effects of vitamin D in a experimental model of colorectal carcinogenesis, reinforcing the importance of primary prevention of CCR in humans.

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EVALUATION OF THE ETHANOL GENOTOXICITY IN S*accharomyces cerevisiae* DURING THE FERMENTATION PROCESS

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Key-words: comet assay, yeast, stress

Demand for energy from fossil sources has been a constant concern due to climate change and the release of toxic gases into the environment, nations have sought to replace these energies with renewable sources for the production of biofuels. In Brazil, ethanol production technology is consolidated due to the use of sugar cane juice and *yeasts* strains, making this country as the second largest producer of ethanol. *Saccharomyces cerevisiae* are the agents of bioconversion of the substrate in product through the fermentative route. Baker's yeasts are used at the start of fermentation because they have high growth capacity, however, they are very sensitive to stress factors, especially at high ethanol concentrations. This compound can cause changes in several cellular mechanisms generating a genotoxic effect. These effects cause transcriptional responses in the yeast genome, causing alterations in the biosynthesis of this metabolite. Studies to understand the mechanisms caused by ethanol in yeast are important to monitor the concentrations in the fermentation medium of this important biotechnological product. The study aims to evaluate the action of genotoxicity in Fleischmann ™ yeast at different concentrations of ethanol. The pre inoculum was carried out in the YPD 2% liquid medium, in which 0.10 g of lyophilized Fleischmann yeast were inoculated and incubated at 30 ºC at 250 rpm. The cells were centrifuged, washed three times, and the biomass obtained was used in 22°Brix must in Erlenmeyer flasks and ethanol concentrations (5, 10 and 15%) were incubated at 30 ºC, 250 rpm 10 hours. In order to evaluate deoxyribonucleic acid-DNA damage, the comet test was used by cell lysis in the presence of the Liticase enzyme (Sigma-Aldrich) at the concentration of 2 mg mL^{-1} and the slides were then immersed in lysis solution (30 mM NaOH, 1M NaCl, 0.1% N-lauroyl sarcosine, 100 mM DMSO, 1% Triton-X100). The slides were electrophoresed in buffer (30 mM NaOH and 2 mM EDTA, pH 13), neutralized in buffer (400 mM Tris-HCl, pH 7.5) and stained with silver nitrate. Changes in DNA were analyzed by optical microscopy and comets were classified according to damage levels from 0 to 4. Results showed that there was damage induction at all ethanol concentrations analyzed, however at 5 and 10% a greater number of changes were observed at levels 2 and 3 and at 15% at levels 3 and 4. The comet test proved to be an effective tool to evaluate the genotoxic action of ethanol in yeast.

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CITOTOXIC ACTION OF ETHANOL IN *Saccharomyces cerevisiae*

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Ethanol, the main biofuel produced in the world, is obtained substantially from the fermentation of the sugarcane juice, which is carried out by *Saccharomyces cerevisiae* yeasts, at the beginning of the fermentation the baking yeasts are used and these are more susceptible to the action of the fermentative stress. Although these microorganisms are adapted to the operating conditions of the industry, the oscillations that exist in this process can lead to cellular stress. Thus, factors such as high concentrations of ethanol can cause physiological changes and interfere with cell growth and viability. Understanding the metabolic adaptations of *Saccharomyces cerevisiae* to stress factors and improving the production process can contribute to improvements in productivity and make the sector even more related to sustainability issues, since these are directly related to acquisition of the products generated by the sugar-energy sector. In this sense, the study aims to evaluate the cytotoxic effects of ethanol on Fleischmann ™ yeasts. To obtain the biomass 0.10 g of lyophilized yeasts were inoculated in the liquid medium YPD 2% and incubated at 28 ºC, 160 rpm until the exponential phase of growth. Subsequently the cells were recovered by centrifugation and 100 µL of the samples were added in 2% YPD with absolute ethyl alcohol PA 99.5% (anhydrous), in the proportions 5%, 8%, 10% and 15% (v / v) incubated for 30, 60 and 90 minutes. After the action of the stressor agent aliquots of 0.5 µL of the cell suspension were applied to Petri dishes containing the solid YPD 2% medium and incubated in an oven at 30 °C for 72 hours. An observation was made regarding the growth capacity in relation to the analyzed ethanol concentrations. All times presented growth in relation to the analyzed concentrations. However, at 60 and 90 min there was inhibition of cell growth at concentrations of 10 and 15% showing that under these conditions the yeast underwent cytotoxic action of ethanol. Thus, it was observed that in the longest times and in the highest concentrations there was greater susceptibility of Fleischmann ™ yeast to ethanolic stress.

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THE INFLUENCE OF POLYMORPHISMS IN DNA DAMAGE, TELOMERE LENGTH AND GLOBAL DNA METHYLATION EVALUATED IN OPEN-CAST COAL MINING WORKERS

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Coal plants represent one of the main sources of environmental pollution due to the combustion process of this mineral and the consequent release of gases and particles capable of penetrating water sources which, in significant quantities, can lead to a potential risk to health and the environment. The susceptibility of individuals to the genotoxic effects of coal mining can be modulated by genetic variations in the xenobiotic detoxification and DNA repair processes. The aim of this study was to evaluate if xenobiotic metabolism polymorphism (GSTP1 *Ile105Val*, GSTT1, GSTM1 and CYP1A1 *Ile462val*), base excision repair polymorphisms (hOGG1 *Ser326Cys* and XRCC1 *Arg194Trp*), and non-homologous end joining repair pathway polymorphism (XRCC4 *Ile401Val*), could modify individual susceptibility to genomic instability and epigenetic alterations induced in workers by occupational exposure to coal. In this study, polymerase chain reaction (PCR – RFLP) was used to examine the polymorphic sites. The reactions, as well as the enzymes used, followed the literature. The sample population comprising 70 coal mine workers and 71 workers non-exposed to coal. The exposed workers were sampled in Candiota (Rio Grande do Sul, Brazil) open-cast coal mine, where they were involved in extraction and transport of coal to storage centers. The non-exposed group consisted of individuals from the same area. Our results demonstrated the effect of individual genotypes on different biomarkers (DI, MN, NBUD and NPB in lymphocytes, buccal MN, telomere length, and % of global DNA methylation) evaluated in the non-exposed and exposed groups.

Significant decrease in % of global DNA methylation were observed in CYP1A1 *Val/-* exposed individuals compared to CYP1A1 *Ile/Ile* individuals (P= 0.0369). Coal workers who carried the XRCC4 *Ile/Ile* genotype showed decrease NBUD frequencies (P= 0.0085), while the XRCC4 *Thr/-* genotype was associated with decrease in Buccal MN cells for the group not exposed (P= 0.0029). No influence of the other polymorphisms studied was observed. Thus, the current study reinforces the importance of considering the effect of metabolizing and repair variant genotypes on the individual susceptibility to incorporate DNA damage, as these processes act in a coordinated manner to determine the final response to coal exposure. Results of this study contribute to the understanding of DNA damage mechanisms induced by complex exposure of open-cast coal miners.

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GENOTOXIC, HISTOPATHOLOGICAL, OXIDATIVE STRESS, AND INFLAMMATORY EFFECTS IN WISTAR RATS EXPOSED TO INHALATION OF SUB-BITUMINOUS COAL FROM A COLOMBIAN MINE

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Inhalation of coal dust is the main cause of pneumoconiosis and chronic obstructive pulmonary disease in workers of coal mines in the world. Several studies suggested the health effects of particulate matter with residues of coal are not restricted to occupationally exposed populations by the levels of circulating particles in the environment. The main goal of this work was to evaluate genotoxic, histopathological, oxidative stress, and inflammatory effects in adults male of Wistar rats with subacute inhalation of sub-bituminous coal from Colombian mine. The animals were divided into 4 experimental groups, including one control group (exposed to inhalation of filtered air). The animals were exposed one hour per day for 14 days. After euthanasia the samples collected were used to alkaline comet assay in blood and a lung, histological examination in lung, liver, kidney, heart, and brain, analysis of enzymatic antioxidant defenses and redox status in biomolecules in liver and lung and finally were measured the concentrations of interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) in plasma samples. The results showed that subacute inhalation of sub-bituminous coal for 14 days increased the levels of DNA damage index in blood, produced histopathological lesions compatible with anthracosis, and accumulation of coal residues in lung tissues. The biochemical analysis showed an increase in antioxidant defenses expressed by elevation of levels of glutathione S-transferase (GST) in lung and superoxide dismutase (SOD) in liver.

In markers of oxidative damage (levels of thiobarbituric acid reactive species (TBARS) and total reduced thiol content (SH) were found increased levels in the lungs. Also, we observed the expression of tumor necrosis factor alpha (TNF-α).

Our data showed the relation between inhalation of sub-bituminous coal dust and generation of genotoxic, histopathological changes, biochemical alterations, and expression of inflammation factors in lungs. These reactions may also be associated with the generation and evolution of diseases in lungs caused by the inhalation of particulate matter from coal mining. This work provides a state of knowledge about the effects of inhalation of sub-bituminous coal from Colombia in terms of DNA damage, histopathological alterations, oxidative stress, and inflammatory response, and invites further studies to search in depth evolution of the initial damage produced by the inhalation of coal.

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EVALUATION OF PURINE OXIDATION AND DNA DAMAGE IN V79 CELLS GENERATED BY THE BITUMINOUS AND SUB-BITUMINOUS COAL FROM COLOMBIA

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Occupational and environmental exposure to open-cast coal mining residues such as Polycyclic Aromatic Hydrocarbons (PAHs) and inorganic elements (IE), has been demonstrated causing a wide range of chronic diseases through the generation of DNA damage and genomic instability. The main goal of this study was to evaluate the cytotoxic and genotoxic effects induced in V79 cells by the exposure to bituminous and sub-bituminous coal samples from two open-pit coal mines located in the states of La Guajira (bituminous coal from El Cerrejon mine coal sample (ECCS)) and Córdoba (sub-bituminous coal from La Guacamaya mine coal sample (LGCS)), in Colombia. The clonogenic assay was used to calculate IC50 values. The alkaline comet assay normal and modified in the high-throughput version was used to detect DNA damage and oxidized purines using enzyme Formamidopyrimidine DNA glycosylase (FPG). Finally, to estimate the potential genotoxic effect, the cytokinesis-block micronucleus cytome assay (CBMN cyt) was used. All experiments were conducted after 3h and 24h of exposition. The clonogenic assay showed significantly increased inhibition of the IC50 values induced by bituminous coal when compared to sub-bituminous coal. The comet assay showed that exposure to both ECCS and LGCS, induced primary DNA lesions after 3h and 24h of exposition in a dose-dependant manner with a significant increase in the percentage of DNA in the tail in cells exposed to ECCS for 3h. When the lesion-specific endonuclease FPG was applied, the percentage increased in cells exposed to LGCS. The CBMN cyt assay showed that exposure to bituminous coal induces the formation of micronuclei (MN) upon 3h of exposure, while sub-bituminous coal only induces MN formation upon 24h. In summary, our results showed that the bituminous coal in ECCS could induce an increase in MN frequency and oxidative damage as early as 3h of exposition at a low dose, whereas the sub-bituminous coal in LGCS only increased MN frequency with the highest dose after 24h.

As the coal samples used in this study are composed by chemical elements in different proportions (bituminous and sub-bituminous coal), they could be capable of producing damage that can or can not be repaired according to the dose and time of exposure.

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EVALUATION OF DNA DAMAGE, DNA-INDUCED DAMAGE AND DNA REPAIR CAPACITY IN VETERINARIAN PROFESSIONALS EXPOSED TO VOLATILE ANESTHETIC

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Key-words: occupational exposure, inhalational anesthetic, comet assay, DNA repair

Inhalational anesthetics have been used for many decades in human and veterinary practice. Cytogenetic studies have shown that professionals working in human operating rooms exposed to chronic inhalational anesthetics may have increase in DNA damage. However, no study has evaluated yet DNA damage, DNA-induced damage and DNA repair capacity in veterinarian professionals occupationally exposed to anesthetic. This study was approved by the ethics committee, and eighteen subjects from both sexes were included in the study and were allocated into two groups: professionals who worked at a Brazilian University Veterinary Hospital exposed to the volatile anesthetic isoflurane and subjects with no history of exposure to inhalational anesthetics (control group). Blood samples were collected and immediately processed under yellow light. Peripheral blood lymphocytes were isolated and basal DNA damage, DNA-induced damage with hydrogen peroxide (100 µM) and DNA repair capacity were assessed using the comet assay by the Comet Assay IV software analysis (tail intensity). For both groups, a significant increase of DNA damage was observed when cells were treated with hydrogen peroxide in relation to basal DNA damage ($p < 0.05$), and a significant decrease occurred when cells were washed and evaluated for repair capacity when compared to hydrogen peroxide treatment ($p < 0.05$) but with no statistical significant difference when compared to basal DNA damage. There were no significant differences for all the evaluations between both groups. Considering the findings, the study suggests that occupational exposure to isoflurane may not induce DNA damage and do not impair the repair capacity in veterinarian professionals. However, the study needs to continue to achieve a greater sample size.

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PRESENCE OF MRN COMPLEX COMPONENTS IN HUMAN MITOCHONDRIA AND THEIR RELATIONSHIP WITH mtDNA REPAIR

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In the nucleus, DNA double strand breaks (DSBs) are predominantly repaired by the Non-Homologous End Joining and the Homologous Recombination repair pathways. In mammals, the MRN complex, formed by the Mre11, Rad50 and Nbs1 subunits, signals for subsequent DNA damage response while binds to and stabilizes DSBs for further processing. While Mre11 has been previously localized in mitochondria, mitochondrial localization of Rad50 and Nbs1 subunits has not been demonstrated and the mechanistic understanding of DSB Repair in mitochondria remains poor. Here we demonstrate, by qPCR analysis, that Hek293T cells can repair mtDNA after treatment with bleomicin, a radiomimetic which induces mostly DSBs.Analysis *in silico* using the IPSORT and Mitofates software indicate that Mre11, Nbs1 and Rad50 do not show canonical mitochondrial localization presequences. However, putative Mitochondrial Processing Peptidases (MPP) cleavage sites and Tom20 - recognizing motifs, a sequence that is recognized by Tom20 subunit of the Translocase of Outer Membrane complex (TOM), were identified in all 3 proteins. *Western Blot* analysis of mitochondria from untreated cells indicated that Nbs1 is found associated with mitochondria, but not in the mitochondrial matrix, raising the possibility that MRN complex subunits stay at intermembrane space and are translocated to the matrix upon DNA damage. Furthermore, a possible mitochondria-specific Nbs1 isoform was identified by *Western Blot* analysis in HeLa mitochondrial extracts, but the nature of this isoform still needs to be confirmed. In conclusion, (i) DSB are efficiently repaired in human mitochondria, as indicated by qPCR analysis; (ii) peptide sequences of MRN subunits indicate possible ways of importing these proteins into mitochondria and (iii) the mitochondrial localization of MRN subunits, in absence or presence of mtDNA damage, needs to be confirmed by *western blot* analysis of highly purified mitochondrial extracts from Hek293T and HeLa cells.

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GENETIC INSTABILITY INDUCED BY OMEPRAZOLE IN PATIENTS UNDER TREATMENT AGAINST GASTRITIS - IN BLOOD SAMPLES

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Key-words: Proton pump inhibitors; Blood samples; Genotoxicity; Oxidative stress; DNA breaks;

Several factors cause damage to gastric mucosa and generate acute inflammation and/or chronic diseases. We performed a clinical biomonitoring of genotoxic, oxidant and apoptotic damages in patients with gastritis under Omeprazole (OME) gastric therapy in attempt to identify possible mechanisms of oxidative damage and correlations among cytogenetic biomarkers, oxidative stress, enzyme defenses and lifestyle. A total of 132 patients volunteers diagnosed for gastritis attended in a Brazilian public hospital was divided into groups. Before endoscopy, questionnaires were applied. Next, from peripheral blood samples we isolated to carry out Cometa assay and and prepared a suspension of red blood cells to analyze free radicals' profile by catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation and nitrite content. Patients receiving OME presented significant genotoxic alterations in PBMC, increase of damage index (ID) and frequency damage (FD) besides apoptosis. Blood samples from patients under OME treatment showed increased levels of CAT, SOD, GSH, lipid peroxidation and nitrite ($p < 0.05$). Pearson's correlation revealed positive association between DI and CAT activity, smoking and nitrite increasing, increase of ID and chemical exposure. So, OME induced chromosomal changes in leukocytes from patients with gastritis probably in a dependent way of oxidative stress. Moreover, DNA damages and apoptosis positively correlated with the increase of CAT, SOD and others, emphasizing the multifactorial risks with omeprazole or other PPIs. Clinical biomonitoring of PPI therapies is important due to high association of developing cancer and indiscriminate use of this class of drugs.

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GENETIC INSTABILITY INDUCED BY OMEPRAZOLE IN PATIENTS UNDER TREATMENT AGAINST GASTRITIS – IN BUCCAL AND GASTRIC MUCOSAL CELLS

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Key-words: Proton pump inhibitors; Mucosa samples; Genotoxicity; Oxidative stress; Apoptosis;

Omeprazole is a gastric therapy drug and its prolonged use can lead to genetic instability. We performed a clinical biomonitoring of genotoxic, apoptotic and oxidative damages in patients with gastrites under Omeprazole (OME) gastric therapy to evaluate toxicogenic effects of OME in oral and gastric mucosa cells (body and antrum), as well as their correlations with risk factors for genetic instability. 137 patients volunteers submitted to upper endoscopy in Getulio Vargas Hospital, Teresina - PI, Brazil, from May to October 2017, were separated into groups: without gastritis (26 patients); gastritis (23 patients); gastritis + *Helicobacter pylori* (*H. pylori*) (16 patients); without gastritis + OME (22 patients); gastritis + OME (25 patients); gastritis + *H. pylori* + OME (25 patients). Patients related used continuous doses of 20 and 40 mg / day of Omeprazole. Health questionnaires were applied. Genotoxic and apoptotic evaluation was done with the Comet Assay of buccal and gastric mucosa samples. Evaluation of the antioxidant profile was performed in blood samples. The majority of participants were female, with a mean age of 47 years and a mean weight of 68 kg. OME induced genotoxicity and a significant increase in the number of apoptosis in cells of antrum, body and buccal mucosa compared to group without gastritis, without differences in relation to *H. pylori* coinfection. These damages can be attributed to oxidative stress, as observed by the increase of superoxide dismutase and catalase and by the statistical correlations of these enzymes with increased DNA damage, as well as the use of drugs, chemicals and alcohol. The results indicate that OME induces genetic instability, and that clinical biomonitoring is a strategy for therapeutic safety in relation to the risks of stomach cancer.

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EVALUATION OF CYTOGENETIC INSTABILITY IN CHILDREN WITH MICROCEPHALY IN RIO GRANDE DO NORTE, BRAZIL

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Microcephaly is a disorder characterized by the reduction of the cephalic or occipitofrontal circumferences. Due to its complex etiology, it requires an adequate maintenance of genomic stability through the activation of cell cycle checkpoints and the DNA damage repair system in order to allow the neurogenesis processes to occur efficiently. Damage to these mechanisms could impair cell viability and promote a reduction on cortical area. Considering the facts presented, the study of the frequency of genome instability biomarkers becomes relevant in order to contribute to the explanation of risk factors associated with the development of this malformation and of chronic degenerative diseases. The present study was performed using the buccal micronucleus cytome assay (BMCyt) in 52 patients with microcephaly and 35 control children. It was observed that compared with the control group, microcephaly children had a higher frequency of basal, binucleated, pyknotic, condensed chromatin and karyorrhectic cells ($p < 0.05$). However, no significant differences were detected on karyolytic cells, micronucleus, nuclear buds and broken eggs ($p > 0.05$). Furthermore, there was a lower frequency of differentiated cells in patients with microcephaly ($p < 0.05$). In addition, there were no association of these biomarkers frequency with sex, age and severity of this birth defect ($p > 0.05$). However, only in the control group it was detected a directly proportional correlation of differentiated cells with age, and a inversely proportional correlation of age with the frequency of karyorrhectic, condensed chromatin, pyknotic, cariolytic cells and nuclear buds ($p < 0.05$). Another fact observed was a correlation of piknotic cells frequency and the mother's abortion history (Rhô Spearman = 0.285, $p < 0.05$). The results suggest that children with microcephaly have an intrinsic high frequency of biomarkers related to impaired cellular proliferative activity, cytokinesis failures and cell death. This fact corroborates with literature data indicating that processes associated with chromosomal segregation failures, cell cycle delays and apoptosis can reduce the number of neuronal cells during the rapid proliferation in neurogenesis. Thus, the data obtained may contribute with new epidemiological and cytogenetic evidence for research on the subject and could be applied in the preventive health care of children with microcephaly.

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GENOMIC MODULATION OF TREATMENT WITH ANTISCORPIONIC SERUM IN ANIMALS INOCULATED WITH SCORPION VENOM *Tityus serrulatus* Lutz & Mello, 1922

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The scorpions are arachnids found in the terrestrial environment over 400 million years ago, allowing them to adapt to different habitats. The species *Tityus serrulatus* has been in contrast for serious accidents caused in Brazil and its huge symptomatology. The only treatment recommended by the WHO is the administration of anti-scorpionic serum. In 2017, we showed for first time, the venom of the *T. serrulatus* scorpion beside the protein damages, causes damages in the DNA molecule too, where these can progress to numerous diseases and complications in cellular system. Based in the effects caused by the venom of this species, the aim of this study was to evaluated the performance of the anti-scorpionic serum used in Brazil in the genomic instability of the venom in different organs through the comet assay. For this study, 120 adult Swiss mice (18-22g with 28-35 days) were used, divided in 20 groups, with six animals. Euthanasia where made in different times after inoculation, disposed in times of 1h, 2h, 6h, 12h and 24h for each group, with a 0h control, to which was administered phosphate-saline buffer, another group where it was inoculated only with venom, another with anti-scorpionic serum, and a last group evaluating the action of venom in conjunction with the treatment of serum. In the different groups, was inoculated subcutaneously ½ DL50 of venom of *T. serrulatus* and right after 10uL of anti-scorpionic serum by the intravenous route. At the end of each time, blood sample was collected by retro-orbital vein and the animals were euthanized, for dissection of the brain, liver, lung and kidney structures for performing the comet assay. The results show the venom of *T. serrulatus* has genotoxic activity at the different evaluated times, and it can be neutralized by action of the anti-scorpionic serum tested. This reduction compared to the venom is significant in most of the structures, after 6h and 12h of poisoning, serum allowed repairs of those damages and preventing future complications due to poisoning and breaks in DNA molecule. It is concluded front this study, regardless of the symptomatology caused by the venom, is possible by means of the treatment with anti-scorpionic serum a neutralization at a molecular level in the several evaluated structures.

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COMPUTATIONAL ANALYSIS OF AMPHIBIAN PHOTOLYASES

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About 40% of existing amphibian species are threatened with extinction, and several hypotheses have been proposed to explain this decline, including the genotoxic effects of increased incidence of solar UV radiation. In order to minimize the harmful effects of UV-induced pyrimidine dimers on their genome, amphibians have mechanisms capable of repairing them, such as photorepair, which involves the action of photolyase enzymes. This work evaluated evolutionary and structural aspects of the primary structures of the family of photolyases [(photolyases (PHR) and cryptochromes (CRY)] of amphibians. First, primary protein sequences of the photolyases family were obtained from the NCBI protein section. After, the BLASTP tool was used to obtain similar protein sequences from the selected protein sequences mentioned above. The RefSeq Protein (RSP) database was used as a "search set" and three species with available RSP sequences were selected in the section "organism section". The TBLASTN tool was used and the database "Transcriptome Shotgun Assembly (TSA)" was marked as a "search set" to search for a nucleotide database in other 25 species of amphibians from our protein sequences. The score of 200 and the *E*-value of 10^{-6} were determined as lower limits for sequence selection in BLAST results. The occurrence of specific enzymes, conservation of residues, and similarity between sequences were evaluated in the obtained sequences. Similar sequences of CRY-DASH, CPD PHR, CRY1, CRY2, 6-4 PHR and CRY4 were found in the amphibian species in the respective percentages: 89, 82, 73, 61, 61 and 25%. In addition, 38% of the analyzed species presented four types of proteins at the same time, although other species presented one (3%), two (14%), three (17%), five (14%) or six (14%) types. In general, sequences of CPD PHR and similar formed a sister group from all the rest (6-4 PHR and CRYs) presenting the biggest difference in the composition of the residues. Additionally, the results indicate that some sequences are poorly annotated. The computational characterization of the evolutionary and structural aspects of the PHR/CRY family in amphibian species presented in this work may shed light on new hypotheses, since several aspects of the PHR/CRY family along the tree of life are controversial and unknown yet.

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DNA REPAIR MECHANISMS THAT COULD BE INVOLVED ON Trypanosoma cruzi MITOCHONDRIA

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Trypanosoma cruzi is a member of the Kinetoplastida and as so, present a single and unique mitochondria, called kinetoplast, which possesses several distinct features in comparison with other eukaryotes mitochondrias. Although several proteins of the DNA repair systems has already being described as present on the parasite mitochondria, there's no much information about the DNA repair pathways involved on the kinetoplast DNA (kDNA) maintenance. In this work we investigate possible DNA repair pathways that could be involved on T. cruzi kDNA metabolism. To first investigate the repair proteins related to the kDNA metabolism we analysed the effect of overexpressing the protein TcMYH, which is involved in base excision DNA repair in *T. cruzi*. As shown by qPCR, the overexpressor strain of TcMYH has more damages on the kDNA , when compared to the wild type strain. Also, when we used a reagent designed to specifically cause damage on the mitochondrial DNA, we could observe that this mutant is more sensitive than the WT control strain, and that the overexpression of the protein generates more AP sites on the parasite organelle. These results suggest that the parasite is able to deal with oxidative damage that attacks the kDNA. One of the unique features of the kinetoplast is the presence of specific proteins called kinetoplast associated proteins (KAP). Although some works described the location of those proteins on the antipodal sites, regions of DNA metabolism on the kinetoplast, their function are not yet clear. In this work we study the the function of the proteinTcKAP7. TcKap7 had its structure predicted using the online software I-Tasser and posseses structural similarities with the Mitochondrial transcription factor A (TFAM). Our results demonstrate that the absence of TcKAP7 is related to a long-term sensitivity to UV radiation, cisplatin and berenil; and a resistence to high levels oxidative stress on the parasite. The same phenotype has been observed for *Angomonas deanei* mutants, another trypanossomatid. Our results demonstrate that the TcKap7 and TcMYH are involved on the DNA damage response for *T. cruzi* and *A. deanei*. This results suggests that those parasites possess proteins related to the maintenance of mitochondrial DNA on T. cruzi.

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XERODERMA PIGMENTOSUM COMPLEMENTATION GROUP C (XPC) FROM *TRYPANOSOMA EVANSI* INTERFERE IN THE FUNCTION OF ITS ORTHOLOGOUS IN *TRYPANOSOMA CRUZI* CELLS

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Keywords: Nucleotide Excision Repair (NER), Xeroderma Pigmentosum complementation group C (XPC), Trypanosomatids.

Nucleotide Excision Repair (NER) is a versatile DNA repair system that acts repairing distortions in DNA double helix. Xeroderma pigmentosum complementation group C (XPC) is involved in recognition of DNA damage in global genome NER and it has been studied in different organisms due of its importance in several cellular processes beyond DNA repair. In *Trypanosoma brucei* the XPC protein plays a role in cell survival and is essential better know this protein in different members of Trypanosomatidae family for understand the biology of these organisms. Thus, the aim of this study was to investigate XPC protein in trypanosomatids using *Trypanosoma evansi* and *Trypanosoma cruzi* as experimental models. Nucleotide sequence of *T. evansi XPC* gene (*TevXPC*) found in TriTrypDB (http://tritrypdb.org) was amplified by PCR and inserted in a cloning vector (pGEM). Later, the gene was inserted in an expression vector (pROCK). Recombinant plasmid pROCK-*TevXPC* was introduced in *T. cruzi* wild type CL Brener strain and its growth was evaluated in comparison to *T. cruzi* cells overexpressing its own *XPC* gene (here named TcXPC cells). Under cisplatin treatment, TcXPC cells were able to recover cell division rates faster than TevXPC cells (*T. cruzi* wild type transfected with *TevXPC* gene). In normal conditions, TevXPC cells showed slower growth in relation to TcXPC and WT (wild type) cells. Therefore, is suspected that exogenous gene may have deleterious effects on TevXPC cells due TevXPC protein interference in TcXPC protein activities or interactions.

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THERAPEUTIC STRATEGIES TARGETING HOMOLOGOUS RECOMBINATION REPAIR BY MIRIN: EFFECT OF MRE11 INHIBITOR ON BREAST CANCER CELLS SURVIVAL

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Cells are able to repair DNA strand breaks to ensure genomic stability. Double strand breaks (DSBs) are the most toxic lesions for DNA, which can be repaired by two main pathways: homologous recombination repair (HR) and non-homologous end joining repair (NHEJ). An interesting tumor treatment strategy has been the pharmacological inhibition of one of these pathways. There are many inhibitors that target HR proteins, but the studies are still limited. One of these is Mirin, which inhibits the exonuclease activity of Mre11 protein, part of the MRN complex (Mre11, Rad50, Nbs1), which is responsible for DSBs signaling. Therefore, inhibiting Mre11 may potentially sensitize cancer cells to DSB lesions such as those induced by ionizing radiation, PARP1 inhibitors and replicative stress. This study aims to test three different therapeutic strategies by treating breast cancer cells proficient in homologous recombination repair with Mirin. Cells were treated with Mirin either alone or in combination with a radiomimetic agent (Zeocin) or a PARP1 inhibitor (Olaparib). Preliminary results indicate that MCF7 and SKBR3 breast cancer cells treated with Mirin (10 to 100 µM) for 24 and 72 hours have a significant reduction of viability from 50 to 100 µM evaluated by neutral red cell incorporation. Cell viability was further reduced after 72 hours of treatment. Cells were also treated with Olaparib (0.1 to 20 µM) for 24 and 72 hours, however no significant sensitivity was detected. A co-treatment with Mirin (25 and 50 µM) and Olaparib (2 and 5 µM) was tested at 24 and 72 hours and no significant additive effect on viability was found. Double strand break kinetics was evaluated after 6, 18, 24 and 48 hour treatment by detection of ϒ-H2AX (pS139) and an increase of signaling was found from 18 to 48 h for Mirin, Olaparib, and the combined treatment. Our results indicate that Mirin induces elevated cytotoxicity in MCF7 and SKBR3 cells, but its action mechanism remains to be investigated. Next, genotoxicity, cell death type and protein expression of breast cancer and normal cells will be investigated after treatment with Mirin to determine drug selectivity and cell sensitivity.

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SIMILARITY ASSESSMENT OF THE EUKARYOTIC NUCLEOTIDE EXCISION REPAIR

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Keywords: evolution, DNA repair, NER pathway

Nucleotide excision repair (NER) is the most versatile DNA repair pathway as it removes different kinds of bulky lesions. Due to its important role for the genome integrity, it appeared earlier in evolution. Nowadays, there is a large amount of information available on genome databases, allowing to retrieve sequences of NER components from many organisms. Starting from these sequences, it is possible to analyze the evolutionary history of NER genes. Therefore, we carried out a similarity assessment for the sequences of ten main proteins and their genes in 13 eukaryotic organisms (*Mus musculus, Gallus gallus, Alligator mississippiensis, Xenopus tropicalis, X. laevis, Danio rerio, Caenorhabditis elegans, Drosophila melogaster, Arabidopsis thaliana, Trypanosoma cruzi, Saccharomyces cerevisiae, and Schizosaccharomyces pombe*). For this, we used *tblastn* to obtain nucleotide and respective protein sequences from each species, using human reference sequences as queries. In order to get the best homologue candidates, we used four criteria as sequence filters: e-value, percent identity, protein domains in common, and protein sequence length. In order to identify the protein domains, we submitted the sequences to *Simple Modular Architecture Research Tool (SMART).* To investigate the evolutionary history of NER pathway, we conducted an ancestral state reconstruction for the protein occurrence on *Mesquite 3.5*. The results were plotted in a phylogenetic tree reconstructed on *Molecular Evolutionary Genetic Analysis 7 (MEGA 7)*, from the concatenated alignment of the proteins found in all the species evaluated. An additional analysis was made in order to evaluate the number of introns of the genes by using *Gene Structure Display Server 2.0 (GSDS 2.0)*. In general, our results show that most of the components analyzed are present in the organisms, but according to our criteria, some of them were not found in certain taxa. We also observed that the protein structure have slight variations in spite of a wide range of alterations in the gene structure, such as an increase in the intron number according to organism complexity. Therefore, this work suggests that further researches are needed to better investigate the real biological effectiveness of NER pathway in some eukaryotic species.

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TOPOISOMERASE II INHIBITORS-INDUCED LESIONS – AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS

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Key-words: *Topoisomerase II inhibitors, NER, double-strand breaks*

Topoisomerase II inhibitors form stabilized drug-DNA-topoisomerase complexes. Processing of these complexes creates DNA double-strand breaks (DSBs). We have already shown that the Nucleotide Excision Repair (NER) pathway is involved in this process. Therefore, the aim of this study was to understand the role of CSB, a NER protein, in response to TopoII inhibitors, and the relation with DSB repair pathways. U2OS cells were transfected with siRNAs for different NER genes, treated with TopoII inhibitors and followed by 53BP1 and $_{\gamma}$H2AX foci IF analysis; the influence of NER knockdowns in the Homologous Recombination (HR) rates was done using a CRISPR based reporter assay. To evaluate the interaction of CSB and TopoII a co-IP was performed in cells treated with the same drugs. A DRIP-qPCR was performed to investigate R-Loops formation in siERCC6 and siTOP2α/β cells with and without the treatments. IF analysis showed an increase in 53BP1 and $_{\gamma}$H2AX foci after MXT treatment, but there was no difference between NER knockdowns (siERCC6, siXPC and siXPA) and the siCTRL. Interestingly, we observed an increase in HR in siXPC cells. We detected a co-IP of TopoII and CSB in response to MXT treatment. In DRIP-qPCR analysis, we found an R-Loops increase in some locus after siTOP2α/β, but not after TopoII inhibition. Our results indicate an interaction between CSB and TopoII, which could explain the implication of CSB in DSBs repair pathway in response to TopoII inhibitors induced lesions.

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INCREASED OXIDATIVE DNA DAMAGE IN INDIVIDUALS AT CARDIOVASCULAR RISK WITH LOW SERUM LEVELS OF VITAMIN A

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Keywords: oxidative damage in DNA, vitamins and minerals antioxidants and cardiovascular risk.

Cardiovascular diseases and overweight have been related to oxidative DNA damage, which is generated by increased oxidative stress. The consumption of food rich in vitamins and minerals can inhibit this type of damage, by potentiating the antioxidant defense of the organism. The objective of this study was to evaluate the levels of oxidative DNA damage in individuals with cardiovascular risk (CR) according to serum vitamins and minerals levels or overweight. Forty-six volunteers with increased waist-hip ratio (WHR) >0,9 for men and >0,85 for women, were evaluated in this cross-sectional study. Blood samples were collected for analysis of serum vitamin and mineral (Vitamin A, C, D and Zinc-Zn) and for the evaluation of oxidative DNA damage. The oxidative damage in DNA was determined by the alkaline comet assay modified with the enzyme formamidopyrimidine glycosylase. To verify the effect of the weight on the damage DNA the individuals were divided in 3 groups: eutrophic, overweight and obese. To assess the effect of vitamin A on DNA damage, participants were divided into 2 groups: vitamin A <0.5 and vitamin A >0.5. The volunteers presented mean age of 40.6±9.2 years (20-59 years), being 52.2% male and 48.7% female. The prevalence of overweight was 82.6%. Serum levels of vitamin A, C, D and Zn did not differ between the groups: eutrophic (vitamin A=0.60±0.1mg/L, C=0.80±0.3mg/L, D=27.1ng/mL), overweight (vitamin A=0.68±0.1mg/L, C=0.73±0.3mg/L, D=28.3±5.5 ng/mL, Zn=81.7±12.7mg/L) and obese (vitamin A=0.65±0.1mg/L, C=0.78±0.3mg/L; D=28.7±6.9ng/mL, Zn=85.7±16.8mg/L), p>0.05. The oxidative DNA damage did not differ between these groups, p>0.05. However, the oxidative DNA damage was higher in the vitamin A group <0.5 mg/L (23.4±5.4) when compared to the group >0.5 mg/L (13.6±1.9; p<0.05). Vitamin A appears to have a protective effect against oxidative DNA damage.

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INVESTIGATING THE BIOLOGICAL ROLE OF OPI1, A REPRESSOR OF PHOSPHOLIPID BIOSYNTHESIS, DURING THE DNA DAMAGE RESPONSE IN *Saccharomyces cerevisiae*

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The DNA damage response (DDR) is a complex network of biological processes that is activated in response to the accumulation of genomic damage, thus preventing genomic instability. It has been shown that during genomic insult occurs an intense metabolic reprogramming of the cells. Interestingly, several proteins involved in inositol metabolism, a precursor of several phospholipids, are phosphorylated during genomic stress by the action of the DDR kinase $Mec1^{ATR}$ suggesting a cross-talk between these two processes. While the regulatory role of Mec1 in cellular processes, such as DNA repair, replication and cell cycle, is largely known, much less is known about its regulation of phospholipid metabolism. We have previously shown that Opi1, a repressor of phospholipid metabolism in budding yeast is phosphorylated by $Mec1^{ATR}$. Therefore, we sought to investigate the contribution of Opi1 for the DDR. Here, we show through a combined genetical/biochemical approach that Opi1 is important for DDR activation. Notably, yeast cells lacking Opi1 are sensitive to genotoxic stress induced by methyl methanesulfonate (MMS), but not to other replication stress inducers such as hydroxyurea (HU). Because Opi1 deletion leads to inositol overproduction, we decided to investigate if inositol accumulation is causing MMS sensitivity. However, addition of increased concentrations of inositol to the cell culture does not lead to MMS sensitivity in wild-type cells. Moreover, deletion of *INO1*, which is responsible for *de novo* synthesis of inositol does not rescue MMS sensitivity of *opi1Δ* cells. Finally, we have shown that MMS treatment induces the translocation of Opi1-GFP from a perinuclear localization to the nucleus even in the absence of inositol, suggesting that Opi1 is repressing genes not related to inositol metabolism during the DDR. Interestingly, we have shown that several strains lacking proteins involved in Mec1 signaling present a moderate inositol auxotrophy that can be averted by *OPI1* deletion. Together, these results indicate that Opi1 is an important regulator of the DDR in budding yeast. However, further investigation is still required to dissect the exact mechanism of Opi1 action during the DDR.

MESENCHYMAL STEM CELLS INTENDED FOR TRANSPLANTATION MAY SUFFER DAMAGE IF EXPOSED TO GENOTOXIC EXTENSIVE MANIPULATION?

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Mesenchymal stem cells (MSCs) are cells of stromal origin, which can be obtained from a variety of tissues, including adipose tissue, which is considered an abundant source and is normally discarded in liposuction processes. In addition, they have immunomodulatory, anti-apoptotic, anti-inflammatory and repair and tissue regeneration properties making them important in clinical therapies. The cryopreservation process enables the storage of cells providing better quality control, standardization and provides an immediate therapeutic supply. However, the integrity of cryopreserved cells has been discussed. Thus, this work had as objective to evaluate the integrity of the genetic material of the cells CTMs in different passages of thawing. Adipose tissue was isolated by the abdominal liposuction procedure of three female individuals aged between 20 and 50 years. For the processing of the liposomated samples and isolation of the CTMs, collagenase was used for enzymatic digestion and the immunophenotyping of the cells was characterized by flow cytometry. To evaluate the cell integrity, the comet assay was used where more than 200 nucleoids per repetition were evaluated using the CometScore program, evaluated the parameters of total comet length, head diameter, percentage of DNA in the tail and Tail Moment (% DNA in the tail multiplied by the length of the tail). We observed that there was an increase in the total size of the comets later thawing, in addition, this increase is followed by the increase of head diameter in the same proportions. However, the tail moment, mainly in the initial's thaws (until 6th passage), remains unchanged with respect to the first pass, and very close to 0, indicating that there is only basal damage from the assay procedure itself. We observed that there was a small, however significant increase of DNA in the tail from the 5th passage, indicating that this may be a limit for cryopreservation of the cells at different passages. Considering that there was an increase in nucleoid diameter and DNA in the tail, we concluded that the use of MSCs is safe and that they have genetic integrity up to the 5th thaw passage.

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MOLECULAR CHARACTERIZATION OF DNA REPAIR IN MOUSE OLFACTORY SENSORY NEURONS

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Key-words: DNA repair, olfactory sensory neurons, neurodegeneration.

Olfactory Sensory Neurons (OSNs) undergo neurogenesis and migration through the olfactory epithelium (OE) during maturation process; therefore, their lifespan is shorter than most neurons within the brain. Olfactory dysfunction has been reported as an early clinical symptom in many neurodegenerative diseases, which are associated with impaired DNA repair. Whether DNA repair mechanisms play a role in OSNs genomic maintenance, however, is still unknown. Our study aims to characterize DNA repair pathways in precursor and mature OSNs. For that, we analyzed data from two different transcriptomes to characterize the expression pattern for DNA repair proteins with age and stage of neuronal differentiation. In order to confirm the results obtained from the in silico analysis, we performed RT-PCR for selected DNA repair targets from newborn (4-7 days) and 3-week-old mice OE samples. The in silico analysis suggested that most DNA repair pathways are reduced in young mice, when compared to newborns. On the other hand, non-homologous end joining (NHEJ) pathway is apparently increased, which may indicate a preference for non-homologous repair pathways for repairing double strand breaks due to lack of homologous sister-chromatids in differentiated cells. However, RT-PCR did not detect significant changes in DNA repair gene expression levels between the ages. Since OSN are constantly exposed to damage by atmospheric oxygen and other toxic molecules present in the air, we hypothesized that these neurons would accumulate more DNA damage than those in the central nervous system (CNS). To test this hypothesis, we performed a long extension PCR assay comparing OE, olfactory bulb (OB) and non-olfactory neural tissue (CE) from 3-week-old mice, but we found no significant differences in the long/short fragment amplification ratio. Although our results are merely descriptive, they have never been reported before, and can be used to support new research on OSN genome maintenance and integrity.

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CHRONIC HYPERGLYCEMIA INDUCES DNA DAMAGE AND OXIDATIVE STRESS WHICH CAN BE DIMINISHED BY DIETARY RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Keywords: DNA damage; Mitochondrial alterations; Hyperglycemia; Dietary restriction; Type 2 diabetes mellitus.

Type 2 diabetes (T2D) is mainly characterized by hyperglycemia, and high glucose levels can lead to several cellular and molecular alterations related to oxidative stress and DNA damage. Considering the impact of obesity in diabetic complications, dietary restrictions (DR) has been recommended for T2D patients. Furthermore, there is evidence that DR positively affects the longevity extension and reduces age-related diseases. The aim of this study was to assess the effects of chronic hyperglycemia on molecular signaling pathways that are linked to oxidative stress, DNA repair, insulin resistance and inflammation, in addition to analyze parameters of oxidative stress at the mitochondrial level and nucleotide pool in mononuclear cells of T2D patients, evaluated before and after a protein-restricted diet (0,8g/kg/day). T2D patients with chronic hyperglycemia (without intervention) compared to healthy individuals were also evaluated. We observed that following a period of 4 weeks, the protein-restricted diet led to decreased levels of DNA damage (comet assay) in patients with T2D (n=7). Regarding the gene expression profiles evaluated for target genes (PCR array), a significant difference was not observed between “before” and “after” the DR intervention. Only a slight tendency of up-regulation was observed for *PI3K*, *PRKAA1* and *ATM* genes. For some patients there was an induction of several genes (*OGG1, MUTYH, SOD1, FOXO3, NUDT1*) with roles in DNA repair and responses to damage. In addition, it was observed that in general, T2D patients with hyperglycemia (without intervention) (n=17) presented increased levels of oxidized bases in the nucleotide pool when compared to controls (n=10). However, the intervention period was not sufficient to exert alterations in patients submitted to DR.

Regarding mitochondrial analysis, significant alterations in the quantification of ROS, membrane potential and mitochondrial mass were not observed between groups of T2D patients (n=15) and healthy individuals (n=15). Thus, chronic hyperglycemia promotes an oxidative stress condition in T2D patients, and DR during 4 weeks was found efficient in significantly reducing DNA damage levels in patients with T2D, in parallel with improvements in glycemic rates, and other clinical parameters, but changes in transcript gene expression and oxidized base levels were not observed after the intervention period.

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ANALYSIS OF A PUTATIVE ROLE FOR RAD52 IN MITOCHONDRIAL FUNCTION AND MITOCHONDRIAL DNA REPAIR IN HUMAN CELLS

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The RAD52 protein is involved in the repair of DNA double-strand breaks (DSB) through homologous recombination (HR), single stranded annealing, or the newly characterized RNA-DNA repair. In mammals, the role of RAD52 is not well characterized. RAD52-knockout mice showed nearly normal phenotype, without significant alterations in HR activity. RAD52, however, may play a back-up role in cells deficient in HR proteins like BRAC1, BRAC2 and RAD51. We have previously demonstrated that RAD52 increases the activity of the oxoguanine DNA glycosylase OGG1, involved in the Base Excision Repair (BER) pathway. In mitochondria, the BER pathway is well characterized and thought to play an important role due to the higher load of oxidized bases in the mitochondrial DNA (mtDNA), as a consequence of exposure to oxidants generated by the oxidative phosphorylation (OXPHOS). Our study intends to analyze the role of RAD52 in mtDNA maintenance, as well as the effects of its absence in cellular bioenergetics. Using two different human cell lines we demonstrated that RAD52 localizes in mitochondria under normal growth conditions, and exposure to hydrogen peroxide induces mitochondrial translocation of at least one of the RAD52 isoforms. To analyze RAD52 functional role in mitochondria we generated HEK293T cells with constitutively knockdown (KD) RAD52 expression via shRNA. Interestingly, RAD52-KD cells showed increased maximal respiration supported by NAD-linked substrates when compared to wild-type or scrambled shRNA control cells, indicative of alterations in the OXPHOS Complex I in these cells. These results may suggest that although the absence of RAD52 does not have significant effects on mouse development, cellular bioenergetics may be altered, and compensatory mechanisms are triggered to maintain cell viability. We are now investigating whether other respiratory complexes are altered in these cells.

Key-words: Mitochondrial DNA Repair, oxidative damage, bioenergetics, RAD52

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HOW XERODERMA PIGMENTOSUM VARIANT PATIENTS CELLS ARE AFFECTED BY TEMOZOLOMIDE?

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Key-words: XP-V cells, Temozolomide, DNA damage, translesion synthesis, DNA repair capacity, cell death

Translesion DNA polymerases are capable of replicating damaged DNA, without removing the lesions, performing translesion synthesis (TLS), a mechanism of DNA damage tolerance. Tumor cells use this mechanism in order to survive the lesions caused by chemotherapy and, therefore, this may be a method of resistance to treatments. Moreover, this process is error-prone and can lead to mutagenesis increasing resistance potential of tumor cells. DNA polymerase eta (pol eta) is a major protein of this group responsible for protection against sunlight-induced tumors. Xeroderma Pigmentosum Variant (XP-V) patients are proficient in nucleotide excision repair (NER), however encoding a mutated pol eta, which leads to a high sensitivity and increased incidence of skin cancer. Little is known about the role of TLS in Temozolomide (TMZ) treatment in cancers. Our aim is to investigate how TMZ affects DNA replication on cells lacking pol eta. Hence, we firstly treated nucleotide excision repair (NER-XP-C) and pol eta deficient cells (XP-V) with TMZ, and evaluated cell viability using a colorimetric assay to analyze cell proliferation (XTT). We observed that TMZ only reduces cell viability in XP-V cells, when compared to DNA repair proficient control cells. We will also perform cell cycle assays in XP-V cells treated with TMZ (using nocodazole, a compound that arrests cells at pro-metaphase during mitosis) to confirm how the treated cells are able to replicate damaged DNA and pass through S-phase, arresting in G2. We also engineered XP-C cells knocking down some of the TLS polymerases, such as pol eta and DNA polymerase iota (pol iota). We expected that XP-C shEta would reduce cell viability when treated with TMZ, but both cells (XP-V shEta and XP-V shIota) showed viability similar to XPC TLS proficient cells. This is being further investigated. Furthermore, we treated XP-V cells and XP-V complemented (XP-Vcomp - which are cells complemented for the TLS function) with two different doses of TMZ and performed HCR assay to detect DNA repair capacity of UVC-treated plasmids. We detected that XP-V cells reduced their DNA repair capacity, when treated with TMZ. In summary, our data indicate that NER mechanisms do not play a response in TMZ treatment, but in TLS deficient cells TMZ probably impairs DNA damage replication and DNA repair capacity, probably as a result of protein oxidation in treated cells.

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UVA LIGHT-INDUCED CELL SENSITIVITY IN XERODERMA PIGMENTOSUM VARIANT PATIENTS CAN BE ASSOCIATED TO DISFUNCTION OF METABOLISM AND AUTOPHAGY REGULATION

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UVA light induces DNA damage, redox stress and participates in skin tumor formation in normal people but specially in Xeroderma Pigmentosum Variant (XP-V) patients. XP-V patients present 1,000-fold higher skin cancer incidence due to mutations in DNA polymerase eta (pol eta), which participates in Translesion Synthesis (TLS) of UV-induced DNA damage. Previous data from our group show that UVA-induced redox stress impairs Nucleotide Excision Repair (NER) proteins and DNA damage removal, which should be normal in XP-V patients. In this context, we wondered why pol eta proficient cells deal better with UVA-induced oxidative stress? Thus, we investigated the role of cellular metabolism in ROS generation, the antioxidant capacity and the role of autophagy in removing damaged molecules. Metabolic stress upon UVA was tested by mitochondrial superoxide production and inhibition of NADPH oxidase (diphenyleneiodonium - DPI), a potential generator of ROS. In fact, superoxide is significantly increased by UVA in both cells, however was more prominent in XP-V cells. Treatment with N-acetylcysteine (NAC) significantly reduced superoxide formation in both cell lines. Significant increase in cell survival was observed in UVA-irradiated XP-V cells treated with DPI. Accordingly, a significant decrease in FPG-sensitive sites by comet assay was observed when DPI was added in both cells. These results suggest that metabolic stress, at least in part, accounts for the generation of ROS after UVA irradiation, mainly in the XP-V cells. In addition, we evaluated NRF2 levels, which responds to oxidized biomolecules and glutathione, a relevant intracellular antioxidant. Western blot demonstrated that XP-V expresses less NRF2 at basal level compared to XP-V comp, as well as after UVA irradiation. GSH levels were lower in XP-V compared to XP-V comp upon UVA. Moreover, chloroquine (inhibitor of autophagy) induced a significant decrease of cellular viability in XP-V comp and XP-V cells after UVA irradiation, indicating that autophagy contributes to cell survival after UVA. We also found that LAMP2A (a protein related to chaperone mediated autophagy) is increased in non- and UVA-irradiated XP-V comp cells. These data suggest that pol eta deficiency generates metabolic stresses and impairment of the detoxification pathways, leading to further DNA, cellular and protein damage and which could be related to cell death and carcinogenesis after UVA light.

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Modulation of mutagenicity and Nutrigenomics (antimutagenesis, anticarcinogenesis, modulation of gene expression by micronutrients)

95 TO 106

30 anos
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MODULATORY EFFECTS OF BETULINIC ACID ON THE MUTAGENICITY INDUCED BY URETHANE IN WING SOMATIC CELLS OF *Drosophila melanogaster*

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Key-words: Antimutagenicity, Cytochrome P450, CYP6A2, Somatic mutation and recombination test, SMART.

Betulinic acid (BA) is a naturally occurring pentacyclic triterpenoid found in several globally distributed plant species. BA has several different properties, such as being antiretroviral, antimalarial, and anti-inflammatory, as well as being a prospect as an anticancer agent, by topoisomerase inhibition. Urethane (URE) is a known promutagen with genotoxic potential in *Drosophila*, being both dose response and dependence associated to metabolic activation. The aim of the present study was to evaluate the mutagenicity and recombinogenicity of different concentrations of BA, in two sets of experiments, as follows: BA alone and BA in combination with URE. Tests were performed by using Standard (ST) and High Bioactivation (HB) crosses of the wing Somatic Mutation and Recombination Test in *Drosophila melanogaster*. In the first experiment, BA (0.0312; 0.0625; 0.125; 0.25 or 0.50 mg/mL) alone was examined for mutagenicity and recombinogenicity, and as for the second one, containing BA plus URE (10 mM), the antimutagenicity property was analyzed. Negative (ultrapure water) and positive (URE 10 mM) controls were included in both experiments. The results showed that BA is metabolized by the Cytochrome P450 enzymes (especially CYP6A2), which are present in the insecticide-resistant strains [OR(R)] at higher concentrations when compared to insecticide-sensitive (flr^3) strains. BA alone did not significantly increase the frequency of mutant spots in both ST and HB crosses. URE significantly increased the frequency of all mutant spot categories in both crosses. URE predominantly acts by inducing mutational events rather than mitotic recombination. BA significantly reduced the total frequency of mutant spots when co-administered with URE, in the three highest concentrations in the ST cross (0.125; 0.25 and 0.50 mg/mL) and in all concentration in the HB cross. The results show that under experimental conditions, BA has modulatory effects on the mutagenicity induced by URE in somatic cells of *D. melanogaster*. We suggest that the modulatory effects of BA may be due to its anti-inflammatory and anti-oxidant capacities, besides its apoptotic induction.

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MODULATORY EFFECTS OF CLONAZEPAM ON THE MUTAGENICITY INDUCED BY DOXORRUBICIN IN WING SOMATIC CELLS OF *Drosophila melanogaster*

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Key-words: Antimutagenicity, benzodiazepines, Cytochrome P450, Somatic mutation and recombination test, SMART.

Clonazepam (CLZ) is one of the most widely consumed drugs in Brazil, belonging to the benzodiazepine class, and acts on the mild inhibition of the central nervous system with anticonvulsive action, sedative, muscle relaxant and tranquilizing effect. Cancer patients being subject to doxorubicin (DXR) therapy typically use CLZ as an adjuvant in order to reduce anxiety and agitation. DXR interferes with the topoisomerase II enzyme and generates free radicals. In this study, the standard (ST) and the high bioactivation (HB) crosses from the wing Somatic Mutation and Recombination Test in *Drosophila melanogaster* were used to assess the mutagenicity of CLZ alone and the modulatory effects of CLZ against DXR-induced mutagenicity. Third-instar larvae trans-heterozygous for two genetic markers, multiple wing hair (*mwh*) and flare (*flr*^{3}), were treated at different concentrations (25; 50 or 100 mM) of CLZ alone or co-administered with DXR (0.4 mM). Negative (ultrapure water) and positive (DXR 0.4mM) controls were included in both experiments. CLZ was not mutagenic in neither crosses. The reference mutagen (DXR) significantly increased the frequency of all mutant spot categories. CLZ significantly reduced the total frequency of mutant spots in all concentrations of the ST and HB crosses. Therefore, under experimental conditions, CLZ had modulatory effects on the mutagenicity induced by DXR in somatic cells of *D. melanogaster*. We suggest that the modulatory effects of CLZ may be due to its anti-oxidant capacity and apoptotic induction through ionic channel pathways.

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DNA METHYLATION AND DIETARY PROFILE OF NON-SYNDROMIC ORAL CLEFT CHILDREN

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Keywords: cleft lip and palate, nutrigenomic, epigenetic, DNA methylation

Non-syndromic cleft lip and/or palate (NSCL/P) is a birth defect with environmental and genetic causes. Epigenetic factors, such as DNA methylation, have been thought to influence heritability of this complex malformation and nutrition is strongly associated with the methylation pattern variability throughout life. Therefore, this work aimed to analyze the DNA methylation pattern of global genome and *BRCA1* promoter in children with oral clefts. In addition, it was investigated the dietary profile of this patients to discuss the association between these factors and the risk of developing chronic diseases. The study was conducted with NSCL/P children attending at the Pediatric Hospital Professor Heriberto Ferreira Bezerra, in Natal/RN, and their mothers; it also included a control group composed by apparently healthy children without clefts and their mothers. Exfoliated buccal cell from all children were collected to perform DNA extraction. To determine global genome methylation, it was performed combined bisulfite restriction analysis of 5-methylcytosine (5-mC) from LINE-1 regions, and methylation specific polymerase chain reaction for *BRCA1* promoter. Besides, an interview were carried out to obtain nutritional, socio-demographic and others information from the participants. The median percentage of LINE-1 5-mC for control and case groups were, respectively, 48% and 45.3%. The difference between this methylation levels were statistically significant (p-value < 0.05). The *BRCA1* promoter was methylated in 40.9% and 59.1% of control and case groups, respectively and in 60% and 40% of the same groups, it was both methylated and unmethylated. Regarding the dietary status, it was observed that NSCL/P group had a high consumption of simple carbohydrates (e.g. refined wheat flour, white rice and sugar) and ultra-processed foods (such as snack, artificial seasoning and candy) together with a low intake of plant based and raw foods (like nuts, grain, vegetables and tubers). This profile signals to a health imbalance and low support for epigenetic regulation required for physiological homeostasis and health maintenance. The results indicate that NSCL/P children have a global hypomethylation pattern in comparison with children not affected and a poor food intake quality. Thereby, it is necessary to improve lifelong health guidelines for these children in order to prevent other chronic metabolic diseases and the inheritance of this phenotype to other generations.

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VITAMIN C AND ACETYLSALICYLIC ACID REDUCE COLON CARCINOGENESIS IN ANIMAL MODEL

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Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, principally in most developed countries. Each year, 1.3 million new cases of CRC are diagnosed and 650,000 people died of CRC. Therefore, chemopreventive actions can be applied or tested in these experimental models, with the purpose of interrupting the change or the course of the pathological process Many compounds chemical have been identified as potential chemopreventive agents, among them, vitamins and synthetic compounds. Among the vitamins stands out vitamin C (VC), that present antioxidant property and contributes to the reduction of oxidative damage in DNA and the development of cancer. In relation the synthetic compounds, stand out the acetylsalicylic acid (ASA), recent epidemiological studies and clinical trials indicate that long term use of ASA could possibly decrease the incidence of certain malignancies, including CRC. Considering the effects of VC and ASA on DNA and in the process of tumorigenesis of many cancers, the aim of the present study was to evaluate the chemopreventive effects of VC and ASA in an animal model of CRC. Were used 50 adult Balb-c mice divided into five groups: G1: Water + Ethylenediaminetetraacetic acid (EDTA); G2: Water + 1,2-Dimethylhydrazine (DMH); G3: VC + DMH; G4: ASA + DMH; and G5: VC + ASA + DMH. The animals received VC in the concentration of 500 mg/L and ASA in the dose 6 mg/kg per day and body weight, both were diluted in the drinking water. To induce colorectal carcinogenesis, the animals received four subcutaneous injections of DMH, 40 mg/kg body weight each injection. At the end of the treatment, were collected blood samples for the realization of comet assay and after the animals were euthanized for the dissection of the liver and colon for the comet assay and aberrant crypt foci assay respectively. The results of the comet assay showed that the ASA and the VC + ASA decreased DNA damage caused by DMH in blood, and in the liver only the ASA presented these decrease. In the aberrant crypt foci assay, the results demonstrated that the ASA and VC + ASA decreased the number of Aberrant crypt foci and the VC, ASA and VC + ASA decrease significantly the number of aberrant crypts in the colon of animals submitted to the colorectal carcinogenesis. In conclusion, the results showed the chemopreventive effects of VC and ASA in an experimental model of colorectal carcinogenesis.

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ASSESSMENT OF THE ANTICARCINOGENIC EFFECT OF THE ETHANOLIC EXTRACT OF *Pyrostegia venusta* (Ker Gawl) *Miers* BY THE EPITHELIAL TUMOR CLONES DETECTION TEST (*WARTS*) IN *Drosophila melanogaster*

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Keywords *Pyrostegia venusta*, *Drosophila melanogaster*, Anticarcinogenic, Antioxidants

Pyrostegia venusta, also called "vine-st. john", "vine-peccary", "vine-tingá" or "lady finger", is popularly known for its medicinal properties, being used to treat vitiligo, diarrhea, cough and respiratory system diseases as bronchitis, influenza and colds; this plant compounds are scientifically recognized for its medicinal properties, including the antioxidative and keratolytics ones, being described antitumor action in melanoma cells. In this context, the present study aimed to assess the effect of alcoholic extract of *P. venusta* in *Drosophila melanogaster* detected by the clone detection of epithelial tumors test (*warts*). To this end, third instar larvae of *D. melanogaster* have undergone treatment with alcoholic extract of the plant in three different concentrations (12.5%, 25% and 50%) isolated and in association with doxorubicin, doxorubicin was also used as positive control at a concentration of 0,4mM and ethanol 5% was used as negative control, as well as, solvent; the adult emerging flies were collected and the tumor frequencies numbered for each treatment performed. The results showed anticarcinogenic effect of the extract *P. venusta* observed by the significant reduction in the frequency of epithelial tumors in co-treated flies, when compared to the positive control at all concentrations tested. Several reports have been released describing ROS scavenger activity by *P. venusta* and apoptosis inducing by intrinsic way. In the present experimental conditions, we conclude that this results are an opportunity for further research to be undertaken in order to reaffirm the antitumor action of compound *P. venusta*. Once the species was beforehand considered antioxidant may in future become a promising therapeutic option to treat cancer patients.

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ANTICARCINOGENIC EVALUATION OF THE ETHANOLIC EXTRACT OF *Hedychium coronarium* IN SOMATIC CELLS OF *Drosophila melanogaster*

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Keywords: antioxidant, chemoprevention, gene *wts*

In traditional folk medicine, Hedychium coronarium extract is used to treat general infections and rheumatism, being its efficiency proven by pharmacological studies that indicate antibacterial and anti-inflammatory activities. The aim of the current study was to evaluate the anticarcinogenic potential of ethanolic extract of *H. coronarium* rhizomes of in somatic cells of *Drosophila melanogaster.* The material was collected in Patos de Minas - MG region (-18,6263 latitude and -46,5523 longitude) and after drying it was submitted to exhaustive extraction using Soxhlet, to obtain the ethanolic extract. The Epithelial Tumour Test (ETT) in *D. melanogaster* was carried out used third-instar larvae descended from the cross between naïve *wts/*TM3 females and *mwh/mwh* males, which were treated with the three different concentrations of ethanolic extract of *H. coronarium* (1.5, 3.0 and 6.0 mg / mL) associated with Doxorubicin (0.4 mM). Were used 1% Tween 80 + 5% ethanol as negative control e Doxorrubicin (0.4mM) as positive control. The anticarcinogenic potential of ethanolic extract was diagnosed by the Mann, Whitney and Wilcoxon nonparametric *U* test, using a $\alpha = 0.05$ level of significance. Statistically significant differences were observed ($p \leq 0.05$) in tumor frequencies in individuals treated with all concentrations of the ethanolic extract when compared to positive control. It was therefore concluded that, in these experimental conditions, ethanolic extract of *H. coronarium* have anticarcinogenic effect in *D. melanogaster*.

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ANTICARCINOGENIC EFFECT OF RUBI GRAPE (*Vitis vinifera*) IN SUPPRESSION OF EPITHELIAL TUMORS INDUCED BY DOXORRUBICIN IN *Drosophila melanogaster*

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Key words: *Vitis vinifera*, *Drosophila melanogaster*, Anticarcinogenic Effect.

Vitis vinifera popularly known as ruby grape has several phenolic compounds, flavonoids and stilbenes which have been pointed out with possible antioxidant, anticarcinogenic, antibacterial and antidiabetic effects, as well as cardioprotective, hepatoprotective and neuroprotective effects. The present work aimed to test the anticarcinogenic potential of *Vitis vinifera* through the test for the detection of clones of epithelial tumors (ETC Test) in *Drosophila melanogaster*. The concentrations tested were determined from the toxicity test. The extract of *Vitis vinifera* was prepared in aqueous medium, aiming at maintaining the properties of *Vitis vinifera* components. Prior to the preparation of the extract, the seeds of *Vitis vinifera* were removed. Such extract has not been subjected to filtration processes. From the total extract (100%), dilutions were made in reverse osmosis water, obtaining concentrations of 50% and 25%. The test for the detection of epithelial tumor clones (ETC Test) in D. melanogaster was carried out using descendant larvae wts / TM3 virgin females with mwh / mwh males, which were chronically submitted to negative controls (reverse osmosis water), positive (doxorubicin - 0.4 mM) and three different concentrations of aqueous extract of *Vitis vinifera* (25%, 50% and 100%). The results showed significant differences in the tumor frequency of the three concentrations of *Vitis vinifera* extract when compared to the positive control. It is concluded that, under current experimental conditions, *Vitis vinifera* extract significantly reduced the formation of tumors in *D. melanogaster* at the three concentrations used, thus exerting anticarcinogenic activity.

Financial support: UNIPAM.

ANTIPROLIFERATIVE AND DNA DAMAGE EFFECTS OF DIPHENYL DITELLURIDE LEADS TO CELL CYCLE ARREST EFFECTS IN HUMAN COLORECTAL CANCER CELLS

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Diphenyl ditelluride (DPDT) is an organotellurium (OT) compound with biological effects, such as potential antioxidant, antigenotoxic and antimutagenic at low concentrations (≤1 µM). DPDT showed cytotoxic effects in mammalian V79 cells by inducing oxidative damage, DNA strand breaks and cell cycle arrest in several models (1 – 10 µM). Colorectal cancer is the one of most concerns in public health and has one of the highest mortality rates in worldwide, the third place in death. Thus, this work aims to investigate the antiproliferative, genotoxic. ROS induction and cell cycle arrest potential of DPDT in human colorectal cancer cells (HCT116) and human fibroblast cells (MRC5). Results showed a decrease in cell viability after DPDT exposures for 72 h in both cell lines, as evaluated by MTT (1 – 20 µM) and clonogenic assays (0.1 – 3 µM). The comet assay detected DNA damage for both cells line in DPDT exposures (3 h and 24 h), with a more pronounced DNA damage index for HCT116. The ROS generations detected by flow cytometry (DCFH-DA), was evidenced in both cell lines with 3 h, but only in HCT116 in 24 h exposure. Furthermore, cell cycle arrest in G2/M phase detected by flow cytometry was more pronounced in the HCT116 than MRC5 cell lines after 72 h of exposure (1 – 10 µM). Taken together, HCT116 colorectal cancer cells were more sensitive to DPDT biological effects than MRC5 cells. This difference may be related to the stronger DNA damage effects induced by DPDT in HCT116 cells, which probably triggered the cell cycle arrest. The action of DPDT apparently occurs in the S phase, taking place to cell cycle arrest in G2/M, leading to the checkpoint activation maybe a mechanism related to a possible oxidative damage generation. The currently ROS inducers have their limitations, therefore investigation of new compounds are required. The employment of genotoxic and checkpoint activators agents that leads to DNA damage response are targets in cancer research for their specificity or immunotherapeutic activity. Thus, the results of this work open up possibilities for future studies on further investigation of possible molecular mechanisms of DPDT in colorectal cancer cells and other cancer models.

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FRUTOSE CONSUMPTION DURING PREGNANCY AND LACTATION CAUSE GENOTOXIC AND BIOCHEMICAL CHANGES IN FEMALE SWISS MICE AND IN ITS OFFSPRING

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Key-words: Fructose; Pregnancy; Lactation; Genotoxicity; Biochemistry.

Fructose, also known as levulose, is a hexose which has a chemical formula expressed by $C_6H_{12}O_6$. The consumption of fructose during the pregnancy can cause hyperglycemia in the pregnant and may stimulate increased production of reactive oxygen species and changes in macromolecules. Based on these findings, the aim of this study was to evaluate the genetic and biochemical effects of fructose consumption on the offspring of Swiss mice treated during pregnancy and lactation. For this, 15 couples of 60-day-old Swiss mice were divided into 3 groups of 5 couples; negative control (G1 - water only) and the fructose groups (G2 and G3 - doses 10%/L and 20%/L, respectively). After birth, the animals were divided into 6 groups: P1 and P2 groups: negative control, male and female respectively, mothers received water during pregnancy and lactation; P3 and P4 groups: Fructose 10%/L, male and female respectively, mothers received 10%/L of fructose during pregnancy and lactation; P5 and P6 groups: Fructose 20%/L, male and female respectively, mothers received 20%/L of fructose during pregnancy and lactation. Fructose was available in the hydration bottles of females throughout all the gestation and lactation periods. These animals were submitted to euthanasia at 30 days of age for genetic and biochemical evaluations. In the gestation and lactation period, the two doses of fructose tested (10%/L and 20%/L) showed genotoxic and mutagenic activity, associated to increase of dietary intake, body weight, lipid profile and fasting glycemia in females. In addition, in the offspring (both males and females), both doses also showed genotoxic activity, but without mutagenic effect. Furthermore, the young presented nutritional and metabolic changes due to the increase in food consumption. The offsprings of females that received fructose during gestation and lactation developed metabolic syndrome throughout life (low HDL, increased triglycerides and fasting glycemia). Thus, it can be suggested that the high consumption of fructose during these periods is harmful for pregnants and their children. In conclusion, there is an awareness of the consumption of fructose among pregnant and lactating women.

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THERMOGENIC STIMULANT SYNEPHRINE INDUCES PRO-OXIDATIVE EFFECTS BY AMPK PATHWAY IN HUMAN GASTROINTESTINAL CELLS *in vitro*

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Key-words: Nutrigenomics; DNA damage; reactive oxygen species; antioxidant defense.

Synephrine (SN) is a natural thermogenic stimulant used to weight loss. SN pharmacological action is similar to sympathomimetic amines and its chemical structure is related to amphetamines. *In vitro* and *in vivo* assays on SN side-effects and genotoxicity have reported inconsistent data. We aimed to investigate the effects of SN in normal gastric mucosa (MNP01) and colorectal intestinal adenocarcinoma (Caco-2) human cells. These cells were used as an *in vitro* model of the gastrointestinal (GI) tract since high concentrations of SN are absorbed after ingestion (30-90%). We evaluated the effects of SN on cell viability, cell proliferation, cell cycle analysis, genomic stability (genotoxicity and mutagenicity), and in the intracellular redox status (reactive oxygen species (ROS) generation, lipid peroxidation and antioxidant enzymes levels). In addition, we evaluated the expression of genes related to the energetic metabolism (AMPK), cell proliferation (PI3K/AKT), inflammation, and DNA damage response (DDR) pathways using reverse transcription quantitative PCR (RT-qPCR). Our results showed that SN did not show cytotoxicity at the concentration of 25-5000 µM. Afterward, SN at 2-200 µM demonstrated no antiproliferative neither genotoxic/mutagenic effects. Gene expression analysis showed no alterations in DDR genes corroborating the results of genotoxic tests in GI cells. Caco-2 cells showed increased cellular ROS levels after SN exposure. Furthermore, AMPK pathway-related genes, known to promote mitochondrial biogenesis, were up-regulated in both cells.These findings highlight the energetic metabolism as a possible mechanism inducing pro-oxidative effects trough AMPK pathway activation upon SN exposure. The expression of cell proliferation/inflammation pathways-related genes were also significantly altered after SN exposure in MNP01 and Caco-2 cells. Interestingly, the possible damage induced by ROS appears to have been prevented due to increased levels of gluthatione in bolth cell lines and decreased levels of catalase in MNP01 cells. This would justify the absence of cytotoxic, genotoxic and mutagenic effects. In summary, this is the first study showing that SN, at relevant concentrations for thermogenic users, promotes ROS overproduction and alter the redox state and gene expression in GI human cells *in vitro,* without inducing hazardous cellular effects.

Taken together, these pro-oxidative effects are mainly related to energetic metabolism stimulation by AMPK pathway.

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THE CORRELATION OF ALUMINUM SERIC CONCENTRATION WITH THE DNA METHYLATION OF SSRP1 GENE IN OBESE WOMEN

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Obesity has been linked to lifestyle and recently have been associated with environmental toxicants, negatively impacting human health. Metals exposure is a very concerning event and Aluminum(Al) is a metal that is commonly used in industry. The contamination occurs mainly by the respiratory (particulate matter) and oral (drinking water and diet) routes. Food packaging processes may contaminate food, canned soft drinks have high Al content due to the leaching of the favored metal at acidic pH. The mechanisms by which these compounds cause disease have not been fully elucidated. DNA methylation changes have been associated with many diseases such as obesity and more recent with Al exposure too. The aim of this study was to evaluate Al seric concentration and DNA methylation profile in obese and normal weight women to evaluate possible associations between metal concentrations and DNA methylation patterns. Materials and Methods: This transversal study enrolled Obese (Ob) and Eutrophic (Eut) women. Anthropometric data: weight (kg) and height (m) and peripheral blood samples for biochemical and genetic analysis were collected. DNA extraction and bisulfite conversion were performed to hybridize the samples on the 450k Infinium Methylation Beadchip. Serum concentration of metals was determined by inductively coupled plasma mass spectrometry (ICP-MS). Statistical analysis included the Shapiro-Wilk, independent t test and Pearson's correlation ($p<0.05$). Results: The study enrolled 32 women, 16 obese (IMC = 45.1 ± 5.4 kg/m^2 p=0.01; Age = 39.3 ± 11.5 years old p=0.72) and 16 normal weight (IMC = 22.5 ± 1.7 kg/m^2; Age = 39.1 ± 13.4 years old). Seric concentration of Al were different between groups (Ob: 83.69 ± 65.60 ug/L, Eut: 43.30 ± 20.05 ug/L, p = 0.02). We found that obese group showed a lower methylation level of the CpG site cg02688348 (TSS200/island) located in the adipogenesis-related gene *SSRP1* than eutrophic women (Δβ = 0.31, FDR=0.02). Also, we observed a significant correlation between gene methylation levels and Al seric concentration (Al-*SSRP1*: r= -0.51, p= 0.01). Conclusion: Obese women were more exposed to Al and this may affect adipogenesis processes.

Keywords: Obesity, Aluminum, DNA methylation, adipogenesis

Financial Support: CNPq, CAPES**,** FAPESP.

IN VITRO APPROACHES FOR MUTAGENICITY AND HEPATOTOXICITY INDUCED BY MARKETABLE PRE-WORKOUT SUPPLEMENTS

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Currently, the practice of physical exercises has been increasing around the world. Many of these practitioners use pre-workout nutrition through dietary supplements because of their effects on the body. However, a sequence of clinical cases of hepatic damages in the hawaiin population brings suspicious about their effect to the consumer. Thus, this project consists on investigating the mutagenic and cytotoxic potential of pre-workout OxyELITE Pro, C4 and Jack3d supplements. The *Salmonella*/Microsome assay was used to determine the mutagenic potential of the supplements investigated. *Salmonella enterica* sorovar typhimurium TA97, TA98 and TA100 strains were used for the 3 products (besides TA102 and TA104 for C4). Assays were performed using five concentrations for Oxyelite and C4 (0.0005, 0.005, 0.05, 0.5, 5 mg/plate) and six concentrations for Jack3d (0.0001, 0.0005, 0.005, 0.05, 0.5, 5 mg/plate), all diluted in DMSO 10% and analyzed with and without metabolic activation (S9 mix 4%). The assays were done in triplicate and the mutagenic effect was considered in those concentrations that had the mutagenicity index (IM = induced revertants/spontaneous revertants) higher than 2. For citotoxicity, the viability assay (WST-1) was used with the human hepatocarcinoma HepG2 cell line and an alkaline phosphatase assay was performed to check for possible hepatic disorders. The percentage of cell survival in WST and the amount of alkaline phosphatase per 10,000 cells in contact with supplements at concentrations of 0 at 5 mg/ml were observed for 24h, 48h, and 72h periods. A positive result was found for mutagenicity for OxyELITE Pro and Jack3d for strains TA98 and TA100, in the presence of metabolization, and for all three supplements for strain TA 97 under the same condition. For the WST assay, cell death occured for all three products at all concentrations after 48h incubation. In the alkaline phosphatase assay, a significant increase of phosphatase in the supernatant was detected when in contact with any of the supplements analyzed after the same period of time. Those results suggest a mutagenic and cytotoxic potential that could cause liver damage.

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Environmental Mutagenesis : Natural Products

BÁRBARA VERENA DIAS GALVÃO

"ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF *Myrciaria cauliflora* LEAF AGAINST *Trypanosoma cruzi* "

ETHNOPHARMACOLOGICAL STUDY ON EFFICACY AND SAFETY OF THE HYDROMETHANOLIC EXTRACT OF *Myrciaria cauliflora* LEAF AGAINST *Trypanosoma cruzi*

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Chagas Disease presents several therapeutic challenges, such as limited action and side effects of available drugs. These reveal the importance of the search, in plants natural products potentially active against protozoa for more effective and safer trypanocidal compounds. Parts of *Myrciaria cauliflora* (Myrtaceae) tree, known as jabuticabeira, have traditionally been used to treat respiratory and digestive disorders. Previous studies have reported the antimicrobial action of its leaves, related to the presence of flavonoids, alkaloids, saponins and tannins. Therefore, considering the importance of the experimental evolution of its biological activity, this study aimed to investigate the safety and efficacy of *M. cauliflora* leaf hydromethanolic extract against *Trypanosoma cruzi* bloodstream trypomastigote form. The mutagenic potential was assessed by the Ames Test, in the absence and presence of metabolic activation, using *Salmonella enterica* serovar Typhimurium standard strains (TA97, TA98, TA100, TA102, TA104). HepG2 cells were assayed for cell viability determination using WST-1 assay. The trypanocidal activity was evaluated against bloodstream trypomastigote form of *T. cruzi* (Y strain, DTU II). Mutagenic effects were detected at the highest concentration tested (500 µg/plate), in the presence of exogenous metabolization in the TA100 strain, indicating base pair substitution damage, from G:C to A:T. The extract exhibited significant trypanocidal activity over a range of safe concentration for HepG2 cells (EC_{50} 2h: 4.8-11.4 µg/mL EC_{50} 24h: 4.7-10.5 µg/mL), about ten times lower than the concentration required to produce a hepatotoxic effect (EC_{50} 24h: 101 µg/mL). Mutagenic and trypanocidal potential may be related to the dose-response dependent redox imbalance of flavonoids, which in high concentrations no longer act as antioxidants and become pro-oxidative agents, damaging the DNA and altering the parasite redox balance. Furthermore, some phenolic compounds found in the extract, might have the ability to complex with parasite macromolecules, such as membrane lipids and proteins, leading to function loss. In summary, the data demonstrate that the *M. cauliflora* leaf extract presented *in vitro* trypanocidal activity in concentrations with high probability of safety for the human consumption, emerging as a potential source of active natural products against Chagas Disease etiological agent.

Financial Support: CNPq, CAPES, FAPERJ, FIOCRUZ, UERJ, UNIRIO.

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Mutagenesis and Human and Animal Health

NATÁLIA CHERMONT DOS SANTOS MOREIRA

"NEURONAL DIFFERENTIATION AND NEUROPROTECTIVE EFFECTS OF NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITORS DESIGNED FOR ALZHEIMER'S DISEASE THERAPY "

NEURONAL DIFFERENTIATION AND NEUROPROTECTIVE EFFECTS OF NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITORS DESIGNED FOR ALZHEIMER'S DISEASE THERAPY

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Alzheimer's disease (AD) is characterized by a progressive loss of episodic memory related to β-amyloid (Aβ) peptide aggregation and abnormal phosphorylation of the tau protein, leading to the loss of cholinergic function. Inhibitors of acetylcholinesterase enzyme (AChEI) are the main class of drugs used in AD therapy. Thus, the objective of this work was to evaluate the neurodifferentiation and neuroprotective effects of two hybrid molecules (TA8Amino and TAHB3, AChEI compounds) of tacrine-donepezil in SH-SY5Y cells. Firstly, cytotoxicity and hepatotoxicity assays were performed at different times (24, 48, 72, and 120h) in SH-SY5Y and HepG2 cells, respectively. Neuronal differentiation assays, protein expression, cell cycle, cell proliferation, mitochondrial changes and oxidative stress were performed on SH-SY5Y cells. Further, viability and cell death assays were performed to evaluate the neuroprotection ability of the hybrids. The compounds did not present cytotoxic effects or high hepatotoxicity and did not alter cell viability at the following concentrations: TA8Amino 0.0035 to 0.112μM and TAHB3 0.088 to 2.84μM, but both compounds were able to induce neuronal differentiation and neuritogenesis, compatible with the increase of β-III-Tubulin expression in cells treated with TA8Amino, which also induced the production of intracellular and mitochondrial ROS. While the hybrids increased SOD1 expression, they did not change the mitochondrial membrane potential and mitochondrial mass, indicating that drug-induced oxidative stress did not generate damage due to mitochondrial dysfunction in differentiated cells. Changes in PTEN $_{(Ser380 / Thr382 / 383)}$, AKT $_{(Ser473)}$ and COX2 protein expression indicate the involvement of the PI3K/ AKT/ COX2 pathway, which is related to neuronal differentiation. Furthermore, TA8amino and TAHB3 showed a neuroprotective effect against the neurotoxic damage induced by the Aβ peptide. In general, the results demonstrate that TA8amino e TAHB3 have advantages as potential drugs, since they are not cytotoxic at concentration levels that inhibit AChE enzyme, besides being able to induce neuronal differentiation, neuritogenesis and neuroprotection, unlike donepezil and tacrine, tested alone.

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GIORGIO SCHREIBER – FUNDAPE PRIZE

YOUNG SCIENTISTS - ORAL PRESENTATION

Genomic Instability and DNA Repair

FRANCIELE BUSATTO

“TOPOISOMERASE II INHIBITORS-INDUCED LESIONS - AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS”

TOPOISOMERASE II INHIBITORS-INDUCED LESIONS – AT THE CROSSROADS BETWEEN NER AND DSB REPAIR PATHWAYS

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Key-words: *Topoisomerase II inhibitors, NER, double-strand breaks*

Topoisomerase II inhibitors form stabilized drug-DNA-topoisomerase complexes. Processing of these complexes creates DNA double-strand breaks (DSBs). We have already shown that the Nucleotide Excision Repair (NER) pathway is involved in this process. Therefore, the aim of this study was to understand the role of CSB, a NER protein, in response to TopoII inhibitors, and the relation with DSB repair pathways. U2OS cells were transfected with siRNAs for different NER genes, treated with TopoII inhibitors and followed by 53BP1 and $_{\gamma}$H2AX foci IF analysis; the influence of NER knockdowns in the Homologous Recombination (HR) rates was done using a CRISPR based reporter assay. To evaluate the interaction of CSB and TopoII a co-IP was performed in cells treated with the same drugs. A DRIP-qPCR was performed to investigate R-Loops formation in siERCC6 and siTOP2α/β cells with and without the treatments. IF analysis showed an increase in 53BP1 and $_{\gamma}$H2AX foci after MXT treatment, but there was no difference between NER knockdowns (siERCC6, siXPC and siXPA) and the siCTRL. Interestingly, we observed an increase in HR in siXPC cells. We detected a co-IP of TopoII and CSB in response to MXT treatment. In DRIP-qPCR analysis, we found an R-Loops increase in some locus after siTOP2α/β, but not after TopoII inhibition. Our results indicate an interaction between CSB and TopoII, which could explain the implication of CSB in DSBs repair pathway in response to TopoII inhibitors induced lesions.

Financial Support: CAPES, CNPq, FAPERGS

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Modulation of Mutagenicity and Nutrigenomics

JESSICA ELLEN BARBOSA DE FREITAS LIMA

"CHRONIC HYPERGLYCEMIA INDUCES DNA DAMAGE AND OXIDATIVE STRESS WHICH CAN BE DIMINISHED BY DIETARY RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS"

CHRONIC HYPERGLYCEMIA INDUCES DNA DAMAGE AND OXIDATIVE STRESS WHICH CAN BE DIMINISHED BY DIETARY RESTRICTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Type 2 diabetes (T2D) is mainly characterized by hyperglycemia, and high glucose levels can lead to several cellular and molecular alterations related to oxidative stress and DNA damage. Considering the impact of obesity in diabetic complications, dietary restrictions (DR) has been recommended for T2D patients. Furthermore, there is evidence that DR positively affects the longevity extension and reduces age-related diseases. The aim of this study was to assess the effects of chronic hyperglycemia on molecular signaling pathways that are linked to oxidative stress, DNA repair, insulin resistance and inflammation, in addition to analyze parameters of oxidative stress at the mitochondrial level and nucleotide pool in mononuclear cells of T2D patients, evaluated before and after a protein-restricted diet (0,8g/kg/day). T2D patients with chronic hyperglycemia (without intervention) compared to healthy individuals were also evaluated. We observed that following a period of 4 weeks, the protein-restricted diet led to decreased levels of DNA damage (comet assay) in patients with T2D (n=7). Regarding the gene expression profiles evaluated for target genes (PCR array), a significant difference was not observed between “before” and “after” the DR intervention. Only a slight tendency of up-regulation was observed for *PI3K*, *PRKAA1* and *ATM* genes. For some patients there was an induction of several genes (*OGG1, MUTYH, SOD1, FOXO3, NUDT1*) with roles in DNA repair and responses to damage. In addition, it was observed that in general, T2D patients with hyperglycemia (without intervention) (n=17) presented increased levels of oxidized bases in the nucleotide pool when compared to controls (n=10). However, the intervention period was not sufficient to exert alterations in patients submitted to DR. Regarding mitochondrial analysis, significant alterations in the quantification of ROS, membrane potential and mitochondrial mass were not observed between groups of T2D patients (n=15) and healthy individuals (n=15). Thus, chronic hyperglycemia promotes an oxidative stress condition in T2D patients, and DR during 4 weeks was found efficient in significantly reducing DNA damage levels in patients with T2D, in parallel with improvements in glycemic rates, and other clinical parameters, but changes in transcript gene expression and oxidized base levels were not observed after the intervention period.

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Environmental Mutagenesis

DIEGO ALEM

"CISPLATIN SENSITIZATION BY VIOLACEIN EXTRACTTED FROM ANTARTIC JANTINOBACTERIUM IN HELA CELLS"

Coautores: Saravia V, Castro-Sowinksi S, and Martinez-Lopez W

CISPLATIN SENSITIZATION BY VIOLACEIN EXTRACTTED FROM ANTARTIC *JANTINOBACTERIUM* IN HELA CELLS

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Cervical cancer is the fourth most common cancer in women around the world, and the seventh overall. Also it is the second most common cancer in women in less developed regions and the fifth cause of death by cancer in these countries. Cisplatin (CDDP) is used as a systemic treatment for advanced, persistent or recurrent cervical cancer, but its high toxicity and production of unwanted side effects (severe kidney problems, allergic reactions, decreased immunity and propensity to infections, gastrointestinal disorders, bleeding and hearing loss, among others), besides the development of resistance to CDDP, turn necessary the search of new drugs with greater therapeutic efficacy and/or fewer side effects. Violacein, a pigment produced by many bacterial strains, has demonstrated its antiproliferative activity in several derived cancer cell lines, therefore, it is important to improve its biotechnological production process. In this respect, it has been optimized the Violacein production from an Antarctic *Jantinhobacterium*, through the evaluation of different temperatures and growth bacteria medium conditions. First we determined the growth temperature for Violacein maximal production, confirming that Violacein should be produced at 20°C. Besides, we evaluated the conditions to obtain the highest yield of Violacein using different amount of TSB media (3 g/L, 15 g/L and 30 g/L) and different potential inducers such as glycerol (1 and 5 %), tryptophan (10 and 25 mM), sodium butyrate (10 mM and 25 mM), Tween 80 (1 and 5 %) and glucose (1.8 and 3.6 g/L), at 20°C and 200 rpm (initial OD of 0.05 at 660nm). Firstly, the temperature and grow medium were selected (3 g/L TSB plus glucose 3.6 g/L), which increased 10.41 times Violacein production. Subsequently, through scaling up to a 5 L bioreactor we were able to obtain a final concentration of 77.3 mg/L. Finally, Violacein anti-proliferative activity in HeLa cells was evaluated reaching an IC 50% = 0.42 µM. Moreover, Violacein in combination with 5 µM CDDP decreased the IC 50% to 0.22 µM. On the other hand, the addition of 0.15 µM of Violacein to CDDP allowed the reduction of the IC 50% to 8.34 µM (being the IC 50% of CDDP alone 17.62 µM). Violacein showed an antiproliferative effect in HeLa cells, acting as well as a sensitizer of HeLa cells to CDDP. These results are promising in the use of Violacein as anticancer or sensitizer to CDDP in cervix cancer.

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Environmental Mutagenesis and Genomics Society

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ALEXANDER HOLLAENDER PRIZE

BEST POSTER

Mutagenesis and Human and Animal Health

ORLANDO SOARES LOUZADA NETO

"EPIDEMIOLOGIC AND IN SILICO DATA SHOWS XRCC4 GENE VARIANT AS A RISK FACTOR TO CHROMOSOMAL REARRANGEMENTS INVOLVED IN LEUKEMOGENESIS IN INFANTS"

Coautores: Oliveira DN, Albano, RM, Pombo-de-Oliveira MS, Rossini A and BCSGIAL

EPIDEMIOLOGIC AND *IN SILICO* DATA SHOWS *XRCC4* GENE VARIANT AS A RISK FACTOR TO CHROMOSOMAL REARRANGEMENTS INVOLVED IN LEUKEMOGENESIS IN INFANTS

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Acute leukemias are the most common and severe group of neoplasms during childhood. Infant acute leukemia (i-AL; up to 24 months old) is characterized by a high frequency of *KMT2A* rearrangements (*KMT2A*-r, previously known as *MLL*-r) both in infant acute lymphoblastic leukemia (i-ALL) and infant acute myeloid leukemia (i-AML). *KMT2A* is an important regulator gene of hematopoietic tissue differentiation during embryogenesis and several evidences point to the origin of rearrangements during fetal life. Previous investigations highlighted maternal exposure to xenobiotic compounds during pregnancy as a risk factor for i-AL and *KMT2A*-r, specially inhibitors of topoisomerase II like flavones and isoflavones of diet, benzoquinones of tobacco smoke and drugs such as quinolones, sodium dipyrone and estradiol. The inhibition of topoisomerase II could lead to double strand breaks (DSBs) on DNA and its incorrect repair may be responsible for chromosomal translocations. Here, we analyzed the risk for i-AL and the non-homologous end joining's (NHEJ) *XRCC4* intron 3 indel (30 bp) polymorphism rs28360071. Using data from the Brazilian Collaborative Study Group of Infant Acute Leukemia (2000-2013) the genotypes were determined by PCR from samples of 592 individuals (up to 24 months old): 292 cases (i-ALL =173; i-AML = 119) and 300 controls. Calculations of allelic/genotypic frequencies and Hardy-Weinberg equilibrium were determined using GenePop 4.2; p-values and odds ratios (OR) were calculated using SPSS Statistics 22.0. Bone marrow expression of *XRCC4* was performed by qPCR. *XRCC4* cDNA and polymorphic region of rs28360071 sequencing were performed on Applied Biosystems 3500 Genetic Analyzer. We found an increase of risk for i-ALL in patients harboring *KMT2A*-r (codominant model IIxID: OR = 2,23, CI: 1.17-4,25, p = 0.014). There was a higher expression of *XRCC4* among patients with ALL than among patients with AML, but this was not related to the genotypes of rs28360071. Human Splicing Finder software pointed to the activation of a cryptic splicing site on intron 3 due to the deletion allele. However, sequencing and melting curve of the amplification fragment of *XRCC4* cDNA didn't show any abnormalities. Our results suggest that *XRCC4* intron 3 indel variant may have a role on *KMT2A* rearrangement generation and leukemogenesis in i-ALL patients.

Financial Support: CNPq, CAPES and FAPERJ

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ALEXANDER HOLLAENDER PRIZE

BEST POSTER

Modulation of Mutagenicity and Nutrigenomics

VICTOR CONSTANTE OLIVEIRA

"MODULATORY EFFECTS OF BETULINIC ACID ON THE MUTAGENICITY INDUCED BY URETHANE IN WING SOMATIC CELLS OF Drosophila melanogaster"

Coautores: Naves MPC, Morais CR, Constante SAR, Orsolin PC, Tavares DC and Spanó MA.

MODULATORY EFFECTS OF BETULINIC ACID ON THE MUTAGENICITY INDUCED BY URETHANE IN WING SOMATIC CELLS OF *Drosophila melanogaster*

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Key-words: Antimutagenicity, Cytochrome P450, CYP6A2, Somatic mutation and recombination test, SMART.

Betulinic acid (BA) is a naturally occurring pentacyclic triterpenoid found in several globally distributed plant species. BA has several different properties, such as being antiretroviral, antimalarial, and anti-inflammatory, as well as being a prospect as an anticancer agent, by topoisomerase inhibition. Urethane (URE) is a known promutagen with genotoxic potential in *Drosophila*, being both dose response and dependence associated to metabolic activation. The aim of the present study was to evaluate the mutagenicity and recombinogenicity of different concentrations of BA, in two sets of experiments, as follows: BA alone and BA in combination with URE. Tests were performed by using Standard (ST) and High Bioactivation (HB) crosses of the wing Somatic Mutation and Recombination Test in *Drosophila melanogaster*. In the first experiment, BA (0.0312; 0.0625; 0.125; 0.25 or 0.50 mg/mL) alone was examined for mutagenicity and recombinogenicity, and as for the second one, containing BA plus URE (10 mM), the antimutagenicity property was analyzed. Negative (ultrapure water) and positive (URE 10 mM) controls were included in both experiments. The results showed that BA is metabolized by the Cytochrome P450 enzymes (especially CYP6A2), which are present in the insecticide-resistant strains [OR(R)] at higher concentrations when compared to insecticide-sensitive (flr^3) strains. BA alone did not significantly increase the frequency of mutant spots in both ST and HB crosses. URE significantly increased the frequency of all mutant spot categories in both crosses. URE predominantly acts by inducing mutational events rather than mitotic recombination. BA significantly reduced the total frequency of mutant spots when co-administered with URE, in the three highest concentrations in the ST cross (0.125; 0.25 and 0.50 mg/mL) and in all concentration in the HB cross. The results show that under experimental conditions, BA has modulatory effects on the mutagenicity induced by URE in somatic cells of *D. melanogaster*. We suggest that the modulatory effects of BA may be due to its anti-inflammatory and anti-oxidant capacities, besides its apoptotic induction.

Financial Support: CNPq, CAPES, UFU, UNIPAM.

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ALEXANDER HOLLAENDER PRIZE

BEST POSTER

Genomic Instability and DNA Repair

FERNANDA TEIXEIRA ROWIES

"MOLECULAR CHARACTERIZATION OF DNA REPAIR IN MOUSE OLFACTORY NEURONS"

Coautores: Malnic, Bettina; Souza-Pinto, Nadja Cristhina

Molecular Characterization of DNA Repair In Mouse Olfactory Sensory Neurons

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Key-words: DNA repair, olfactory sensory neurons, neurodegeneration.

Olfactory Sensory Neurons (OSNs) undergo neurogenesis and migration through the olfactory epithelium (OE) during maturation process; therefore, their lifespan is shorter than most neurons within the brain. Olfactory dysfunction has been reported as an early clinical symptom in many neurodegenerative diseases, which are associated with impaired DNA repair. Whether DNA repair mechanisms play a role in OSNs genomic maintenance, however, is still unknown. Our study aims to characterize DNA repair pathways in precursor and mature OSNs. For that, we analyzed data from two different transcriptomes to characterize the expression pattern for DNA repair proteins with age and stage of neuronal differentiation. In order to confirm the results obtained from the in silico analysis, we performed RT-PCR for selected DNA repair targets from newborn (4-7 days) and 3-week-old mice OE samples. The in silico analysis suggested that most DNA repair pathways are reduced in young mice, when compared to newborns. On the other hand, non-homologous end joining (NHEJ) pathway is apparently increased, which may indicate a preference for non-homologous repair pathways for repairing double strand breaks due to lack of homologous sister-chromatids in differentiated cells. However, RT-PCR did not detect significant changes in DNA repair gene expression levels between the ages. Since OSN are constantly exposed to damage by atmospheric oxygen and other toxic molecules present in the air, we hypothesized that these neurons would accumulate more DNA damage than those in the central nervous system (CNS). To test this hypothesis, we performed a long extension PCR assay comparing OE, olfactory bulb (OB) and non-olfactory neural tissue (CE) from 3-week-old mice, but we found no significant differences in the long/short fragment amplification ratio. Although our results are merely descriptive, they have never been reported before, and can be used to support new research on OSN genome maintenance and integrity.

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MUTATION RESEARCH PRIZE

BEST POSTER

Environmental Mutagenesis

FRANCISCO CARLOS DA SILVA JUNIOR

"RETENE, A NON-PRIORITY HYDROCARBON, IS ABLE TO GENERATE OXIDATIVE STRESS, MUTAGENIC EFFECTS, AND CELL DEATH"

Coautores: Peixoto MS, de Oliveira Galvão MF, Roubicek DA, de Oliveira Alves N and Batistuzzo de Medeiros SR

RETENE, A NON-PRIORITY HYDROCARBON, IS ABLE TO GENERATE OXIDATIVE STRESS, MUTAGENIC EFFECTS, AND CELL DEATH

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Retene (1-methyl-7-isopropylphenanthrene; RET) is the most abundant polycyclic aromatic hydrocarbon (PAH) released upon the burning of cellulose, although it is not considered as one of the priority PAHs and is not included for risk assessments by the US Environmental Protection Agency (US-EPA). Despite its abundance, there are only a few studies elucidating the toxic effects of RET. Therefore, the aim of this work was to evaluate whether RET plays a crucial role in the induction of oxidative stress through the generation of reactive oxygen species (ROS) by showing mutagenic activity *in vitro* and, in cell death processes (apoptosis and necrosis). Thus, human lung adenocarcinoma A549 cells were treated with different concentrations of RET as follows: 3.3 ng/mL, 10 ng/mL and 30 ng/mL for 24 and 72 hours. The oxidative stress analysis was performed using flow cytometry. In addition, cytokinesis-block micronucleus assay (CBMN) was adopted to verify the frequency of micronuclei (MN), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs). Cell death was investigated by labeling Annexin V and propidium iodide (PI). Traditional plate-incorporation *Salmonella* mutagenicity assay was performed to evaluate the RET mutagenicity using the *Salmonella* strains TA98, TA100, TA97a and TA102, with or without activation metabolic (S9 mix). Our results revealed that RET was able to hyperpolarize the mitochondrial membrane and increase the contents of mitochondria, owing to an increase in the production of mitochondrial superoxide and intracellular ROS. Moreover, RET does not induce base-pair substitution (TA100) and frameshift (TA98 and TA97a) and transition/transversion (TA102) mutations. However, treatment with RET led to a significant increase in the frequency of the formation of MN, NPBs, and NBUDs. There was no significant difference in the viability of cells exposed to RET for 24 hours. In contrast, 72 hours of treatment with RET was adequate to decrease viability, mainly due to necrosis. Taken altogether, the results of our study provide new evidence suggesting that RET promotes oxidative stress, contributes to the processes of genomic instability and favors necrosis. Thus, we highlight the importance of including RET in routine environmental analyses in the future as a potential risk factor involved in complex diseases and carcinogenesis. Studies concerning the ability of RET to transform cells are being conducted.

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MUTATION RESEARCH PRIZE

BEST POSTER

Environmental Mutagenesis

MELISSA ROSA DE SOUZA

"THE INFLUENCE OF POLYMORPHISMS IN DNA DAMAGE, TELOMERE LENGTH AND GLOBAL DNA METHYLATION EVALUATED IN OPEN-CAST COAL MINING WORKERS"

Coautores: Kahl VFS, Rohr P, Kvitko K, Cappetta M, Lopes WM, Simon D, and Da Silva S.

THE INFLUENCE OF POLYMORPHISMS IN DNA DAMAGE, TELOMERE LENGTH AND GLOBAL DNA METHYLATION EVALUATED IN OPEN-CAST COAL MINING WORKERS

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Coal plants represent one of the main sources of environmental pollution due to the combustion process of this mineral and the consequent release of gases and particles capable of penetrating water sources which, in significant quantities, can lead to a potential risk to health and the environment. The susceptibility of individuals to the genotoxic effects of coal mining can be modulated by genetic variations in the xenobiotic detoxification and DNA repair processes. The aim of this study was to evaluate if xenobiotic metabolism polymorphism (GSTP1 *Ile105Val*, GSTT1, GSTM1 and CYP1A1 *Ile462val*), base excision repair polymorphisms (hOGG1 *Ser326Cys* and XRCC1 *Arg194Trp*), and non-homologous end joining repair pathway polymorphism (XRCC4 *Ile401Val*), could modify individual susceptibility to genomic instability and epigenetic alterations induced in workers by occupational exposure to coal. In this study, polymerase chain reaction (PCR – RFLP) was used to examine the polymorphic sites. The reactions, as well as the enzymes used, followed the literature. The sample population comprising 70 coal mine workers and 71 workers non-exposed to coal. The exposed workers were sampled in Candiota (Rio Grande do Sul, Brazil) open-cast coal mine, where they were involved in extraction and transport of coal to storage centers. The non-exposed group consisted of individuals from the same area. Our results demonstrated the effect of individual genotypes on different biomarkers (DI, MN, NBUD and NPB in lymphocytes, buccal MN, telomere length, and % of global DNA methylation) evaluated in the non-exposed and exposed groups. Significant decrease in % of global DNA methylation were observed in CYP1A1 *Val/-* exposed individuals compared to CYP1A1 *Ile/Ile* individuals (P= 0.0369). Coal workers who carried the XRCC4 *Ile/Ile* genotype showed decrease NBUD frequencies (P= 0.0085), while the XRCC4 *Thr/-* genotype was associated with decrease in Buccal MN cells for the group not exposed (P= 0.0029). No influence of the other polymorphisms studied was observed.

Thus, the current study reinforces the importance of considering the effect of metabolizing and repair variant genotypes on the individual susceptibility to incorporate DNA damage, as these processes act in a coordinated manner to determine the final response to coal exposure. Results of this study contribute to the understanding of DNA damage mechanisms induced by complex exposure of open-cast coal miners.

Financial Support: CNPQ, FAPERGS, ULBRA

MUTATION RESEARCH PRIZE

BEST POSTER

Mutagenesis and Human and Animal Health

LUIZA MARTINS LONGARETTI

"EVALUATION OF ANTIGENOXIC AND ANTIMUTAGENIC EFFECTS OF PRE-TREATMENT WITH MELATONIN IN A MODEL OF MELANOMA SKIN CANCER IN MICE"

Coautores: Luciano JA, Rigo FK, Steiner BT, Strapazzon G, Damiani AP and Andrade VM

EVALUATION OF ANTIGENOXIC AND ANTIMUTAGENIC EFFECTS OF PRE-TREATMENT WITH MELATONIN IN A MODEL OF MELANOMA SKIN CANCER IN MICE

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Key-words: Melanoma; B16F10 cells; Melatonin; DNA damage; antioxidant.

Melanoma is an aggressive skin cancer, originating of the melanocytes, and can be disseminated by the primary tumor, with a risk of metastasis to lung, liver and cortex, causing mutagenic effects on DNA. The melatonin is an endogenous hormone, identified as a free radical scavenger, with an ability to protect the DNA, and has antiproliferative effects in melanoma cells. Thus, the aim of the present study was to evaluate the effects of B16F10 melanoma cells and the effects of melatonin supplementation on genotoxic parameters in mice submitted to animal model of melanoma cancer. In this study thirty-two male C57Bl/6 mice, were divided in four groups: PBS + vehicle (n=6), melanoma + vehicle (n=10), PBS + melatonin (n=6) and melanoma + melatonin (n=10). The melanoma groups received a B16F10 cell injection and the melatonin was administered during 60 days. After treatment, measurement of tumor and metastasis were evaluated. The DNA damage was determined in peripheral blood, lung, liver and cortex using the comet assay and bone marrow for micronucleus test. Results demonstrated there was no founded metastasis in lung, liver and cortex, this result may be attributed to the type of analysis performed because we only evaluated metastasis by visual observation. The B16F10 cells were effective to induce DNA damage in all tissues analyzed and melatonin supplementation decreased these damages in blood, liver and cortex it is because this hormone exerts strong anti-tumor activity via several mechanisms, including anti-proliferative, pro-apoptotic effects and antioxidative properties. However, was not found the same results in the lung, the hypothesis is because the melatonin can be induced apoptosis in cancer cell, effect not evaluated by the comet assay. In conclusion, this study has evidence B16F10 cells is effective to induce genotoxicity and mutagenicity and melatonin can reduce the damages.

Financial support: FAPESC, UNESC.

MUTATION RESEARCH PRIZE

BEST POSTER

Modulation of Mutagenicity and Nutrigenomics

MAIARA PEREIRA

"VITAMIN C AND ACETYLSALICYLIC ACID REDUCE COLON CARCINOGENESIS IN ANIMAL MODEL"

Coautores: Beretta A C L, Schneider V O, Ostermann R A B, Longaretti L M, Bazo A P, Frajacomo F T T and Andrade V M

VITAMIN C AND ACETYLSALICYLIC ACID REDUCE COLON CARCINOGENESIS IN ANIMAL MODEL

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Key-words: Aberrant crypts; Acetylsalicylic Acid; Chemoprevention; Colorectal Cancer; DNA damage; Vitamin C.

Colorectal cancer (CRC) is one of the major causes of morbidity and mortality, principally in most developed countries. Each year, 1.3 million new cases of CRC are diagnosed and 650,000 people died of CRC. Therefore, chemopreventive actions can be applied or tested in these experimental models, with the purpose of interrupting the change or the course of the pathological process Many compounds chemical have been identified as potential chemopreventive agents, among them, vitamins and synthetic compounds. Among the vitamins stands out vitamin C (VC), that present antioxidant property and contributes to the reduction of oxidative damage in DNA and the development of cancer. In relation the synthetic compounds, stand out the acetylsalicylic acid (ASA), recent epidemiological studies and clinical trials indicate that long term use of ASA could possibly decrease the incidence of certain malignancies, including CRC. Considering the effects of VC and ASA on DNA and in the process of tumorigenesis of many cancers, the aim of the present study was to evaluate the chemopreventive effects of VC and ASA in an animal model of CRC. Were used 50 adult Balb-c mice divided into five groups: G1: Water + Ethylenediaminetetraacetic acid (EDTA); G2: Water + 1,2-Dimethylhydrazine (DMH); G3: VC + DMH; G4: ASA + DMH; and G5: VC + ASA + DMH. The animals received VC in the concentration of 500 mg/L and ASA in the dose 6 mg/kg per day and body weight, both were diluted in the drinking water. To induce colorectal carcinogenesis, the animals received four subcutaneous injections of DMH, 40 mg/kg body weight each injection. At the end of the treatment, were collected blood samples for the realization of comet assay and after the animals were euthanized for the dissection of the liver and colon for the comet assay and aberrant crypt foci assay respectively. The results of the comet assay showed that the ASA and the VC + ASA decreased DNA damage caused by DMH in blood, and in the liver only the ASA presented these decrease. In the aberrant crypt foci assay, the results demonstrated that the ASA and VC + ASA decreased the number of Aberrant crypt foci and the VC, ASA and VC + ASA decrease significantly the number of aberrant crypts in the colon of animals submitted to the colorectal carcinogenesis. In conclusion, the results showed the chemopreventive effects of VC and ASA in an experimental model of colorectal carcinogenesis.

Financial support: UNESC and CAPES.

MUTATION RESEARCH PRIZE

BEST POSTER

Genomic Instability and DNA repair

JAMES EDUARDO LAGO LONDERO

"COMPUTATIONAL ANALYSIS OF AMPHIBIAN PHOTOLYASES"

Coautores: Feltrin RS, Segatto ALA, and Schuch AP

COMPUTATIONAL ANALYSIS OF AMPHIBIAN PHOTOLYASES

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About 40% of existing amphibian species are threatened with extinction, and several hypotheses have been proposed to explain this decline, including the genotoxic effects of increased incidence of solar UV radiation. In order to minimize the harmful effects of UV-induced pyrimidine dimers on their genome, amphibians have mechanisms capable of repairing them, such as photorepair, which involves the action of photolyase enzymes. This work evaluated evolutionary and structural aspects of the primary structures of the family of photolyases [(photolyases (PHR) and cryptochromes (CRY)] of amphibians. First, primary protein sequences of the photolyases family were obtained from the NCBI protein section. After, the BLASTP tool was used to obtain similar protein sequences from the selected protein sequences mentioned above. The RefSeq Protein (RSP) database was used as a "search set" and three species with available RSP sequences were selected in the section "organism section". The TBLASTN tool was used and the database "Transcriptome Shotgun Assembly (TSA)" was marked as a "search set" to search for a nucleotide database in other 25 species of amphibians from our protein sequences. The score of 200 and the *E*-value of 10^{-6} were determined as lower limits for sequence selection in BLAST results. The occurrence of specific enzymes, conservation of residues, and similarity between sequences were evaluated in the obtained sequences. Similar sequences of CRY-DASH, CPD PHR, CRY1, CRY2, 6-4 PHR and CRY4 were found in the amphibian species in the respective percentages: 89, 82, 73, 61, 61 and 25%. In addition, 38% of the analyzed species presented four types of proteins at the same time, although other species presented one (3%), two (14%), three (17%), five (14%) or six (14%) types. In general, sequences of CPD PHR and similar formed a sister group from all the rest (6-4 PHR and CRYs) presenting the biggest difference in the composition of the residues. Additionally, the results indicate that some sequences are poorly annotated. The computational characterization of the evolutionary and structural aspects of the PHR/CRY family in amphibian species presented in this work may shed light on new hypotheses, since several aspects of the PHR/CRY family along the tree of life are controversial and unknown yet.

Financial Support: CNPq, CAPES

ROYAL SOCIETY OF CHEMISTRY

MUTAGEN-BRASIL PRIZE

BEST POSTER

Environmental Mutagenesis

LUIZA ARAÚJO DA COSTA XAVIER

"ANALYSIS OF GENOME OXIDATIVE DAMAGE IN INHABITANTS OF A MEDIUM-HIGH BACKGROUND RADIATION AREA"

Coautor: Amaral VS

ANALYSIS OF GENOME OXIDATIVE DAMAGE IN INHABITANTS OF A MEDIUM-HIGH BACKGROUND RADIATION AREA

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Keywords: 8-oxo-guanosine, ionizing radiation, exposome

In the state of Rio Grande do Norte, Brazil, the Lajes Pintadas (LP) city has high concentrations of radon (Rn), a noble gas derived from the uranium decay chain, has a half-life of 4 days and is concentrated in closed places. When inhaled, the alpha particles emitted by this gas and its decay products interact with the lung cells, can cause DNA damage and generate oxidative oxygen species from the radiolysis of the water present in cells. Ionizing radiation is a known mutagenic and carcinogenic agent. However, more epidemiological and molecular studies are needed to analyze in an integrated way several variables that make up the exposome of target populations in order to better represent and evaluate the effects and risks of chronic exposure to environmental ionizing radiation in humans. Therefore, the aim of this work is to analyze the levels of genome oxidative damage in inhabitants of Lajes Pintadas/RN, considered a medium-high background radiation area. Thus, it was performed a quantification of 8-hydroxy-2'-deoxyguanosine (8-oxoG) with the DNA/RNA Oxidative Damage ELISA Kit. For this test, it was collected urine samples from 82 adults from LP and 46 from people who live in a normal background radiation area as a control group; also, they all were interviewed with a questionnaire to obtain socio-demographic status, health history and environmental and occupational exposures information. The 8-oxoG median (and interquartile range) of the control and LP groups were, respectively, 129.33 (91.04) and 136.11 (155.08) ng/mL. The difference of 8-oxoG concentration between the groups was not significant (p-value = 0.188) and this variable was not correlated with radon levels (Rho = -0.171, p-value = 0.403). When testing 8-oxoG as dependent variable in a multiple linear regression, it was obtained a significant model with buccal cell's nuclear buds frequency (B = -0.234, p-value = 0.045), buccal binucleated cells frequency (B = -0.287, p-value = 0.019) and platelets concentration (B = 0.255, p-value = 0.032). These variables explained 25% of 8-oxoG variance [R^2 = 0.25, F (4, 59) = 4.911, p-value = 0.002]. It can be concluded that high Rn levels did not significantly influenced the genome oxidative metabolism of the subjects analyzed in this study. Only variables representing the internal exposome was capable to explain part of 8-oxoG levels. More studies are necessary to investigate which other factors are mainly responsible for genome oxidative damages.

Financial Support: *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES).

MUTAGEN-BRASIL PRIZE

BEST POSTER

Mutagenesis and Human and Animal Health

MIRLEY ALVES VASCONCELOS

“LACK OF MUTAGENICITY AND CARCINOGENICITY OF VITAMIN D3 SUPPLEMENTATION IN SOMATIC CELLS OF Drosophila melanogaster”

Coautores: Orsolin PC, Oliveira VC, Morais CR, Bonetti AM and Spanó MA

LACK OF MUTAGENICITY AND CARCINOGENICITY OF VITAMIN D3 SUPPLEMENTATION IN SOMATIC CELLS OF *Drosophila melanogaster*

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Keywords: cholecalciferol, epithelial tumor test, somatic mutation and recombination test

Vitamin D deficiency is associated with several chronic diseases, such as obesity, diabetes, rheumatic arthritis, Parkinson's, Alzheimer's, osteomalacia, osteoporosis, and cancer. Vitamin D supplementation has increased and also been associated to antineoplastic properties, with diverse effects on cancer development and progression. The metabolism and functionality of vitamin D are changed in different types of cancer though. Such changes allow a greater resistance to antitumorigenic effects of the vitamin D and contribute to the development and progression of cancer. The aim of the current study was to evaluate the mutagenic and carcinogenic potential of cholecalciferol, commonly called as Vitamin D3 (VD3), a fat-soluble secosteroid compound. Mutagenic effects were evaluated using the somatic mutation and recombination test (SMART) on wing cells of *Drosophila melanogaster*. Third-instar larvae from standard (ST) and high bioactivation (HB) crosses were treated with different concentrations of VD3 (6.25, 12.5, 25, 50, 100 mM). Water (Reverse Osmosis), 1% Tween 80 and doxorubicin (DXR 0.4 mM) were used, respectively, as negative control, solvent control and positive control. The results showed that VD3 did not induce mutation at any of the concentrations used in both crosses. The carcinogenic effect of VD3 was assayed through the Epithelial Tumour Test (ETT) in *D. melanogaster*. Third-instar larvae from the cross between naïve *wts/*TM3 females and *mwh/mwh* males, were treated with the same concentrations used in the SMART. Water (Reverse Osmosis), 1% Tween 80 and doxorubicin (DXR 0.4 mM) were used, respectively, as negative, solvent and positive controls. The results showed no significant increase in tumor, when compared to the solvent control. Therefore, our results showed that under experimental conditions, VD3 supplementation had no mutagenic and carcinogenic effects in *D. melanogaster*.

Financial support: FAPEMIG; CAPES; CNPq, UFU; UNIPAM.

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BEST POSTER

Genomic Instability and DNA Repair

BRUNO MARÇAL REPOLÊS

"DNA REPAIR MECHANISMS THAT COULD BE INVOLVED ON Trypanosoma cruzi MITOCHONDRIA"

Coautores: Morini FS; Tahara EB; Motta, MC; Franco GR; Macedo, AM; Pena, SDJ; Fragoso, SP and Machado CR

DNA REPAIR MECHANISMS THAT COULD BE INVOLVED ON Trypanosoma cruzi MITOCHONDRIA

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Key words: *Trypanosoma, cruzi,* mitochondria, dna repair, dna damage

Trypanosoma cruzi is a member of the Kinetoplastida and as so, present a single and unique mitochondria, called kinetoplast, which possesses several distinct features in comparison with other eukaryotes mitochondrias. Although several proteins of the DNA repair systems has already being described as present on the parasite mitochondria, there's no much information about the DNA repair pathways involved on the kinetoplast DNA (kDNA) maintenance. In this work we investigate possible DNA repair pathways that could be involved on T. cruzi kDNA metabolism. To first investigate the repair proteins related to the kDNA metabolism we analysed the effect of overexpressing the protein TcMYH, which is involved in base excision DNA repair in *T. cruzi*. As shown by qPCR, the overexpressor strain of TcMYH has more damages on the kDNA , when compared to the wild type strain. Also, when we used a reagent designed to specifically cause damage on the mitochondrial DNA, we could observe that this mutant is more sensitive than the WT control strain, and that the overexpression of the protein generates more AP sites on the parasite organelle. These results suggest that the parasite is able to deal with oxidative damage that attacks the kDNA. One of the unique features of the kinetoplast is the presence of specific proteins called kinetoplast associated proteins (KAP). Although some works described the location of those proteins on the antipodal sites, regions of DNA metabolism on the kinetoplast, their function are not yet clear. In this work we study the the function of the proteinTcKAP7. TcKap7 had its structure predicted using the online software I-Tasser and posseses structural similarities with the Mitochondrial transcription factor A (TFAM). Our results demonstrate that the absence of TcKAP7 is related to a long-term sensitivity to UV radiation, cisplatin and berenil; and a resistence to high levels oxidative stress on the parasite. The same phenotype has been observed for *Angomonas deanei* mutants, another trypanossomatid. Our results demonstrate that the TcKap7 and TcMYH are involved on the DNA damage response for *T. cruzi* and *A. deanei*. This results suggests that those parasites possess proteins related to the maintenance of mitochondrial DNA on T. cruzi.

Finnancial Support: FAPEMIG, CNPq, CAPES

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BEST POSTER

Modulation of Mutagenicity and Nutrigenomics

DIEGO LUÍS RIBEIRO

"THERMOGENIC STIMULANT SYNEPHRINE INDEUCES PRO-OXIDATIVE EFFECTS BY AMPK PATHWAY IN HUMAN GASTROINTESTINAL CELLS IN VITRO"

Coautores: Machado ART, Machado CS, Aissa AF, Santos PW, Barcelos GRM and Antunes LMG

THERMOGENIC STIMULANT SYNEPHRINE INDUCES PRO-OXIDATIVE EFFECTS BY AMPK PATHWAY IN HUMAN GASTROINTESTINAL CELLS *in vitro*

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Synephrine (SN) is a natural thermogenic stimulant used to weight loss. SN pharmacological action is similar to sympathomimetic amines and its chemical structure is related to amphetamines. *In vitro* and *in vivo* assays on SN side-effects and genotoxicity have reported inconsistent data. We aimed to investigate the effects of SN in normal gastric mucosa (MNP01) and colorectal intestinal adenocarcinoma (Caco-2) human cells. These cells were used as an *in vitro* model of the gastrointestinal (GI) tract since high concentrations of SN are absorbed after ingestion (30-90%). We evaluated the effects of SN on cell viability, cell proliferation, cell cycle analysis, genomic stability (genotoxicity and mutagenicity), and in the intracellular redox status (reactive oxygen species (ROS) generation, lipid peroxidation and antioxidant enzymes levels). In addition, we evaluated the expression of genes related to the energetic metabolism (AMPK), cell proliferation (PI3K/AKT), inflammation, and DNA damage response (DDR) pathways using reverse transcription quantitative PCR (RT-qPCR). Our results showed that SN did not show cytotoxicity at the concentration of 25-5000 µM. Afterward, SN at 2-200 µM demonstrated no antiproliferative neither genotoxic/mutagenic effects. Gene expression analysis showed no alterations in DDR genes corroborating the results of genotoxic tests in GI cells. Caco-2 cells showed increased cellular ROS levels after SN exposure. Furthermore, AMPK pathway-related genes, known to promote mitochondrial biogenesis, were up-regulated in both cells.These findings highlight the energetic metabolism as a possible mechanism inducing pro-oxidative effects trough AMPK pathway activation upon SN exposure. The expression of cell proliferation/inflammation pathways-related genes were also significantly altered after SN exposure in MNP01 and Caco-2 cells. Interestingly, the possible damage induced by ROS appears to have been prevented due to increased levels of gluthatione in bolth cell lines and decreased levels of catalase in MNP01 cells. This would justify the absence of cytotoxic, genotoxic and mutagenic effects. In summary, this is the first study showing that SN, at relevant concentrations for thermogenic users, promotes ROS overproduction and alter the redox state and gene expression in GI human cells *in vitro,* without inducing hazardous cellular effects. Taken together, these pro-oxidative effects are mainly related to energetic metabolism stimulation by AMPK pathway.

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Environmental Mutagenesis

BRUNA COGO BORIN

"CHARACTERIZATION OF GENOTOXIC POTENTIAL OF SUNLIGHT IN SANTA MARIA, RS, BRAZIL"

Coautores: Londero JEL and Schuch AP

CHARACTERIZATION OF GENOTOXIC POTENTIAL OF SUNLIGHT IN SANTA MARIA, RS, BRAZIL

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Key-words: UV radiation, solar incidence, genotoxicity, DNA lesions, biological dosimetry

The ultraviolet (UV) radiation emitted by the sun differs in three different types: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm). Only UVA rays and part of UVB rays cross the stratospheric ozone layer and affect the Earth's surface. Exposure of organisms to UV radiation leads to the formation of different types of DNA lesions, especially photoproducts known as Cis-syn-cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts. In addition, UV radiation can generate oxidative damage such as 8-oxoguanine (8-oxoG). These DNA lesions form the basis of mutagenic processes which can lead to development of skin cancer. In this work, the quantification of DNA lesions induced after exposure to sunlight was correlated with data of incidence of solar UV radiation in a specific locality of southern Brazil. Measurements of solar UVB and UVA radiation were taken through the use of specific radiometers, in the municipality of Santa Maria/RS (29º S). Exposure of plasmid DNA to sunlight was performed with the DNA dosimeter system, which was exposed continuously to the sun between 10:00 a.m. to 4:00 p.m., on sunny days of the different seasons of the year. For detection and quantification of sunlight-induced DNA lesions the exposed samples were incubated with the following DNA repair enzymes at 37° C for 60 minutes: T4-endonuclease V, which cleaves CPD lesions; Formamidopyrimidine-DNA glycosylase, which cleaves oxidized purines; and Endonuclease III, which cleaves oxidized pyrimidines. After electrophoresis migration in agarose gels it was possible to observe the separation of damaged DNA from undamaged DNA. The results indicate that the formation of CPD lesions is higher than the formation of oxidative damage. In general, the frequency of formation of these lesions is directly related to the dose of solar UV radiation incident on the surface, which varies according to the seasons. Therefore, the variation of solar UV radiation that reaches the Earth’s surface may result in different genotoxic impacts in exposed organisms, as well as in human health.

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Mutagenesis and Human and Animal Health

GABRIELA DOS SANTOS MARTINS

"MELATONIN HEPATOPROTECTIVE EFFECT IN THE EXPERIMENTAL MODEL OF NON-ALCOHOLIC ESTEATE-HEPATITIS"

Coautores: Miguel FM·, Marroni NP, Silva J, Picada JN, Silva JB

MELATONIN HEPATOPROTECTIVE EFFECT IN THE EXPERIMENTAL MODEL OF NON-ALCOHOLIC ESTEATE-HEPATITIS

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Melatonin (MLT) has been widely cited in different experimental models as a potent antioxidant. The term non-alcoholic fatty liver disease (NAFLD) comprises a broad spectrum of conditions associated with accumulation of lipids in the liver, ranging from steatosis to non-alcoholic steatohepatitis (NASH), and may progress to more severe forms such as fibrosis, cirrhosis and hepatocellular carcinoma. NASH is defined by the presence of inflammatory infiltrate, presenting a hepatocellular lesion (balonization). The NASH experimental model is performed with methionine and choline deficient diet (MCD). The objective of this study was to evaluate the effect of MLT on the hepatic tissue in mice with NASH, induced by MCD diet. Thirty-two male mice of the C57BL6 lineage were divided into four groups: Control (CO), CO+MLT, NASH, NASH+MLT. The animals of the NASH and NASH + MLT groups received MCD diet for 4 weeks. MLT (20 mg/kg) was administered intraperitoneally (i.p) from day 15 of the of experiment after induction of NASH until the end of the experiment. After cardiac puncture blood was collected for comet assay, hepatic tissue for histological analysis (HE and Picrossiruis), and to evaluate oxidative stress. In the evaluation of lipoperoxidation by thiobarbituric acid reactive substances (TBARS), a significant increase of the NASH group was observed when compared to the control groups and a significant reduction in the NASH + MLT group. The enzyme catalase (CAT) had a significant increase of the NASH group in relation to the control groups and a significant decrease in the NASH + MLT group. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes present a reduction in the NASH group in relation to the control groups and a significant increase in the NASH + MLT group. In the histological analysis of the liver of animals with NASH, a hepatic parenchyma, inflammatory infiltrate and presence of fibrosis are observed. In the comet assay the NASH group presented a higher frequency of DNA demage when compared to the control groups, and the NASH + MLT group presented a significant reduction in relation to the NASH group. The MCD-induced NASH generated cellular and tissue damage in the same exposed animals, observed by the increase in lipoperoxidation, altered activity of antioxidant enzymes and changes in histological evaluation. Melatonin was able to attenuate the damages oxidative stress and genotoxicity caused in this experimental model.

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Genomic Instability and DNA Repair

LAÍS YOSHIE MORIKAWA MUTA

"PRESENCE OF MRN COMPLEX COMPONENTS IN HUMAN MITOCHONDRIA AND THEIR RELATIONSHIP WITH MTDNA REPAIR"

Coautor: Souza-Pinto NC

PRESENCE OF MRN COMPLEX COMPONENTS IN HUMAN MITOCHONDRIA AND THEIR RELATIONSHIP WITH mtDNA REPAIR

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In the nucleus, DNA double strand breaks (DSBs) are predominantly repaired by the Non-Homologous End Joining and the Homologous Recombination repair pathways. In mammals, the MRN complex, formed by the Mre11, Rad50 and Nbs1 subunits, signals for subsequent DNA damage response while binds to and stabilizes DSBs for further processing. While Mre11 has been previously localized in mitochondria, mitochondrial localization of Rad50 and Nbs1 subunits has not been demonstrated and the mechanistic understanding of DSB Repair in mitochondria remains poor. Here we demonstrate, by qPCR analysis, that Hek293T cells can repair mtDNA after treatment with bleomicin, a radiomimetic which induces mostly DSBs.Analysis *in silico* using the IPSORT and Mitofates software indicate that Mre11, Nbs1 and Rad50 do not show canonical mitochondrial localization presequences. However, putative Mitochondrial Processing Peptidases (MPP) cleavage sites and Tom20 - recognizing motifs, a sequence that is recognized by Tom20 subunit of the Translocase of Outer Membrane complex (TOM), were identified in all 3 proteins. *Western Blot* analysis of mitochondria from untreated cells indicated that Nbs1 is found associated with mitochondria, but not in the mitochondrial matrix, raising the possibility that MRN complex subunits stay at intermembrane space and are translocated to the matrix upon DNA damage. Furthermore, a possible mitochondria-specific Nbs1 isoform was identified by *Western Blot* analysis in HeLa mitochondrial extracts, but the nature of this isoform still needs to be confirmed. In conclusion, (i) DSB are efficiently repaired in human mitochondria, as indicated by qPCR analysis; (ii) peptide sequences of MRN subunits indicate possible ways of importing these proteins into mitochondria and (iii) the mitochondrial localization of MRN subunits, in absence or presence of mtDNA damage, needs to be confirmed by *western blot* analysis of highly purified mitochondrial extracts from Hek293T and HeLa cells.

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Genomic Instability and DNA repair

EDUARDO KENNEDY CARRÃO DANTAS

"IN VITRO APPROACHES FOR MUTAGENICITY AND HEPATOTOXICITY INDUCED BY MARKETABLE PRE-WORKOUT SUPPLEMENTS"

Coautor: Ferreira CLS; Zanenga LF Ferraz ERA; Aiub CAF; Araujo-Lima CF and Felzenswalb I

IN VITRO APPROACHES FOR MUTAGENICITY AND HEPATOTOXICITY INDUCED BY MARKETABLE PRE-WORKOUT SUPPLEMENTS

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Currently, the practice of physical exercises has been increasing around the world. Many of these practitioners use pre-workout nutrition through dietary supplements because of their effects on the body. However, a sequence of clinical cases of hepatic damages in the hawaiin population brings suspicious about their effect to the consumer. Thus, this project consists on investigating the mutagenic and cytotoxic potential of pre-workout OxyELITE Pro, C4 and Jack3d supplements. The *Salmonella*/Microsome assay was used to determine the mutagenic potential of the supplements investigated. *Salmonella enterica* sorovar typhimurium TA97, TA98 and TA100 strains were used for the 3 products (besides TA102 and TA104 for C4). Assays were performed using five concentrations for Oxyelite and C4 (0.0005, 0.005, 0.05, 0.5, 5 mg/plate) and six concentrations for Jack3d (0.0001, 0.0005, 0.005, 0.05, 0.5, 5 mg/plate), all diluted in DMSO 10% and analyzed with and without metabolic activation (S9 mix 4%). The assays were done in triplicate and the mutagenic effect was considered in those concentrations that had the mutagenicity index (IM = induced revertants/spontaneous revertants) higher than 2. For citotoxicity, the viability assay (WST-1) was used with the human hepatocarcinoma HepG2 cell line and an alkaline phosphatase assay was performed to check for possible hepatic disorders. The percentage of cell survival in WST and the amount of alkaline phosphatase per 10,000 cells in contact with supplements at concentrations of 0 at 5 mg/ml were observed for 24h, 48h, and 72h periods. A positive result was found for mutagenicity for OxyELITE Pro and Jack3d for strains TA98 and TA100, in the presence of metabolization, and for all three supplements for strain TA 97 under the same condition. For the WST assay, cell death occured for all three products at all concentrations after 48h incubation. In the alkaline phosphatase assay, a significant increase of phosphatase in the supernatant was detected when in contact with any of the supplements analyzed after the same period of time. Those results suggest a mutagenic and cytotoxic potential that could cause liver damage.

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