

XII Congresso da MutaGen-Brasil

Associação Brasileira de Mutagênese
e Genômica Ambiental

PROGRAMAÇÃO Resumos (*Abstracts*)

28-30 de janeiro de 2016
Hotel Fazenda Quatro Estações
Indaiatuba, S.P.



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XII Congresso da MutaGen-Brasil

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<http://www.hotelfazenda4estacoes.com.br/>

Indaiatuba, S.P.

28 a 30 de Janeiro de 2016

Homepage do evento: <http://www.congressomutagen.org>

Organização: MutaGen-Brasil (Associação Brasileira de Mutagenese e Genômica Ambiental, antiga SBMCTA)

<http://mutagen-brasil.org.br>

Suporte e apoio:



MutaGen-Brasil
Associação Brasileira de Mutagenese e Genômica
Ambiental



Comissão Organizadora:

Nadja C. Souza Pinto (IQ-USP, São Paulo, SP)
Jenifer Saffi (UFCSPA, Porto Alegre, RS)
Elza Tiemi Sakamoto Hojo (FFCLRP-USP, Ribeirão Preto, SP)
Fabio Luis Forti (IQ-USP, São Paulo, SP)
Deborah Arnsdorff Roubicek (CETESB, São Paulo, SP)
César Koppe Grisolia (UNB, Brasília, DF)

Comissão científica:

Nadja C. Souza Pinto (IQ-USP, São Paulo, SP)
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César Koppe Grisolia (UNB, Brasília, DF)
Carlos F. M. Menck (ICB-USP, São Paulo, SP)
João A. P. Henriques (UFRGS, Porto Alegre, RS)
Carlos Renato Machado (UFMG, Belo Horizonte, MG)
Gisela de Aragão Umbuzeiro (UNICAMP, Piracicaba, SP)
Lucia Regina Ribeiro (UNESP, Botucatu, SP)
Catarina Satie Takahashi (FFCLRP-USP, Ribeirão Preto, SP)
Daisy Maria Favero Salvadori (UNESP, Botucatu, SP)
Israel Felzenszwalb (UERJ, Rio de Janeiro, RJ)
Vera Maria Ferrão Vargas (UFRGS, FEPAM), Porto Alegre, RS)
Juliana da Silva (ULBRA, Canoas, RS)
Vanessa Moraes de Andrade (UNESC, Criciúma, SC)
Lucymara Fassarella Agnez Lima (UFRN, Natal, RN)
Ilce Mara de Syllos Cólus (UEL, Londrina, PR)

Temas do evento

Respostas a danos induzidos no DNA; Mecanismos de reparo do DNA; Genotoxicidade de substâncias mutagênicas; Agentes mutagênicos físicos e nanomateriais; Antioxidantes, anti-mutagênese e nutrigenômica; Fatores genéticos e ambientais associados ao envelhecimento e doenças; Epigenética, mutações e câncer; Oncologia translacional e estratégias terapêuticas; Genômica ambiental e exposição dos organismos a contaminantes diversos; Ecogenotoxicologia e ensaios em organismos-modelo.

Apresentação do XII Congresso MutaGen-Brasil

O XII Congresso Brasileiro da Associação Brasileira de Mutagênese e Genômica Ambiental – Mutagen-Brasil (antiga SBMCTA) é o 12º evento organizado por essa associação científica sem fins lucrativos, a qual foi fundada em setembro de 1989 e, desde essa data, os congressos nacionais têm sido realizados regularmente a cada dois anos. Os três últimos eventos aconteceram em Ouro Preto, MG (2009), São Pedro, SP (2011) e Foz do Iguaçu, PR (2013), sendo este último, concomitante com o evento internacional 11ª ICEM (11th *International Conference on Environmental Mutagens*). Os participantes são em geral oriundos de universidades públicas e privadas, institutos de pesquisa além de empresas e órgãos dedicados a assuntos regulatórios do meio ambiente.

O evento tem caráter importante na disseminação do conhecimento e formação de profissionais brasileiros na área de Mutagênese, Carcinogênese e Genômica Ambiental, priorizando e incentivando a participação de alunos de graduação e pós-graduação, bem como de pós-doutorandos, os quais têm a oportunidade de apresentação de seus trabalhos científicos, bem como de discussões com pesquisadores conceituados e de grande prestígio na área.

Várias abordagens em pesquisa são contempladas no evento, principalmente aquelas relacionadas à ação e efeitos de contaminantes ambientais (exposição ocupacional e acidental), bem como caracterização das respostas celulares e dos organismos, além de mecanismos estudados ao nível molecular. A exposição a muitos desses agentes tem sido associada ao risco a várias doenças e ao comprometimento da saúde humana e animal, o que também será abordado no evento.

Assim, o XII Congresso da Mutagen-Brasil tem o objetivo de contribuir para a atualização e disseminação do conhecimento científico sobre temas relevantes no campo da pesquisa básica e aplicada em mutagênese, carcinogênese e genômica ambiental. Ressalta-se ainda, a importância e relevância do evento quanto à contribuição na qualidade da formação de profissionais brasileiros, ampliando suas oportunidades de sucesso profissional.

O congresso visa também oferecer aos seus pesquisadores participantes a oportunidade destes interagirem com conferencistas conceituados, o que poderá promover estudos colaborativos entre grupos de pesquisa (em âmbito nacional e internacional), contribuindo desta forma para o aprimoramento de profissionais e desenvolvimento científico.

A comissão organizadora tem a honra de externar os agradecimentos sinceros aos pesquisadores palestrantes e deseja um excelente congresso e convívio agradável a todos os participantes.

Cordialmente,
Comissão organizadora

PROGRAMAÇÃO

28/janeiro – Quinta-feira

Manhã:

- 9:00 – 12:00 – Minicurso 01 (Métodos alternativos ao uso de animais para avaliação de mutagenicidade)
- 11:00 – 12:00 – Reunião do Conselho – MutaGen-Brasil

Tarde:

- 14:00 – 15:00 – Escola MutaGen (História da mutagênese no Brasil)
- 15:30 – Abertura
- 16:00 – Homenagem ao Prof. Álvaro Leitão (UFRJ)
- 17:30 – Conferência 1 (Roger Woodgate: Posttranslational Regulation of Human DNA Polymerase I)
- 19:00 – Poster session 1 + Cocktail

29/janeiro – Sexta-feira

Manhã:

- 8:00 – 9:00 – Escola MutaGen (Mecanismos de reparo de DNA/Ensaio para medir lesões e reparo de DNA)
- 8:00 – 9:00 – Minicurso 2 (O desafio da gestão científica para o investigador acadêmico iniciante)
- 9:00 – 11:00 – Simpósio 1 (Honoring Christophe Cazaux, Coordinator: Jean-Sebastien Hoffmann)
- 9:00 – 11:00 – Simpósio 2 (Environmental contaminants, Coordinator: Deborah Roubicek)
- 9:00 – 11:00 – Simpósio 9 (MutaGen Young Talents I, Coordinator: Nadja Souza Pinto)
- 11:10 – 12:00 – Conferência 2 (Jean-Sebastien Hoffmann: High expression of POLQ, the gene encoding the DNA replication timing factor Pol θ , is strongly associated with poor clinical outcome of patients with colon, breast and lung cancers)
- 11:10 – 12:00 – Conferência 3 (Gisela Umbuzeiro: Effect directed analysis: linking cause and effect)

Almoço: 12 – 13:30

Tarde:

- 13:30 – 15:30 – Simpósio 3 (Genotoxic effects of endogenous and exogenous exposure, Coordinator: Elza T. Sakamoto Hojo)
- 13:30 – 15:30 – Simpósio 4 (DNA damage and applications of the comet assay, Coordinator: Vanessa Moraes de Andrade)
- 15:30 – 16:00 – Coffee break
- 16:00 – 17:00 – Conferência 4 (Jesper B. Andersen: Genetic analysis of mutations in human biliary tract disease and translational treatment aims)
- 16:00 – 17:00 – Conferência 5 (Guenter Speit: The Comet Assay in Human Biomonitoring – Expectations and Limitations)
- 17:30 – 18:30 – Escola MutaGen (Uso de testes de mutagenicidade como ferramenta 1 e 2)
- 19:00 – 21:00 – Poster session 2

30/janeiro – Sábado

Manhã:

- 8:00 – 9:00 – Escola MutaGen (Ciclo celular e análises por citometria de fluxo em células expostas a agentes genotóxicos/Avaliação de citotoxicidade e detecção de morte celular em resposta a danos no DNA)
- 8:00 – 9:00 – Minicurso-02 (O desafio da gestão científica para o investigador acadêmico iniciante)
- 9:00 – 11:00 – Simpósio 5 (Translational genomics and therapeutic strategies in cancer, Coordinator: Wilner Martínez-López)
- 9:00 – 11:00 – Simpósio 6 (DNA metabolism in neglected tropical disease, Coordinator: Carlos Renato Machado)
- 9:00 – 11:00 – Simpósio 10 (MutaGen Young Talents II, Coordinator: Nadja Souza Pinto)
- 11:10 – 12:00 – Conferência 6 (Ludmil Alexandrov: Signatures of mutational processes in human cancer)
- 11:10 – 12:00 – Conferência 7 (Richard McCulloch: Antigenic variation in *Trypanosoma brucei* shows a greater association with DNA replication timing than with DNA double strand break formation)

Almoço: 12:00 – 13:30

Tarde:

- 13:30 – 15:00 – Assembléia MutaGen
- 15:00 – 15:50 – Conferência 8 (Carlos F. M. Menck: Mechanisms of resistance to genotoxic chemotherapeutic agents in the fight against tumor cells)
- 15:50 – 16:15 – Coffee break

- 16:15 – 18:00 – Simpósio 7 (Kinases and DNA damage response, Coordinator: Jenifer Saffi)
- 16:15 – 18:00 – Simpósio 8 (Proteomics of proteins involved in genomic maintenance, Coordinator: Fabio Forti)
- 18:00 – 19:00 – Encerramento e entrega de prêmios

Ementas dos mini-cursos e Escola MutaGen

Minicurso 01 – Métodos Alternativos a experimentação animal em genética toxicológica – *Isabel Villela (InnVitro), Miriana Machado (UFRGS), Rhaul de Oliveira (UNB)*

Ementa: O curso irá abordar os desafios e possibilidades para a substituição de testes in vivo em estudo de genética toxicológica, incluindo o uso de modelos animais inferiores, como Zebrafish. Os tópicos abordados incluirão a situação regulatória quanto à genética toxicológica e ensaios validados e recomendados pelos órgãos reguladores. Esse tema é altamente relevante e atual, uma vez que o Brasil está implementando, de acordo com recomendação do CONCEA e aprovação da ANVISA, a substituição gradual de testes toxicológicos em mamíferos.

Minicurso 02 – O Desafio da Gestão Científica para o Investigador Acadêmico Iniciante - *Lucia R. Ribeiro (UNESP), André L.V. Sávio (UNESP), Fábio H. Fernandes (UNESP)*

Ementa: Destacar a importância da capacidade de liderança e de administração para o início da carreira científica/acadêmica e fornecer informações práticas que irão contribuir para o êxito no planejamento e gerenciamento de programas de pesquisa e ensino.

Justificativa: É esperado que um Investigador Iniciante seja capaz de selecionar pessoal, estabelecer um laboratório, planejar um programa de pesquisa coerente, obter financiamento e publicar os seus artigos nas melhores revistas de destaque internacional, além de, quase sempre, atuar como professor. Enquanto isto, ele é colocado sob uma enorme pressão para produzir "papers". Assim, o Investigador Iniciante precisará de habilidades especiais para atender a todas essas expectativas. É improvável que o mesmo tenha recebido instruções explícitas, em qualquer uma destas habilidades, na Graduação, no Mestrado, no Doutorado e no Pós doutorado. Em geral, o pouco que se aprende é, provavelmente, por "tentativa e erro", assistindo aos professores e conversando com os orientadores, mentores e colegas.

Escola MutaGen – Tópicos essenciais em metabolismo de DNA e genotoxicologia – *Catarina Takahashi (USP), Carolina Berra (USP), Daniela T. Soltys (USP), Deborah A. Roubicek (CETESB), Vera M. F. Vargas (Fundação Estadual de Proteção Ambiental Henrique Luís Roessler), Ana Paula L. Montaldi (FFCLRP-USP), Paulo Roberto D. V. Godoy (FFCLRP-USP)*

Ementa: A Escola MutaGen foi estruturada com o objetivo de fornecer conceitos básicos em mecanismos moleculares de mutagênese, formação de lesões em DNA e respostas celulares à essas, bem como apresentar e discutir as metodologias utilizadas para avaliar esses efeitos tanto no contexto de pesquisa acadêmica quando aplicados ao monitoramento ambiental. A Escola é destinada a alunos de graduação e pós-graduação, bem como a novos pesquisadores na área.

Conferências

Opening ceremony: Honoring Prof. Álvaro Leitão

João A. P. Henriques (UFRGS, Brazil)

Álvaro Leitão (UFRJ, Brazil)

Conference 1:

Roger Woodgate (NIH, USA): Posttranslational Regulation of Human DNA Polymerase δ

Conference 2:

Jean-Sebastien Hoffmann (INSERM, France): “High expression of POLQ, the gene encoding the DNA replication timing factor Pol θ , is strongly associated with poor clinical outcome of patients with colon, breast and lung cancers”

Conference 3:

Gisela Umbuzeiro (UNICAMP, Brazil): “Effect directed analysis: linking cause and effect”

Conference 4:

Jesper B. Andersen (University of Copenhagen, Denmark): “Genetic analysis of mutations in human biliary tract disease and translational treatment aims”

Conference 5:

Guenter Speit (University Clinic Ulm, Germany): “The Comet Assay in Human Biomonitoring – Expectations and Limitations”

Conference 6:

Ludmil Alexandrov (Los Alamos National Lab., USA): “Signatures of mutational processes in human cancer”

Conference 7:

Richard McCulloch (University of Glasgow, Scotland): “Antigenic variation in *Trypanosoma brucei* shows a greater association with DNA replication timing than with DNA double strand break formation”

Conference 8:

Carlos F. M. Menck (USP, Brazil): “Mechanisms of resistance to genotoxic chemotherapeutic agents in the fight against tumor cells”

Simpósios

Symposium 1: Honoring Christophe Cazaux

Coordinator: Jean-Sebastien Hoffmann

1. *Valérie Bergoglio* (CNRS, France): “Human DNA polymerase η prevents delayed replication at common fragile site and telomeres”
2. *Marie-Jeanne Pillaire* (CNRS, France): “Chk1 stability requires DNA polymerase κ in response to replication stress”
3. *Ana Paula C. Brandalize* (UCS, Brazil): “Genetic variations in translesional DNA polymerases $\text{pol}\theta$ and $\text{pol}\eta$: implications for hereditary cancer predisposition syndromes”
4. *Carlos Renato Machado* (UFMG, Brazil): “Role of *Trypanosoma cruzi* Rad51 in repairing DNA double strand breaks and oxidative lesions in in vitro and in vivo”

Symposium 2: Environmental contaminants

Coordinator: Deborah Roubicek

1. *Silvia Regina Batistuzzo de Medeiros* (UFRN, Brazil): “Mutagenic effects of products generated by cashew nuts processing”
2. *Juliana da Silva* (ULBRA, Brazil): “DNA damage, telomere length and global methylation profile as genomic instability markers in rural workers exposed to pesticides”
3. *Vera Vargas* (Fundação Estadual de Proteção Ambiental Henrique Luís Roessler, Brazil): “Biomonitoring children and the environment as a means of characterizing contaminated and reference areas”
4. *Israel Felzenszwalb* (UERJ, Brazil): “Genotoxic risk of workers exposed to heavy air pollution”

Symposium 3: Genotoxic effects of endogenous and exogenous exposure

Coordinator: Elza T. Sakamoto Hojo

1. *Siamak Haghdoost* (Stockholm University, Sweden): “Genotoxic effect of Reactive Oxygen Species- the role of intracellular nucleotide pool”
2. *Ilce Mara S. Colus* (UEL, Brazil): “Characterizing medicinal plants as therapeutic agents”
3. *Andre Passaglia Schuch* (UFMS, Brazil): “DNA damage induced by sunlight and its effects on amphibian models”
4. *Jesus Blasquez* (Instituto di Biomedicina di Sevilla, Spain): “Hypermutation and hyperrecombination as adaptative strategies in bacteria”

Symposium 4: DNA damage and applications of the comet assay

Coordinator: Vanessa Moraes de Andrade

1. *Andrew Collins* (University of Oslo, Norway): “Application of the comet assay in human biomonitoring”

2. *João Paulo Fernandes Teixeira* (Instituto Nacional de Saúde, Portugal): “The comet assay in human exposure biomonitoring”
3. *Vanessa Moraes de Andrade* (UNESC, Brazil): “Role of functional food consumption on DNA damage”
4. *Elisângela P.S. Lacerda* (UFG, Brazil): “Cytotoxicity of ruthenium(II)bipyridine complexes against Ehrlich tumor cells”

Symposium 5: Translational genomics and therapeutic strategies in cancer

Coordinator: Wilner Martínez-López

1. *Douglas V. N. P. Oliveira* (University of Copenhagen, Denmark): “Molecular pathways and therapeutic design in cholangiocarcinoma”
2. *Wilner Martínez-López* (IIBEC, Uruguay): “In vitro testing of HDACi for anti-cancer therapy”
3. *Alessandra L. Pelegrini* (USP, Brazil): “Development of a model for silencing XPF and ERCC1 genes to increase cisplatin toxicity for human tumor cells”
4. *Andre L. M. Juchem* (UFRGS, Brazil): “Antiproliferative effect of the diphenyl ditelluride in human cancer colon cells”

Symposium 6: DNA metabolism in neglected tropical disease

Coordinator: Carlos Renato Machado

1. *Maria Carolina Elias* (Insituto Butantã, Brazil): “Is replication firing control working for genomic maintenance in *Trypanosoma brucei*?”
2. *Luiz R. O. Tosi* (USP, Brazil): “Functional compartmentalization of Rad9 and Hus1 reveals diverse assembly of the 9-1-1 complex components during the DNA damage response in *Leishmania*”
3. *Catarina A. Marques* (University of Glasgow, Scotland): “Highly unorthodox chromosome duplication revealed by mapping origins of DNA replication in *Leishmania*”
4. *Isabela C. Mendes* (UFMG, Brazil): “The roles of XPC and CSB genes in DNA repair and cell cycle in *Trypanosoma cruzi*”

Symposium 7: Kinases and DNA damage response

Coordinator: Jenifer Saffi

1. *Jörg Kobarg* (UNICAMP, Brazil): “The kinases of the human Nek family: integrating DNA damage response, primary cilia function and cell cycle checkpoints”
2. *Jennifer Ann Stortz* (University of Glasgow, Scotland): “Multiple protein kinases provide diverse DNA repair-associated functions in *Trypanosoma brucei*”
3. *Dinara Moura* (UFCSPA, Brazil): “The short, but newsworthy, history of Kin3 protein from *Saccharomyces cerevisiae*”
4. *Michele Lima* (Sorbonne University, France): “Dual inhibition of ATR and ATM potentiates the activity of trabectedin and lurbinectedin by perturbing the DDR and homologous recombination repair”

Symposium 8: Proteomics of genomic maintenance mechanisms

Coordinator: Fabio Forti

1. *Lucymara F.A. Lima* (UFRN, Brazil): “Proteomic analysis in meningitis animal model submitted to adjuvant therapy with vitamin B6”
2. *Eros Lazzerini-Denchi* (Scripps Res. Institute, USA): “Telomere dysfunction and chromosome instability”
3. *Opher Gileadi* (University of Oxford, UK): “Structural biology of DNA repair: Helicases, nucleases and therapeutic opportunities”
4. *José Renato R. Cussiol* (Cornell University, USA): “A crucial and conserved role for Dpb11^{TopBP1} in DNA end resection”

Symposium 9: MutaGen Young Talents I

Coordinator: Nadja Souza Pinto

1. *Vivian F. S. Kahl* (ULBRA, Brazil): “Telomere dynamic and epigenetic status are altered in tobacco farmers”
2. *Rodrigo A. P. Martins* (UFRJ, Brazil): “*In vivo* roles of Smc1a in developing nervous system”
3. *Natalia C. Moreno* (USP, Brazil): “UVA light induces DNA damage and mutagenesis in normal cells and Xeroderma pigmentosum variant patients cells”
4. *Carolina M. Berra* (USP, Brazil): “Is mouse mitochondrial DNA protected from alkylation damage by AAG?”
5. *Francine I. Vacchi* (USP, Brazil): “Genotoxicity assessment of environmental samples containing disperse dyes and aromatic amines”
6. *Jeyson C. Lopes* (UFU, Brazil): “Assessment of genotoxic effect of copper (II) complex of 1,10-Phenanthroline and Doxycycline (CuDoxPhen) in somatic cells of *Drosophila melanogaster*”

Symposium 10: MutaGen Young Talents II

Coordinator: Nadja Souza Pinto

1. *Macelle C. G. Giron* (UNICAMP, Brazil): “Use of diagnostic strains of *Salmonella*/microsome assay to compare the mutagenicity of atmospheric particulate matter from Limeira and Stockholm”
2. *Carolina A. Lisboa* (UNB, Brazil): “Are zebrafish embryos good models for embryotoxicological assessment of antidepressants? The case of study of amitriptyline”
3. *Sinara S. Jardim* (USM, Brazil): “Transposition mechanism of *mariner-mos1* under stress conditions”
4. *Gustavo S. Kajitani* (USP, Brazil): “Ultraviolet-induced DNA damage: photorepair in the skin of DNA repair deficient mice on cell proliferation and inflammation”
5. *Danilo J. Xavier* (USP, Brazil): “Blood mononuclear cells of type-2 diabetes mellitus and Alzheimer disease patients show alterations in common related to inflammation and DNA damage response”
6. *Fernanda M. Munari* (UCS, Brazil): “Pso2 interactions with DNA damage response genes after exposure to interstrand crosslink-inducing agents in *Saccharomyces cerevisiae*”.

RESUMOS (ABSTRACTS)

CONFERÊNCIAS E SIMPÓSIOS

Conference 1

Posttranslational Regulation of Human DNA Polymerase ι

Roger Woodgate

Laboratory of Genomic Integrity, NICHD

Human DNA polymerases (pols) η and ι are Y-family DNA polymerase paralogs that facilitate translesion synthesis (TLS) past damaged DNA. Both pol η and pol ι can be monoubiquitinated *in vivo*. Pol η has been shown to be ubiquitinated at one primary site. When this site is unavailable, three nearby lysines, may become ubiquitinated. In contrast, mass spectrometry analysis of monoubiquitinated pol ι revealed that it is ubiquitinated at over 27 unique sites. Many of these sites are localized in different functional domains of the protein, including the catalytic polymerase domain, the PCNA-interacting region, the Rev1-interacting region, as well as its Ubiquitin Binding Motifs, UBM1 and UBM2. Pol ι monoubiquitination remains unchanged after cells are exposed to DNA damaging agents such as UV-light (generating UV-photoproducts), ethyl methanesulfonate (generating alkylation damage), mitomycin C (generating interstrand crosslinks), or potassium bromate (generating direct oxidative DNA damage). However, when exposed to naphthoquinones, such as menadione and plumbagin, which cause indirect oxidative damage through mitochondrial dysfunction, pol ι becomes transiently polyubiquitinated via K11- and K48-linked chains of ubiquitin and subsequently targeted for degradation. Polyubiquitination does not occur as a direct result of the perturbation of the redox cycle, as no polyubiquitination was observed after treatment with rotenone, or antimycin A, which inhibit mitochondrial electron transport. Interestingly, polyubiquitination was observed after the inhibition of the lysine acetyltransferase, KAT5/p300. We hypothesize that the formation of polyubiquitination chains attached to pol ι occurs via the interplay between lysine acetylation and ubiquitination of ubiquitin itself at K11- and K48- rather than oxidative damage *per se*.

Conference 2

High expression of POLQ, the gene encoding the DNA replication timing factor Pol θ , is strongly associated with poor clinical outcome of patients with colon, breast and lung cancers*Jean-Sébastien Hoffmann*

ONCOPOLE Toulouse – CRCT INSERM U1037, Jean-sebastien.hoffmann@inserm.fr

We have recently discovered that the human DNA polymerase θ (Pol θ) functions during the earliest steps of DNA replication and influences the timing of replication initiation¹. Pol θ binds to chromatin during early G1 of the cell cycle, interacts with the Orc2 and Orc4 components of the Origin recognition complex and controls the association of MCM helicase proteins with origins. Pol θ -depleted cells exhibit a normal density of activated origins in S phase, but Early-to-Late and Late-to-Early shifts are observed at a number of replication domains. This novel Pol θ function could be particularly important within the context of cancer as we found that excess Pol θ , strongly associated with poor clinical outcome of patients with colon, breast and lung cancers²⁻⁴, resulted mostly in a delayed timing of multiple chromosomal domains.

I will discuss how abnormal temporal control of replication might represent an adaptive mechanism responsible for cell survival upon high level of replication stress, a driving force of cancer progression, and explain therapeutic resistance towards multiple anticancer agents that target DNA replication forks.

Conference 3

Effect Directed Analysis – linking cause and effect

Gisela A. Umbuzeiro

Faculdade de Tecnologia, UNICAMP, Brazil

Effect Directed Analysis (EDA) is an approach that combines biotesting, separation and chemical analysis to identify bioactive chemicals in complex mixtures. It is one of the best ways to disclose new priority pollutants. Bioassays, like the Salmonella/microsome assay (Ames test), have been used successfully in EDA studies. But sometimes there are limitations when the samples are not potent enough to be fractionated and re-tested. With the advance of the chemical techniques in the non-target analysis, the ability to identify potential chemical pollutants in water increased substantially. As a consequence new ways of doing EDA has emerged. Virtual EDA (vEDA) is one of those approaches. The basic concept of vEDA is to combine information derived from mass spectral and bioinformatics analyses and a bioassay using multivariate statistics to isolate compounds or peaks, in case of non-target analysis that co-vary with the biological responses. It has the advantage of avoiding the first fractionation step and reducing the number of tests. We believe that linking this information with the mutagenicity results obtained from diagnostic strains that responds differently for specific classes of mutagens can be a powerful way of finding new priority pollutants. Some examples applied to air and water evaluation will be presented and discussed.

Conference 4

Identification of Unique Genetic Aberrations with known Driver Mutation Directs Targeted Therapy in Cholangiocarcinoma

Chirag Nepal, Colm J. O'Rourke, Douglas NVP Oliveira, Andrzej Taranta and Jesper B. Andersen

Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark.

Introduction: Cholangiocarcinoma (CCA) is a treatment-refractory malignancy with heterogeneous pathobiology and dismal prognosis. Several disease-causing mutations, e.g., *IDH1R132G* and *KRASG12D*, have been shown to “drive” development of this malignancy. However, a complex issue remains as to account for the heterogeneous pool of “backseat” aberrations, which in chromosomal proximity to the causative variant likely are to influence drug response. This emphasizes the urgency for understanding the molecular heterogeneity of the disease to prompt patient stratification and advance therapeutic options.

Methods: To comprehensively characterize the genomic landscape in intrahepatic CCA, we analyzed the tumors of 297 patients. Whole-exome sequencing (WES) was completed on a total of 142 patients. In the discovery screen, we performed an ultra-high coverage (>150X) analysis on paired tumor and surrounding liver tissues from 15 iCCA patients, enriched for driver mutations in *IDH1* and *KRAS*. The genetic variation (SNVs, InDels, copy number (CN) and chromosomal rearrangements etc.) was determined by three independent tools to identify highconfidence recurring variants.

Results: In the pursuit of novel treatment options, we report a comprehensive annotation of the somatic landscape in iCCA. We identified in range 20-170 somatic deleterious mutations per patient, validated the prevalent hotspot mutations (*KRASG12D* and *IDH1R132G*) and identified novel aberrations in genes e.g., *CDC27*, *PABPC3*, *BCLAF1*, *KIR2DL3*, *POTEF*, *FRG1* and *CCR5*, with no prior association to iCCA. New hotspot reoccurring mutations were found in *BCLAF1N627S*, *FRG1K258R*, *KIR2DL3C270S*. Next, we performed unsupervised clustering on patients with mutations in *KRASG12D* and *IDH1R132G* and identified a unique prevalence (~70% in total) of mutated genes specific to each driver, suggesting that this set of unique “passenger genes” distinguish subsets of patients. Observations from our WES analysis were further confirmed in a public validation set of 127 iCCA. Next, we performed a prevalence screen of 48 cancer-related genes on additional 155 iCCA patients and confirmed the deregulation of multiple potential causal pathways, including *FGFR2*, *PI3K/mTOR*, *TP53*, *CDKN2A* and *Notch1*, which were all determined in our discovery screen.

Conclusion: Our results suggest that association of “passenger” genes with a known diseasecausing alteration may reveal unique patient subsets and multiple putative novel target genes, highlighting the potency of genomics-based directions for novel therapeutics.

Conference 5

The Comet Assay in Human Biomonitoring – Expectations and Limitations

Günter Speit

Ulm University, Institute of Human Genetics, Ulm, Germany

The alkaline comet assay (single cell gel electrophoresis) is the most widely used method for measuring DNA damage in eukaryotic cells. It detects DNA strand breaks, alkali-labile sites and incisions in the course of excision repair. The comet assay is a valuable tool in basic research into mechanisms of DNA damage and repair and an accepted method in genotoxicity testing. It is also frequently used in human biomonitoring as a marker of occupational and environmental exposures to genotoxic agents. In human biomonitoring, positive results were reported for various kinds of exposures. Frequently, conflicting results were published for the same type of exposure. Experimental variation is a main reason for the large heterogeneity of comet assay data and frustrates attempts to compare studies carried out in different laboratories. This presentation will review the biological basis of the comet assay and discuss protocol features and confounding factors that may affect results, including practical issues related to sample collection, management and storage. Requirements for an appropriate assay performance in human biomonitoring will be proposed and the limitations of the assay will be critically analyzed.

Conference 6

Signatures of mutational processes in human cancer.

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All cancers are caused by somatic mutations. These mutations may be the consequence of the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA, or defective DNA repair. In some cancer types, a substantial proportion of somatic mutations are known to be generated by exposures, for example, tobacco smoking in lung cancers and ultraviolet light in skin cancers, or by abnormalities of DNA maintenance, for example, defective DNA mismatch repair in some colorectal cancers. In this talk, I will present analysis encompassing 15,131 samples across 40 distinct types of human cancer revealing more than 30 different signatures of mutational processes. Some signatures are present in many cancer types, notably a signature attributed to the APOBEC family of cytidine deaminases, whereas others are confined to a single cancer class. Certain signatures are associated with age of the patient at cancer diagnosis, known mutagenic exposures or defects in DNA maintenance, but many are of cryptic origin. The results reveal the diversity of mutational processes underlying the development of cancer, with potential implications for understanding of cancer etiology, prevention and therapy.

Conference 7

Antigenic variation in *Trypanosoma brucei* shows a greater association with DNA replication timing than with DNA double strand break formation

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Survival of the African trypanosome, *Trypanosoma brucei*, in its vertebrate hosts depends upon periodic changes in the composition of a protective Variant Surface Glycoprotein (VSG) coat. Continual switching of surface antigens is termed antigenic variation and has evolved in many pathogens to evade host adaptive immunity. In *T. brucei* several molecular processes elicit VSG switching, though the major route is recombination of VSG genes from a large silent archive into a small number of telomeric transcription loci, termed VSG expression sites. The generalist repair strategy of homologous recombination is used to catalyse VSG switching, meaning how the reaction is initiated is important, as this step may provide species-specificity that drives switching at high rates. To examine this, we have analysed the function of a RecQ-like helicase, RECQ2, which we show is recruited to and acts in the repair of DNA breaks, both genome-wide and within the VSG expression site. In contrast, loss of RECQ2 does not impair VSG switching, but causes an elevated rate of the reaction, driven by increased recombination. These data are incompatible with models for VSG switch initiation based on the direct formation of DNA DSBs; indeed, we show that an induced DSB in the VSG expression site inefficiently elicits VSG switching by recombination. To test what processes might lead to VSG switching, we mapped replication dynamics, revealing that the active VSG expression site is, uniquely among all such telomeric sites, early replicating. Moreover, we show that this differential replication timing is specific to the life cycle stage in which VSG is expressed. The specific association between VSG activity and replication timing reveals a new, plausible model for antigenic variation based on replication-derived DNA fragility of the transcribed VSG expression site.

Conference 8

Mechanisms of resistance to genotoxic chemotherapeutic agents in the fight against tumor cells

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Most of the drugs employed in tumor therapy involve the induction of DNA lesions, which are processed in the tumor cells by different cell pathways. The induction of DNA damage and their processing may restore tumor cell resistance to the drugs, and the knowledge of these processes may help us to improve the efficiency of tumor chemotherapy. We have been investigating different processes to how glioma and breast cancer cells respond to genotoxic agents such as temozolomide (TMZ), chloroethylating agents, cisplatin (CP) and doxorubicin (DOXO). Malignant glioma is a severe type of brain tumor with a poor prognosis and few options for therapy. TMZ and CP damage DNA upon different pathways, although we observed that glutathione availability on tumor cells has a decisive role on resistance to both agents. Modulating glutathione levels with BSO (a glutathione inhibitor) or NAC (a glutathione precursor) provided demonstration that it protects cells from DNA damage and cell death. Also, using *in vivo* nude mice model, we showed that the combination of BSO and low dose of TMZ and CP greatly inhibited tumor progression. In addition, we have evidence that NFR2, a transcription factor that controls the cells response to oxidative stress plays an important role on cells resistance to these agents. On the other hand, the influences of extracellular matrix (ECM) and three-dimensional (3D) microenvironment were investigated on how human breast cancer cells respond to DOXO. Interestingly, autophagy was compromised in the 3D cultures, which was associated with the increased cytotoxicity of DOXO to these cells. Indeed, autophagy inhibition potentiated DOXO-induced cell death under the 2D culture conditions, while the autophagy inducer rapamycin improved the resistance of 3D-cultured cells to this drug. We further demonstrated that autophagy resistance observed in monolayer cells depends on the p53- and DRAM-1 expression. Therefore, 3D tissue microenvironment controls breast-tumor cell sensitivity to DOXO treatment by preventing p53-DRAM-autophagy axis activation.

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Symposium 1: Honoring Christophe Cazaux

Human DNA polymerase η prevents delayed replication at common fragile site and telomeres.

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Human DNA polymerase η (Pol η) is best known for its role in responding to genome damage that arises when a cell is exposed to UV irradiation, and for its ability to replicate efficiently past UV pyrimidine dimers. We have recently uncovered that Pol η also operates in response to low replicative stress, extending the task of Pol η to undamaged DNA replication in cycling cells. I will present how Pol η is necessary for common fragile site but also telomere stability. We demonstrated that pol η is physically present on these difficult to replicate regions during S phase and its absence resulted in increased FANCD2 focus formation in mitosis and accumulation of large 53BP1 nuclear body formation in G1 containing with these specific regions of the genome. Consistent with this accumulation of un-replicated, or under-replicated DNA in absence of pol η we also observed increase of EdU incorporation in late G2/M phase in the vicinity of the FANCD2 staining, suggesting that completion of DNA replication is delayed in G2 or even in M phase. This observation led us to study more generally the process of delayed DNA replication after replication stress in human cells and obtain more insight into the mechanism by which completion of replication occurs. We explored how and when late EdU incorporation takes place and determined which DNA polymerase is able to perform delayed DNA synthesis.

Chk1 stability requires DNA polymerase Kappa in response to replication stress

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To maintain genomic stability cells have evolved two major mechanisms to deal with the constant challenge of DNA replication fork arrest during the S-phase of the cell cycle: the induction of ATR replication checkpoint pathway and the recruitment of specialized DNA polymerases to perform translesion synthesis¹. We have recently reported that the human Pol Kappa, the most conserved specialized DNA polymerases², is required for an optimal Chk1 phosphorylation in response to replication stress. We have found that Pol Kappa is implicated in the synthesis of short DNA intermediates at stalled forks facilitating the recruitment of the PCNA-like clamp, the 9-1-1 checkpoint clamp composed by Rad9, Rad1 and Hus1 proteins³. We showed that the absence of Pol Kappa impedes fork restart after replication stress, as evidenced by DNA spreading experiments.

Our recent findings give molecular insights into the precise mechanism of the checkpoint function of Pol Kappa. We use two different approaches (immunoprecipitation and proximity ligation assay) to show that Pol Kappa interacts with Chk1 in response to replication stress. In absence of Pol Kappa, the protein level of Chk1 is decreased meaning that Pol Kappa plays an important role in the Chk1 regulation, role that is not shared by Pol eta, one of the closest relative of Pol Kappa. We observed that the ubiquitin hydrolase USP7 : interact with Pol Kappa ; is required to stabilize Pol Kappa in the nucleoplasm after hydroxyurea treatment and catalyse Pol Kappa deubiquitination. Finally we found that Pol kappa is important for the interaction between USP7 and Chk1. Taken together these data highlight a central role of Pol Kappa not only in the fork restart or the recruitment of Rad9 after replication stress but also in the regulation of Chk1 stability through the Ubiquitine hydrolase USP7.

Role of *Trypanosoma cruzi* Rad51 in repairing DNA double strand breaks and oxidative lesions in in vitro and in vivo.

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Rad51 is a central enzyme of homologous recombination. Here we described different roles in DNA repair provided by Rad51 from *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. The effect on DNA double strand break repair was assessed by gamma radiation treatment. Epimastigotes overexpressing TcRad51 presented intensely TcRad51 labeled foci before treatment and a faster growth recovery while partially knocked out for TcRad51 showed less intense foci, retarding growth recovery. Overexpression of TcRad51 also conferred increased resistance to hydrogen peroxide treatment, while the partial knockout of TcRad51 increased sensitivity, indicating a role in repair of oxidative lesions. In contrast, Rad51 was not involved in the repair of crosslink lesions due to UV light and cisplatin, as no difference was seen amongst different Rad51 mutant cells. Besides roles in epimastigotes, TcRad51 is also important during mammalian infection, as shown by increased numbers of TcRad51 overexpressing amastigotes and diminished numbers of partial TcRad51 knockout amastigotes after infection of fibroblasts and macrophages. TcRad51 overexpressing parasites also presented increased parasitemia and greater resistance to Benzonidazol in infected mice. Thus, TcRad51 is a critical protein in the repair of oxidative lesions and DNA double strand breaks in two crucial life cycle stages of *T. cruzi*.

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Genetic variations in translesional DNA polymerases pol θ and pol η : implications for hereditary cancer predisposition syndromes

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Deficient DNA repair is one of the most prominent risk factors for tumor development and genetic variations in DNA repair genes have been shown to play an important role in the carcinogenesis of breast and colorectal cancers. Taken into account the possible involvement of *POLQ* in single and/or DSB repair, we hypothesized that variations in this DNA repair gene could drive the development of breast cancer. Analysing the contribution of *POLQ* to the development of both sporadic and hereditary breast cancer, a specific SNP in the promoter region of *POLQ* was associated with the phenotype of hereditary breast and ovarian cancer syndrome and/or with bilateral breast cancer, but not sporadic breast cancer. Our data contributes to previous evidence suggesting that downregulation or absence of *POLQ* expression leads to inaccurate DSB repair. Thus *POLQ* could be considered as an important player in breast carcinogenesis, acting in this context as a tumor suppressor gene due to its important role in DNA repair. At the same time, the involvement of pol η in the mismatch repair system by interacting directly with the msh2 and msh6 proteins was recently described. Lynch Syndrome (LS) is associated with increased risk of colorectal cancer and other tumors at young age, representing about 5% of all CRC diagnoses. It is mostly caused by germline mutations in one of the mismatch repair genes responsible for the correction errors in base pairing during DNA replication. Recently our group identified germline *POLH* variants that were present in 32% of individuals with clinical criteria for LS. The ability to distinguish a growing proportion of the 5-10% of all cancers that develop in individuals who have inherited a genetic mutations conferring heightened susceptibility to hereditary cancer syndromes may permit new efforts in cancer surveillance and prevention, as well as understand the molecular mechanisms involved in this increased susceptibility.

Symposium 2: Environmental contaminants

Mutagenic effects of products generated by cashew nuts processing

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The present study conducted an assessment of the occupational risk associated to artisanal cashew nut roasting by the use of exposure and effect biomarkers, as well as the quantification, characterization and dispersion analysis of the released particulate matter (PM). A real-time airborne particle monitor was used to quantify PM_{1.0}, PM_{2.5} and PM₁₀ concentrations. Furthermore, the PM was sampled using a Handi-vol sampler and the physicochemical characteristics were determined by gravimetric and SEM-EDS analysis. Trajectories, dispersion and deposition of the emitted material were calculated using the NOAA-HYSPLIT model. Urinary 1-hydroxypyrene (1-OHP) levels were analyzed by high-performance liquid chromatography. DNA damage, chromosomal instability and cell death were measured by the buccal micronucleus cytome assay (BMCyt assay). The PM concentration for all measurements in the exposed area was higher than in the non-exposed area ($p < 0.0001$). Furthermore, it was observed that the control area yielded a higher prevalence of coarse particles, while a higher prevalence of fine particles was observed in the exposed area. The SEM-EDS analyses showed a wide variety of irregular particles in the samples, such as tar balls, smooth-surfaced and mineral particles. Biomass burning tracers of K, Cl, S and Ca were the major inorganic compounds found. The dispersion analysis suggested that the PM_{2.5} can reach neighboring regions to a distance of 40 kilometers. PAH exposure was confirmed by increases in urinary 1-OHP levels in cashew nut workers. The frequencies of BMCyt biomarkers were higher in the exposed group ($p < 0.0001$) than in the control group. The influence of factors such as age range, smoking status and family history of cancer on the MN frequency was evidenced and a correlation ($r = 0.61$; $p < 0.0001$) between the exposure (1-OHP) and effect (MN) biomarker was observed. The physico-chemical and dispersion analyses of the PM showed typical particles from biomass burning with the potential to reach neighboring regions. The PAH exposure and genotoxic potential among cashew nut workers were confirmed by the increase in urinary 1-OHP levels and BMCyt biomarkers, with a positive significant correlation. The uses of exposure and effect biomarkers were therefore efficient in assessing the occupational risk associated with artisanal cashew nut roasting. The high rates of PM_{2.5} are considered a potential contributor to this effect.

DNA damage, telomere length and global methylation profile as instability markers in rural workers exposed to pesticides

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Telomeres are genomic structures consisting of hexamer repeats (TTAGGG)_n. These DNA portions reflect mitotic history, biochemical trauma to the genome and are considered a useful biomarker of cellular aging. Pesticide exposure has been shown to be genotoxic via oxidative damage and is shown to be associated with adverse health outcomes. In our study we explored an association between telomere length (TL), global DNA methylation and DNA damage in tobacco farmers exposed to pesticides. The content of inorganic elements was measured from plasma samples using the particle-induced X-ray emission (PIXE) technique. Individuals occupationally exposed to mixture of pesticides at tobacco fields showed significantly shorter telomere. We also observed significant decrease in global DNA methylation and p16 hypermethylation in exposed individuals. DNA damage is increased in farmers than controls. Several inorganic elements such as Na, S, P, Cl and K usually found in pesticides formulations, have significantly elevated plasma levels in tobacco farmers than controls. The results suggest that long term occupational pesticide exposure in tobacco farmers is associated with increased DNA damage, shorter telomeres and altered DNA methylation.

Pointing genetic markers: biomonitoring children and the environment as a means of characterizing contaminated and reference areas.

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Exposure to environmental chemical compounds in liquid, solid or gaseous wastes from industrial or domestic discharges is of increasing concern, as they represent a potential hazard to the ecosystems, and ultimately to human health. The use of genetic biomarkers, along with the determination of specific chemicals in environmental samples, is a good strategy to investigate the cause-effect relationships in those complex mixtures. Since 2005, our group has been working on the association of this information with genetic biomonitoring of human populations, as biomarkers of human chronic exposure to genotoxins. Mutagenic compounds found in air particulate matter, drinking water, soil and domestic dust are used as biomarkers of environmental stress. The focus of this investigation is on the organic fraction of the samples, especially on polycyclic aromatic hydrocarbons. Lately, human biomonitoring studies have increasingly prioritized children instead of adults, as they are more sensitive. In our studies, the evaluation of genetic environmental markers with the *Salmonella*/microsome assay is done with stains which are sensitive to PAHs and their nitro-derivative. The frequency of micronuclei, nuclear buds and other anomalies in exfoliated buccal cells, as well as primary DNA damage in peripheral lymphocytes with the Comet assay, are being used in human biomonitoring. Recently, the micronucleus test in human peripheral lymphocytes has been used to improve the assessment of human exposure. This presentation will focus on our latest studies on the characterization of contaminated areas, reference sites and human exposure to those complex mixtures.

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Genotoxic risk of workers exposed to heavy air pollution

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Epidemiological studies found an increased risk of cancers in occupants exposed to traffic air pollution. PM_{2.5} are toxic and can enter into the respiratory tract and circulatory system, can adsorb various substances, such as polycyclic aromatic hydrocarbons (PAHs) nitro-PAHs. The study was carried out with Rebouças tunnel workers (exposed group) and 11 healthy men (control group). Samples of buccal mucosa cells and peripheral blood were evaluated using micronucleus (MN) assay. Urine samples were used to estimate the concentration of 1-hydroxyprene (1-HOP) and 2-naphthol (2-NAP). A significantly higher frequency of MN in buccal cells and binucleated lymphocytes was observed for the exposed workers than for the control group. Higher concentrations of 1-HOP and 2-NAP were detected in the exposure group. In conclusion, damage to the genetic material and the high concentrations of metabolites of PAHs detected in the biological samples taken from control group can be related to daily exposure to pollutants.

Symposium 3: Genotoxic effects of endogenous and exogenous exposure

Genotoxic effect of Reactive Oxygen Species- the role of intra cellular nucleotide pool

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Cells are constantly exposed to reactive oxygen species (ROS), produced either endogenously during metabolism or exogenously by environmental factors and drugs. ROS exert their mutagenic effect by modifying DNA base in the DNA and nucleotide pool (dNTP), which may lead to mutation during replication. While ROS-modified DNA bases are repaired by base excision repair pathway, modified dNTPs can be incorporated into the DNA during replication and give rise to mutations. The dominant forms of ROS- induced dNTP modification include 8-oxo-dGTP and 2-OH-dATP. Different types of mutations can arise depending on which dNTP has been modified and then incorporated in to the DNA. The cells are equipped with the nucleotide pool sanitization enzymes (ex. MTH1) to avoid incorporation of modified dNTPs into the DNA. MTH1 which belongs to the nucleotide pool sanitization system and dephosphorylates 8-oxo-dGTP/2'-OH-dATP to 8-oxo-dGMP/2'-OH-dAMP and inhibit their incorporations of 8-oxo-dGTP into the DNA. Once modified bases are incorporated into the DNA, other repair enzymes, e.g. OGG1 and MYH, will remove the modified bases from the DNA. For instants, MYH removes mispaired adenine with 8-oxo-dG. We have studied mutation frequency, mutational spectra, clonogenic survival, the level of micronuclei and the levels of 8-oxo-dG in cytoplasm and in the cell culture medium in TK6 cells with normal as well as knockdown in MTH1, MYH and combined MTH1/MYH proteins in order to study the mutagenic effect of ROS-induced dNTP modifications. The cells were exposed to low doses of ionizing radiation and UVA radiation as ROS inducers. In the presentation, the genotoxic role of the nucleotide pool modifications in the MTH1 and MYH knockdown cells exposed to UV (UVA, UVB and UVC) and low doses of gamma radiation will be discussed.

Characterizing medicinal plants as therapeutic agents

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The Brazilian savanna known as "cerrado" has an enormous biodiversity, which contains numerous native plants used in the treatment of various popular diseases as gastrointestinal disorders. Experimental and epidemiological studies have shown that consumption of plant extracts may promote chemopreventive action and / or be useful in treating diseases. However, it can be harmful to take herbal medicines without being aware of their potential adverse effects. The RE90/04 ANVISA-Brazil determines the performance of cytotoxicity and genotoxicity tests for the regulation of herbal medicines. Considering this resolution, we are investigating the possible cytotoxic and mutagenic properties of native plants from Brazilian flora as from standardized herbal medicines in normal and tumor human cell lines. The extracts that do not present cytotoxic and mutagenic effects are evaluated for their antimutagenic activities and their protective action mechanisms are investigated using molecular biology tools. Interesting and promising results were found. One example are the different biological responses obtained when cell lines were submitted to treatments with extracts of four species of herbal medicines belonging to the genus *Byrsonima*. Extracts of these plants are commonly used as medicine and have proved gastroprotective and antidiarrheal activities, but there is no report on its safety or toxicity. Extracts of *B. correaifolia* and *B. verbascifolia* were mutagenic, while the *B. intermedia* and *B. fagifolia* did not show this effect and also protected the cells against normal and tumor DNA damage induced by benzo[a]pyrene (B[a]P). This finding directs to the use of the first two species as chemotherapeutic and the last two as chemopreventive agents. The fact that plants belonging to the same genus have so discrepant results serves as a warning for those who use them indiscriminately for medicinal purposes and encourages future studies on other biological activities of these extracts.

DNA Damage Induced by Sunlight and its Effects on Amphibian Models

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The increased incidence of solar ultraviolet radiation (UV) associated with the depletion of the stratospheric ozone layer has been proposed as an environmental stressor, which may help to explain the enigmatic decline of amphibian populations worldwide. In this work, influential events concerning the Antarctic ozone hole were identified in a dataset containing 35 years of ozone measurements over southern Brazil. The effects of environmental doses of UVB and UVA radiation were addressed on the morphology and development of treefrog tadpoles, as well as on the induction of apoptosis and the micronucleus in blood cells. These data were complemented by the detection of UV-induced DNA lesions and their removal from the genome by DNA repair pathways. Additionally, the ability of tadpoles to perceive and escape from UV rays was evaluated as another mechanism of photoprotection. 72 ozone depletion events over southern Brazil were identified from 1979 to 2013. Interestingly, their yearly frequency increased three-fold during the last 17 years. The results show that treefrog tadpoles are very sensitive to UVB light, which reduces their survival and developmental rates. On the other hand, they were resistant to UVA, which may be explained by the activation of photolyases during UVA irradiation. Consequently, the rates of micronucleus formation and apoptosis induction by UVB were considerably higher compared to those by UVA, although both treatments were able to induce malformations after conclusion of metamorphosis. Surprisingly, a sensory mechanism that triggers the escape of tadpoles from UVB and UVA light complements the low efficacy of DNA repair pathways, avoiding generation of DNA damage and maintaining the genomic integrity. These results demonstrate the genotoxic impact of both UVB and UVA radiation on treefrogs and its significance for further studies to assess the UV-induced biological effects on declining amphibian species.

Hypermutation and hyperrecombination as adaptive strategies in bacteria

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Evolutionary success of bacteria relies on the genetic variation, which optimizes their adaptability to ever-changing environments. Bacteria may evolve by 2 main ways: i) through horizontal gene transfer (HGT) and through mutation and/or recombination. In absence of HGT, when variation is limited by the mutation supply rate, natural selection favours increased mutation rates (hypermutable). Bacterial hypermutator strains in nature arise by allelic variation of the genetic mechanisms of mutation avoidance, mainly mismatch repair (MMR) and prevention of 8oxoG-based mutation (GO) systems. Homologous recombination can also promote adaptation by allowing the integration of foreign DNA, importing new alleles or even new functions, or via intrachromosomal gene shuffling. Mutator alleles have been extensively studied in *E. coli* and other bacterial species, where a large diversity of mutation rates can be found among natural isolates. However, much less is known about the existence, causes and diversity of high recombination rates in bacteria.

I will describe the causes and consequences of hypermutation and hyperrecombination (if known) in bacteria, mainly based on their effects on antibiotic resistance and adaptation to pathogenic life style.

Symposium 4: DNA damage and susceptibility assessment

Application of the comet assay in human biomonitoring

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The comet assay – single cell gel electrophoresis – is a simple method for measuring DNA strand breaks, readily applied to isolated white blood cells, and so eminently suitable for use in human biomonitoring. The inclusion in the assay of digestion of DNA with bacterial repair endonucleases, formamidopyrimidine DNA glycosylase (FPG) or endonuclease III, allows the additional detection of modified bases. The assay (with or without enzymes) has been applied in numerous studies of occupational/environmental exposure, to, for example, asbestos and other fibres, pesticides, benzene and other volatile chemicals, traffic fumes, anticancer drugs, radiation, and heavy metals. It has also been used to study nutritional effects on genome stability, for example in intervention trials with phytochemicals or antioxidant-rich foods.

While peripheral blood mononuclear (PBMN) cells are the most common samples analysed with the assay, other materials such as buccal epithelial cells, nasal and tear-duct cells have sometimes been used. The need to isolate PBMN cells from fresh blood limits the number of samples that can be collected at one time; however, it is also possible to isolate white cells from small portions of frozen whole blood. The comet assay itself is labour-intensive, and so high throughput versions (based on 12 mini-gels on a glass slide, or 96 on a GelBond film) have been developed.

Although damage to DNA is known to initiate carcinogenesis, DNA damage measured in blood cells is best regarded as a marker of exposure to genotoxins rather than as an indicator of cancer risk, as there are many processes that intervene in the progression from DNA lesions to cell transformation. Almost all damage is in fact repaired. DNA repair – recognised as a likely factor in determining cancer susceptibility – can be measured by monitoring the removal of damage from cells treated with a genotoxic agent; but for human biomonitoring, it is more convenient to use in an *in vitro* assay on an extract of PBMN cells. In this assay, the accumulation of DNA breaks (repair intermediates) in a given time is a measure of individual repair capacity. The assay has also been adapted for use with extracts from frozen tissues.

Experimental variability – particularly variation between laboratories – has been a problem. It can be reduced by adoption of standard protocols, and controlled by the use of reference standards. Pooling of data from different laboratories is necessary to obtain definitive answers to questions relating to effects of genetic variation, smoking, age, sex, nutrition, lifestyle etc. on DNA damage and repair, and the ComNet project was set up with this in mind: we will collect as much as possible of the thousands of individual comet assay data that exist for rigorous statistical analysis.

The use of comet assay in human biomonitoring studies

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In the last decades, the comet assay has been widely used as a molecular biomarker to detect DNA damage in several human biomonitoring studies. This technique provides information on the genotoxic potential of chemicals (genotoxicity testing) as well as the chemoprotective properties of others (antigenotoxicity testing); its application on suitable organisms can provide data on genetic damage due to environmental contamination (ecological monitoring or ecogenotoxicology); another main application concerns human biomonitoring that examines the effects of occupational and environmental exposure to different agents.

The high sensitivity of the Comet assay makes it a very useful biomarker particularly in cases of low dose exposures. It is, in most cases, accepted that the range of damage detectable with the comet assay is roughly from one hundred to several thousand breaks per human. This interval covers physiologically relevant levels of damage, ranging from the normal background levels of damage and damage levels induced by non-lethal doses of cell-damaging agents.

The relevance of the endpoints measured with the alkaline comet assay is still not fully understood as they mirror a temporary strand break which under normal circumstances, will be repaired in a short period before being fixed as a mutation and often constitutes an error free DNA repair process. Nevertheless, several studies have been showing a very good agreement between DNA damage assessed by alkaline version of the comet assay and other cytogenetic tests that detect fixed damage (chromosomal aberrations).

In the present work, the advantages and disadvantages of the use of comet assay in human biomonitoring studies will be presented, as well as some studies that have used this biomarker to characterise the genetic effects of both environmental and occupational exposure to different xenobiotics, including pesticides, formaldehyde and heavy metals.

Understanding assay limitations, both experimental and biological, is essential to improve both data quality and data relevance obtained and to guarantee that comet assay continues to provide enhanced reliability as a biomarker in human biomonitoring studies.

Role of functional food consumption on DNA damage

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The environment that we live, the lifestyle we adopt and our genetic information may be risk factors for the development of chronic noncommunicable diseases (NCDs). The NCDs were traditionally related to population in developed countries, but today has a global importance. In 2008, 63% of all deaths worldwide were due to NCDs, principally cardiovascular diseases, diabetes mellitus type 2 (DM2), cancer and chronic respiratory diseases. More and more evidence suggested that high consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, and age-related functional decline. A wide variety of fruits and vegetables provide different nutrients and a range of bioactive compounds including vitamins (vitamin C, folate, vitamin D and provitamin A), minerals (potassium, calcium, and magnesium), phytochemicals (flavonoids, phenolic acids, alkaloids, and carotenoids), and fibers. When these compounds provides benefits to the body additional to adequate nutrition to either improve health and well-being or reduce the risk of disease they are named functional food. To be classified as functional food, the foods must remain as foods rather than pills or capsules and must demonstrate their effects in amounts that can normally be consumed in the diet. In this study, the benefits of the use of functional foods in reducing the risk of DNA damage will be discussed. In our laboratory we have been working with functional foods such as kale, acerola, vitamin D, caffeine, yerba mate and Brazil nuts in many research related to genomic instability evaluation in animals and humans.

CYTOTOXICITY OF RUTHENIUM(II)/BIPYRIDINE COMPLEXES AGAINST EHRlich TUMOR CELLS

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Key-words: ruthenium, DNA damage, apoptosis.

Among the anticancer agents derived from metals, the most promising are ruthenium compounds for demonstrating antimetastatic properties, low toxicity to normal cells and high selectivity for tumor cells. This study aimed to determine the potential cytotoxic, genotoxic and elucidate the signaling pathway involved in cell death process of Ru(II) complexes bipyridine, Ru8, Ru21 and Ru26, *in vitro*, in cell line murine breast carcinoma, Ehrlich tumor. To assess the cytotoxicity of the complexes was performed MTT assay. IC₅₀ values were: for Ru8 of 21µM, Ru21 and Ru26 of the 8.52µM and 14.93µM. The complex with better cytotoxic activity and potential selective for tumor cells was selected for further study. The complex with better cytotoxic activity and potential selective for tumor cells was selected for further study. To assess the genotoxicity of Ru21 was held the comet test. After 24 hours, the Ru21 at concentrations of 4, 8 and 16 µM induced DNA damage of 44, 53 and 44, respectively. After 48 hours, Ru21 induced DNA damage in the concentrations of 4µM of ID=48 in 8µM concentration of ID=45 and 16µM ID=42.5% compared to the negative control 16.5. Digite um texto ou endereço de um site ou traduza um documento.

To evaluate the effect of Ru21 complex on the cell cycle kinetics was performed testing the cell cycle by flow cytometry. After 24 hours of treatment, an increase in the percentage of cells in G0/G1 phase and a considerable reduction of cells in S phase and G2/M was observed, considering a cycle arrest. In addition, an increased number of cells in sub-G1 was observed, featuring a DNA fragmentation. To evaluate the type of induced death Ru21 from morphology, apoptosis detection test/necrosis by fluorescent microscopy was conducted. After 24 hours of treatment with Ru21 at concentrations of 4, 8 and 16µM were observed 33%, 42% and 30% respectively of the initial cells in apoptosis compared to negative control 11%. In the treatment of 48 hours at concentrations of 4, 8 and 16µM were observed 50%, 64% and 60% of cells in early apoptosis respectively compared to the negative control. When assessed the expression of the Tp53 genes Bax and Caspase 9 by qPCR, we observed a significant increase in the rate of expression of the Tp53 gene and Bax. The new complex of ruthenium (II), tested is promising because it has low IC₅₀, selectivity for tumor cells, and induce DNA damage, cell cycle arrest and cell death by apoptosis.

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Symposium 5: Translational genomics and therapeutic strategies in cancer

Molecular pathways and novel therapeutic design in cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a dismal type of cancer, which accounts for 20% of tumor incidence in the liver. Liver tumors are highly metastatic, treatment-refractory and currently the second most common cause of cancer-related deaths, following lung carcinoma. Yet, to date there are no drug therapies approved, despite some trials with mixed outcomes. Moreover, although DNA damaging agents are the clinical mainstay in oncology, this drug class has not been rigorously tested and adopted in CCA therapy, due to lack of sufficient research in the area. In parallel, with the advent of new, faster and cheaper high-throughput methods, this scenario is experiencing a drastic change in unraveling new mechanistic pathways that govern the progression of cancers. Recently, next-generation sequencing of CCA has identified a 47% variant frequency in genes coding for crucial factors of the DNA damage response (DDR)-associated chromatin remodeling SWI/SNF family. Thus, a novel approach to understand the molecular mechanisms of the DDR system in CCA is urgent to improve therapy and holds great promise on advancing patient outcome. Here, give this encouraging new viewpoint, which highlights the potential benefit in adopting DNA damage-based chemotherapeutics to advance CCA patient prognosis, we are interested in characterizing the role of the DDR machinery in CCA. In order to do so, we first ran a mutational landscape of our panel of established CCA cell lines. As observed in patients, those cell lines are highly heterogeneous in frequency of mutations, with no significant hotspots throughout their entire genome. We also performed a high-throughput shRNA-based screening in all known members of the SWI/SNF family. Interestingly, some members showed impairment in proliferation itself, and more importantly, they were more sensitive to treatment. We are now introducing a new reporter assay into our system in order to observe specifically DNA damage response on CCA cells. In all, our focus is to broaden the understanding of such a peril disease by dissecting its underlying pathways. By doing so, we can specifically target those pathways and explore such perspective into novel therapeutic outcomes.

***In vitro* testing of HDACi for anti-cancer therapy**

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Epigenetic gene regulation can contribute to generate genetic alterations during cancer development. Mainly demethylating agents as well as histone deacetylase inhibitors (HDACi) are in use in clinical trials and few of them just approved to be used for specific cancer treatments. The underlying mechanisms of HDACi sensitization to classical cancer treatments has been related to increased acetylated lysine in histone tails. In this respect, we have been studied, in human bladder cancer derived cell lines, the sensitization to etoposide treatment by using the HDACi sodium butyrate. Cell survival assays, cell cycle analysis by flow cytometry as well as the measurement of genetic damage by comet assay were carried out to determine the effect of different concentrations of sodium butyrate (1mM and 5mM) in the sensitization to etoposide. Contrary to expectations, 1mM of Sodium Butyrate was more effective in sensitizing cell lines to etoposide treatment evidenced through comet assay. Flow cytometry analysis showed that the higher dose of sodium butyrate produce a cell cycle delay respect to the lower dose, which could explain the refractory effect, as topoisomerase II, the etoposide target, is expressed in cycling cells. Therefore, it is of the utmost importance to properly know the target for chemotherapy as well as the appropriate dose of HDACi to obtain the desired effect on tumor cells with the epigenetic therapy. Besides, we are testing new synthesized HDACi produced by the group of Medicinal Chemistry from our Faculty of Sciences (Uruguay). Some phenazines derivatives were designed as specific HDACi (mainly for the inhibition of group II HDACs, as determined by protein-protein interaction docking analysis). In this respect, we have determined the cytotoxicity of 10 new specific HDACi as well as their capacity to inhibit HDACs activities, by means of MTT technique and western blot, respectively, in two bladder cancer cells lines (T24 and 253J). The anti-proliferative assay determined that two compounds were able to inhibit selectively the tumor cell lines. One of them showed an increase in the histone H4 acetylation activity evidenced by western blot at the same level as the effect produced by the general HDACi trichostatin A or TSA. Besides, the sensitizing capacity to classical chemotherapy was also tested, treating T24 or 253J cells to cisplatin in the presence of selected (by their anti-proliferative effect or HDACi activity) new synthesized HDACi or the general HDACi TSA. The chromosomal aberrations test showed that two new compounds out of 10 statistically increase the chromosomal aberrations induced by cisplatin alone, suggesting that its HDACi activity could be responsible for the sensitizer properties of these new compounds to cisplatin. Further analysis on the expression of specific HDACs or its target proteins (such as the hypoxic inducible factor or HIF-1 due to its effect on tumor adaptation to hypoxic conditions) will be carried out to confirm our assumptions of the inhibitory tumor cells growth capacity for these compounds.

DEVELOPMENT OF A MODEL FOR SILENCING OF XPF AND ERCC1 GENES TO INCREASE CISPLATIN TOXICITY FOR HUMAN TUMOR CELLS.

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Keywords: cisplatin, lung cancer, XPF-ERCC1

Cisplatin is a powerful agent used clinically to treat a wide variety of tumors such as ovarian, testis and lung. However, the efficacy of the treatment can be reduced due to the resistance development. There are multiple suggested mechanisms for cisplatin resistance in tumors and the increase of DNA repair is proposed to be one of the most relevant. Cisplatin acts by forming DNA adducts, which include monoadducts, intra- and interstrand DNA crosslinks (ICLs). The ICLs are highly toxic lesions that can inhibit and block DNA replication and transcription, which may lead to cell death. ERCC1–XPF is a structure-specific endonuclease that is required for the repair of these lesions through the Nucleotide Excision Repair and Interstrand Crosslink Repair pathways. It has been suggested that expression of ERCC1 correlates with cisplatin drug resistance in non-small cell lung cancer (NSCLC) and other kinds of tumors and the silencing of these proteins can alter the expression levels of other. In this work, different strategies were used to obtain silenced strains for these two genes to test the effect on the cytotoxicity of cisplatin in lung cancer cells (A549) and lung fibroblasts (MRC5 and IMR90). For this purpose, RNA interference techniques have been applied, with transient and permanent silencing by short RNA sequences (siRNA) and lentiviral transduction (shRNA), respectively. We also used the CRISPR/Cas9 technique to inactivate these genes. These cells, silenced for XPF, ERCC1 or both, were analyzed by different tests and compared with fibroblasts extracted from patients deficient in XPF for their sensitivity to cisplatin and other ICLs inducing agents. Interestingly, XPF silencing appears to also reduce the expression of ERCC1. In general, it has been demonstrated that silenced cells present higher sensitivity to these agents. Moreover, glutathione (GSH) inside the cells functions as barrier to cisplatin cytotoxicity. Our results show that addition of a GSH synthesis inhibitor (BSO) to the cisplatin treatment induced a strong increase in cisplatin sensitivity. Thus, XPF-ERCC1 silencing combined with cisplatin and BSO appears to be an interesting therapeutic strategy for improving the clinical protocol against lung cancer.

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ANTIROLIFERATIVE EFFECT OF THE DIPHENYL DITELLURIDE IN HUMAN CANCER COLON CELLS

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Diphenyl ditelluride (DPDT) is an organotellurium (OT) compound with pharmacological effects, such as potential antioxidant, antigenotoxic and antimutagenic in low concentrations. On the other hand, higher concentrations of DPDT showed cytotoxic action in mammalian V79 cells by induced oxidative damages, DNA strand breaks, cell cycle arrest and topoisomerase I inhibition. In this sense, the cytotoxicity of other OT compounds has been reported, and their employment in anticancer therapy was suggested. Thus, the objective of this study is to investigate the antiproliferative potential of this molecule in human colon cancer cells (HCT116) and human fibroblast cells (MRC5). For this, the cells lines were exposed to DPDT in concentrations range from 0.1 to 10 μ M. To evaluate cell viability we perform the MTT and clonogenic assay, for the period of 3 (with 24h cellular recovery), 24, 48 or 72 h. In 3, 24 and 48h of exposure to DPDT showed similar cytotoxicity to both cell lines. In contrast, DPDT was able to induce decrease in the cell viability to 72h of exposure in MTT and clonogenic assay. The IC₅₀ values are 13 and 3 μ M to MRC5 and HCT116, respectively in MTT assay. Consistently, clonogenic assay showed similar values of IC₅₀ (MRC5 = 8 μ M and HCT116 = 3 μ M) in 72 h of exposure in relation to MTT. To evaluate genotoxicity we perform the alkaline comet assay with cell exposure of 3 and 24 h. For 3h, 10 μ M of DPDT induced similar DNA damage index (DI) increase to both cell lines. However, concentrations >5 μ M of DPDT increase the DNA strand breaks occurrence only to HCT116 cells, in 24 h of exposure. The results showed that HCT116 cells are more sensitive than MRC5 to DPDT and this difference in cell viability between both lines may be due to genotoxic effects induced by this compound. Therefore, DPDT is an interesting molecule for colon cancer cells antiproliferative approach with selective cytotoxic potential, but more studies must be done.

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Symposium 6: DNA metabolism in neglected tropical disease**Is replication firing control working for genomic maintenance in *Trypanosoma brucei*?***M Carolina Elias*

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Trypanosoma brucei is an ancient eukaryote that presents a high genomic integrity compared with other trypanosomatids. The study of genomic maintenance is important to understand the molecular bases of a fundamental biological process of the etiological agent of African trypanosomiasis, especially because the switch between activated or blocked DNA replication is directly related to ability to infection. Moreover, the reduced complexity of this unicellular organism compared to multicellular makes *T. brucei* a good model system for investigating at least some aspects of genomic maintenance. Eukaryotic DNA replication starts in multiple sites of chromosomes named replication origins, and firing of origins might be accurately controlled. Since endogenous obstacles can interfere with a moving replisome and either stalls it, activation of dormant (backup) origins is important to allow the progression of a converging fork up to the site of fork arrest. Otherwise, lacking of dormant origins makes cell complete replication in the presence of stress using fork-restart mechanisms generating mutations. Blockage of origin firing, on the other hand, is important in the presence of DNA damage. We could verify that *T. brucei* is able to activate dormant origin after hydroxyurea treatment, suggesting that the system of backup origin activation is present in this organism. We are now investigating how *T. brucei* deals with the double strand breaks (DSB) damages. We found that intra-S checkpoint is activated in procyclic forms after phleomycin treatment and that this checkpoint remains activated 3 h after DSB repair. Whether this intra-S checkpoint arrest is related with blockage of late S origin firing is under study, preliminary results suggest cell treated during early S phase have their late origins inactivated. Our data show that ATPase activity of Orc1Cdc6, the replication origin bound protein, is fundamental for the recruitment of the helicase complex into origin. Therefore, modulation of Orc1Cdc6 could be involved in the switch control of replication origins in *T. brucei*.

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Functional compartmentalization of Rad9 and Hus1 reveals diverse assembly of the 9-1-1 complex components during the DNA damage response in *Leishmania*

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The Rad9-Rad1-Hus1 (9-1-1) complex is a key component in the coordination of DNA damage sensing, cell cycle progression and DNA repair pathways in eukaryotic cells. This PCNA-related ring-shaped trimer is loaded onto RPA-coated single stranded DNA and interacts with ATR kinase to mediate effective checkpoint signaling to halt the cell cycle and to promote DNA repair. Mounting evidence suggests that a broader range of function ensures structural diversification of the 9-1-1 clamp. The protozoan parasite *Leishmania major* is an early-branching eukaryote with a remarkably plastic genome. Such plasticity hints at the existence of peculiar genome maintenance mechanisms in this organism. We investigated the existence of the 9-1-1 complex in *Leishmania* and found that the subunits LmRad9, LmRad1 and LmHus1 form a complex *in vivo* and associate with chromatin in response to replication stress. Similar to LmHus1, LmRad9 participates in telomere homeostasis and is required in the cell response to both replication stress and double strand breaks. However, LmRad9 and LmHus1 deficient cells presented marked opposite phenotypes, which suggest their functional compartmentalization. Remarkably, LmRad9 and LmHus1 are found outside the 9-1-1 complex; LmRad9 forms an alternative complex, and LmHus1 exists also as a monomer. We propose that the diverse assembly of the *Leishmania* 9-1-1 subunits mediates their functional compartmentalization, which has a direct impact on the response to genotoxic stress.

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Highly unorthodox chromosome duplication revealed by mapping origins of DNA replication in *Leishmania*

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DNA replication is initiated at genomic sites termed origins of replication, which in eukaryotes are designated by the binding of the origin recognition complex (ORC), a key initiator of replication. All eukaryotic nuclear chromosomes examined to date are replicated from multiple origins, which are selected from a larger pool of ORC binding sites and vary in activation timing or strength. Mapping binding of ORC1/CDC6 (an ORC-like initiator) and origin location shows that *Trypanosoma brucei* nuclear chromosome replication conforms to this paradigm: though origins are more widely spaced than in other eukaryotes, origin number correlates with chromosome size and origins are found at ~20% of core ORC1/CDC6-binding sites. However, what distinguishes origin-active ORC1/CDC6 sites from inactive sites is unknown. We have now mapped replication origins genome-wide in *Leishmania*. These data reveal an unprecedented strategy for replication, since only a single origin can be found in each chromosome, irrespective of size. Indeed, single origins are found in two *L. mexicana* chromosomes that are each syntenic with two *L. major* chromosomes, showing that origin singularity survives chromosome fusion or fission. All mapped origins fire with equal efficiency, suggesting variation in origin strength and activation following a replication timing programme is a facet of chromosome origin multiplicity. Like in *T. brucei*, *Leishmania* origins localise to the boundaries of the transcription units and, indeed, show substantial location conservation. Unlike in *T. brucei*, however, *Leishmania* origin-active loci can be clearly distinguished from inactive loci. Overall, the observation of origin singularity in a eukaryote has implications for the evolution of origin multiplicity and associated controls, and for *Leishmania* genome maintenance.

The roles of XPC and CSB genes in DNA repair and cell cycle in *Trypanosoma cruzi*.

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Nucleotide Excision Repair (NER) is a versatile DNA repair pathway, responsible for detecting and removing distorting lesions in the DNA double helix. The pathway is divided into two subpathways, global genome repair (GGR) and the transcription coupled repair (TCR). In most eukaryotes, XPC performs distortion detection throughout the genome in GGR, while CSB is recruited by stalled RNA polymerase II in actively transcribed genes during TCR. *Trypanosoma cruzi* is the etiological agent of Chagas' disease, a tropical infirmity that affects 10 million people in tropical regions of the globe. Like all kinetoplastids, *T. cruzi* displays highly unusual gene expression, with virtually all its nuclear genes transcribed in multigenic gene clusters and little evidence for control of gene expression at the small number of poorly defined promoters. In this study, we show that NER in *T. cruzi*, similar to *T. brucei*, is organized in a different way from most characterized eukaryotes. Depletion and overexpression of *T. cruzi* XPC and CSB proteins revealed no evidence that XPC is involved in UV- or cisplatin- induced damage, but depletion of XPC results in delayed cell cycle progression and multinucleated cells. Depletion of CSB causes increased sensitivity to UV, even though the lesions induced are slowly repaired. In addition, depletion of CSB increases sensitivity to cisplatin and MMS, which are rapidly repaired. Overexpression of CSB caused elevated mortality at high levels of UV in an ATM/ATR-dependent manner, since that death can be abolished in presence of caffeine. These results indicate the predominant use of TCR in *T. cruzi*, perhaps due to the transcriptional processes in the parasite.

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Symposium 7: Kinases and DNA damage response

"The kinases of the human Nek family: integrating DNA damage response, primary cilia function and cell cycle checkpoints"

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Aside from Polo and Aurora, a third but less studied kinase family involved in mitosis regulation is the Neks [never in mitosis-gene A (NIMA)-related kinases]. The founding member of this family is the sole member NIMA of *Aspergillus nidulans*, which is crucial for the initiation of mitosis in that organism. All 11 human Neks have been functionally assigned to one of the three core functions established for this family in mammals: (A) centrioles/mitosis (Nek2,6,7,9); (B) primary ciliary function/ciliopathies (Nek1,4,8); and (C) DNA damage response (DDR) (Nek 1,8,10 and 11). Recent findings, especially on Nek 1 and 8, showed however, that several Neks participate in parallel in at least two of these contexts: primary ciliary function and DDR. We performed extensive interactomics studies on the majority of the human Neks to obtain further insights in the functional contexts they are involved in. As expected many of the new interacting proteins we identified point to the same core functions cited above. One of the major findings was the fact that more Neks than initially suspected seem to participate in the the DNA damage response, including Nek7, Nek4, Nek5 and Nek10. Furthermore, additional new functional contexts were identified that may also apply to more than one Nek family member. We showed recently an involvement of Nek4 and 5 in mitochondrial functions. Nek1 had been previously reported to regulate mitochondria mediated cell death. As another group recently showed for Nek2 we also found an involvement of Nek4 in mRNA splicing related events. And finally we found a new context of Nek5 in the regulation of a complex biochemical process called polyglutamylation, which targets mainly tubulins, but also regulatory proteins. Nek5 exerts an inhibitory effect on the activity of some of the members of the large family of enzymes that mediate this process. In summary, our data suggest important roles of several Nek family members in the regulation of different aspects of the cell cycle, primary cilia function and DNA damage response and furthermore point to them as interesting new target candidates in cancer therapy.

Multiple protein kinases provide diverse DNA repair-associated functions in *Trypanosoma brucei*.

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In eukaryotic cells, it is essential to efficiently and effectively repair damage to the genome in order to maintain cell viability. In all eukaryotes proteins kinases (PKs) contribute to repair, acting to prevent or limit cell cycle progression under damage stress, to co-ordinate the activity of repair proteins and to restart cell growth when damage is resolved. *Trypanosoma brucei* possess approximately 183 PKs, many of which remain unstudied and none of which have been shown to act as DNA repair PKs. A genome wide RIT-seq screen was performed, comparing growth in the presence and absence of methyl methanesulphonate (MMS), which generates widespread genome damage. Eight PKs were revealed that showed >2 fold loss of read mapping after RNAi in the presence of MMS relative to absence. Four of these PKs, none of which have been shown to act in repair in any organism, were validated by growth in the presence and absence of MMS, cell cycle analysis, as well as by measuring levels of phosphorylated histone H2A. In addition, a tousel-like kinase and ATR, two known eukaryotic repair PKs, were shown to mediate the *T. brucei* response to MMS damage, though both are essential, limiting their detection in the RITseq screen. Perhaps surprisingly, ATM shows no evidence for a role in MMS repair. Epitope tagging of the novel repair PKs reveals both nuclear and non-nuclear, region-specific subcellular localisation, indicating diverse functions, some of which appear to link repair or damage sensing with downstream cell division processes.

The short, but newsworthy, history of Kin3 protein from *Saccharomyces cerevisiae**Dinara Jaqueline Moura*

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The genome integrity of the cells is kept by DNA damage response pathways which ensure the faithful maintenance and replication of the genome. The activation of these pathways leads to gene transcription, temporary cell cycle arrest, activation of DNA repair, senescence or cell death. Checkpoints play a critical role in maintaining genetic stability by ensuring that DNA repair mechanisms correct DNA damage before replication or segregation of damaged chromatids. Once DNA lesions are detected, the checkpoint transducers transmit and amplify the checkpoint signal to downstream targets such as the DNA-repair apparatus and the cell-cycle machinery. Often, protein modifications by phosphorylation are involved in the transmission of the signal or activation of this response by influencing the stability or activity of proteins that are implicated in checkpoint maintenance or cell-cycle progression. The eukaryotic cell cycle is highly conserved, and this conservation has allowed the identification of common regulators in a diverse set of eukaryotes ranging from yeast to humans. Among the many protein kinase families described, members of the NIMA (*Never-In Mitosis, gene A*)-related kinases (Nrk), identified as participating in the control of cell cycle, are less well functionally characterized. Kin3 protein, an ortholog of the *Aspergillus nidulans* protein kinase NIMA, is a nonessential serine/threonine protein kinase of the budding yeast *Saccharomyces cerevisiae*. We have uncovered a role for the Kin3 protein in the DNA damage response by showing that *KIN3* deficient yeast cells are more sensitive to the effects of DNA adducts induced by methyl methane sulfonate, cisplatin, doxorubicin and nitrogen mustard than is its isogenic wild-type strain. The expression level of the *KIN3* gene and its protein are upregulated after genotoxic stress. Moreover, in this work we suggested that the Kin3 protein can be involved in DNA strand-break recognition, thereby mediating G2 cell cycle arrest, as *kin3Δ* cells fail to arrest properly at G2/M phase checkpoints in response to DNA adduct damage, and this happens in a Tel1/Mec1-dependent pathway. Also, we showed that the Kin3 protein interacts genetically (epistatic mutant interaction) and physically (two-hybrid assay) with each protein of the MRX complex. In this scenario, *KIN3* synthesis does not require MRX complex formation, suggesting that Kin3 might be activated in a previous step to MRX complex formation. In addition we demonstrate that Kin3, as other Neks, localizes to the nucleus and also to the spindle pole bodies (microtubule-organizing centers) of the cells and that the expression of a mutated protein, in which an aspartate residue was replaced by a glycine residue at the region responsible for binding of the ATP molecule, did not cause an increased sensitivity to genotoxic treatment, suggesting that its kinase activity may not be crucial for its role in DNA-damage response. More recently, *in silico* methods based on homology between proteins are being used for determining the structure of KIN3 protein, as well as analyses of the molecular dynamics simulations to indicate the structural features of Kin3 in a cellular environment.

DUAL INHIBITION OF ATR AND ATM POTENTIATES THE ACTIVITY OF TRABECTEDIN AND LURBINECTEDIN BY PERTURBING THE DDR AND HOMOLOGOUS RECOMBINATION REPAIR

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Trabectedin (Yondelis®, ecteinascidin-743, ET-743) is a marine-derived natural product approved for treatment of advanced soft tissue sarcoma and relapsed platinum-sensitive ovarian cancer. Lurbinectedin is a novel DNA minor groove binder structurally related to trabectedin. The structural variation of lurbinectedin is accompanied by important modifications of the pharmacokinetic and pharmacodynamic properties in cancer patients although the preclinical activities of this drug remain close to those observed for trabectedin. Both ecteinascidins generate DNA double-strand breaks that are processed through homologous recombination repair (HRR), thereby rendering HRR-deficient cells particularly sensitive. Until now, no strategy has been evaluated to inhibit or to perturb this repair pathway although this approach is likely to improve the activity of ecteinascidins by mimicking HRR deficiency. In this study, we characterize the DNA damage response to trabectedin and lurbinectedin in human carcinoma cell lines. Our results show that trabectedin and lurbinectedin activate the ATM/Chk2 (ataxia-telangiectasia mutated/checkpoint kinase 2) and ATR/Chk1 (ATM and RAD3-related/checkpoint kinase 1) pathways. Pharmacological inhibition of Chk1/2, ATR or ATM is not accompanied by any significant improvement of the cytotoxic activity of the ecteinascidins. Interestingly, simultaneous inhibition of both ATM and ATR strongly potentiates the activity of both ETs against human cervical and ovarian carcinoma cells by efficiently blocking the foci formation of HRR proteins following exposure to ecteinascidins, resulting in extensive chromosome damage. Together, our data identify ATR and ATM as central coordinators of the DDR to ecteinascidins and provide a mechanistic rationale for combining these compounds with ATR and ATM inhibitors.

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Symposium 8: Proteomics of genomic maintenance mechanisms

Proteomic analysis in meningitis animal model submitted to adjuvant therapy with vitamin B6

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Bacterial meningitis caused by *Streptococcus pneumoniae* leads to death in up to 30% of patients. More than half of the survivors had neurological impairments due to cell damages caused by inflammatory process. Despite the efficiency of the antibiotics treatment, the host inflammatory response against pneumococcal meningitis is the main cause of hippocampal apoptosis, which is associated with learning and memory deficits. The oxidative stress induced by inflammatory response is the main cause of cell death. Activation of base excision DNA repair enzymes, as APE1 and OGG1, has been demonstrated in meningitis animal model and the depletion of NAD⁺ due to PARP-1 activity was associated to apoptosis. Adjuvant treatment with vitamin B6 (vitB6) has been proposed as an alternative to preservation of the cellular energetic status and reduction of apoptosis. Vitamin B6 is a hydrosoluble molecule involved in a wide range of biochemistry reactions. Several studies demonstrated vitB6 is able to decrease the accumulation of neurotoxic metabolites, preserve cellular energy stores and chelate reactive oxygen species (ROS). This study aims to assess the role of vitB6 on global neuroprotection during pneumococcal meningitis. Eleven-day-old Wistar rats were infected with *S. pneumoniae* following antibiotic treatment and with vitB6 supplementation or saline buffer at two time points and after dissected brains were submitted to further analysis. Results showed lower numbers of *S. pneumoniae* colony-forming units (CFU) in cerebrospinal fluid (CSF), animal death prevention and maintenance of corporal mass in the vitB6-supplemented group. Protein expression of apurinic/aprimidinic endonuclease 1 (APE-1) and apoptosis-inducing factor (AIF) were analyzed in the hippocampus (HP) and cortex (CX) tissues. The supplementation of vitB6 induced the decrease of cortical AIF expression and prevented the formation of truncated form of APE1 (34kDa) in HP. Additionally, significant reduction of glutamate concentration and protein carbonyl content were seen in the CX while no changes were seen at PARP activity in the HP. Proteomic analysis of HP revealed that proteins involved in inflammation and apoptosis were down-regulated while proteins related to energy process were mostly up-regulated after vitB6 supplementation. This study demonstrated that dietary supplementation with vitamin B6 could induce changes in the cellular protein balance and prevent protein oxidation during intense neuroinflammation induced by immunological processes. Since the nervous system exhibits a lower antioxidant activity, the features of vitB6 demonstrated in the present study are important to improve the mechanisms of neuroprotection during acute neuroinfection.

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Telomere dysfunction and chromosome instability

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When telomeres become critically short DNA damage response factors are recruited at chromosome ends initiating a cellular response to DNA damage. Using complementary proteomic approaches, we defined the changes in chromatin composition that occur upon onset of acute telomere dysfunction triggered by depletion of the telomere-associated factor TRF2. This unbiased purification of telomere-associated proteins in functional or dysfunctional conditions revealed the dynamic changes in chromatin composition that take place at telomeres upon DNA damage induction. Based on our results, we identified novel factors involved in telomere homeostasis as well as novel factors involved in the processing of dysfunctional telomeres.

Structural biology of DNA repair: helicases, nuclease and therapeutic opportunities

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RecQ helicases are a ubiquitous family of DNA unwinding enzymes required to preserve genome integrity, thus preventing premature aging and cancer formation. The five human representatives of this family play non-redundant roles in the suppression of genome instability using a combination of enzymatic activities that specifically characterize each member of the family. These enzymes are in fact not only able to catalyze the transient opening of DNA duplexes, as any other conventional helicase, but can also promote annealing of complementary strands, branch migration of Holliday junctions and, in some cases, excision of ssDNA tails. Remarkably, the balance between these different activities seems to be regulated by protein oligomerization. This review illustrates the recent progress made in the definition of the structural determinants that control the different enzymatic activities of RecQ helicases and speculates on the possible mechanisms that RecQ proteins might use to promote their multiple functions.

A CRUCIAL AND CONSERVED ROLE FOR Dpb11^{TopBP1} IN DNA END RESECTION*Cussiol JR¹, Liu Y¹, Dibitetto D², Freire R³, Pellicoli A² and Smolka MB¹*¹Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA²Department of Biosciences, University of Milan, Milano, Italy³Unidad de Investigación, Hospital Universitario de Canarias, Instituto de Tecnologías Biomédicas Tenerife, SpainE-mail: jr867@cornell.eduKeywords: DNA end resection, DNA repair, Dpb11^{TopBP1}, Rad9^{53BP1}

During DNA replication, cells are particularly susceptible to accumulate genomic instability, as replication forks often stall and collapse, leading to the generation of DNA double strand breaks (DSBs) and gross chromosomal rearrangements. Proper repair of DSBs in S-phase relies on homologous recombination (HR)-based mechanisms, which use the information from the undamaged sister chromatid as a template. In the absence of a functional HR-machinery, such as in cancer-prone *BRCA1* mutations, cells repair DSBs via non-homologous end joining (NHEJ), which is a highly mutagenic repair mechanism, especially during DNA replication. Despite the importance of properly controlling the use of HR and NHEJ to prevent genomic instability and cancer, how cells regulate repair pathway choice during the cell cycle is not well understood. A critical step in triggering NHEJ is the recruitment of the 53BP1 protein (Rad9 in *Saccharomyces cerevisiae*) to sites of lesions and the consequent block of DNA end resection, which otherwise commits to HR repair. Here, using a synthetic biology approach, we characterize in budding yeast a central role for the multi-BRCT domain protein Dpb11 in the modulation of DNA end resection. Dpb11 licenses the recruitment of pro-resection (Slx4-Rtt107) and anti-resection (Rad9) factors for DNA lesion. Targeting of Rad9 to the 9-1-1 complex by fusing Rad9 to a BRCT domain of Dpb11 that interacts with 9-1-1 results in a severe block of DNA end resection and reduction in Rad52 foci, indicative of a strong defect in HR repair. Moreover, cells expressing the Dpb11 BRCT-Rad9 chimera show enhanced sensitivity to genotoxins and Rad53 hyperactivation, consistent with the notion that Rad53 signaling has a major role in regulation of DNA end resection in yeast. Importantly, a point mutation that disrupts the BRCT domain from the chimera restores all the phenotypes described above to wild type levels, implying that recruitment of Rad9 to the 5' recessed end of a ssDNA::dsDNA junction where the 9-1-1 complex is loaded is a key step for resection control. Finally, we present evidence that this mechanism is evolutionary conserved as TopBP1, the human ortholog of Dpb11, interacts with pro-resection (CTIP and BRCA1) and anti-resection factors (53BP1) in different stages of the cell cycle. Our results, place Dpb11^{TopBP1} as a regulator of the DNA repair pathway choice of the cell with implications for anticancer therapies.

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Symposium 9: MutaGen Young Telents I

TELOMERE DYNAMIC AND EPIGENETIC STATUS ARE ALTERED IN TOBACCO FARMERS

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Telomeres are genomic structures at the ends of the chromosomes consisting of hexamer repeats (TTAGGG)_n that shorten at a regular rate during cell replication. These DNA portions may reflect biochemical trauma to the genome. Pesticide exposure has been shown to be genotoxic and associated with adverse health outcomes, including cardiovascular and neurological diseases and several types of cancer. In the present study, we explore if telomere dynamic and epigenetic status are related in tobacco farmers. We measured TL using quantitative polymerase chain reaction (qPCR) assay. Histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities were analyzed as epigenetic status, such as 5-methyl-2'-deoxycytidine (5mdC) assay was used as a marker of global genomic DNA methylation. Total antioxidant activity (TEAC) and thiobarbituric acid reactive species (TBARS) were analyzed as oxidative stress parameters. The content of inorganic elements was measured from plasma samples using particle-induced X-ray emission (PIXE) technique. Individuals occupationally exposed to mixture of pesticides at tobacco fields showed significantly shorter telomeres (P<0.0001). We also observed significant DNA hypomethylation in exposed individuals (P=0.0007). Tobacco farmers also had increased HDAC activity (P=0.02) and levels of TBARS (P<0.05), which also presented a correlation with global DNA methylation in farmers (P=0.02). TL was significantly shorter for smokers and ex-smokers in control group when compared to never smokers (P<0.05). Several inorganic elements such as Na, S, P, Cl and K usually found in pesticides formulations, were significantly elevated in tobacco farmers compared to controls (P<0.05). For the entire population, HDAC was positively correlated with TL (P=0.02) and global DNA methylation (P=0.006). We did not observe any influence of gender or alcohol consumption on any of the measured parameters. The results suggest that smoking can affect TL. Coherently, deacetylation of histones is inversely correlated with global hypomethylation, but also with TL, showing that such epigenetic changes may influence TL dynamics on entire population. Nevertheless, long-term occupational pesticide exposure in tobacco farmers is associated with shorter telomeres and epigenetic status changes, probably due to increase in oxidative stress. Pesticides are known for increasing oxidative stress and for being toxicants that modify epigenetic states, affecting human health.

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In vivo roles of Smc1a in developing nervous system

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Smc1a, Smc3 and Rad21 are the structural components of the cohesin complex that crucial for maintenance of sister chromatids cohesion during the S-phase of the cell cycle until the final sub phases of mitosis. Phosphorylation of Smc1a by Atm and Atr kinases is important for DNA damage signaling and repair. In addition, in postmitotic cells, the cohesin complex regulates chromatin conformation and gene transcription. Mutations in Smc1a or other cohesin genes lead to a group of diseases known as cohesinopathies. Cornelia de Lange Syndrome (CdLS) is one of these and is characterized by developmental defects, including facial malformation, neurodevelopmental delay, mental retardation and ophthalmological problems.

First, we analyzed mRNA (real-time RT-PCR) and protein expression (western blotting) patterns of Smc1a, Smc3 and Rad21. The three core components of cohesin complex are expressed throughout retinal development. To understand the role of Smc1a in developing nervous system *in vivo*, we developed a conditional knockout mice (cKO) in which exons 2 and 3 of *Smc1a* gene were flanked by lox sequences. Two different lines of Cre mice were used to inactivate Smc1a in developing CNS (*Pax6-Cre* and *Nestin-Cre*). Inactivation of Smc1a in the retina impaired eye growth. In Smc1a deficient-retinas, we observed a decrease in the number of mitotic cells, an increase in cell death and p53 stabilization. Inactivation of both p53 and Smc1a (*Smc1a^{Nes-Cre}; p53^{-/-}*) reverted the cell death induced by Smc1a loss. Transcriptome analysis revealed profound differences in the gene expression patterns of Smc1a-deficient retinas. Moreover, Smc1a loss in retinal progenitor cells (*Pax6-Cre*), not only induced cell death during embryogenesis, but also led to the degeneration of post-mitotic photoreceptors cells during postnatal development.

We have seen for the first time that Smc1a regulates the survival of proliferating cells during CNS development *in vivo*. These findings demonstrate that Smc1a is essential brain and eye development and corroborate to the understanding of the malformations caused by the loss of function of cohesin complex, as observed in human syndromes.

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UVA LIGHT INDUCES DNA DAMAGE AND MUTAGENESIS IN NORMAL CELLS AND XERODERMA PIGMENTOSUM VARIANT PATIENTS CELLS

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Keywords: UVA light, Xeroderma Pigmentosum Variant, DNA damage, mutagenesis.

Ultraviolet radiation A (UVA) is an important environmental agent that reaches the earth's surface (about 95% of sunlight) and induces DNA damage that may participate in the skin cancer induction. The cells have several mechanisms that prevent or tolerate DNA damage caused by UV. Xeroderma Pigmentosum Variant (XP-V) patients present increased risk to skin cancer due to mutations in DNA polymerase eta, an involved in Translesion Synthesis (TLS). The aim of this work is to evaluate the genotoxic and mutagenic effects of UVA light in XP-V cells in comparison to repair proficient cells. Flow cytometry was used to detect if UVA light is able to induce damage and cell cycle arrest (propidium iodide for cell cycle and γ H2AX for DNA damage processing). Moreover, the cells were also treated with caffeine, which normally increase XP-V cells sensitivity to UVA. Mutagenesis was identified by exome sequencing of transformed cells cloned after exposure. The results showed that UVA light induces DNA damage in the transformed cells, nevertheless repair proficient cells were able to solve it more efficiently. A cell cycle arrest (mainly in S-phase) and a significant increase of cell death was observed in XP-V cells. This phenotype is exacerbated by the use of caffeine. Moreover, the permanence of lesions during replication might lead to collapse of replication fork, inducing double strand breaks and consequently cell death. Concerning to primary cells, the UVA light led to increased levels of γ H2AX immediately after irradiation in all lineages tested, but the staining was apparently more persistent in XP-V cells. However, caffeine did not lead to an increase of γ H2AX in the irradiated cells revealing that kinases ATR/ATM (inhibited by caffeine) may participate in γ H2AX formation. Besides, XP-V primary cells also presented a strong cell cycle arrest and increased apoptosis (sub-G1 cells). The results also indicated that UVA induced-mutagenesis was higher in XP-V cells, when compared to control cells. The identified mutations were mainly C:G \rightarrow T:A (probably due to CPDs) and G:C \rightarrow T:A (related with oxidative stress). These results showed that in absence of DNA polymerase eta, UVA light compromises the bypass of lesions resulting in cycle arrest, cell death and mutagenesis, which indicate that UVA light may play a role in skin cancer induction in XP-V patients.

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IS MOUSE MITOCHONDRIAL DNA PROTECTED FROM ALKYLATION DAMAGE BY AAG?

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Key-words: BER, alkylation base damage, mitochondria.

DNA repair systems are ubiquitous in all living organisms and crosstalk with several cellular mechanisms in order to preserve genome integrity from damage generated by a variety of exogenous and endogenous agents. Due to its localization, close to the reactive oxygen species generating electron transport chain, mitochondrial DNA (mtDNA) accumulates more damage than nuclear DNA. The base excision repair (BER) pathway is the main repair mechanism for single strand breaks and DNA base modifications, and is the predominant repair system in mitochondria. In mammals, 3-methyladenine DNA glycosylase (AAG) initiates BER of alkylated bases, playing an important role in protecting against the genotoxic effects of alkylating agents, such as several molecules of clinical relevance in chemotherapy. Mitochondrial localization of AAG was recently described in human cells. It was also reported that mice overexpressing AAG are hypersensitive to alkylating agents while knockout animals and cells are resistant, indicating that alkylation sensitivity can paradoxically be a direct result of AAG-initiated repair on alkylated substrates, influencing cellular homeostasis. Thus, we evaluated the presence of AAG in mouse mitochondria. *In silico* prediction of murine AAG (mAAG) targeting to mitochondria and their mitochondrial targeting sequences (MTSs), by the MitoProt II and iPSORT softwares, indicated that mAAG did not display a canonical MTS, and have a low probability for mitochondrial localization (score = 0.6634). Western blotting analysis, using anti-AAG monoclonal antibody, did not detect AAG in C2C12 mitochondrial extracts, while it was clearly detected in HeLa mitochondrial and nuclear extracts. The mitochondrial extracts were shown to be free of nuclear contamination using anti-Cox4 and anti-PCNA antibodies as mitochondrial and nuclear markers, respectively. Also, mAAG could not be co-localized with mitochondria in C2C12 by immunofluorescence using anti-AAG monoclonal antibody and MitoTracker Orange CMTMRos as mitochondria marker. Together, these data suggest that mAAG does not localize in mitochondria. Further analysis by fluorescence-based incision assay will investigate whether an incision activity toward alkylated bases is detected in murine mitochondria.

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RESUMOS (ABSTRACTS)

SESSÃO DE POSTERS

GENOTOXICITY ASSESSMENT OF ENVIRONMENTAL SAMPLES CONTAINING DISPERSE DYES AND AROMATIC AMINES

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Key-words: disperse dyes, aromatic amines, genotoxicity

Surface water and effluents under the influence of textile discharges can exhibit mutagenic activity. In ecotoxicology, in vitro genotoxicity testing often relies on prokaryotic organisms such as in the well standardized Salmonella/microsome assay. Many studies have demonstrated that primary DNA damage measurement with the comet assay represents a very early and sensitive genotoxicity biomarker in aquatic species. For in vitro studies, fish cell lines are considered an interesting eukaryotic model retaining specific fish physiological characteristics. The aim of this study was to identify dye and aromatic amines in samples under the influence of textile discharges; and evaluate the genotoxic responses of those samples in Salmonella/microsome assay using TA98 and YG1041 and in Fpg-modified comet assay using RTL-W1. Samples were collected in Piracicaba River (upstream and downstream), Wastewater Treatment Plant treated effluent and Quilombo River at Americana city, São Paulo State, Brazil. We identified 9 disperse dyes and 11 aromatic amines, including ones prohibited in European Community. Genotoxicity comet assay shows the presence of compounds producing reactive oxygen species in all the sites (similar response among them). Standard comet assay performed without Fpg better indicates a contribution of the discharges in the genotoxicity of the river downstream. But this is much more evident with the Salmonella/microsome assay results. Mutagenicity was not detected with TA98 with and without S9, except for the Quilombo River. But when YG1041 was included, mutagenicity was detected in all sites with a very different profile comparing upstream and the other sites. The response of the Salmonella/microsome assay strongly indicates that aromatic amines or other compounds that require S9 metabolization to become active, as some azo disperse dyes, are contributing to the observed mutagenic activity downstream, which was corroborated by the chemical analysis. It is possible to conclude that without the use of the diagnostic strain YG1041 this would not be revealed. The influence of the textile discharges was also confirmed by chemical analysis, because most part of dyes and aromatic amines were found in the river downstream. As a conclusion it is important to use assays based on complementary endpoints to better characterize the mutagenicity of environmental samples with the advantage of the indication of what classes of compounds are responsible for the effect.

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Assessment of genotoxic effects of Copper (II) complex of 1,10-Phenanthroline and Doxycycline (CuDoxPhen) in somatic cells of *Drosophila melanogaster*.

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Key-words: CuDoxPhen, free radicals, direct mutagenic

Metal synthetic nucleases can be useful in the treatment of cancer because they can block genetic expression. An inconvenience is that usually the cleavage of DNA requires the concomitant addition of an external reagent, such as thiols and hydrogen peroxide. Certain copper complexes that break DNA strands by an oxidative pathway were reported to be cytotoxic against tumor cells, many studies have shown that copper (II) complex of 1,10-phenanthroline and doxycycline (CuDoxPhen) cleave the DNA strands by an oxidative mechanism involving the generation of ROS, suggesting mechanism of cytotoxic action. This work aims to evaluate the genotoxic effects of a copper (II) complex CuDoxPhen, *in vivo* using the *Drosophila melanogaster* as organism model. In the present study, the Somatic Mutation And Recombination Test in *Drosophila melanogaster* (SMART) was employed to determine the genotoxic effects of CuDoxPhen. Chronic treatments with CuDoxPhen were performed with 3-day-old larvae of the standard (ST) cross of the wing spot test at concentrations of 6,25; 12,5 and 25mg/L. In addition, the carcinogen doxorubicin was administered at 0,04 mM, as a positive control, as negative control was employed osmosis reverse water. Somatic spots on normal wings from marker heterozygous (MH) flies were scored to determine mutational events in somatic cells for each compound. The results showed mutagenic effects of CuDoxPhen at the 6,25 and 25mg/L concentrations in the ST cross, when compared with the negative control. In addition, at the concentration of 12,5mg/L the CuDoxPhen showed non-mutagenic effect, when compared with the negative control. However, when associated with DXR, CuDoxPhen enhanced the doxorubicin effects at all concentrations. In view of this experimental conditions and results it was concluded that CuDoxPhen was associated with direct mutagenic effects and had a synergic action with doxorubicin, once CuDoxPhen and doxorubicin promote the formation of free radicals, explaining the enhanced mutagenic effects, if used during chemotherapy it could enhance the effects of the chemotherapeutic used. However, Further studies can improve the comprehension about the mechanisms of CuDoxPhen action.

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Symposium 10: MutaGen Young Talents II

USE OF DIAGNOSTIC STRAINS OF SALMONELLA/MICROSOME ASSAY TO COMPARE THE MUTAGENICITY OF ATMOSPHERIC PARTICULATE MATTER FROM LIMEIRA AND STOCKHOLM

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Key-words: total particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), nitro and oxy-PAHs

Chemical composition of atmospheric particulate matter (PM) organic fraction is mainly influenced by pollution sources, temperature and solar radiation. In the winter the average temperatures in Limeira and Stockholm are 20.4 and -2 °C and sunlight are 11 and 2 h/day, respectively. This difference in environmental conditions may result in differences in the PM composition and mutagenicity. Although the contribution of PAHs for PM mutagenicity is important, nitro and oxy-PAHs seem to be the main mutagenic components. Photochemical activity during the daytime promotes chemical changes in PAHs and is the main cause of day to night variations in mutagenic compounds concentrations. Some PAH-derivatives have been recognized mutagens that do not require S9 to be mutagenic in the Salmonella assay and, in general, are more active than the parent PAHs that require S9 activation. The use of diagnostic strains may help the characterization of the mutagenic chemical composition of PM. The aim of this work was to compare the mutagenicity profile of PM extracts from pooled total PM samples collected during the winter in Limeira and Stockholm using the strains TA1538, TA98, YG5185, YG1021, YG1024, and YG1041. Both PM samples were collected, processed and tested using the same protocols, which allow direct comparisons. Samples were extracted by accelerated solvent extraction with toluene. The Salmonella/microsome microsuspension assay was used in dose-response experiments with and

without metabolic activation (S9). We present only data from TA1538, TA98 and YG5185. Tests with the other strains are still being conducted. The mutagenic profiles obtained so far for both sites were very similar, despite differences in environmental conditions and mutagenic potencies. Limeira presented mutagenic activities around 7 (-S9) and 9 (+S9) times higher than Stockholm for the tested strains. Both sites presented higher mutagenicity -S9, suggesting the major contribution of nitro and oxy-PAH type of mutagens. The addition of S9 reduced the mutagenicity from the both cities around 3 times for TA1538 and YG5185, and 10 times for TA98, suggesting that non-substituted PAHs, although present in PM, have a small contribution to the mutagenicity. We anticipate that the responses obtained with strains more sensitive to nitro and oxy-PAHs will detect a different profile from Limeira and Stockholm because more photochemical reactions are expected in cities with higher temperature and sunlight.

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ARE ZEBRAFISH EMBRYOS GOOD MODELS FOR EMBRYOTOXICOLOGICAL ASSESSMENT OF ANTIDEPRESSANTS? THE CASE OF STUDY OF AMITRIPTYLINE

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Keywords: model organisms, *Danio rerio*, teratology, psychiatric compounds.

A trend of increase in the use of psychiatric drugs has been suggested by governmental authorities worldwide. The psychiatric drugs are a diverse group of chemicals (i.e. antidepressants, anxiolytics, mood stabilizers, antiepileptic) which acts on central nervous system. The antidepressants are one of the most prescribed pharmaceuticals being administrated for the treatment of different depressive disorders. Amitriptyline is a tricyclic antidepressant which has an inhibitory effect on the serotonin and norepinephrine uptake in the presynaptic nerve endings, thereby reducing the hyperactivity of the hypothalamus–pituitary–adrenocortical axis. In this study, the Fish Embryo Toxicity (FET) Test OECD No. 236 is proposed as a tool to evaluate the effects of an antidepressant drug – Amitriptyline. Test started with newly fertilized eggs exposed to amitriptyline concentrations of 0; 0.1; 0.28; 0.79; 2.23; 6.3; 17.5 and 50 mg/L. Sixteen eggs per treatment, divided in 03 replicates, were selected and distributed in 24-wells microplates, one per well. The tests were carried out at 26 ± 1 °C conditioned in a climatic chamber during 168 h. Embryos were daily observed under a stereomicroscope (Stemi 2000-C, Zeiss, Germany). In the embryo phase, were evaluated: egg coagulation, otolith formation, eye and body pigmentation, somite formation, tail circulation and hatching; after hatching: oedema, equilibrium, undersize, spine deformation and mortality. At 48 h, the results showed that amitriptyline significantly reduced the hatching time of embryos. Regarding mortality, at 72 h of exposure a LC₅₀ of 17.2 mg/L was obtained. Several teratologies were observed in concentrations higher than 17.5 mg/L, including abnormal development of the tail. Behavior alterations were also observed including loss of equilibrium, paralysis and abnormal posture (embryos side-lying in the bottom of the microplate well). In summary, the present study aimed to evaluate the usefulness of zebrafish embryos for the embryotoxicity assessment of antidepressants, namely amitriptyline. Effects on behavior might be explained by the modulation of target neurotransmitters of amitriptyline, serotonin and norepinephrine, or indirect effects on cholinergic pathways. Considering the high sensitivity of embryos and the wide range of responses (teratologies, behaviour and mortality) triggered by amitriptyline exposure FET test seems to be a promising tool for the toxicity assessment of psychiatric drugs.

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TRANSPOSITION MECHANISM OF *mariner-mos1* UNDER STRESS CONDITIONS

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Key-words: stress, *mariner*, *Mos1*, transposition somatic, cell cycle.

The mobility is a principal key feature of transposon elements (TEs). This capacity of moving within a host genome gives to TEs the role of contributing to mutagenesis and genetic variability. However, the mechanisms that trigger the activation of TEs under stress condition are not well understood. The *mariner-Mos1* DNA transposon mobilizes both somatic as well as in germline cells in the *Drosophila simulans white-peach* genome. This mutant lineage allows the phenotypic study of this transposon activity through the formation of mosaic eyes. First, this mutant lineage was exposed to different stresses: ultraviolet radiation (UVC, 25J/m²), mild heat stress (28°C) and oxidative stress (Paraquat, 1mM and 2mM). Then, the *mariner-Mos1* and positive control *Hsp70* and superoxide dismutase gene expression profiles were evaluating by RT-qPCR. The *mariner-Mos1* mobilization activity was determined based on the number of red spots in the eyes of flies submitted to the same stresses, and the impact of each stress on cell cycle was also evaluated by flow cytometry. The UVC treatment had no effect in the *mariner-Mos1* gene expression, as well as in the formation of mosaic eyes. In contrast, the expression of *Hsp70* increased after UVC stress suggesting that *mariner-Mos1* expression is not directly shaped in response to this heat shock gene. Furthermore, the treatment with 28°C increased the expression of both genes and the number of red spots in the eyes of flies. After the UVC exposure, it was observed a long delay in the development of flies, as well a transient arrest in the cell cycle progression with an accumulation of cells in the G1 phase. On the other hand, the flies treated with 28°C showed a reduced time of development and a faster cell cycle progression. These results indicate that heat induces the increase of transcription and mobilization of *mariner-Mos1*, but UVC only induces the expression of *Hsp70* gene, suggesting that the mechanism of activation of *mariner-Mos1* transposition must be coupled to conditions that promote DNA replication and cell cycle progression. The effects of oxidative stress in *mariner-Mos1* transposase gene expression are in progress, although the exposures to Paraquat does not induced the formation of red spots in the eyes of flies, which indicates that genotoxic agents does not induce somatic transposition.

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ULTRAVIOLET-INDUCED DNA DAMAGE: PHOTOREPAIR IN THE SKIN OF DNA REPAIR DEFICIENT MICE ON CELL PROLIFERATION AND INFLAMMATION

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Keywords: Ultraviolet irradiation, photolyases, DNA repair, inflammation.

Ultraviolet (UV) irradiation is considered one of the most genotoxic agents present in our environment. It damages DNA molecules, inducing mainly cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4PP). These lesions interfere in essential cellular processes, such as transcription and replication, promoting severe effects in the skin, such as inflammation, dysplasia and cancer. In placental mammals, UV-induced lesions are repaired by the Nucleotide Excision Repair (NER) pathway. This pathway is subdivided into two recognition pathways, the Transcription Coupled Repair (TCR) and the Global Genome Repair (GGR). In this work, we used XPA knockout (KO), NER deficient and CSA KO, TCR deficient mice. Both mice strains transgenically expressed either CPD or 6-4PP photolyases (enzymes that repair specifically CPD or 6-4PPs through a light dependent mechanism) in order to assess the *in vivo* effects of the photoremoval of each of these lesions after low, chronic UVB exposure. In CSA KO mice, the removal of CPD resulted in a reduction of hyperplasia and cell proliferation, while 6-4PP removal did not change these effects. In the XPA KO mice, the removal of CPD completely prevented the UV hyperplasia effect, while the 6-4PP removal promoted only partial reduction. These data suggest that CPD is the main lesion triggering hyperplastic processes, both in TCR and NER deficient mice, with 6-4PP having a minor role in NER deficient mice. We also studied the effect of CPD or 6-4PP removal in XPA KO mice in the induction of the inflammatory process by *in vivo* imaging of ICAM-1 and MPO expression after a single, high UVB dose. The removal of either type of lesion was able to prevent ICAM-1 and MPO expression 6 hours after UVB irradiation and reduce the expression of MPO after 24 hours. These results indicate that both lesions have a major role in UVB induced skin inflammation.

Financial support: FAPESP, CAPES and CNPq (Brazil).

BLOOD MONONUCLEAR CELLS OF TYPE-2 DIABETES MELLITUS AND ALZHEIMER DISEASE PATIENTS SHOWS ALTERATIONS IN COMMON RELATED TO INFLAMMATION AND DNA DAMAGE RESPONSE

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Key-words: Type 2 Diabetes Mellitus, Hyperglycemia, Alzheimer's disease, Microarrays, MicroRNA prediction

Both type 2 Diabetes Mellitus (T2D) and Alzheimer's disease (AD) cases are increasing worldwide, causing a great impact on public health. The first is characterized by hyperglycemia, related to several metabolic complications, comorbidities and increased DNA damage, while the last is a type of senior dementia, whose development has also been associated with accumulation of DNA damage. Evidences in the literature shows that T2D patients have increased risk to develop AD and *vice-versa*, supporting a connection between AD and T2D, manly related to inflammation, impaired glucose metabolism and increased oxidative stress. In the present work, we aimed to compare the differentially expressed genes in both diseases, expecting to find altered gene functions in common between them, including pathways related to DNA damage and stress responses. Firstly, we compared the transcriptional expression (mRNA - Microarray technique) displayed by peripheral blood mononuclear cells (PBMCs) from hyperglycemic T2D patients (T2D-H, n=14), non-hyperglycemic T2D patients (T2D-N, n=15), and healthy non-diabetic individuals (n=16). The same comparison was carried out between a group of AD patients (n=25) and age-matched healthy individuals (n=15). After bioinformatics analysis, the differentially expressed genes from each comparison were crossed, to search for common differentially expressed genes. 41 differentially expressed genes were found in common between the two comparisons. When submitted to DAVID

functional enrichment tool, inflammatory response was the enriched term indicated by this analysis. Accordingly, *IL8*, *CCL3L3* and *CXCL1* genes were all significantly upregulated in T2D and AD patients. Comparing the gene set enrichment and gene set analyses in both data sets, altered pathways such as regulation of DNA repair and superoxide response were found in DM2, while DNA damage response pathways were found in AD. In addition, after using the MirWalk 2.0 tool to predict the MicroRNAs that may regulate those 41 genes, apart from MicroRNAs that were already implicated in T2D and AD (such as miR-29b) we found microRNAs (hsa-miR-224 and hsa-miR-148a), which were not yet described for both diseases including. The present results provide support for the involvement of inflammation process in the pathogenesis of AD and T2D, and point out new MicroRNAs that might be altered in this context. We also demonstrate evidence that DNA damage responses may be compromised in T2D and AD.

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PSO2 INTERACTIONS WITH DNA DAMAGE RESPONSE GENES AFTER EXPOSURE TO INTERSTRAND CROSSLINK-INDUCING AGENTS IN *SACCHAROMYCES CEREVISIAE*

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Keywords: DNA repair, PSO2/SNM1, DNA double strand breaks, 8-methoxypsoralen, bifunctional chemotherapeutic drugs, MRX complex

Bifunctional mutagenic agents are largely used as chemotherapeutics and produce interstrand crosslinks (ICLs), which covalently link both DNA strands, effectively blocking replication and transcription. ICL processing may cause DNA double strand-breaks (DSBs). Pso2 protein (mammalian orthologs SNM1A, SNM1B/Apollo and SNM1C/Artemis), a member of the highly conserved metallo- β -lactamase super family of nucleases, plays a central role in ICL repair in yeast. In this study, we aimed to extend the characterization of Pso2 function in ICL repair through the identification of interacting proteins, using the two-hybrid system (THS) in yeast. In addition, the genetic interaction of *PSO2* with genes involved in early stages of ICL repair was investigated. Nine fusion protein products were isolated for Pso2p using THS, among them the Sak1 kinase, which interacted with the C-terminal β -CASP domain of Pso2p. Comparison of mutagen-sensitivity phenotypes of *pso2 Δ* , *sak1 Δ* and *pso2 Δ sak1 Δ* disruptants revealed that SAK1 is necessary for complete WT-like repair. The epistatic interaction of both mutant alleles suggests that Sak1p and Pso2p act in the same pathway of controlling sensitivity to DNA-damaging agents. We also observed that Pso2p is phosphorylated by Sak1 kinase *in vitro* and co-immunoprecipitates with Sak1p after photoactivated 8-methoxypsoralen (8-MOP+UVA) treatment. Survival data after treatment of *pso2 Δ* , *yku70 Δ* and *yku70 Δ pso2 Δ* with nitrogen mustard, *PSO2* and *SAK1* with *YKU70* or *DNL4* single-, double- and triple mutants with 8-MOP+UVA indicated that ICL repair is independent of YKu70p and DNL4p in *S. cerevisiae*. Furthermore, a non-epistatic interaction was observed between *MRE11*, *PSO2* and *SAK1* genes after ICL induction, indicating that their encoded proteins act on the same substrate, but in distinct repair pathways. In contrast, an epistatic

interaction was observed for *PSO2* and *RAD52*, *PSO2* and *RAD50*, *PSO2* and *XRS2* genes in 8-MOP+UVA treated exponentially growing cells. Taken together, these results showed that Sak1 kinase plays an important role in contribution to Pso2 nuclease in the repair of ICL-induced DSBs. According to the proposed model in this work, Xrs2p could be directing the DSB to Pso2p or to MRX complex for further processing as *PSO2* showed non-epistatic interaction with *MRE11* in repair of 8-MOP+UVA-induced ICL, and epistatic interaction with *XRS2*.

Abstracts

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TITANIUM DIOXIDE NANOPARTICLES INDUCES REDOX IMBALANCE IN THE GOLDEN MUSSEL *Limnoperna fortunei*

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Introduction: Nanomaterials (1 to 100 nm) exhibit high surface area and small volume, resulting in an increased bioactivity and reactivity. Titanium dioxide nanoparticles (TiO₂-NP) have been used in the production of paints, pharmaceutical and cosmetic products, such as in the manufacture of sunscreens. Consequently, these industrial uses lead to the release of nanomaterials in the environment. In this context, the implications of TiO₂-NP for human and environment health should be assessed. This study aimed to evaluate the effects of TiO₂-NP on the antioxidant enzymes superoxide dismutase (Sod) and catalase (Cat) profiles in a biomonitor organism golden mussel *Limnoperna fortunei*.

Material and Methods: The TiO₂-NP was characterized and TiO₂-NP exposure was performed at concentrations of 1, 5, 10 and 50 µg mL⁻¹ for 2 and 4 h. After these periods soft bodies was processed and Sod and Cat activities measured.

Results/Discussion: TiO₂-NP crystalline and size analysis demonstrated anatase and rutile phase and an average size of about 20 nm. In biological experiments, the results showed that TiO₂-NP destabilizes the cell redox system of Golden mussels. Antioxidant activities of Sod and Cat enzymes were significantly decreased under all treatments at 2h exposure. TiO₂-NP could be generating high levels of superoxide anion radical (O₂^{•-}), thus increasing redox imbalance in mussel cells. At 4h TiO₂-NP exposure, the effective enzymatic antioxidant response restores the activity of Sod and Cat enzymes.

Conclusions and Acknowledgments: The exposure to TiO₂-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 Golden mussel as a potential biomonitor organism for TiO₂-NP in the fresh water compartment.

We thank FAPERGS, CNPq, CAPES, the University of Caxias do Sul and the University of Rio Grande do Sul.

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CYTOTOXICITY AND GENOTOXICITY INDUCED BY COAL AND COAL FLY ASH PARTICLES SAMPLES IN V79 CELLS

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Keywords: Coal, coal fly ash, DNA oxidative damage, comet assay, micronucleus assay.

Coal mining is an activity with a high potential to causing human health and environmental impacts. Several studies have shown that exposure to coal or coal combustion products can cause harmful effects in *in vitro* and *in vivo* systems, mainly by the induction of oxidative damage. The aim of this work was to assess cytotoxic and genotoxic effects using the V79 cell line treated with coal and coal fly ash particles derived from a coal power plant located in Santa Catarina-Brazil. Four samples, two coal samples (COAL11 and COAL16) and two coal fly ash samples (CFA11 and CFA16) were included in this study. COAL16 was co-firing with a mixture of fuel oil and diesel oil. Cytotoxicity was assessed by clonogenic assay. The cytokinesis-block micronucleus test was used to assess endpoints of genotoxicity. The standard comet assay as well as a modified comet assay protocol using endonucleases for the detection of oxidative DNA lesions were used to measure the induction of primary DNA lesions. The quantification of polycyclic aromatic hydrocarbons (PAH) in the samples was conducted by HPLC/UV/Vis. The comet assay data showed that exposure of V79 cells to coal and coal fly ash particles induced primary DNA lesions. Application of lesion-specific endonucleases (FPG and ENDO III) demonstrated increased DNA effects indicating the presence of high amounts of oxidative DNA lesions. The Cyt-CBMN analysis showed that exposure of V79 cells to high concentrations of coal and coal fly ash particles induced cytotoxic effects (apoptosis and necrosis) and chromosomal instability (nucleoplasmic bridges, nuclear buds and MN formation). The

effects observed may be associated with compounds contained in the surface of the particles as hazardous elements, oxides and PAH which were detected in the samples.

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GENETIC DAMAGE IN COAL MINERS EVALUATED BY BUCCAL MICRONUCLEUS CYTOME ASSAY

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Keywords: Coal, coal miners, buccal micronucleus cytome assay, inorganic elements, occupational exposure.

During coal mining activities, large quantities of coal dust, ashes, polycyclic aromatic hydrocarbons and metals are released into the environment. This complex mixture presents one of the most important occupational hazards for health of workers. The aim of the present study was to evaluate the genetic damage together with the presence of inorganic elements, in an exposed workers population to coal mining residues of Guajira-Colombia. Thus, 100 exposed workers and 100 non-exposed control individuals were included in this study. To determine genetic damage we assessed the micronucleus (MN) frequencies and nuclear buds in buccal mucosa samples (BMCyt) assay, which were significantly higher in the exposed group than non-exposed control group. In addition, karyorrhectic and karyolytic cells were also significantly higher in the exposed group (cell death). No significant difference was observed between the exposed groups engaged in different mining activities. No correlation between age, alcohol consumption, time of service and MN assay data were found in this study. However, the content of inorganic elements in blood samples analyzed by a Particle-induced X-ray emission technique (PIXE) showed higher values of silicon (Si) and aluminum (Al) in the exposed group. In this study we discuss the possibility of DNA damage observed in the mine workers cells be a consequence of oxidative damage.

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Why *Trypanosoma cruzi* does not have the catalase gene which is so important to the oxidative stress resistance?

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Trypanosoma cruzi, the causal agent of Chagas disease, is exposed to oxidative stress situations during its complex life cycle. To deal with this stress, this parasite has an efficient and peculiar antioxidant system, which is dependent of trypanothione. A curious fact is that the antioxidant pathway of *T. cruzi* does not include the catalase enzyme, which decomposes the H₂O₂ into oxygen and water and is found in almost all aerobic organisms. Our hypothesis is that *T. cruzi* may have suppressed the catalase gene as a strategy to better signal the oxidative environment. We have showed that CL Brener *T. cruzi* epimastigotes which were transfected with *E. coli* catalase gene (katE) have an increased resistance to the H₂O₂ treatment in relation to the wild type parasites (WT). Moreover, pretreat the parasites with a low dose of H₂O₂ before the treatment makes the WT cells as resistant to H₂O₂ as the cells expressing catalase. Despite this, *T. cruzi* expressing catalase did not show any apparent difference after the pretreatment. We have also verified that the catalase expression decreases the trypanothione reductase and increases the superoxide dismutase levels, suggesting a change in the *T. cruzi* antioxidant system that leads to an increased H₂O₂ levels. Nevertheless, macrophages from C57BL/6 WT and Phox KO (deficient in the gp91phox subunit of NADPH oxidase) mice were infected with each studied *T. cruzi* strain. Our results showed that parasites expressing catalase have an improved growth in macrophages when compared to the WT. After a previous H₂O₂ treatment, WT parasites have an increased parasitism in Phox KO, but this is not so evident in the parasites expressing catalase. Furthermore, *in vivo* assays showed no difference between the parasitemia of both parasites in mice. Preliminary results suggest that the modified parasites have a higher survival rate in the invertebrate host. All these data indicate that the loss of the catalase gene was important to better signal the oxidative stress in *T. cruzi*.

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WOLAND: A TOOL FOR ANALYSIS OF POINT MUTATION PATTERNS FROM RESEQUENCING GENOMICS DATA.*De Souza TA¹, Menck CFM¹*

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Keywords: bioinformatics, NGS, mutagens, DNA repair

The arising of high-throughput sequencing technologies in last decade has been a revolution in the study of individuals and even single cells genomes. In the context of this emerging age of individualized genomic information and big data, it is critical to developing methods and tools to enable reliable testing for insightful biological hypotheses. Here we present WOLAND, a multiplatform tool to analyze point mutation patterns using resequencing data from any organism or cell. WOLAND is being implemented as a Perl and R tool using as inputs filtered unannotated or annotated SNV lists, combined with its correspondent genome sequences. WOLAND can provide the number and frequency of nucleotide type changes, detection of regions enriched in mutations alongside the genome (hotspots) and extraction of sequence-context sequences of each SNV. It is also possible to count established mutational motifs associated with environmental mutagens and DNA-repair mechanisms and calculation of transcriptional strand bias of mutations linked to its mutational motifs. WOLAND is being tested with simulated and real data both for whole-genome and exome approaches. These potentially suitable applications for WOLAND, some of them already tested, are the study of the genome-wide impact of both endogenous and exogenous mutagens on organisms and cells, such as UV radiation and oxidative stress and analysis of mutagenic profiles of DNA repair-associated diseases. Also, mechanisms of molecular mutational processes involving specific target proteins and identification of potential hazardous mutagens in environmental samples can be profiled using WOLAND. The output can be used in third-party tools as Genome Browser or WebLogo, facilitating the addition of specific annotation as CpG content and nucleotide context frequency. Currently, WOLAND is being developed and adapted to a Galaxy web platform to allow easy multi-sampling load and generation of reports by users. In summary, we believe that WOLAND could help to bring some light into the increasing complexity and growing demand for the study of mutation dynamics genome-wide.

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TP53 STATUS CAN MODULATED THE ANTIPROLIFERATIVE ACTIVITY OF SILIBININ (*Silybum marianum*) IN BLADDER CANCER CELLS

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Key-words: bladder cancer, gene expression, silibinin, *TP53*

Silibinin is a natural phenol derived from the flavonolignan family and found in seeds of milk thistle. Recent data have shown its effectiveness for preventing/treating bladder tumors, making this natural compound as a promising antineoplastic agent. It is known *TP53* mutations are the most common alterations in bladder cancer cells, and seem to be related to the tumor response to therapies. Thereby, in this study we investigated the cytotoxic and toxicogenetic activity of silibinin in bladder cancer cells with different *TP53* status. Two bladder urothelial carcinoma cell (UCC) lines were used: RT4, with the wild-type *TP53* gene; and T24, with mutated *TP53* gene. Silibinin was tested at concentrations of 50, 75, 100, 115, 130, 135, 150, 200 and 250 μ M. Cell proliferation, clonogenic survival, apoptosis rates, genotoxicity (comet assay) and relative expression profile of *FRAP/mTOR*, *FGFR3*, *AKT2* and *DNMT1* genes and of miR-100 and miR-203 were evaluated. The results showed decreased proliferation and increase of late apoptosis in *TP53* mutated cells. Increased early apoptosis rates and primary DNA damage, and decrease of cell colonies in the clonogenic survival assay were detected in both RT4 and T24 cell lines. Downregulation of *FRAP/mTOR*, *AKT2*, *FGFR3* and *DNMT1* gene expression occurred in RT4 cells. No significant differences were detected for miR100 and miR203 expression in the two cell lines. In conclusion, despite the reduction of clone formation in both cell lines, the toxicogenomic effect of silibinin on *FRAP/mTOR*, *AKT2*, *FGFR3* and *DNMT1* was dependent on the *TP53* status. Taken together, the data confirmed the role of silibinin as an antiproliferative compound, whose mechanism of action was related to the *TP53* status.

Financial Support: FAPEMIG, CNPq, UFOP

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**INTEGRATING TOOLS FOR THE MUTAGENIC EVALUATION OF ENVIRONMENTAL SAMPLES
- PRELIMINARY RESULTS***Mazzini F, Soares CM, Rech CM, Suzuki CF and Roubicek DA*

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Key-words: environmental samples, Ames test, micronucleus test

The information that only a small part of the chemical compounds produced by man are adequately evaluated for their ability to cause mutations, and the diversity of complex mixtures that can be formed when these compounds are released in the environment leads us to reflect on the relevance of existing tools to provide answers on a fast and efficient manner. Mutagenicity tests are generally used to determine the mutagenic/genotoxic potential of individual compounds. However, many of the existing tests can be used on an environmental assessment strategy, in which they serve as indicators of the presence of these compounds in the environment, without chemical identification. In the present study we intended to establish a tiered approach, combining the Ames test and the *in vitro* micronucleus test in V79 cells to analyze the mutagenic potential of surface waters. The results given by both assays were compared in order to evaluate if the information provided amplifies the diagnosis of the environmental mutagenicity. Organic extraction of 20 water samples was performed with SPE-DEX 4790[®] automated extraction system using acetone, ethyl acetate and 1% NH₄OH in methanol as solvents. For the Ames test, *Salmonella enterica* ser. Typhimurium TA98 and TA100 strains were used, with and without metabolic activation. For the micronucleus test, 2.10⁴ cells were seeded and treated without metabolic activation. Two thousand cells were analyzed for each concentration and controls. We calculated the minimum effective dose (MED) at 1.5, i.e. the lowest dose that produces a 1.5 fold effect increase when compared to the negative control. This approach allows the comparison of the results of the two assays. Fourteen of the 20 samples analyzed showed a 1.5 increase compared to the negative control in the Ames test with TA98 strain, and 4 samples induced micronuclei. We could not detect any mutagenicity in 4 samples, and only two samples induced both gene mutations and micronuclei. MED values were similar in one of them, and to the other sample, the dose capable of inducing micronuclei was much lower than the required to induce a 50% increase of revertants in the Ames test. Our preliminary results suggest that the Ames test in conjunction with the *in vitro* micronucleus test may be a good strategy to investigate the mutagenic potential of water samples, since they take into account the essential mutagenic endpoints: gene and chromosome mutations.

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MICRONUCLEI INDUCED BY WATER-SOLUBLE AND ORGANIC EXTRACTS OF URBAN PARTICULATE MATTER IN HUMAN LUNG EPITHELIAL A549 CELLS*Palacio IC^{1,2}, Barros SBM² and Roubicek DA¹*¹Dept. Environmental Analyses, São Paulo State Environmental Agency, CETESB,²Dept. Clinical Analyses and Toxicology, Univ. São Pauloisabel.palacio@gmail.com

Particulate matter, micronuclei, A549, biomonitoring

Particulate matter (PM) has been considered as an air pollutant that plays an important role in diverse health effects. PM is a complex and heterogeneous mixture, highly variable in time and space, and its detrimental biological effects depend not only on the PM size, but also on its chemical and gravimetric composition. Several researches have demonstrated the genotoxicity of organic and water soluble extracts of urban PM. Studies *in vitro* with mammalian cells have shown that exposure to PM can result in an increased cell death, production of reactive oxygen species (ROS), DNA strand breaks, DNA adducts and mutagenicity. Given the known health effects of PM and the relationship with the chemical composition, we have investigated the *in vitro* genotoxic effect of organic and water-soluble fraction of PM₁₀ with the cytokinesis blocked micronucleus test in human alveolar carcinoma cells A549, with the aim of assessing MN test as a suitable tool for genotoxic PM biomonitoring. The samples were collected in different seasons of the year in the state of São Paulo and fifteen soluble metals and the sixteen EPA's priority polycyclic aromatic hydrocarbons, both types of compounds described as mutagenic for humans were chemically determined. Our results show that the water-soluble and the organic fraction of PM₁₀ are both important in the production of the DNA damage. Of the 24 samples analyzed, five water-soluble and seven organic extracts presented a genotoxic response, although PM₁₀ concentrations were below the limit recommended by the Brazilian legislation. The polycyclic aromatic hydrocarbons (PAHs) prevailing in our samples were fluoranthene (Flt), benzo (ghi)perylene (BghiP) and Benzo(b)fluoranthene (BbF), presenting higher concentrations in winter. In the water-soluble extracts, the highest concentrations of the elements studied were found for zinc, iron, and copper in the three places of collection. It was confirmed that MN induction is an efficient early biomarker of the health impairment that long-term PM exposure could produce, and that the total concentration of PM and the chemical analyses alone are not sufficient for the prognosis of biological effects in exposed populations. Thus, together with analytical methods, the implementation of biomarkers can be used as a relatively simple tool for the control of air pollution and the protection of populations at risk.

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GENETIC DAMAGE IN COAL MINERS EVALUATED BY BUCCAL MICRONUCLEUS CYTOME ASSAY

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Keywords: Coal, coal miners, buccal micronucleus cytome assay, inorganic elements, occupational exposure.

During coal mining activities, large quantities of coal dust, ashes, polycyclic aromatic hydrocarbons and metals are released into the environment. This complex mixture presents one of the most important occupational hazards for health of workers. The aim of the present study was to evaluate the genetic damage together with the presence of inorganic elements, in an exposed workers population to coal mining residues of Guajira-Colombia. Thus, 100 exposed workers and 100 non-exposed control individuals were included in this study. To determine genetic damage we assessed the micronucleus (MN) frequencies and nuclear buds in buccal mucosa samples (BMCyt) assay, which were significantly higher in the exposed group than non-exposed control group. In addition, karyorrhectic and karyolytic cells were also significantly higher in the exposed group (cell death). No significant difference was observed between the exposed groups engaged in different mining activities. No correlation between age, alcohol consumption, time of service and MN assay data were found in this study. However, the content of inorganic elements in blood samples analyzed by a Particle-induced X-ray emission technique (PIXE) showed higher values of silicon (Si) and aluminum (Al) in the exposed group. In this study we discuss the possibility of DNA damage observed in the mine workers cells be a consequence of oxidative damage.

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INFLUENCE OF NUCLEOTIDE EXCISION REPAIR ON MITOXANTRONE CYTOTOXICITY*Rocha JC^{1,2}, Busatto FF^{1,2}, Souza LK¹, Saffi J^{1,2}*

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Keywords: Mitoxantrone; Topoisomerase II inhibitors; Nucleotide Excision Repair; CSB; XPC

Mitoxantrone (MXT) is an anticancer drug structurally related to anthracyclines, like doxorubicin (DOX), used in treatment of leukemia, non-Hodgkin lymphoma and breast and prostate cancer. These drugs belong to the class of Topoisomerase II inhibitors, acting by formation of stabilized cleavable complexes (TopoII-DNA complexes). Indeed, they can form lesions like DNA adducts, reactive oxygen species (ROS) and interstrand crosslinks (ICL). Studies have shown that nucleotide excision repair (NER) is involved in repair of DOX-induced lesions. Considering the similarities between MXT and DOX, the aim of this work was evaluate the influence of NER on MXT cytotoxicity. For this purpose, were used human fibroblasts proficient (MRC5) or deficient in NER proteins (XPA, XPD, CSB and XPC), kindly provided by Dr. Alain Sarasin (Institute Gustave Roussy – France) and CSB deficient cells stably expressing CSB or empty vector, kindly provided by Dr. Carlos F. M. Menck (USP – Brazil). Cells treated with MXT were evaluated for cell viability (by XTT assay and Annexin-V staining), DNA damage induction (by Comet assay and gamma-H2AX induction), cell cycle profile and DNA synthesis (by propidium iodide and BrdU incorporation), ROS production (using 2'-7'-dichlorodihydrofluorescein diacetate DCFH-DA) and TopoII-DNA complexes formation (by TARDIS assay – trapped in agarose DNA immuno staining). Results showed that NER-deficient cells are highly sensitive to MXT. However, cells deficient in each NER sub pathway - transcription coupled repair (deficient in CSB protein) and global genome repair (deficient in XPC protein) demonstrate differences in sensitive each other. XPC-deficient cells are slightly more resistant than CSB-deficient cells, and in the same way as MRC-5 proficient cells, showed G2/M arrest, normal DNA synthesis rate and a pattern of complexes formation similar to proficient cells. CSB-deficient cells, in turn, showed accumulation of cells in S phase, reduced DNA synthesis and a more intense signal for complexes formation. No ROS production was observed in experimental conditions used. Complementation of CSB-deficient cells with CSB rescue MXT-induced sensitivity and also decreased TopoII-DNA complexes signal, suggesting that resolution of these lesions would take place. These results indicate that NER proteins are implicated in response to MXT-induced lesions and that CSB protein has a role in resolution of MXT-induced TopoII-DNA

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TELOMERE DYNAMIC AND EPIGENETIC STATUS ARE ALTERED IN TOBACCO FARMERS

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Telomeres are genomic structures at the ends of the chromosomes consisting of hexamer repeats (TTAGGG)_n that shorten at a regular rate during cell replication. These DNA portions may reflect biochemical trauma to the genome. Pesticide exposure has been shown to be genotoxic and associated with adverse health outcomes, including cardiovascular and neurological diseases and several types of cancer. In the present study, we explore if telomere dynamic and epigenetic status are related in tobacco farmers. We measured TL using quantitative polymerase chain reaction (qPCR) assay. Histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities were analyzed as epigenetic status, such as 5-methyl-2'-deoxycytidine (5mdC) assay was used as a marker of global genomic DNA methylation. Total antioxidant activity (TEAC) and thiobarbituric acid reactive species (TBARS) were analyzed as oxidative stress parameters. The content of inorganic elements was measured from plasma samples using particle-induced X-ray emission (PIXE) technique. Individuals occupationally exposed to mixture of pesticides at tobacco fields showed significantly shorter telomeres ($P < 0.0001$). We also observed significant DNA hypomethylation in exposed individuals ($P = 0.0007$). Tobacco farmers also had increased HDAC activity ($P = 0.02$) and levels of TBARS ($P < 0.05$), which also presented a correlation with global DNA methylation in farmers ($P = 0.02$). TL was significantly shorter for smokers and ex-smokers in control group when compared to never smokers ($P < 0.05$). Several inorganic elements such as Na, S, P, Cl and K usually found in pesticides formulations, were significantly elevated in tobacco farmers compared to controls ($P < 0.05$). For the entire population, HDAC was positively correlated with TL ($P = 0.02$) and global DNA methylation ($P = 0.006$). We did not observe any influence of gender or alcohol consumption on any of the measured parameters. The results suggest that smoking can affect TL.

Coherently, deacetylation of histones is inversely correlated with global hypomethylation, but also with TL, showing that such epigenetic changes may influence TL dynamics on entire population. Nevertheless, long-term occupational pesticide exposure in tobacco farmers is associated with shorter telomeres and epigenetic status changes, probably due to increase in oxidative stress. Pesticides are known for increasing oxidative stress and for being toxicants that modify epigenetic states, affecting human health.

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OCCUPATIONAL RISK LEADS TO GENOTOXIC DAMAGE IN TOBACCO FARMERS*Souza RS¹, Kahl VFS¹, Simon D² and Da Silva J¹*

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Brazil is the largest tobacco exporter worldwide and 90% of Brazilian tobacco fields are in the Rio Grande do Sul state. Cultivation of tobacco is an important economic activity for the country and, consequently, there is a large number of tobacco farmers routinely exposed to pesticides and nicotine (present in tobacco leaves). These substances can be absorbed by the body through contact with the skin, swallowed or inhaled. The aim of this study was to analyze tobacco farmers chronically exposed to low doses of pesticides and to nicotine in relation to possible genotoxic and mutagenic effects arising from occupational exposure, through Comet Assay (CA), Cytokinesis Block Micronucleus Assay (CBMN) in cultured lymphocytes and Buccal Micronucleus Cytome Assay (BMCyt) in buccal cells. Seventy-six individuals were evaluated: 45 were non-exposed (control group) and 31 were exposed individuals (exposed group), with an average of 28.7 (\pm 15.4) years of work at tobacco fields. When cell death and nuclear division index were compared, through CBMN, there was no significant difference between groups. However, there were increased cells with micronucleus (MN; $P= 0.0103$) and with nucleoplasmatic bridges ($P= 0.001$) for exposed individuals when compared to control ones. BMCyt analysis showed a significant increase of MN ($P<0.01$), bud, broken-egg and binucleated cells ($P<0.001$), parameters of DNA damage; and also significant increase of picnotic ($P<0.05$) and chromatin condensed cells ($P<0.001$), indicatives of cell death. It was not observed a correlation between MN found on CBMN of cultured lymphocytes and on BMCyt of buccal cells. When damage index was compared, through CA, it was verified a significative increase ($P<0.0001$) of DNA damage for exposed group in relation to control group. Also, there was a positive correlation ($P= 0.0011$) of genotoxicity on tobacco farmers related to years of work at farming. These results show that occupational exposure to pesticides and nicotine and exposure time are related to increase of DNA damage verified, regardless gender. Pesticides are known for molecularly interact with DNA. Tobacco farming has suffered challenges regarding improvements as to excessive use of pesticides and to great physical effort that demands from farmers, besides making them constantly undergo some kind of occupational exposure. Therefore, it becomes increasingly indispensable studies to alert the scientific community and guidance to these workers.

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CLASTOGENICITY OF *Melissa officinalis* METHANOLIC EXTRACT AND ABSENCE OF CLASTOGENIC AND ANTICLASTOGENIC ACTIVITY OF ITS ETHANOLIC EXTRACT IN HUMAN LYMPHOCYTES

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Key-words: lemon balm, chromosomal aberration, cytotoxicity

Popularly known in Brazil as “erva cidreira” or “melissa”, the species *Melissa officinalis* L. is widely used worldwide – as part of the folk medicine, through the use of teas, infusions and oils – as well as by food and pharmaceutical industries. Its antiviral, anxiolytic and antitumoural potential has been scientifically proved, while studies about its mutagenic and antimutagenic potential are scarce, thereby requiring further investigation. Thus, this study aims at analysing the cytotoxicity, clastogenicity and anticlastogenicity of the methanolic (ME) and ethanolic (EE) extracts of *Melissa officinalis* in human lymphocytes, by trypan blue exclusion test and the chromosomal aberration assay (CA), respectively. Therefore, the cell culture was prepared with 0,5 mL of peripheral blood of 06 volunteers (both genders) and incubated at 37 °C for 48 hours, the following treatments were performed: negative control (dimethylsulfoxide – DMSO – 30 µL); positive control (Methyl Methanesulfonate – MMS – 1µg/mL); ME (100 µg/mL); EE (100 µg/mL); ME + MMS; e EE + MMS, and incubated for another 24 hours. Colchicine (0,016%) was added to the cultures, 1 hour and 30 minutes before the process was finished, to obtain cells in metaphase. Around 10 µL of the cell suspension culture were collected for the cell viability analysis, which was the result of the non-stained cells (living) divided by the total of counted cells (stained and non-stained). The slides for CA were prepared by dripping and stained with Giemsa 8%. The analysis was carried out under a light microscope (1000x magnification), counting 100 metaphases with 46 chromosomes per volunteer/treatment, verifying chromosomal number and integrity. The results of the trypan blue showed cell viability >99%, indicating the inexistence of cytotoxicity of both extracts in human lymphocytes, which is in accordance with literature, since *M. officinalis* has been reported to have cytotoxic activity only over tumoural cells, not on healthy ones. The results of the test of CA indicated clastogenicity of ME (p=0,04), suggesting that this extract may potentially cause harm to the DNA, possibly due to toxicity of methanol. Nevertheless, no results were statistically significant

for the verification of anticlastogenicity, revealing that both methanolic and ethanolic extracts do not protect the DNA against chemically-induced damage, which is consistent with the literature.

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EVALUATION OF GENOTOXICITY OF DESFLURANE ANESTHESIA IN PATIENTS WHO UNDERWENT MINIMALLY INVASIVE SURGERY*Nogueira FR¹, Braz LG¹, Aun AG¹, Souza KM¹, Braz JR¹, and Braz MG¹*

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Key-words: inhalation anesthetics; DNA damage; comet assay; elective surgical procedures.

Many anesthetic agents are used in anesthesiology field, often without full understanding of the potential effects on the patients' health. There are controversial results about genotoxic effects of volatile anesthetics. Desflurane is one of the newest volatile halogenated agents that have been used for general anesthesia maintenance. However, scarce literature is available concerning possible genotoxicity effect of desflurane in clinical studies. Thus, the aim of the study was to evaluate the genotoxic potential of anesthesia maintained with desflurane in 15 non-smoking adult patients without comorbidities, of both sexes, who underwent minor surgery (septoplasty) lasting at least 90 min. Patients enrolled in the study received desflurane anesthesia (1.0 minimum alveolar concentration – MAC; 6%), and blood samples were collected before anesthesia induction (T0), 90 min after the beginning of anesthesia (T1) and on the following day of surgery (T2). Lymphocytes were isolated and DNA damage was assessed by the alkaline comet assay. Coded slides were analyzed using Comet Assay IV system and data were expressed as tail intensity. Results showed statistically significant increase in T2 compared to T0 ($p=0.04$). The findings suggest that desflurane anesthesia can induce DNA strand breaks and alkali-labile sites on the day after minimally invasive surgery in healthy patients. Since the MAC for desflurane is much higher than the other volatile halogenated anesthetics, this high MAC can contribute for genotoxicity. Further investigations are required to investigate the possible mechanisms of desflurane-induced DNA damage. Thus, the current study contributes to add new information about the possible toxic effects of anesthetics at the molecular level.

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NEW POSSIBLE MOLECULES FOR CHAGAS DISEASE TREATMENT

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Key-words: Chagas disease, nitroimidazoles, genotoxicity, mutagenicity, trypanocidal activity

Chagas disease is an important tropical disease caused by *Trypanosoma cruzi* that has no effective drug treatment available besides the benznidazol which has toxic activity and limited effect. New drugs are needed for its treatment. The Institute of Pharmaceutical Technology at FIOCRUZ has designed three new nitroimidazole compounds: PNING 47-13 (4-cyclopropyl-1-(1-methyl-4-nitro-1*H*-imidazol-5-yl)-1*H*-1,2,3-triazole), PNING 43-14 (ethyl 1-(1-methyl-4-nitro-1*H*-imidazol-5-yl)-1*H*-1,2,3-triazole-4-carboxylate) and PNING 39-14 (1-(1-methyl-4-nitro-1*H*-imidazol-5-yl)-4-(4-pentylphenyl)-1*H*-1,2,3-triazole). This work investigates the anti-trypanosomal, cytotoxic, mutagenic and genotoxic activities of these compounds. Bloodstream trypomastigotes of the Y strain was incubated with them to evaluate their trypanocidal activity (IC₅₀). The compounds showed trypanocidal activity: 5.4 μ M (PNING 47-13), 45.3 μ M (PNING 43-14) and 12.9 μ M (PNING 39-14). The mutagenicity was evaluated performing the *Salmonella* microsome assay with *Salmonella enterica* serovar Typhimurium strains (TA97, TA98, TA100, TA102), including metabolically competent ones (YG1021, YG1024), which overexpress nitroreductases and acetyltransferases. The nitroimidazoles showed dose-dependent mutagenicity for at least one strain, with and without S9 mix at high doses (up to 100 μ g/plate). It was observed a correlation among nitroreductases, acetyltransferase, S9 mix and the compounds mutagenic activity. RAW264.7 macrophage rat cell line and HepG2 human hepatocarcinoma cell line were used for cytotoxic and genotoxic analyses. The cell proliferation WST-1 assay and LDH release assay were performed. WST-1 assay showed positive result only for PNING 39-14 and RAW, after 24 h of treatment (5.0 and 2.5 μ g/well). There was no cytotoxic activity with the LDH assay (up to 5.0 μ g/well). The genotoxicity was evaluated performing the Micronucleus (MN) test. The MN formation in cells was observed for PNING 47-13 and PNING 43-

14, incubated either with HepG2 or RAW (3 and 24 h; up to 100 μ g/well). PNING 39-14 did not show genotoxicity, although it might be due to the cytotoxicity previously observed in the WST-1 assay. Mutagenic and genotoxic activities started to display at high concentrations showing that a safe concentration interval with trypanocidal activity is accessible. Our results suggest that advances can be made in the development of new safe nitroimidazole compounds for Chagas disease treatment.

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MUTAGENIC ACTIVITY OF *Cinnamomum camphora* AND A POSSIBLE COLCHICINE INHIBITION, *IN VITRO*.*Santos, JC^{1,2} and Bellini MF¹.*¹ Laboratory of Molecular Biology and Cytogenetics, University Sagrado Coração, Bauru-SP² Studies Center of Education and Health, Faculty of Philosophy and Sciences, Paulista State University (UNESP, Campus II), Marília-SP.

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Key-words: camphor, cytotoxicity, chromosomal aberration, micronucleus.

Cinnamomum camphora (camphor) is a terpenoid ketone, prescribed for the treatment of diseases related to inflammation and also used in cosmetics. The Brazilian Sanitary Surveillance Agency (ANVISA) and the Food and Drug Administration (FDA) established 3% as maximum camphor content in products for external use due to its toxicity. Therefore, this study aimed to determine the cytotoxic and mutagenic potential of camphor in propylene glycol (PG) and essential fatty acid (EFA) solutions in human lymphocytes culture (approved by the Institutional Ethics Committee) at Trypan Blue Exclusion Method, micronucleus test in binucleated cells (CBMN) and chromosomal aberrations (CA). To perform the test, peripheral blood was collected from six healthy volunteers and this added 0.5 ml in culture medium containing RPMI 1640 + Hepes, fetal bovine serum, phytohemagglutinin A and antibiotics and incubated at 37°C, by 6 hours. After 6 hours were performed treatments with camphor in PG and e EFA solutions with and without damage-inducing agent (MMS); re-incubated for 42h (colchicine (0.016%) - 1h30 before the end) for CA test; or for 72 hours after exposure and 44 hours before the end of CBMN test was added cytochalasin B (6µg / mL) to the culture. The cell suspension was reserved for the cytotoxicity assay by Trypan Blue Exclusion Method. The cytotoxicity test in both trials showed a cell viability > 50. In the CA test, chromosome analysis was possible only on negative control treatment, so it is believed that camphor can prevent the action of colchicine, making it impossible to obtain metaphase spreads. CBMN test showed an increase in micronucleus formation in camphor treatment EFA solution (10.17 ± 4.793 ; $p = 0.0012$), which showed mutagenic activity compared to the negative control (4.24 ± 3.599), but no mutagenic or antimutagenic activity were observed for camphor in PG solution. Thus, a worrying situation is found in the cosmetic market and Brazilian pharmaceutical, where the products are not consistent with the maximum concentration suggested by ANVISA and FDA, and the present data suggest lower concentrations since the concentration of 1% was mutagenicity. However, more studies in the genetics-toxicological of *Cinnamomum camphora* are needed to confirm and obtain more conclusive results about its use.

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In vivo roles of Smc1a in developing nervous systemGabriel E. Matos-Rodrigues¹, Gabriel R. Cavaleiro¹, Pierre-Olivier Frappart² andRodrigo A. P. Martins¹¹Programa de Biologia Celular e do Desenvolvimento, ICB, Universidade Federal do Rio de Janeiro, Brazil and ²Clinical Cooperation Unit Neuropathology, DKFZ, Germany.

Smc1a, Smc3 and Rad21 are the structural components of the cohesin complex that crucial for maintenance of sister chromatids cohesion during the S-phase of the cell cycle until the final sub phases of mitosis. Phosphorylation of Smc1a by Atm and Atr kinases is important for DNA damage signaling and repair. In addition, in postmitotic cells, the cohesin complex regulates chromatin conformation and gene transcription. Mutations in Smc1a or other cohesin genes lead to a group of diseases known as cohesinopathies. Cornelia de Lange Syndrome (CdLS) is one of these and is characterized by developmental defects, including facial malformation, neurodevelopmental delay, mental retardation and ophthalmological problems.

First, we analyzed mRNA (real-time RT-PCR) and protein expression (western blotting) patterns of Smc1a, Smc3 and Rad21. The three core components of cohesin complex are expressed throughout retinal development. To understand the role of Smc1a in developing nervous system *in vivo*, we developed a conditional knockout mice (cKO) in which exons 2 and 3 of *Smc1a* gene were flanked by lox sequences. Two different lines of Cre mice were used to inactivate Smc1a in developing CNS (*Pax6-Cre* and *Nestin-Cre*). Inactivation of Smc1a in the retina impaired eye growth. In Smc1a deficient-retinas, we observed a decrease in the number of mitotic cells, an increase in cell death and p53 stabilization. Inactivation of both p53 and Smc1a (*Smc1a^{Nes-Cre}; p53^{-/-}*) reverted the cell death induced by Smc1a loss. Transcriptome analysis revealed profound differences in the gene expression patterns of Smc1a-deficient retinas. Moreover, Smc1a loss in retinal progenitor cells (*Pax6-Cre*), not only induced cell death during embryogenesis, but also led to the degeneration of post-mitotic photoreceptors cells during postnatal development.

We have seen for the first time that Smc1a regulates the survival of proliferating cells during CNS development *in vivo*. These findings demonstrate that Smc1a is essential brain and eye development and corroborate to the understanding of the malformations caused by the loss of function of cohesin complex, as observed in human syndromes.

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GENOTOXICITY OF MODERN WASTE ANESTHETIC GASES EVALUATED BY BUCCAL MICRONUCLEUS CYTOME ASSAY IN ANESTHESIOLOGISTS*Souza KM, Braz LG, Nogueira FR, Aun AG, Braz JR and Braz MG*

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Key-words: environmental air pollutants, anesthetic gases, occupational exposure, micronucleus assay

Occupational exposure to waste anesthetics gases (WAG) may result in adverse health effects of professionals who work in the operating rooms (OR). There are no data available in Brazil regarding the concentrations of the WAG in the OR. In addition, no study so far has evaluated the buccal micronucleus cytome (BMCyt) assay, a minimally invasive method to detect DNA damage, chromosomal instability, cell death, and the regenerative potential of buccal mucosal tissue, in professionals occupationally exposed to the most modern WAG. Thus, the current study evaluated the BMCyt assay in professionals who worked in Botucatu Medical Hospital at UNESP, and determined the concentrations of WAG in the OR in the mentioned hospital. Exfoliated buccal cells were collected from 60 physicians, as follows: anesthesiologists (n=30) exposed to WAG for at least 2 years, and physicians (n=30) with no occupational exposure, matched by age, sex and lifestyle. Coded samples were immediately processed and slides were stained with Feulgen-Fast green. The analyses included basal and differentiated cells, binucleated cells, micronucleus (MN), nuclear buds, and karyorrhexis, pyknosis, karyolysis and condensed chromatin. The concentrations of isoflurane, sevoflurane and desflurane (halogenated anesthetics) and nitrous oxide (anesthetic gas) were determined in the OR using a portable infrared spectrophotometer. The results showed that the exposed group had higher frequencies of MN and two cell death parameters (karyorrhexis and pyknosis), and lower frequency of basal cells when compared to the controls ($p < 0.05$). The means of WAG found in ORs were above 5 parts per million (ppm) for the halogenated anesthetics and above 150 ppm for nitrous oxide. In conclusion, the findings indicated that the occupational exposure to the most used WAG cause genomic instability, detected by the BMCyt, in anesthesiologists exposed to the concentrations found in the current study, suggesting that OR professionals can be considered in potential risk for harmful genetic effects. Moreover, the BMCyt assay seems to be a sensitive biomarker to detect genotoxicity in professionals occupationally exposed to WAG.

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Quantification of the Cytotoxic, mutagenic and genotoxic effects of Methyl Methanesulfonate on *Oryza sativa* L. seeds*Dantas AF1, Lopes RM2, Jose SCBR3, Pádua JG3, Gimenes MA3, and Grisolia CK4*

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Key-words: *Oryza sativa* L., Methyl Methanesulfonate, Cytogenetic tests, Comet test

The knowledge about the genomic stability of stored seeds in genebanks is highly important for understanding their viability for germination. For this, it is necessary a better understanding on the genetic integrity of important varieties for human diet, as common rice, *Oryza sativa* L. ($2n = 2x = 24$). Despite its great importance, studies about effects of mutagens on common rice are scarce. This study aimed to evaluate possible cytotoxic, mutagenic and genotoxic effects of a known mutagen, methyl methanesulfonate (MMS), on accessions of common rice seeds (BRS Formoso and BGA008070 Primavera) from Embrapa's genebanks, through cytogenetic and comet tests. Seeds of the two accessions of *O. sativa* were exposed to three concentrations of MMS (5, 10 and 15 mg/L) for three periods (4, 8 and 24 h) to cytogenetic tests and for 24 h to comet test. In cytogenetic tests were evaluated mitotic index (cytotoxicity), frequencies of chromosome aberrations and micronuclei (mutagenicity) of meristematic root cells of the two accessions of *O. sativa*. For the comet assay, analysis were performed from embryos nucleoids of the two accessions of *O. sativa* using the software comet assay 4.3.1 being the tail intensity parameter chosen to measure DNA damage (genotoxicity). The results of cytogenetic tests demonstrated that only BGA008070 Primavera accession was sensitive to the effects of MMS, by significantly increasing the frequency of chromosome aberrations at concentration of 10 mg/L for 8 and 24 h (* $P < 0.05$ and ** $P < 0.01$, respectively), as well as caused significant decrease in the mitotic index at concentration of 15 mg/L for 4 h (* $P < 0.05$), compared to negative control. Meanwhile, MMS caused DNA damage, increasing the tail intensity of nucleoids in both accessions for the three tested concentrations through comet assay, when compared to the negative control. It was not observed increased frequency of micronuclei and cytotoxic effects at concentration of 15 mg/L for exposure greater than 4 h to BGA008070 Primavera accession indicating that the effects caused by

MMS are subject to repair. Furthermore, DNA damages induced by comet assay, as well as damages induced by MMS, can be repaired by the cells. In fact, BRS Formoso accession was not sensitive to the genotoxic effects of MMS suggesting that there are genetic, physiological and physical differences between these two tested accessions.

Acknowledgments: Brazilian Agricultural Research Corporation (EMBRAPA), University of Brasilia (UnB) and Brazilian National Council for Research and Biotechnology (CNPq)

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Translesion DNA Polymerases and genome maintenance in *Trypanosoma brucei*Zurita-Leal AC, Prorocic M and McCulloch R

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Key- words: Repair, Translesion polymerases

The genome of every organism is subject to damage, which is tackled either by repair or tolerance. Many DNA repair pathways have been documented in *Trypanosoma brucei* but less attention has been paid to damage tolerance, a reaction in which lesion bypass is needed, in particular to ensure continued genome replication. Such bypass is promoted by translesion DNA polymerases (TLS Pols), of which five putative examples can be found in *T. brucei*. We have used RNAi to examine the function of four of these proteins in bloodstream forms of *T. brucei*. Loss of PolN (Nu) was shown to be severely detrimental to growth, with accumulation of cells showing aberrant nuclei, suggesting a critical role in nuclear genome maintenance. RNAi of PolZ (zeta) did not impair growth, but resulted in increased sensitivity to methyl methanesulphonate (MMS) damage, suggesting a role in the response to alkylation. The sequence of PolQ (theta) suggests that the predicted protein may not be a joint polymerase-helicase like in other eukaryotes, but only a helicase. RNAi revealed that loss of the factor did not affect growth, nor did it result in increased MMS sensitivity. Despite this, we provide evidence that PolQ interacts with BRCA2, a key component of *T. brucei* homologous recombination and a factor whose loss leads to erosion of the variant surface glycoprotein gene archive. RNAi of PolH (eta) also does not impair growth, and study into the potential contribution of this enzyme to the damage response is ongoing. Taken together, these data reveal widespread and variant functions for four of five putative TLS DNA polymerases in *T. brucei* genome biology.

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Dissecting the kinome of *T. brucei*: RIT-seq of cell cycle sorted *T. brucei* identifies kinases involved in the regulation of nuclear DNA replication

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The coordinated replication and segregation of the genome to daughter cells is an integral cellular process to ensure inheritance and the preservation of life. Several protein kinases (PKs) have been documented to regulate multiple steps in nuclear DNA replication in yeast and other eukaryotes. However, though information is emerging about the machinery and coordination of *T. brucei* nuclear replication, nothing is known about the putative PK regulation of the reaction. Identification of how nuclear replication is regulated would not only provide insight into the evolution of this essential process, but may open up new avenues for the therapeutic intervention of the diseases caused by *T. brucei* and other kinetoplasts. To address this, we pooled all bloodstream form *T. brucei* cell lines that individually target every PK (183 in total) by inducible RNAi (Jones et al., PLOS Pathogens, 2014). The pool was then sorted, with and without RNAi induction, according to their cell cycle stage based on DNA content (G1, S-phase and G2/M) and relative read depth mapped (RITseq) over time and per cell cycle stage. This screen revealed PKs already known to be involved in cell cycle progression (e.g. transition from G1 to S: CRK1 and CRK2), as well as several novel PKs. We are characterizing several of the PKs in detail by examining cellular localization and the effect of their RNAi on DNA synthesis and replication factor localisation. In doing this, we have begun to dissect the PK network involved in *T. brucei* nuclear DNA replication.

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THE GENOTOXIC EFFECT OF ANESTHETIC GASES IN MEDICAL RESIDENTS

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Key-words: inhalation anesthetics, DNA damage, comet assay

The genetic material is exposed to a several agents that can induce damage. Among the exogenous agents, the volatile anesthetics have been evaluated about their possible genotoxic effect, especially in hospital professionals exposed for a long-term. However, it is unknown whether a shorter period of exposure to anesthetic gases can triggers DNA damage. Taking into consideration the importance of occupational exposure to anesthetic gases assessment, the aim of this study was to evaluate genetic damage in physicians at the end of their Medical Residency Program. After the approval of the study from the Ethical Committee, the study was conducted at the Hospital das Clínicas, Faculdade de Medicina de Botucatu - UNESP in 40 medical residents, who were allocated in two groups of 20, as follows: the exposed group consisted of medical residents who worked in operating rooms (Anesthesiology and Surgery areas) and were exposed to anesthetic gases (isoflurane, sevoflurane and nitrous oxide) for a three-year period, and a control group, consisting of medical residents of Internal Medicine. Blood samples were collected, lymphocytes were isolated and then processed for the comet assay. Coded slides were analyzed using the Comet Assay IV to measure DNA damage. There were no significant differences between groups regarding demographic data ($p > 0.05$). The results showed that the exposed group had a significant increase of DNA strand breaks and alkali-labile sites compared to the control group ($p = 0.027$). Therefore, despite the short period of exposure, medical residents exposed to anesthetic gases have increased genetic damage detected by the alkaline comet assay. The findings highlight the genetic risk in young professionals exposed to volatile anesthetics.

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EVALUATION OF THE CYTOTOXIC, GENOTOXIC AND MUTAGENIC POTENTIAL OF THE MELITTIN ON THE HUMAN FIBROBLAST CELL CULTURE (HFF-1).*Berreta MP1, Hoshina MM1 and Marin-Morales MA1.*

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Key words: natural products, MTT test, comet assay, micronucleus, bee venom component.

Currently, it is popular knowledge that natural products can provide more health benefits if compared with synthetic chemicals. An example of this is the bee venom that was always been used on traditional oriental medicine on treatment of arthritis and pain. Recently the bee venom, which has as major active principle the melittin, has been used in the cosmetic industry as a potential agent on wrinkle reducing. The melittin acts increasing blood circulation and, consequently, the production of collagen and elastin, eliminating dead cells and reducing wrinkles. Nowadays, it is known that it also acts on healthy cells, causing their rupture because the melittin binds to plasmatic membrane creating water pores or increasing cell permeability. Thus, the present study aimed to evaluate the cytotoxic, genotoxic and mutagenic potentials of the melittin on human fibroblast cells (HFF-1). For this, the MTT test, comet assay and micronucleus with cytokinesis block were performed. For the MTT test (cytotoxic test), the concentrations 50.0 µg/ml, 25.0 µg/ml, 10.0 µg/ml, 5.0 µg/ml, 1.0 µg/ml, 0.5 µg/ml, 0.05 µg/ml, 0.005 µg/ml, 0.0005 µg/ml, 0.00005 µg/ml, 0.000005 µg/ml of melittin were tested. Of these, only the concentrations of 50.0 µg/ml, 25.0 µg/ml, 10.0 µg/ml, 5.0 µg/ml were cytotoxic to the cells. The other concentrations, despite not showing significant values comparing to the negative control, did not show a cytotoxic effect by having a high cell viability (over than 75%). Thus, the chosen concentrations for performing the comet assay were 1.0 µg/ml, 0.05 µg/ml and 0.0005 µg/ml, which viabilities were respectively 89.1%, 86.9% and 89.8%. None of the studied concentrations showed genotoxic potential for the cells, which allow us to conclude that these concentrations were not able to induce damage to the HFF-1 cells or, if had damage inducing, the cells were able to recover in the 24 hours period that they were exposed to melittin. When using the same concentrations to perform the MN test with the HFF-1 cell line, none of them showed mutagenic potential, probably due to the characteristic of the cell that responds only to the exposed product. Despite of the melittin did not induce cytotoxic, genotoxic and mutagenic effects on fibroblast cells we cannot guarantee a safe use to it, once there is a few studies about melittin and no conclusive information about its effects, before or after passing through the cell metabolism.

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UVA LIGHT INDUCES DNA DAMAGE AND MUTAGENESIS IN NORMAL CELLS AND XERODERMA PIGMENTOSUM VARIANT PATIENTS CELLS*MORENO NC 1,2, GARCIA CCM 4, SOUZA TA 2,3, MENCK CFM 2*

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Keywords: UVA light, Xeroderma Pigmentosum Variant, DNA damage, mutagenesis.

Ultraviolet radiation A (UVA) is an important environmental agent that reaches the earth's surface (about 95% of sunlight) and induces DNA damage that may participate in the skin cancer induction. The cells have several mechanisms that prevent or tolerate DNA damage caused by UV. Xeroderma Pigmentosum Variant (XP-V) patients present increased risk to skin cancer due to mutations in DNA polymerase eta, an involved in Translesion Synthesis (TLS). The aim of this work is to evaluate the genotoxic and mutagenic effects of UVA light in XP-V cells in comparison to repair proficient cells. Flow cytometry was used to detect if UVA light is able to induce damage and cell cycle arrest (propidium iodide for cell cycle and γ H2AX for DNA damage processing). Moreover, the cells were also treated with caffeine, which normally increase XP-V cells sensitivity to UVA. Mutagenesis was identified by exome sequencing of transformed cells cloned after exposure. The results showed that UVA light induces DNA damage in the transformed cells, nevertheless repair proficient cells were able to solve it more efficiently. A cell cycle arrest (mainly in S-phase) and a significant increase of cell death was observed in XP-V cells. This phenotype is exacerbated by the use of caffeine. Moreover, the permanence of lesions during replication might lead to collapse of replication fork, inducing double strand breaks and consequently cell death. Concerning to primary cells, the UVA light led to increased levels of γ H2AX immediately after irradiation in all lineages tested, but the staining was apparently more persistent in XP-V cells. However, caffeine did not lead to an increase of γ H2AX in the irradiated cells revealing that kinases ATR/ATM (inhibited by caffeine) may participate in γ H2AX formation. Besides, XP-V primary cells also presented a strong cell cycle arrest and increased apoptosis (sub-G1 cells). The results also indicated that UVA induced-mutagenesis was higher in XP-V cells, when compared to control cells. The identified mutations were mainly C:G→T:A (probably due to CPDs) and G:C→T:A (related with oxidative stress). These results showed that in absence of DNA polymerase eta, UVA light compromises the bypass of lesions resulting in cycle arrest, cell death and mutagenesis, which indicate that UVA light may play a role in skin cancer induction in XP-V patients.

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CYTOGENETIC BIOMONITORING IN WOMEN UNDERGOING CHEMOTHERAPY*Souza ACF¹, Da Silva VHP¹, Scudeller TT², Amaral MTP² and Ribeiro DA¹*¹ Department of Biosciences, Federal University of São Paulo - UNIFESP, São Paulo –SP, Brazil.² Department of Health Care Management, Federal University of São Paulo - UNIFESP, São Paulo – SP, Brazil.E-mail: carolflygare@yahoo.com.br

Key-words: breast cancer, chemotherapy and micronucleus

Cancer (CA) is the leading cause of mortality from people around the world. In addition to surgery, one of the most treatments against cancer is chemotherapy. To date, chemotherapy is considered efficient to eradicate the disease; however, the therapy may induce damage to the genetic material. Thus, the aim of this study was to evaluate putative cytotoxic and mutagenic effects induced by chemotherapy in women diagnosed to breast cancer. For this purpose, a cross-sectional study consisted of 42 women aged 18 to 70 years old, allocated according to the diagnosis and stage of breast CA treatment: control group (healthy) (n =15), chemotherapy group (n=11) and post-chemotherapy group (n=16). Cytotoxicity and mutagenicity were analyzed by micronucleus test in buccal mucosa cells. Statistically significant differences ($p<0.05$) in the frequency of micronucleus were detected in the chemotherapy and post-chemotherapy groups when compared to control. Also, statistically significant differences ($p<0.05$) were detected in the frequency of karyorrhexis between chemotherapy and control groups. Taken together, our results indicate that chemotherapy induces mutagenicity and cytotoxicity in buccal mucosa cells, being persistent after the treatment.

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**CONNECTIONS BETWEEN RHO GTPASE AND DNA DAMAGE RESPONSE:
AN EXPERIMENTAL AND PREDICTION APPROACH***Magalhaes YT¹, Espinha G¹ and Forti FL¹*

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Key-words: RhoA, DNA damage response, ultraviolet radiation, cell signaling

RhoA is a member of the Ras superfamily of small GTPases. It is overexpressed or overactivated in many types of aggressive cancers, such as breast and prostate, and regulates cytoskeleton, cell cycle, and cell death. RhoA has been implicated in the DNA double strand break damage promoted by bacterial toxins and gamma radiation, its roles in genomic stability needs more cellular and molecular investigations. Here we investigated the influence of RhoA activity in HeLa and MeWo cancer cell lines exposed to different UV-radiation treatments. By transfecting both cells either with the RhoA inhibitor C3 toxin or with RhoA-N19 mutant (dominant negative), we analyzed some molecular and cellular responses to DNA damage triggered by UV stress. We also correlated the obtained experimental results with physical protein-protein interaction networks from databanks in order to unveil the RhoA mechanism of action in response to DNA damage. Clonogenic assays showed that in both cell lines RhoA inhibition promote a significantly reduced survival compared to parental cells, submitted or not to UV treatments. In alkaline comet assays, again the RhoA inhibition in both MeWo and HeLa cell lines exhibited increased DNA damage and a delayed DNA repair 6 hours after the UV treatments. Immunoblotting experiments evaluating phosphorylation kinetics of MAPK showed a delay in ERK1/2 phosphorylation in dominant negative clones, indicating that RhoA inactivation interferes with ERK activity, thus providing lower resistance to UV-radiation effects. Additionally, RhoA inhibition in HeLa and MeWo cells led to a decrease in levels of phosphorylated H2AX, and a delay in p53 phosphorylation, whereas the levels of phospho-Chk1 are increased 15 minutes earlier than in parental cells. These results suggest that RhoA activity is important for DNA damage recognition and cell cycle arrest for repair initiation, with consequent tumor progression. Analysis of RhoA interaction networks led to construction of possible indirect mechanisms of this enzyme in regulation of proteins involved in DNA damage response, as H2AX, p53 and Chk1, very likely through Ubiquitin, JNK1 and ATM proteins. Since it has not been described roles of RhoA in UV-mediated DNA damage repair, our data suggest that this protein is significantly involved in signaling pathways for DNA damage recognition and for the repair can be efficiently accomplished.

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1044.

ANTIMUTAGENIC POTENTIAL OF PURPLE CARROT EXTRACT (*DAUCUS CAROTA L. SSP. SATIVUS VAR. ATRORUBENS ALEF.*) IN RAT BLOOD CELLS

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Keywords: 4-Nitroquinoline-1-oxide, *daucus carota*.

Nowadays, it is important to evaluate if some dietary components exert antimutagenic effects against environmental mutagens and carcinogens. The aim of this study was to evaluate the antimutagenic potential of purple carrot extract in blood cells. A total of 20 male Wistar rats were distributed into 4 groups (n=5 per group): Group 1 - negative control group (non-treated group); Group 2 – experimental group (received 4NQO in drinking water and treated with purple carrot extract incorporated to the commercial diet during 12 weeks); Group 3 – 4NQO control group (received 4NQO in drinking water during 12 weeks only); Group 4 – purple carrot extract control (received purple carrot extract incorporated to the commercial diet for 12 consecutive weeks only). Micronucleus test in bone marrow as performed for evaluating mutagenicity. Purple carrot extract was able to decrease the number of micronucleated cells in rats exposed to 4-nitroquinoline 1-oxide control group. In conclusion, our results demonstrate that purple carrot extract is an anti-mutagenic compound in rat blood cells.

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EFFECTS OF AIR POLLUTION ON THE RISK OF CONGENITAL ANOMALIES IN SÃO LUIZ CITY, MARANHÃO ISLAND.*Pires RCR1 and Silva VC2*

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Keywords: air pollution, congenital anomalies, environmental teratogenesis.

Air pollution not only contributes to global warming but also has deleterious effects on the human health. Recent epidemiologic studies in different countries have indicated association between ambient air pollution and adverse birth outcomes, such as preterm birth, low birth weight and intrauterine growth retardation. Other studies have shown that living in areas with industrial pollution is associated with higher rates of congenital anomalies. Congenital anomalies are recognized to be a major risk factor of stillbirth and of neonatal and infant mortality. Therefore, we investigated whether maternal exposure to air pollution is associated with elevated birth defect risk in infants delivered between 2008 and 2012 in the metropolitan region of São Luiz, Maranhão Island. We conducted an ecological design and data on birth defects was collected from the Information System of Live Births of the city. Air contaminants data for sulfur dioxide (SO₂), matter with diameter less than 10 µm (PM₁₀), carbon oxide (CO), ozone (O₃) and nitrogen dioxide (NO₂) were monthly provided from Department of Environment. Cases with single or multiple congenital defects were registered according to the International Classification of Diseases, version 10. Odds Ratio (OR) was used to measure the effect of ambient air pollution on birth defects. It was estimated by logistic regression. Rate of prevalence of congenital anomalies ranged from 6.25 per 1,000 live births (2009) to 5.35 live births (2013). The most frequent birth defects involved congenital malformation and deformities of the musculoskeletal system (60.95%) and congenital malformations of the nervous system (16.03%). However, our results were not able to relate the presence of air pollutants on birth defects, except for PM₁₀ and SO₂ and cardiovascular defects. Air pollutants could directly exert adverse effects as pro-oxidants binding to lipid and proteins, therefore promoting oxidative stress and the production of free radicals, a process that may elicit a variety of diseases or defects. In addition, there is recent evidence that air pollutants can contribute to epigenetic changes, including alteration of DNA methylation. Such epigenetic modifications during pregnancy could impair normal embryo development and lead to birth defects. Further studies with other designs are needed to confirm the hypothesis of the effect of air pollution on the occurrence of congenital defects.

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THE FOOD SUPPLEMENT SYNEPHRINE AND ITS CITOTOXICITY, OXIDATIVE POTENTIAL AND GENOTOXICITY IN HUMAN CELLS *IN VITRO*

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Key Words: synephrine, cytotoxicity, DNA damage, reactive species.

Synephrine (*p*-synephrine) is a primary protoalkaloid derived from the phenylethylamines group that is naturally found in the immature fruit of bitter orange (*Citrus aurantium*), is added to food supplements to stimulating thermogenic effects. The abusive consumption of food supplements with ergogenic and weight loss purposes has been observed worldwide. The safety of these products has been questioned and adverse events were associated with supplements containing synephrine. In addition, the cytotoxicity and genotoxicity of this has not previously been assessed. The aim of this study was evaluated the possible cytotoxicity, antiproliferative, genotoxicity, mutagenicity and oxidative activities in primary cultures of gastric cells and Caco-2 (human intestinal adenocarcinoma cells) *in vitro*, and then showed possible consequences of the hazard of this substance to human health. Initially, cytotoxicity of ten concentrations of synephrine (25-5000 μ M) were tested by MTT and Neutral Red assay (NR). After that, three non-cytotoxic concentrations (2, 20 and 200 μ M) were chosen to analyze the effects on cell proliferation (total protein content), genotoxicity (comet assay), mutagenicity (cytome assay) and the generation of intracellular reactive species (REs) using CM-H₂DCFDA probe. In all assays, the negative, positive and solvent controls were included. The results obtained in the MTT assay showed no cytotoxic effects of synephrine in any concentration when compared to the negative control; however, in the NR assay, concentrations higher than 800 μ M in Caco-2 cells demonstrated cytotoxic activity. After, concentrations of 2, 20 and 200 μ M were selected and in cell proliferation curves, no reduction of cell growth was observed in both cell lines. Concerning the evaluation of oxidative activity using CM-H₂DCFDA probe, only gastric cells increased the amount of intracellular RS in the higher concentration tested (200 μ M). When evaluating the possible genotoxic effects using the comet assay and the mutagenic effects by micronucleus test, synephrine did not induce DNA damage. Therefore, it can be concluded, so far, the results indicate that synephrine has no effects on the viability and on DNA stability of human cells lines *in vitro* used, but since this is the first study which assessed its potential hazard, more data about alterations in biochemical parameters as well as changes in gene expression (RT-qPCR) will be obtained for better understanding the mechanisms of action of this supplement.

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TOXICITY INDUCED BY ALOE VERA GLYCOLIC EXTRACT AND ULTRAVIOLET RADIATION IN EUKARYOTIC CELLS

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Keywords: Babosa, UV radiation, toxicity, anthraquinones.

Aloe vera is a plant that belongs to the Liliaceae family, being the common name for over 400 plant species. Among the large variety of known species of Aloe, *Aloe barbadensis* Miller (Aloe vera) is the most used by the population because of its folk medicinal properties and by the cosmetic industry in order to compose shampoos, creams and other cosmetics. They are succulent and xerophytic plants, whose leaves store a gelatinous substance that is extracted and incorporated into those products. However, recent studies have shown that components of the aloe vera leaf gel containing photochemical properties that in the presence of ultraviolet A radiation, would be responsible for adverse effects. Among these components, two anthraquinones, aloe-emodin and aloin, have been identified as capable of absorbing UV radiation and produce reactive oxygen species. Thus, this study aims to evaluate the toxicity induced by a commercial glycolic extract of Aloe vera, in combination with UVA radiation, in eukaryotic cell line (A549 - human adenocarcinoma alveolar basal epithelial cells). The aloe vera glycolic extract was donated by Mapric (São Paulo) and it was obtained from the gelly-like substance found in the inner part of the plant leaves. The cytotoxicity of the extract and its association with UVA are being evaluated by the WST-1 cell proliferation assay; Trypan blue exclusion test and clonogenic recovery assay. Initially, the aloe vera glycolic extract proportions chosen were 1.56, 3.13, 12.5, 25 and 50%, and the cells were exposed to it during the times 2, 6, 12, 24 and 48 hours. Data obtained showed that the lower proportions of aloe vera (1.56, 3.13 and 6.25%) were not cytotoxic, while the proportions of 12.5, 25 and 50% induced a significant decrease in cell survival. Following, A549 cell cultures were incubated with aloe vera glycolic extract (1.56, 3.13 and 12.5%) associated with UVA exposition (10W/s) during 30, 60, 90 and 120 minutes. Once more the extract proportions of 1.56, 3.13 and 6.25% showed to be safe to the cells. The 12.5% Aloe vera glycolic extract proportion, associated with UVA radiation, induced a slight difference in cell death, suggesting that the aloe vera glycolic extract could be harmful to cells when associated to UVA rays and people should be careful to use dermatological products with aloe vera under the sunlight.

Financial support: CAPES, UERJ, CNPq, FAPERJ

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CYTOTOXICITY ASSESSMENT OF THE PARTITION ETHYL ACETATE METHANOLIC EXTRACT- ABAJERU IN A549 LINEAGE CELLS

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Key-words: *C. icaco* L., column chromatography, cytotoxicity

The plants are used in medical practice for treatment of various diseases, despite little scientific knowledge about the potential toxic, they have aroused curiosity due to the pharmacological, economic and cultural activities. In this way, several studies have been developed to ensure the safety and effective use of various species. Among them, *Chrysobalanus icaco* L., or abajeru as it is popularly known, has many biological activities, highlighting the antiviral and hypoglycemic effects. It was isolated from the methanolic extract a triterpenoid (pomolic acid) capable of inhibiting growth and inducing apoptosis in a erythroleukemic cell lineage. The aim of this study was to evaluate the cytotoxic potential of the partitions 1 and 2 of ethyl acetate of the methanol extract of *C. icaco* leaves in A549 lineage (lung carcinoma). The samples were collected in the Parque das Dunas, Cabo Frio, RJ, and the extract was prepared from leaves immersed in methanol. Subsequently, the extract was placed on a chromatographic column where in the stationary phase comprises silica gel and the mobile phase was the organic solvent ethyl acetate. The cell lineage (A549) was seeded in 96 well plates and treated with different concentrations of the partitions 1 and 2. Following the 48 hour treatment period, the cytotoxic activity was assessed using the WST-1one colorimetric assay which evaluates the ability of mitochondria to transform the tetrazolium salt in formazam dye through the global activity of the mitochondrial dehydrogenase. The cytotoxic effect on cells subjected to treatment with two partitions of ethyl acetate was observed. In one partition, there was a reduction in cell viability when treated with the concentration 1µg / mL compared to other concentrations, moreover, this concentration was not statistically different ($p > 0.05$) from treatment with doxorubicin. In the partition 2, concentrations of 1 and 10µg / mL were significantly different from negative control ($p < 0.05$) and IC 50 was observed at a concentration of 10µg / ml. This work shows the cytotoxic activity of ethyl acetate partition of the methanol extract and its antitumor activity also points to the need for further studies on the chemical composition of the partition 2 in order to identify which one (s) agent (s) generated this activity.

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CYTOTOXICITY STUDY OF BUTANOL PARTITION FROM METHANOLIC EXTRACT OF *CHRYSOBALANUS ICACO* IN CELL LINE A549

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Key-words: Abajeru, toxicity, Antineoplastic

Chrysobalanus icaco L. belongs to the family Chrysobalanaceae characterized by the presence of flavonoids, triterpenes, diterpenes, steroids and tannins. Polyphenols found in abajeru extract perform numerous biological activities such as anti-inflammatory, antibacterial, antiviral, anesthetic, antileukemic, among others. A fraction of the methanol extract of abajeru leaves was identified and isolated a substance, pomólic acid, a triterpenoid with anticancer activity. Biological assays showed this effect by induction of programmed cell death (apoptosis) in tumor cells of various origins, including those expressing multidrug resistance characteristics. This work aims to study the cytotoxic potential of two butanol partition of the methanol extract of *Chrysobalanus icaco* L. leaves in A549 lineage (lung carcinoma). For the preparation of butanol partition extract was employed a glass column which have a stationary phase comprising silica gel and a mobile phase composed of butanol, then immediately add the extract of interest in the top of the column and the organic solvent butanol and at the end of the column we obtained the solvent metabolites that have increased affinity with it, after which the solvent butanol process is again placed on the column and the process is repeated. To assess cell viability of the A549 lineage, following exposure of the same two partitions butanol extract was determined by WST-1 assay. After incubation with different concentrations, WST-1 reagent was added for further evaluation colorimetric plate reader. From the assessment of the partition 1 results, it was observed that the concentrations of 1µg / ml and 5µg / mL show a significant difference ($p < 0.05$) from the control and were not significantly different compared to doxurrubicina positive control. In the partition 2, at concentrations of 1µg / ml and 5µg / ml showed a significant difference from the control ($p < 0.05$), and the IC₅₀ was the concentration of 5µg / mL, proving more cytotoxic than doxurrubicin 25nM. The next step is the high-performance liquid chromatography in order to discover the metabolites responsible for cytotoxic activity.

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SILICA EXPOSURE BIOMARKERS: A NEW APPROACH IN THE DISEASE DETECTION

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Keywords: Genotoxicity, GPX, RT-qPCR

Silica is a mixture of silicon and oxygen elements, found in amorphous and crystalline forms, being the last one more abundant on Earth. Inhalation of silica crystals may result in a serious lung disease known as silicosis, with high prevalence worldwide due to occupational exposure in activities such as mining, sand blasting and construction. The disease is progressive and irreversible, initiated by an inflammatory process with ROS production and results in pulmonary fibrosis and even in cancer development. As silicosis is not curable, it is of great interest and practical consequence the possibility of using physiological responses as prospective biomarkers, which may indicate initial exposure to crystalline silica. Thus, this study sought to establish biomarkers on cellular and molecular levels, which may point, as early as possible, silica exposure, and also the emergence of silicosis. Comet assay, evaluation of the GPX enzyme activity, and assessment of the level expression of TGF- β , IFN, IL-4, IL-5, IL-12 and CCL-5 genes were assayed in biological matrices (blood or lung) of mice, between 1 and 7 days after silica exposure. Significant difference was observed between control and exposed animals due to the rate of DNA damage throughout the studied period. GPX enzyme seems to show an activity peak after 4 days of exposure, significantly different from all other evaluated times. The TGF- β gene expression was increased after 1 day. IFN, IL-4, IL-12 showed higher expression in the intermediate period, between 3 and 5 days after silica exposure. The CCL-5 gene also showed increasing expression in 3 to 5 days after exposure, but it dropped on day 7. IFN, IL-4, IL-12 showed no expression at day 7. It was not observed the IL-5 gene expression in the evaluated period. Data obtained in this study indicate that the use of the comet assay, coupled with the activity of GPX enzyme and evaluation of the expression of genes related to inflammatory process generated by silica exposure is a promising strategy for risk assessment, monitoring and prevention of silicosis.

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MUTAGENIC AND GENOTOXIC EVALUATION OF LICOPENE EXTRACTED BY IONIC LIQUID IN RAT BLOOD AND LIVER CELLS*Da Silva VHP¹, Laranjeiras PM¹, Margonato CC¹, de Rosso VV¹ and Ribeiro DA¹*

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Key-words: ionic liquid, licopeno, mutagenicity, genotoxicity

The ionic liquid or melted salt 1-Butyl-3-methylimidazolium is an alternative to the process of extraction of natural pigments such as carotenoids. Lycopene represents about 80-90% of total of carotenoids presents in tomato. The aim of this study was to evaluate genotoxic and mutagenic potential of lycopene extracted by ionic liquid *in vivo*. A total of 20 male Wistar rats were distributed into four groups ($n=5$), as follows: **control group**; received corn oil for 7 days by gavage, **ionic liquid group** (IL), received 10 mg Kg⁻¹ for 7 days by gavage; **10 mg lycopene group**, received 10 mg Kg⁻¹ for 7 days by gavage; and **500 mg lycopene group**, received 500 mg Kg⁻¹ for 7 days by gavage. Genetic damage in peripheral blood cells was found for IL, 10 mg and 500 mg groups when compared to control group. Micronucleus test in bone marrow cells revealed increase of micronucleated cells at group exposed to 500 mg of Kg⁻¹ of lycopene. No significant statistically differences were noticed to micronucleus test among groups in liver cells. Taken together our results demonstrate that lycopene extracted by ionic liquid 1-Butyl-3-methylimidazolium at higher doses is genotoxic and mutagenic in rat blood cells.

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IS MOUSE MITOCHONDRIAL DNA PROTECTED FROM ALKYLATION DAMAGE BY AAG?*Berra CM¹, Meira LB² and Souza-Pinto, NC¹*¹Depto. de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil.²Faculty of Health and Medical Sciences, University of Surrey, Surrey, UK.E-mail: cmberra@gmail.com

Key-words: BER, alkylation base damage, mitochondria.

DNA repair systems are ubiquitous in all living organisms and crosstalk with several cellular mechanisms in order to preserve genome integrity from damage generated by a variety of exogenous and endogenous agents. Due to its localization, close to the reactive oxygen species generating electron transport chain, mitochondrial DNA (mtDNA) accumulates more damage than nuclear DNA. The base excision repair (BER) pathway is the main repair mechanism for single strand breaks and DNA base modifications, and is the predominant repair system in mitochondria. In mammals, 3-methyladenine DNA glycosylase (AAG) initiates BER of alkylated bases, playing an important role in protecting against the genotoxic effects of alkylating agents, such as several molecules of clinical relevance in chemotherapy. Mitochondrial localization of AAG was recently described in human cells. It was also reported that mice overexpressing AAG are hypersensitive to alkylating agents while knockout animals and cells are resistant, indicating that alkylation sensitivity can paradoxically be a direct result of AAG-initiated repair on alkylated substrates, influencing cellular homeostasis. Thus, we evaluated the presence of AAG in mouse mitochondria. *In silico* prediction of murine AAG (mAAG) targeting to mitochondria and their mitochondrial targeting sequences (MTSs), by the MitoProt II and iPSORT softwares, indicated that mAAG did not display a canonical MTS, and have a low probability for mitochondrial localization (score = 0.6634). Western blotting analysis, using anti-AAG monoclonal antibody, did not detect AAG in C2C12 mitochondrial extracts, while it was clearly detected in HeLa mitochondrial and nuclear extracts. The mitochondrial extracts were shown to be free of nuclear contamination using anti-Cox4 and anti-PCNA antibodies as mitochondrial and nuclear markers, respectively. Also, mAAG could not be co-localized with mitochondria in C2C12 by immunofluorescence using anti-AAG monoclonal antibody and MitoTracker Orange CMTMRos as mitochondria marker. Together, these data suggest that mAAG does not localize in mitochondria. Further analysis by fluorescence-based incision assay will investigate whether an incision activity toward alkylated bases is detected in murine mitochondria.

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TOXICITY INDUCED BY *Petiveria alliacea* L. ETHANOLIC EXTRACT FROM LEAVES IN *Escherichia coli*

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Keywords: cytotoxicity, prokaryote and ethnobotany.

Petiveria alliacea L. is a shrub belonging to *Phytolaccaceae* family, native from the Amazon region and known popularly as amansa-senhor, piu-piu and guiné. It is widely used in folk medicine for treatment of various therapeutic purposes such as, analgesic, antibacterial, antiprotozoal, antifungal and hypoglycemic properties. However, their toxicological effects are not well elucidated, requiring further studies in this area. Thus, the aim of the present study was to evaluate the toxicity of ethanolic extract of *P. alliacea* leaves. Leaves were collected and dried at 45°C for 48 hours and submitted to the preparation of ethanolic extract by infusion, followed by rotatory evaporation. In order to evaluate the cytotoxic extract potential, wild type *E. coli* cultures, in exponential growth phase, were incubated with different concentrations of the plant extract, during 60 minutes. The obtained results indicate that samples incubated with 1; 5; 10 and 15 mg/mL of *Petiveria alliacea* leaves extract showed no variation in survival fractions compared to the control ($p > 0,05$). On the other hand, in higher concentration assayed (20; 25 and 30 mg/mL) the cell survival decreased as time incubation function ($p < 0,05$). Together, data obtained suggest that *P. alliacea* extract has a cytotoxic potential *in vivo*, depending on the concentration and exposure time. Its genotoxic feature is under investigation in an *in vitro* model with plasmid DNA, with the goal to better understanding the action of *P. alliacea* in cells and molecules.

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TRANSPOSITION MECHANISM OF *mariner-mos1* UNDER STRESS CONDITIONSJardim SS¹, Schuch AP^{1,2}, Pereira CM¹ and Loreto ELS^{1,2}

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Key-words: stress, *mariner*, *Mos1*, transposition somatic, cell cycle.

The mobility is a principal key feature of transposon elements (TEs). This capacity of moving within a host genome gives to TEs the role of contributing to mutagenesis and genetic variability. However, the mechanisms that trigger the activation of TEs under stress condition are not well understood. The *mariner-Mos1* DNA transposon mobilizes both somatic as well as in germline cells in the *Drosophila simulans white-peach* genome. This mutant lineage allows the phenotypic study of this transposon activity through the formation of mosaic eyes. First, this mutant lineage was exposed to different stresses: ultraviolet radiation (UVC, 25J/m²), mild heat stress (28°C) and oxidative stress (Paraquat, 1mM and 2mM). Then, the *mariner-Mos1* and positive control *Hsp70* and superoxide dismutase gene expression profiles were evaluating by RT-qPCR. The *mariner-Mos1* mobilization activity was determined based on the number of red spots in the eyes of flies submitted to the same stresses, and the impact of each stress on cell cycle was also evaluated by flow cytometry. The UVC treatment had no effect in the *mariner-Mos1* gene expression, as well as in the formation of mosaic eyes. In contrast, the expression of *Hsp70* increased after UVC stress suggesting that *mariner-Mos1* expression is not directly shaped in response to this heat shock gene. Furthermore, the treatment with 28°C increased the expression of both genes and the number of red spots in the eyes of flies. After the UVC exposure, it was observed a long delay in the development of flies, as well a transient arrest in the cell cycle progression with an accumulation of cells in the G1 phase. On the other hand, the flies treated with 28°C showed a reduced time of development and a faster cell cycle progression. These results indicate that heat induces the increase of transcription and mobilization of *mariner-Mos1*, but UVC only induces the expression of *Hsp70* gene, suggesting that the mechanism of activation of *mariner-Mos1* transposition must be coupled to conditions that promote DNA replication and cell cycle progression. The effects of oxidative stress in *mariner-Mos1* transposase gene expression are in progress, although the exposures to Paraquat does not induced the formation of red spots in the eyes of flies, which indicates that genotoxic agents does not induce somatic transposition.

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THE GENOTOXIC AND MUTAGENIC EFFECTS IN LETTUCE (*Lactuca sativa* L.) GROWN IN GARDEN BUILT ON THE TAILING COAL*Rohr P¹, Martins M¹, Teixeira KO¹, Jesus MM¹, Borges GD¹ Gonçalves CD¹ and Andrade VM¹.*

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Key-words: coal, comet assay, MN, PIXE

Coal mining leads to a significant environmental commitment, since the landscape changes until soil contamination by potentially polluting wastes. The sites of these rejects deposits were, until recently, offered to the population as landfill, being used for planting vegetables. Due to the presence of genotoxic and mutagenic substances in coal and its waste, the present study aimed to evaluate the genotoxic and mutagenic potential of lettuce (*Lactuca sativa* L.) grown in garden built on the tailings coal. For this, 36 animals divided in 3 groups according to treatment: negative control (NV), lettuce juice mine (LJM) and organic lettuce juice (OLJ), each group was subdivided in acute and chronic treatment. Acute treatment corresponded to a single administration of the substance by gavage and after 3h, 6h and 24h of ingestion blood samples were collected. 24 hours after administration, the animals were killed by decapitation and bone marrows were collected. While the chronic treatment the animals received gavage dosing of the substance 1 once daily for 30 days, blood samples from these animals were collected on days 2, 5, 10, 20 and 30 on the 30th day the animals were euthanized for removal bone marrow. The identification and quantification of heavy metals in soil samples from the gardens were performed using the PIXE technique. In acute treatment, the OLJ group showed no significant difference in any of the evaluated parameters and LJM group had higher damage index (DI) and frequency (DF) in relation to the NC group 6h after administration ($p < 0,05$), with no significant change the frequency of MN. In chronic treatment, the OLJ group again showed no significant difference in the parameters evaluated, and the LJM group had higher DI and DF compared to CN and OLJ groups ($p < 0.01$) at all time points assessed, no differences in MN frequency. The land mine showed higher amounts of Ca, Mg, P, Mn, S, Zn and Cu, into the soil of organic growing ($p < 0,05$). We conclude that lettuce grown on coal waste has genotoxic potential and can be a good indicator for detecting and bioaccumulation of heavy metals in the ecosystem.

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CYTOTOXICITY AND MUTAGENICITY OF ORGANOMETALLIC DERIVATIVES OF VALPROIC ACID IN *SACCHAROMYCES CEREVISIAE*

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Valproic acid (VPA) is an important antiepileptic drug that has been largely used in humans for 40 years in the treatment of partial or generalized seizures, bipolar disorder and schizophrenia. Furthermore, it was demonstrated that VPA has an antiproliferative action against different types of tumor cells, but the mechanisms of its antitumoral action are not well known, although it is suggested that VPA can modulate the deacetylation of histones and change the expression of genes related to cell cycle and apoptosis. However, clinical studies have reported several side effects due to its high toxicity. Thus, new VPA derivatives have been synthesized to obtain less toxic and more effective VPA compounds. The objective of this study was to investigate the biological activities of VPA and VPA's organometallics derivatives Cu₂Valp₄, CuValp₂Phen and MgValp₂Phen in yeast. The cytotoxic and mutagenic potentials of the VPA and VPA's derivatives were evaluated using the BY4741 and XV185-14c *Saccharomyces cerevisiae* strains, respectively. In the survival tests, cells were exposed to all compounds (10 - 150 mM sodium valproate (SV); 0.5 - 2 mM Cu₂Valp₄, 0.5 - 2 mM CuValp₂Phen and 0.1 - 0.5 mM MgValp₂Phen) for 24 h in stationary, exponential and growing conditions. The results showed that all substances were able to decrease the survival only when cells were exposed to them in the presence of complete media (growing condition). Thus, the VPA derivatives presented higher cytotoxic effects when compared to sodium valproate, with a cytotoxicity profile of MgValp₂Phen > CuValp₂Phen > Cu₂Valp₄. In the mutagenicity assays, cells were exposed to all compounds (12.9 - 51,6 mM sodium valproate, 0.5 - 2 mM Cu₂Valp₄, 0.45 - 1.85 mM CuValp₂Phen and 0.07 - 0.3 mM MgValp₂Phen) for 24 h in growing condition. In this assay, VPA was not mutagenic while VPA derivatives were able to induce punctual mutations verified by the increase of revertant colonies in the auxotrophic phenotype for histidine and lysine aminoacids. Interestingly, neither compounds induced reversion mutation in the homoserine locus, in growing condition, suggesting that the compounds do not cause frameshift mutations. These results demonstrate that the VPA Cu and Mg derivatives are more cytotoxic and mutagenic than the VPA prototype compound in *S. cerevisiae*. Further studies are needed to understand the mechanism related to toxic effect of these compounds.

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BASE EXCISION REPAIR IMBALANCE IS ASSOCIATED WITH UNFAVOURABLE CLINICAL OUTCOMES AND MODULATES RESPONSE TO CHEMOTHERAPY IN SPORADIC COLORECTAL CANCER*Leguisamo-Meirelles, N¹, Gloria, HC¹, Kalil, AN^{1,2}, Martins, TV², Meira, LB³ and Saffi, J¹*Email: nmleguisamo@gmail.com

Keywords: Colorectal cancer; base excision repair; prognosis; energy metabolism.

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Inappropriate base excision repair (BER) and mismatch repair (MMR) activities are known to contribute in 5-fluoracil (5-FU) and temozolomide (TMZ) resistance. Imbalances in different steps of BER can influence cancer cells' fate in different ways, such as through metabolic intermediates disruption, which may have a determinant role in patients' survival. In this study, we characterized BER and MMR expression profiles in colorectal tumours and its association with clinical and pathological features and exploited, *in vitro*, the possible mechanisms behind tumour aggressiveness and response to chemotherapy. Study design includes two arms: (1) clinical: Seventy pairs of sporadic colorectal tumours and matched adjacent mucosal specimens were assessed for BER (MPG, OGG1, APE1, Pol β , XRCC1) and MMR (MLH1 and MSH2) gene (qPCR) and protein expression (immunohistochemistry) and its association with pathological and clinical features. (2) *in vitro*: MMR-deficient colon cancer cells overexpressing MPG and XRCC1 and treated with 5-FU and TMZ were evaluated for viability and energy metabolism. Overexpression of BER components was associated with more aggressive tumour features and poor pathological outcomes in colorectal cancer patients (presence of lymphatic and perineural invasion; poorly-differentiated tumours; advanced TNM staging). However, only 5 of all cases presented overexpression of the entire BER set, which configures that most of the study subjects presented imbalance of this pathway. MMR-low expressers tumours (24%) were associated with reduction in MPG, OGG1 and PARP1 mRNA levels. Overexpression of MPG, but not XRCC1, in MMR-deficient colon cancer cells increased sensitivity to 5-FU and TMZ through ATP depletion and lactate accumulation. We concluded that heterogeneity in BER gene and protein expression levels, which can be considered a pathway imbalance, is associated with unfavourable prognosis in colorectal cancer patients. Since the overexpression of a BER upstream (MPG), but not downstream (XRCC1), component is associated with higher sensitivity to 5-FU and TMZ in colon cancer cells

due to ATP depletion and lactate accumulation, we suggest that the levels of both MPG and XRCC1 in colorectal tumours might be used to predict 5-FU effectiveness in MMR-deficient neoplasms. Moreover, we propose more investigations on TMZ efficacy in colorectal cancer treatment, by exploiting the crosstalk between MMR and BER and the metabolism disruption.

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EVALUATION OF CLASTOGENICITY AND ANTICLASTOGENICITY OF *Cymbopogon citratus* AND *Lippia alba* ALCOHOLICS EXTRACTS IN HUMAN LYMPHOCYTES*Pellegatti AGS¹, Porfirio MD¹, Queiroz TB², Neves FTA³, and Bellini MF¹.*

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Key-words: lemon balm, chromosomal aberration, cytotoxicity

Cymbopogon citratus (DC.) Stapf and *Lippia alba* (Mill.) N. E. Brown are two species of Lemon balm most commonly used in popular medicine. However, studies of its toxic potential are scarce, thereby putting the human population health at risk, bearing in mind the indiscriminate use of this species. In light of the foregoing, this study aims at analysing the cytotoxicity, clastogenicity and anticlastogenicity of the methanolic (EM) and ethanolic (EE) extracts of *Cymbopogon citratus* and *Lippia alba* in human lymphocytes, by trypan blue exclusion test and the chromosomal aberration assay (AC), respectively. Therefore, the cell culture was prepared with 0,5 mL of peripheral blood of 3 volunteers (both genders) and incubated at 37 °C for 48 hours, the following treatments were performed: negative control (dimethylsulfoxide – DMSO – 30 µL); positive control (Methyl Methanesulfonate – MMS – 1µg/mL); EM (100 µg/mL); EM *C. citratus* (100 µg/mL); EM *L. alba* (100 µg/mL); EE *C. citratus* (100 µg/mL); EE *L. alba* (100 µg/mL); EM *C. citratus* + MMS; EM *L. alba* + MMS; EE *C. citratus* + MMS; and EE *L. alba* + MMS, and incubated for another 24 hours. Colchicine (0,016%) was added to the cultures, 1 hour and 30 minutes before the process was finished, to obtain cells in metaphase. Around 10 µL of the cell suspension culture were collected for the cell viability analysis, which was the result of the non-stained cells (living) divided by the total of counted cells (stained and non-stained). The slides for AC were prepared by dripping and stained with Giemsa 8%. The analysis was carried out under a light microscope (1000x magnification), counting 100 metaphases with 46 chromosomes per volunteer/treatment, verifying chromosomal number and integrity. The results of the trypan blue showed cell viability >99%, indicating the inexistence of cytotoxicity of methanolic and ethanolic extracts of both species in human lymphocytes. The analyses of clastogenicity and anticlastogenicity did not show statistically significant results (p<0,05). Thus, this partial results suggest that both *Cymbopogon citratus* and *Lippia alba* methanolic and ethanolic extracts do not cause DNA damage, just as they do not protect the DNA against chemically-induced damage.

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CAFFEINE REDUCES DNA DAMAGE IN PERIPHERAL BLOOD AND NEURAL TISSUE OF OLD MICE

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Keywords: caffeine, DNA damage, aging

Biologic aging is a process that starts at birth and continues until the death of the individual, it is a period of changes related to the passage of time that causes deleterious effects in the organism. Aging can be described as gradual changes in the physiology of the cell, which causes a decrease in its normal function. Errors in DNA sequences are regular events throughout the lifetime of any given organism. Together with the aging process, arise degenerative diseases which are characteristic of this phase of life, however, studies using caffeine which is a psychoactive substance, present in various products consumed daily, have demonstrated negative correlations in the development of these diseases. The aim of present study was to evaluate the level of DNA damage in peripheral blood and neural tissue of old mice treated with caffeine. For the present study, 40 Albinos swiss male mice (20 animals between 3-4 months and 20 animals between 13-16 months) were divided into 4 groups: Young Adults-water, young adults - caffeine, older adults -water and older adults- caffeine. Groups of young and old caffeine received caffeine solution (0.3 g/L) in bottle of water (free access) during four weeks. The others received only water during the experimental time. After the treatments were collected blood samples from animals through an incision at the tail end for performing of Comet Assay and after the animals were euthanized by cervical dislocation for the dissection of the hippocampus and femurs for performing the comet assay and micronucleus test, respectively. The comet assay for blood and hippocampus no showed significant difference between the groups of animals in the same age, demonstrating that caffeine showed no genotoxic activity. However, when comparing young and old animals observed a significant difference to blood and hippocampus, the old animals showed higher damage levels in relation to young who ingested the same substances, showing that caffeine alone was not able to reverse the damage caused by aging detected by comet assay. In the micronucleus test we observe an increase in the frequency of EPCMNs in old animals that received water compared to the young. The old animals receiving caffeine showed a decrease in the frequency of MN in EPC compared with animals of the same age who did not ingest this substance, showing that caffeine did

not show mutagenic activity and was able to reverse the mutagenic damage caused by aging. With our results conclude that caffeine was not genotoxic neither mutagenic at the doses tested. Although caffeine alone was not able to reverse the genotoxicity caused by aging detected by comet assay, in the micronucleus test, caffeine was able to reverse the mutagenic damage caused by aging.

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METHOXYAMINE SENSITIZES CANCER CELL LINES TO PHOTODYNAMIC THERAPY INDUCED BY CHLOROALUMINUM PHTHALOCYANINE NANOEMULSION*Franchi LP^{1,3}, Primo FL¹, Amantino CF¹, Montaldi APL², Takahashi CS^{1,2} and Tedesco AC^{1,2}*

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Keywords: photosensitizers; APE1 inhibitors and cell death.

The photodynamic therapy (PDT) has become an emergent anticancer therapy used in the last years. The PDT can use the aluminum phthalocyanine chloride incorporated into a nanoemulsion (CIAIPc/NE) as a photosensitizer. The CIAIPc are molecules excited by light that generates singlet oxygen culminating in oxidative stress and, consequently, cell death. Methoxyamine (MX) is a small organic amine that blocks DNA Base Excision Repair (BER) by tightly interacting with the DNA apurinic/apyrimidinic sites. These sites are then processed by protein APE1. APE1 is overexpressed in numerous solid cancers. Thus, we hypothesized that the indirect inhibition of APE1 by MX would increase the potential cytotoxicity of oxidative stress induced by PDT. Consequently, this combination would be able to sensitize resistant cancer cell lines to cell death. We first evaluated the expression of APE1 protein by Western Blot (WB) in two different cancer cell lines (A549 – human lung adenocarcinoma; and HeLa – human cervical carcinoma) and one human normal fibroblast cells (GM07492A). Then, we used fluorescence microscopy to detect the uptake of CIAIPc/NE (600 nM, 3 h-treatment) into the cell lines studied. Next, the cells were photoactivated by 0.5 J/cm² dose using laser at 670 nm; and again the APE1 protein was assayed by WB to detect its levels after photostimulation. Following, the cell death was detected using flow cytometry (ViaCount assay) after the photostimulation. Finally, co-treatments with MX (40 mM) and PDT (600 nM, 3 h-treatment) were realized. We detected high levels of APE1 protein in cancer cell lines (A549 3-fold and HeLa 2.6-fold increased) compared to normal fibroblasts. The CIAIPc were mainly found into cytoplasm and at nuclear periphery of the cells. After photostimulation of CIAIPc the APE1 protein expression was clearly increased in HeLa cells; while the levels remain almost unaltered in A549 cells. Moreover, a dose dependent ($p < 0.05$) induction of cell death was observed to all cell lines 24 h after PDT. The cell death population reached ~60% to A549 and ~65% to HeLa at the dose tested of 0.5 J/cm²; while an index of 34% was detected to GM07492 at the same dose. The co-treatment with MX and PDT increased the HeLa cell death index to ~90% ($p < 0.05$). However, only a mild increase could be observed to A549 cells (cell death index of ~70%). Thus, focusing HeLa cells, the impairment of BER by MX can be considered as a strategy to improve the efficacy of CIAIPc/NE-induced PDT.

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ROLE OF HUS1 GENE IN THE REPLICATIVE STRESS IN *TRYPANOSOMA CRUZI*Silva HMC¹, Alves CL¹, Franco GR¹, Macedo AM¹, Pena SDJ¹ and Machado CR¹

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The DNA damage response is a coordinated mechanism of damage signaling, in which the detection of the lesion leads to cell cycle arrest and recruitment of the repair machinery. Hus1 is a widely conserved gene that encodes a protein involved in this pathway. The Hus1 protein forms a trimeric complex with Rad1 and Rad9 (9-1-1 complex) in response to halt replication, facilitating the activation of the ATR kinase. Studies in *Leishmania major* suggest that Hus1 increases the capacity of this parasite in dealing with replicative stress. The mechanisms which are involved in the repair generated by this type of stress are unclear in *Trypanosoma cruzi*. Thus, this work aimed to study the importance of the Hus1 gene in the replicative stress in *T. cruzi*, the parasite that causes Chagas disease. To survey TcHus1 function, it was generated a *T. cruzi* strain with increased levels of this gene. Thereby, epimastigotes CL Brener strain were transfected with the pROCK vector containing the TcHus1 gene. The transfection was confirmed by quantitative real time PCR reaction and showed that in the pROCK-Hus1 parasites the Hus1 levels are two times higher than the strain which were transfected with the empty vector. The analysis of the overexpressor parasite growth profile under normal conditions suggested that overexpression of this gene does not compromise the basic functions of the cell, once we have verified that the pROCK-Hus1 and pROCK parasites have the same growth rate in the absence of genotoxic agents. The growth profile of the parasites which were treated with agents that cause replicative stress was analyzed. The treatment with benznidazole, cisplatin, camptothecin, hydroxyurea and methyl methane sulfonate (MMS) showed no difference in the growth profile of the Hus1 overexpressor in relation to the control cells. However, the RT-qPCR of cells subjected to treatment with MMS showed that only pROCK-Hus1 parasites are able to increase Hus1 expression in response to replicative stress generated by MMS. This may be due to the absence of gene expression control in these cells. These results have not been conclusive to elucidate if the function of TcHus1 gene is promote the activation of the ATR kinase, as in other eukaryotes. Further studies are necessary to better understand the role and the importance of this pathway in the maintenance of the genome integrity in this protozoan.

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Investigating Machine Learning Methods to Characterize Origins of DNA Replication in Kinetoplastid GenomesSamantha J. Campbell, Catarina A. Marques, Richard McCulloch and Nicholas J. Dickens

Origins of DNA replication in the genomes of *Trypanosoma brucei* and *Leishmania* species are not well characterized at the sequence level. They lack readily identifiable motifs using conventional sequence-similarity methods; similarities that may be obfuscated by the G- or GC-richness of the strand switch regions (SSR) in which they are located. However, it is essential to investigate this sequence further to increase our understanding of the DNA replication in Kinetoplastids and also provide more detailed comparative analysis that may highlight potential mechanisms of genome plasticity in *Leishmania* species.

We are using machine learning techniques, such as k-means clustering and support vector machines to identify the sequence features that may underpin the correct classification of origin and non-origin sequence. Although it may not be possible to determine a specific motif, the inter-species conservation of locations of the origins of DNA replication in *Trypanosoma brucei* and *Leishmania* species indicates that there is something specific related to these sequences that is required for a SSR to act as an origin. Machine learning approaches can be used to provide biologically relevant classification of origins of DNA replication and predict these origins in new species or strains. Furthermore, the analysis can be expanded to compare regions of genome plasticity in the *Leishmania* genus and perhaps understand the relationship between this plasticity and genome replication.

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MINIATURIZATION OF THE SALMONELLA/MICROSOME ASSAY IN MICROSUSPENSION*Zwarg JRRM1, Morales DA1, and Umbuzeiro GA1*

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Key-words: mutagenicity; 3R ; ames test

The Salmonella/microsome assay is the most used mutagenicity test both for evaluation of chemicals and environmental samples. There are several versions of protocols available in the literature. Miniaturization of toxicological tests has been a tendency in compliance with the concept of the 3Rs (Replacement, Reduction and Refinement). MPF is a successful miniaturized version of the Salmonella/microsome assay, uses liquid medium and has a limited window of response. When quantification of the mutagenic response is important and strains with very different spontaneous reversion rates are used the assays has some limitations. Recently a protocol that uses 24 well agar microplates was developed by Molecular Toxicology Inc and similar results were obtained when compared to the regular Ames test (plate incorporation version). The objective of this study was to miniaturize of the microsuspension Salmonella/microsome assay using agar microplates under the concept of the 3R and different strains with spontaneous revertants rates (low, TA1538, medium, TA98 and high, YG1041). Following the same principle of the microsuspension Salmonella/microsome assay. We began the tests of miniaturization replacing the conventional plates for microplates with 24 well, aiming to miniaturize the assay by 20 times. However, preliminary results point to a problem with the strains TA98 and TA1538, which have alower spontaneous revertants rates. The negative controls for these strains showed a tendency of blank wells (no revertants). To correct this problem we decided to change the microplates to 12 well-ones. This time, the miniaturizing of the microsuspension assay reduced by 10 times and optimistic results were obtained for TA1538 and TA98. Experiments with YG1041 are still being performed. In this new procedure a reduction of 4 times were obtained for sample and metabolic activation when compared with the microsuspension protocol. Both protocols MPA and Microsuspension provided similar sensitivity for the strains analyzed so far. The Miniaturization procedure is less laborious and uses less sample, materials and reagents. The MPA procedure seems to be a promising tool specially to test environmental samples for mutagenic activity when quantity of sample is usually a limiting factor.

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ASSESSMENT OF CYTOTOXICITY AND GENOTOXICITY INDUCED BY CELLULOSE BIOMEMBRANES CONTAINING STANDARDIZED EXTRACT OF PROPOLIS IN WISTAR RATS*Ozelin SD¹, Furtado RA¹, Rinaldi Neto F¹, Souza LDR¹, Dias FGG¹, Jorge AT¹, Berretta AA² and Tavares DC¹.*¹Universidade de Franca, Franca, SP.²Apis Flora Comercial e Industrial LTDA., Ribeirão Preto, SP.

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Burns are among the leading external causes of deaths that are registered in Brazil. Burned skin is exposed to microbial invasion, which may lead to serious infections, which are the main determinant of deaths due to burns. In this sense, a biological membrane containing standardized extract of propolis with antimicrobial and healing activity was developed as an alternative therapy for burn victims. The present work aimed the toxicogenetic study this biomembrane as part of the safety assessment in its use. Therefore, it was employed the micronucleus assay in Wistar rats peripheral blood. The animals were anesthetized and a circular lesion on the back of each one was created using a punch of 6 mm in diameter, and a 10 mm in diameter of membrane fragment was carefully added on the lesion and it was maintained for 14 days. Negative (no lesion), positive (methyl methanesulfonate, 40 mg/kg body weight) and lesion controls groups were included. On the 2nd, 7th and 14th day after the beginning of treatment, peripheral blood samples were taken from blood vessels in the tail of each animal. The genotoxicity was evaluated by micronucleus frequency in polychromatic erythrocytes (PCEs). The ratio of PCEs/total red blood cells (RBC) was employed to evaluate the cytotoxicity of the treatments. The results demonstrated that the animals whose lesions were treated with biomembranes did not show micronucleus frequencies significantly different when compared to the negative control group. The PCEs/total RBC ratio did not show significant statistically differences between different treatments and negative control groups. Thus, under these experimental conditions, the membranes did not display genotoxic and cytotoxic effects.

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THE USE OF YG5185 DIAGNOSTIC STRAIN FOR DETECTING POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) ASSOCIATED MUTAGENICITY IN TOTAL AIR PARTICULATE MATTER (PM)

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Key-words: non-substituted PAHs, benzo[a]pyrene (B[a]P), Salmonella/microsome assay

Although nitro and oxy-PAH are the main chemical classes related to mutagenic activity of PM samples collected in different countries, the presence of non-substituted PAHs continues to be of great concern due to the carcinogenic potential of some of its congeners. The diagnostic strain YG5161 was developed to have higher sensitivity to PAHs because of a plasmid carrying the *dinB* gene encoding DNA polymerase IV but its high sensitivity to nitro-compounds and aromatic amines makes the not useful for the analysis of atmospheric samples. YG5185 presented similar characteristics and sensitivity to the YG5161; however the genes *nfsB* and *oat*, responsible for the expression of NR and OAT enzymes were disrupted. Our objective was to evaluate the YG5185 ability to discriminate mutagenicity related to non-substituted PAHs present in PM extracts. PAHs were chemically determined by liquid chromatography-gas chromatography/mass spectrometry. The Salmonella/microsome microsuspension protocol was performed in dose-response experiments with metabolic activation (S9) using TA1538, TA98 and YG5185. To calculate the contribution of B[a]P in the mutagenicity detected by the used strains, this compound was also tested. Limeira presented the highest mutagenic activities with potencies of 89, 18, and 9 rev/m³ for TA1538, TA98, and YG5185 respectively. Followed by Kyoto (13, 3, and 3 rev/m³) and Stockholm (10, 2, and 2 rev/m³). The B[a]P mutagenic potencies were 0.2, 0.1 and 6 rev./ng for TA1538, TA98, and YG5185, respectively. The highest concentration of B[a]P was also found in Limeira (0.82 ng/m³), followed by Stockholm (0.08 ng/m³) and Kyoto (0.07 ng/m³). The B[a]P contribution for TA1538 was about 0.2%, and for TA98 0.4%. As expected for YG5185, B[a]P contributed with 56% for Limeira,

25% for Stockholm, and 13% for Kyoto. The strain YG 5185 was able to better distinguish the activity of non-substituted PAHs, such as B[a]P, present in PM extracts. However, to understand the remained mutagenicity it will be necessary to test other individual PAHs present in the extracts.

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CYTOTOXICITY ASSESSMENT OF VALPROIC ACID ORGANOMETALLIC DERIVATIVES IN V79 CELLS*Furtunato TVO¹, de Oliveira IM¹, Machado MS¹, Moura e Silva S², Ely MR⁴, Dumas F³, Henriques JAP.^{1,4}*

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Valproic Acid (VPA) is a short chain carboxylic acid used in the treatment of various forms of epilepsy and psychiatric disease, such as bipolar disorder and schizophrenia. Furthermore, the VPA has antiproliferative activity on different tumor cell lines, such as gliomas, neuroblastomas and leukemia by inhibition of histone deacetylase and induction of changes in the expression of many genes related to cell cycle and apoptosis. Although already widely used in clinical practice, there are limitations in its use by patients due to its known systemic toxicity. Thus, the development of VPA derivatives with low toxicity and similar efficacy would be of great importance in the treatment of psychiatric diseases. In this way, new compounds were synthesized by the inclusion of copper (Cu) in VPA molecule and their possible cytotoxic effects in Chinese hamster lung fibroblast - V79 cell line were analyzed by MTT and Clonogenic assays. For this, V79 cells were exposed to NaValp (500 to 2000 μ M), Cu₂Valp₄ (50 to 200 μ M), CuValp₂Phen (1.0 to 10 μ M – MTT; 0.5 to 7.5 μ M-Clonogenic), e CuValp₂Bipy (50 to 200 μ M – MTT; 25 to 200 μ M-Clonogenic), for 72 hours. The concentration ranges used were chosen based on the solubility of each compound. MTT and Clonogenic results showed that all NaValp derivatives were able to induce decrease in the cell viability in a dose-response manner. However, the prototype compound NaValp demonstrated lower cytotoxicity effects and only at high concentrations (>1000 μ M), mainly in MTT assay. Taken together, the VPA's Cu derivatives showed higher cytotoxicity compared to VPA. In addition, based on the IC₅₀ values, the observed cytotoxicity profile was CuValp₂Phen > CuValp₂Bip > Cu₂Valp₄ > VPA.

Support: CNPq, FAPERGS, CAPES.

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AUTOPHAGY AND MITOCHONDRIAL DYSFUNCTION IN LUNG CELLS EXPOSED TO PARTICULATE MATTER FROM BIOMASS BURNING IN AMAZON*Simões Peixoto M1, de Oliveira Alves N2, Vessoni AT3, Fortunato RS4, Artaxo P5 Hacon SS6, Menck CF3 and Batistuzzo de Medeiros SR1*

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Keywords: Autophagy, oxidative stress, particulate matter

Biomass burning emissions in the Amazon region drastically change the composition of the atmosphere and cause negative effects on human health, such as increased incidences of respiratory diseases. Mitochondrial dysfunction and oxidative stress are often implicated in the pathology of these illness and damage of lung cells following exposure to particulate matter; nevertheless, the role of autophagy in this scenario remains unclear. Here we investigate the potential effects of particulate matter smaller than 10 μ m (PM₁₀) from Amazonian biomass burning on autophagy and oxidative stress in human lung cells (A549). We incubated A549 cells with organic PM₁₀ for 24h. Oxidant production by 2',7'-dichlorofluorescein diacetate (DCF) and MitoSOX fluorescent assay were performed for evaluation of oxidative stress and mitochondrial function; while autophagy activities were determined by analyzing the autophagosome formation, which was shown as microtubule-associated protein light chain 3 (LC3) by western blot and by visualization of vesicles in A549 GFP-LC3 transfected cells. Notably, the PM₁₀ organic fraction induced higher levels of reactive oxygen species (ROS) in comparison with negative control, increases in a concentration-dependent manner. Furthermore, the results showed that mitochondrial superoxide was significantly increased after 24 h of PM₁₀ exposure, resulting in a 2.4-fold increase of fluorescence intensity. In addition, organic PM₁₀ augments the levels of LC3 and LC3-positive vesicles, demonstrating activation of autophagy machinery. These results taken together suggest the possible role of oxidative stress in PM-induced autophagy in A549 cells, which may contribute to development of respiratory diseases.

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USE OF DIAGNOSTIC STRAINS OF SALMONELLA/MICROSOME ASSAY TO COMPARE THE MUTAGENICITY OF ATMOSPHERIC PARTICULATE MATTER FROM LIMEIRA AND STOCKHOLM

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Key-words: total particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), nitro and oxy-PAHs

Chemical composition of atmospheric particulate matter (PM) organic fraction is mainly influenced by pollution sources, temperature and solar radiation. In the winter the average temperatures in Limeira and Stockholm are 20.4 and -2 °C and sunlight are 11 and 2 h/day, respectively. This difference in environmental conditions may result in differences in the PM composition and mutagenicity. Although the contribution of PAHs for PM mutagenicity is important, nitro and oxy-PAHs seem to be the main mutagenic components. Photochemical activity during the daytime promotes chemical changes in PAHs and is the main cause of day to night variations in mutagenic compounds concentrations. Some PAH-derivatives have been recognized mutagens that do not require S9 to be mutagenic in the Salmonella assay and, in general, are more active than the parent PAHs that require S9 activation. The use of diagnostic strains may help the characterization of the mutagenic chemical composition of PM. The aim of this work was to compare the mutagenicity profile of PM extracts from pooled total PM samples collected during the winter in Limeira and Stockholm using the strains TA1538, TA98, YG5185, YG1021, YG1024, and YG1041. Both PM samples were collected, processed and tested using the same protocols, which allow direct comparisons. Samples were extracted by accelerated solvent extraction with toluene. The Salmonella/microsome microsuspension assay was used in dose-response experiments with and without metabolic activation (S9). We present only data from TA1538, TA98 and YG5185. Tests with the other strains are still being conducted. The mutagenic profiles obtained so far for both sites

were very similar, despite differences in environmental conditions and mutagenic potencies. Limeira presented mutagenic activities around 7 (-S9) and 9 (+S9) times higher than Stockholm for the tested strains. Both sites presented higher mutagenicity -S9, suggesting the major contribution of nitro and oxy-PAH type of mutagens. The addition of S9 reduced the mutagenicity from the both cities around 3 times for TA1538 and YG5185, and 10 times for TA98, suggesting that non-substituted PAHs, although present in PM, have a small contribution to the mutagenicity. We anticipate that the responses obtained with strains more sensitive to nitro and oxy-PAHs will detect a different profile from Limeira and Stockholm because more photochemical reactions are expected in cities with higher temperature and sunlight.

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DNA interaction activity of Cu(II) complexes with Valproic acid containing or not 1,10-phenanthroline and 2,2'-bipyridine.

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Key-words: Plasmid DNA cleavage, metallic complexes, circular dichroism, DNA interaction.

Pathways as catalytic centers in enzymes. The copper (II) chemical specie is a Lewis acid and participate in numerous cellular redox reactions. The research on metals coordinates with organic molecules used regularly as drugs has increased in the last years. In accordance with its features and affinity for biological goals, these organometallic complexes may be more effective than the precursor organic molecules. Three new Cu II complexes using valproic acid, a anticonvulsant drug used in the treatment of epilepsy, were produced using different ligands. **Objective:** The aim of this work is to characterize the interference with DNA of the Cu_2Valp_4 (1), $CuValp_2$, 1,10phenanthroline (2) and $CuValp_2$, 2,2'bipyridine. **Material and Methods:** Complexes were synthesized as described in the literature. All the chemicals were purchased from Sigma Aldrich. The plasmid pBSK II, was extracted and purified using Qiagen Plasmid Maxi Kit. The plasmid cleavage assay was performed as described in the literature in absence of light (AL), visible light (VL) and ultraviolet-B light (UV) in pH 7.0, 7.5 and 8.0. Tests were performed in presence of DMSO an OH radical scavenger to evaluate the dependence from this ROS. Kinetic studies were performed at 50 μ M concentration in AL, VL and UV. Spectrophotometric UV-VIS assays were realised at 50 μ M complexes with different DNA concentrations. Circular Dichroism (CD) was performed using CT-DNA at 50 μ M and different

complexes concentrations. **Results:** The complexes were tested to their ability to cleave supercoiled plasmid DNA (F I) forming circular open (F II), linear (F III) or even breaking the DNA almost completely. All complexes in all conditions presented DNA cleavage ability but are partially inhibited at concentration of 500 μ M or higher. The UV-B light exposure demonstrated increase in the cleavage ability observed in 1, 2 and 3 but VL do not, as demonstrated by kinetic studies. DMSO co-incubation demonstrated that 3 produces OH radicals while 1 and 2 cleave DNA by other ways. UV-VIS spectrophotometrical and CD studies Demonstrated that 1 is a groove or external binder, 2 is an intercalation agent and 3 possibly acts a groove binder that reacts forming covalent ligations with the DNA or other molecules. The three complexes reacts with DNA and can be photoactivated but 2 and 3 are far more active than 1 and probably more suitable for modelling anticancer drugs.

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Cytotoxicity and genotoxicity of Zinc organometallic complex derived from Valproic Acid in Chinese hamster lung fibroblasts cells

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Key-words: Valproic Acid, Organometallic derivades, Citotoxicity.

Valproic Acid (VPA) is a drug used in treatment of epilepsy and bipolar disorders. In addition, experimental and clinical trials using VPA have been demonstrated an anti-cancer action in different tumors and cell lines, such as gliomas, neuroblastomas and leukemia by inhibition of histone deacetylase. In contrast, there are limitations in its use by patients due because VPA may cause adverse effects, like as hepatotoxicity and haematotoxicity. In this sense, the development of VPA organometallic complexes may be more effective and could have better stability, safety and lower toxicity than the precursor compound. Thus, new compounds were synthesized by the inclusion of zinc (Zn) and ligand (phennantroline – Phen) in VPA molecule ($ZnValp_2Phen$) and their cytotoxic and genotoxic properties were determined in Chinese hamster lung fibroblast (V79). For this, V79 cells were exposed to sodium valproate (NaValp) and $ZnValp_2Phen$ (ZincValproatePhennantroline) in different times of exposition (3, 24 and 72 hours). After, the cytotoxicity was determined by MTT, clonogenic assay and population doubling time (PDT) and genotoxicity by comet assay. For PDT and Comet Assay were used proportional concentration of $ZnValp_2Phen$ and VPA correspondent to approximately IC_{80} , IC_{50} and IC_{25} determined in survival tests with 72 hours of exposition ($ZnValp_2Phen$ 1, 2.5 and 5 μM and VPA 200, 1000 and 2000 μM). Cellular viability test were time and dose dependent and showed that $ZnValp_2Phen$ derivate induced more sensibility than VPA. Interestingly, PDT test demonstrated a possible cytostatic action induced by treatment with the compounds ($ZnValp_2Phen > NaValp$). The genotoxicity evaluated by comet assay showed that

treatment with ZnValp₂Phen and VPA induced damage when compare to negative control. In conclusion, ZnValp₂Phen induced cytotoxicity and damage in DNA.

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INCREASED FREQUENCIES OF MICRONUCLEATED CELLS IN MICE TREATED WITH DISPERSE RED 1 AZO DYE

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Key-words: environmental mutagenesis, micronucleus assay, water contaminants

Textile industry is responsible for the discharge of large amounts of dyes and related compounds in water bodies. The mutagenic activity observed in water samples under the influence of textile activity has been attributed to the presence of dyes, especially from the azo group (N = N). However, studies on the mutagenic potential of these dyes on *in vivo* models are still scarce. Among these compounds, we highlight the CI Disperse Red 1 (DR1), due to its widespread use, genotoxic and mutagenic *in vitro* properties and occurrence in water surface waters. The aim of this study was to investigate *in vivo* mutagenic activity of DR1 dye in bone marrow cells of Swiss mice. The animals (n = 8 / group) were distributed into five groups, namely: Negative Control (NaCl 0.9%); Positive Control (MNU, n-methyl-n-nitrosourea - 50 mg / kg body weight) and three groups treated with DR1 at concentrations of 0.5, 50 and 500 mg/kg of bw. Treatments were done by single oral dose (gavage - 0.3 ml / animal). MNU was intraperitoneally administered. Micronucleus test in mouse bone marrow cells was performed after euthanasia, 24 h after dye administration. A total of 1,000 polychromatic erythrocytes (EPC) was analyzed per animal, in order to obtain the frequency of micronucleated polychromatic erythrocytes (MNPCE). For statistical analysis, the Poisson distribution was used. The results (mean \pm SD) showed significant increase ($p < 0.05$) in the frequency MNPCE in those animals treated with DR1 at doses of 0.5 and 50 mg/kg bw. The absence of mutagenic effect in the group treated with the highest dose (500 mg/kg bw) could be explained by a cytotoxic effect. In conclusion, the results suggest that *in vivo* exposure to the dye Disperse Red 1 is capable of inducing mutagenicity in bone marrow cells of Swiss mice.

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AEROBIC EXERCISE REDUCES DNA DAMAGE BY INCRASING BDNF LEVEL IN BRAIN OF OLD RATS*Vilela TC¹, Damiani AP¹, Macan TP¹, Muller AP², Pinho RA² and Andrade VM¹*

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Key-words: Aerobic exercise, strength exercise, brain, BDNF, DNA damage.

Aging is a complex process that involves alterations in brain structure and function. These alterations may generate cognitive impairment, such as reduction of memory and learning. Brain-derived neurotropic factor (BDNF) is produced by neurons in an activity-dependent manner and plays important roles in synaptic and behavioral plasticity and cell survival. Furthermore, it is known that DNA is progressively damaged during aging and it has been observed that BDNF could enhance neuronal DNA repair. In this context, studies has showed that physical exercise increases the expression of BDNF and this could reduce the DNA damage. Therefore, the aim of the present study was evaluate the effect of two physical training models on BDNF levels and DNA damage in hippocampus and cerebral cortex of old rats. Eighteen 24-month-old male Wistar rats were divided into untrained, aerobic training and strength training. The rats were subjected to strength or treadmill training for 8 weeks. The BDNF levels were measured by Western Blotting and the Comet Assay was utilized to assess DNA damage levels. The BDNF levels increased in the hippocampus in the aerobic and strength groups, but in the cerebral cortex these levels were higher only in the strength group. Brain analysis of the aerobic group showed decreased DNA damage in relation to untrained group however similar results was not observed in the strength group. Taken together, these findings suggest a protector effect of exercise on brain DNA damage, possibly BDNF-dependent manner induced by the exercise aerobic training. The mechanisms involved on these results are unclear and future studies are needed.

Supported by: UNESC, CAPES

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INVESTIGATION OF THE CYTOTOXICITY, GENOTOXICITY AND CELL CYCLE PROGRESSION OF THE ALKALOID SANGUINARINE IN MCF-7 HUMAN BREAST CANCER CELLS*Almeida IV¹, Fernandes LM¹, Yoshimoto M¹, Buzo MG¹, and Vicentini VEP¹.*¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá - UEM, Maringá, PR.

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Keywords: apoptosis, benzo[c]phenanthridine alkaloid, breast cancer, chemotherapy, necrosis.

Breast cancer is an emerging public health problem. This disease is quite aggressive and highly resistant to conventional therapies and it is related to a high female mortality rate around the world. Chemotherapeutic agents coming from plants and derivatives have been proven effective in the treatment and prevention of this disorder. Sanguinarine is a benzo[c]phenanthridine plant alkaloid extracted from the roots and aerial parts of *Sanguinaria canadensis*, *Chelidonium majus* and *Macleaya cordata*, which has been widely studied for their cytotoxic, genotoxic, antiproliferative and apoptotic properties in several human tumor cell lines. The present study aimed to evaluate the cytotoxicity, genotoxicity and influence on cell cycle of this compound in human breast adenocarcinoma cells (MCF-7). Therefore, the following protocols were carried out: MTT assay, which measures the mitochondrial activity and cell viability; DNA fragmentation by comet assay; and the cell cycle progression, by flow cytometry analysis. For these tests, MCF-7 cells (10^5 /mL) were seeded in 96-well plates (MTT assay, for 24 and 48 hours), 25 cm² flasks (comet assay, 3 hours) or 6-well plates (cell cycle, for 24 hours), evaluating different alkaloid concentrations. Three independent experiments were performed and the results were submitted to analysis of variance followed by Dunnett's test ($\alpha=0.05$). It was observed that sanguinarine was cytotoxic to MCF-7 cells at 7.5 μ M, effectively reducing cell viability (less than 80%) at 10 μ M (24 hours) and at 7.5 μ M (48 hours), by the MTT test, possibly reducing mitochondrial activity, increasing oxidative stress and activating the intrinsic apoptosis pathway. Furthermore, analysis of the comet assay indicated that the plant alkaloid was genotoxic for breast cancer cells, with a significant increase in the damage index at a concentration of 10 μ M, when compared to the control. Cell cycle analysis also showed that the same concentration of sanguinarine induced a sub-G1 population of MCF-7 cells due to a significantly greater percentage of apoptotic or necrotic cells compared to the control. Thus, the results of this study suggest that sanguinarine alkaloid has a potential chemotherapeutic action for the treatment of breast cancer, and can contribute to the development of new therapies related to cancer.

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CYTOTOXICITY, GENOTOXICITY AND CELL CYCLE ANALYSES OF THE PLANT ALKALOID CHELERYTHRINE IN MCF-7 HUMAN BREAST CANCER CELLS*Almeida IV¹, Fernandes LM¹, Lucio FT¹, Heck MC¹, and Vicentini VEP¹.*

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Keywords: apoptosis, benzo[c]phenanthridine alkaloid, breast cancer, chemotherapy, necrosis.

The increasing recurrence of tumors in humans and the severe side effects of conventional chemotherapeutic agents reduce the clinical efficacy of several anticancer drugs in clinical routine. Therefore, there is always a constant requirement to develop and/or discover alternative drugs for cancer treatment. Chemotherapeutic agents coming from plants and derivatives have proven effective in the treatment and prevention of this disease. Chelerythrine is a benzo[c]phenanthridine alkaloid extracted from the roots and aerial parts of plants such as *Macleaya cordata*, *Sanguinaria canadensis* and *Chelidonium majus*, which has been widely studied because of their cytotoxic, genotoxic, antiproliferative and apoptotic activities in different human cancer cell lines. The present study aimed to evaluate the cytotoxicity, genotoxicity and influence on cell cycle progression of this compound, in human breast adenocarcinoma cells (MCF-7). Therefore, the following protocols were carried out: MTT assay, which measures the mitochondrial activity and cell viability; DNA fragmentation by comet assay; and the cell cycle progression, by flow cytometry analysis. MCF-7 cells (10^5 /mL) were seeded in 96-well plates (MTT assay, for 24 and 48 hours), 25 cm² flasks (comet assay, 3 hours) or 6-well plates (cell cycle, for 24 hours), evaluating different alkaloid concentrations. Three independent experiments were performed and the results were submitted to analysis of variance followed by Dunnett's test ($\alpha=0.05$). It was observed that chelerythrine was not cytotoxic to MCF-7 cells at concentrations from 0.1 to 20 μ M, in both time periods (24 and 48 hours), with cell viability greater than 90%, by MTT test. Furthermore, the comet assay analysis also indicated the absence of genotoxicity for this alkaloid, since there were no statistically significant differences between the treatments and control group. Likewise, flow cytometry analyses showed no cell cycle arrest or induction of sub-G1 adenocarcinoma cells. Thus, the results of this study suggest that the plant alkaloid chelerythrine may have low therapeutic efficacy in breast cancer treatment, despite of its efficacy for other tumor cell lines, as related in the literature. Otherwise, this substance can contribute to the development of new therapies related to cancer.

Financial Support: Fundação Araucária (277/14-Prot.39392).

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ALDA-1 CAN REDUCE SLC27A2 GENE EXPRESSION IN CARDIOMIOCYTES INHIBITING THE METABOLIC DYSFUNCTION INDUCED BY THE ANTINEOPLASTIC DOXORRUBICINSouza LCS¹, Fernandes FH, Munari CC, Marcondes JPC, Presti PT, Ferreira ALA, Salvadori DMF

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Key-words: doxorubicin, gene expression, fatty acid metabolism, Alda-1

Because of its broad-spectrum of antineoplastic activity, doxorubicin (DOX) is one of the most potent drugs currently used. However, despite of DOX effectiveness, several studies have shown its dose-dependent and cumulative cardiotoxicity. It has been proposed that this toxicity is caused by reactive oxygen species (ROS) and their interaction with some cellular constituents. Lipoperoxidation of phospholipids from biological membranes can lead to consequent production of toxic aldehydes that affect fatty acids metabolism. Free fatty acids are the main source of energy in adult heart and the inhibition of their metabolism results in toxic intermediates with deleterious effects on the heart tissue and decreased cardiac function. Toxicity can be reduced by the aldehyde dehydrogenase (ALDH), what require elevated mitochondrial oxidative capacity to generate ATP. In cardiotoxicity conditions ALDH2 (mitochondrial isoform) is dephosphorylated, increasing the oxidative stress and mitochondrial impairment. This scenario can be changed by the Alda-1 action, which interacts with ALDH2, allowing the enzyme to remain in a state suitable for substrate catalysis. In view of the relationship between fatty acids dysfunction and DOX cardiotoxicity, and the possible Alda-1 protective potential, this study investigated the expression profile of 84 genes involved in the metabolism of fatty acids. mRNA from cardiomyocytes of male Wistar adult rats (n=24) under DOX and Alda-1 treatment were evaluated by real-time PCR-Array. The animals were euthanized 24 hours after intraperitoneal injections. Data showed significant increase ($p<0.05$) of *Fabp4* (fatty acid binding protein 4, adipocyte) and *Slc27a2* (solute carrier family 27 member 2) expression after DOX treatment. Contrarily, the association DOX + Alda-1 decreased the *Slc27a2* expression. This reduction can be interpreted as beneficial to the cardiomyocytes considering that *Slc27a2* overexpression is associated with metabolic dysfunctions.

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EFFECT OF CHRONIC ADMINISTRATION OF L-TYROSINE ON DNA DAMAGE IN RAT BRAIN TREATED WITH OMEGA-3

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Introduction: The tyrosinemia type II is an inborn error of metabolism caused by a mutation in a gene encoding the enzyme tyrosine aminotransferase leading to a accumulation of tyrosine in the body. Studies have shown that excessive production of reactive oxygen species can cause damage to macromolecules, such as DNA, lipids and proteins. Whereas the accumulation of tyrosine promotes oxidative stress, the main aim of this study was to investigate the effects of chronic administration of tyrosine on DNA damage in brain of rats treated with omega-3. Methods: The animals were divided into 4 groups: control (tween + water), L-tyrosine (L-tyrosine + water), omega-3 (omega-3 + tween) and L-tyrosine + omega-3. The treatment were realized in the 7th to the 28th day of life of the animal, being administered L-tyrosine (500 mg/kg body weight) intraperitoneally in 12/12 hours and omega-3 (0.8 g/kg body weight) by gavage only once a day. Twelve hours after the last administration, the animals were euthanized and the structures, cortex, hippocampus, striatum and cerebellum were separated for analysis. Results: In all the structures analyzed were observed an increase the DNA damage frequency and damage index in the L-tyrosine group and the omega-3, single or in combination with L-tyrosine, it was reversed DNA damage in all structures. Conclusions: This study demonstrated that DNA damage occurs in brain of the animal models of tyrosinemia. The DNA lesions could to explain the neurological dysfunction in patients with tyrosinemia type II.

Keywords: tyrosinemia type II; brain; Omega 3; Comet assay; DNA.

Financial support: UNESC; CAPES; CNPq; INCT-TM.

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ROLE OF mTOR IN THE REGULATION OF DNA REPAIR ACTIVITIES IN HUMAN MITOCHONDRIA*Faria CMPB1 and Souza-Pinto NC1*

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Key-words: DNA repair, mTOR, base excision repair, BER mitochondria, mitochondrial DNA

The mammalian target of rapamycin (mTOR) regulates and coordinates the balance between growth, proliferation and autophagy. Inhibition of mTOR has been demonstrated to increase health- and life-span in mice, making it an important target of research aiming at increasing human health-span and in postponing age associated diseases. Aging is a systemic decrease in organismal function, which primary cause is not completely understood. One of the proposed causes of aging is the accumulation of alterations in nuclear and/or mitochondrial DNA caused by increased damage formation and/or decreased repair. Despite the fact that both the mTOR pathway and DNA repair pathways have proposed implications in aging, their relationship has not been satisfactorily explored yet. This study has the objective of trying to elucidate the possible effects that mTOR inhibition has in the base excision repair (BER) pathway. For that, human HEK293T cells with decreased mTOR protein levels, via shRNA knockdown, were submitted to a series of experiments. Our results indicate that the mTOR knockdown cells have lower basal oxygen consumption rate and extracellular medium acidification rate, which are indicatives of lower mitochondrial content and overall lower metabolic rates. Western blot of DNA repair enzymes has revealed that (at least in whole cell extracts) most DNA repair proteins investigated do not show altered levels. On the other hand, APE1 protein levels were decreased in mTOR knockdown cells. Nonetheless, flow cytometry analysis indicates that mTOR knockdown cells are protected from cell death, measured using sub-G1 population as a proxy, induced by methylene blue, a mitochondrial DNA damaging agent. Despite this apparent protection, mTOR knockdown cells exhibited a higher basal sub-G1 population in untreated controls. Concluding, the results so far appear to indicate that the increase in healthspan and lifespan promoted by mTOR inhibition might not be due to an increase in DNA repair promoted by the BER pathway. The decrease in the levels of APE1 corroborates this, since APE1 also functions in signaling and is regulated by oxidation in cysteine residues. We hypothesize that a lower basal metabolic rate might be generating less reactive oxygen species, leading to fewer damages in DNA and less APE1 signaling, resulting in overall less BER activity.

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CYTOTOXIC AND GENOTOXIC ACTION OF PLANT ALKALOID SANGUINARINE IN HUMAN HEPATOMA CELLS (HepG2/C3A)*Almeida IV¹, Fernandes LM¹, Lucio FT¹, Heck MC¹, and Vicentini VEP¹.*

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Keywords: benzo[c]phenanthridine alkaloid; cell viability; DNA fragmentation; MTT assay.

Natural products, in particular plant alkaloids, occupy a key position between the drugs employed in cancer therapy. Sanguinarine is a natural benzo[c]phenanthridine alkaloid, derived from the roots of *Sanguinaria canadensis* and *Chelidonium majus*, and aerial parts of *Macleaya cordata*, in addition to other plant species of the Papaveraceae family. This compound is known to possess several biological properties, including cytotoxicity, genotoxicity, antiproliferative and apoptotic activity against different human tumoral cell lines, which guarantees a high chemotherapeutic potential. With the purpose of analyze the cytotoxic and genotoxic effect of sanguinarine on tumoral metabolizing human hepatoma cells - HepG2/C3A, the MTT assay were performed to assess mitochondrial activity and cell viability, as well as DNA fragmentation by comet assay. The cells (10^5 /mL) were seeded in 96-well plates and 25cm² flasks in the MTT and comet assay, respectively, to evaluate different alkaloid concentrations. Three independent experiments were performed and the results were submitted to analysis of variance followed by Dunnett's test ($\alpha=0.05$). In the evaluation of cytotoxicity, all concentrations of sanguinarine (0.1 to 20 μ M) significantly reduced the absorbance compared to the control, for 24 hours, in the same way that concentrations of 2.5 to 20 μ M, for 48 hours, possibly reducing mitochondrial activity, increasing oxidative stress and activating the intrinsic apoptosis pathway. However, cell viability was only less than 80% at the concentration of 5 μ M, in both time. For the comet assay, analysis of the damage index revealed genotoxic activity statistically significant for concentrations of 1.0 and 2.5 μ M, with a predominance of class 1 comets, compared to the control. DNA fragmentation is probably a consequence of the DNA-binding properties exerted by the alkaloid. The results of this study indicate that the alkaloid sanguinarine presents cytotoxic and genotoxic activity to tumoral metabolizing HepG2/C3A cells, which qualifies this compound as a potential agent for the development of new therapies related to the combat of cancer.

Financial Support: Fundação Araucária (277/14-Prot.39392).

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CYTOTOXIC AND GENOTOXIC EFFECTS OF PLANT ALKALOID CHELERYTHRINE IN HUMAN HEPATOMA CELLS (HepG2/C3A)*Almeida IV¹, Fernandes LM¹, Yoshimoto M¹, Heck MC¹, and Vicentini VEP¹.*¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá - UEM, Maringá, PR.

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Keywords: benzo[c]phenanthridine alkaloid; cell viability; DNA fragmentation; MTT assay.

Natural plant compounds determine the odor and add flavor to vegetables, and may have beneficial or harmful effects to human health, which promotes an important basis for pharmaceutical research. Chelerythrine, a benzo[c]phenanthridine alkaloid, is the main active component of the medicinal plant *Macleaya cordata*, also present in other plants of the Papaveraceae family, whose pharmacological properties include antimicrobial, antifungal, anti-inflammatory and apoptotic actions, which currently is widely studied for their anti-cancer properties. Chelerythrine inhibits protein kinase C, which plays a crucial role in cell growth, differentiation and apoptosis; inhibit antiapoptotic proteins, promoting programmed cell death; and inhibits mitochondrial succinate dehydrogenase enzyme, favoring the production of reactive oxygen species. With the purpose of analyze the cytotoxic and genotoxic effect of chelerythrine on tumoral metabolizing human hepatoma cells - HepG2/C3A, the MTT assay were performed to assess mitochondrial activity and cell viability, as well as DNA fragmentation by comet assay. The cells (10^5 /mL) were seeded in 96-well plates and 25cm² flasks in the MTT and comet assay, respectively, to evaluate different alkaloid concentrations. Three independent experiments were performed and the results were submitted to analysis of variance followed by Dunnett's test ($\alpha=0.05$). In the evaluation of cytotoxicity, it was observed that chelerythrine is cytotoxic to human hepatoma cells, reducing cell viability in a dose-dependent manner (less than 80%) at the concentration of 12.5 μ M, for 24 and 48 hours, when compared to the control, by the MTT test. For the comet assay, analysis of the damage index revealed statistically significant genotoxic activity for the same concentration, with a predominance of class 1 comets, compared to control. The results of this study indicate that chelerythrine alkaloid presents cytotoxic and genotoxic activity to tumoral metabolizing HepG2/C3A cell line, which qualifies this compound as a potential agent for the development of new therapies related to the combat of cancer.

Financial Support: Fundação Araucária (277/14-Prot.39392).

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CYTOTOXICITY, GENOTOXICITY AND APOPTOSIS-INDUCING EFFECTS OF SANGUINARINE ALKALOID IN MRC-5 HUMAN LUNG FIBROBLASTS, *IN VITRO**Almeida IV¹, Lucio FT¹, Fernandes LM¹, Heck MC¹, and Vicentini VEP¹.*¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá - UEM, Maringá, PR.

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Key-words: alkaloid benzo[c]phenanthridine, non-tumor cell line, DNA fragmentation, MTT assay, cell viability.

Sanguinarine is a benzo[c]phenanthridine alkaloid which is found in many species of plants from the *Papaveraceae*, *Fumariaceae*, *Ranunculaceae* and *Rutaceae* families. This chemical is typically found in roots and rhizomes of *Sanguinaria canadensis*, roots of *Chelidonium majus* and aerial parts of *Macleaya cordata*. This compound causes hyperhidrosis when ingested by humans, and is also related to the increase of cytotoxicity, genotoxicity and mutagenicity in mice, mainly because of the generation of reactive oxygen species and DNA binding interactions. Besides, sanguinarine has been known to present cytotoxic, antiproliferative and apoptotic effect, which guarantees its highest chemotherapeutic potential. The presented study aimed to evaluate the possible cytotoxic, genotoxic, apoptotic and antiproliferative effects of sanguinarine in a MRC-5 cell line (normal human fetal lung fibroblasts) using MTT cytotoxicity assay to determine mitochondrial activity and cell viability; comet assay to DNA fragmentation analysis; in addition to its apoptosis-induction by annexin V flow cytometry detection. For these tests, cells were plated in 96-well plates at a density of 10^5 cells/well (MTT assay for 24 and 48 hours), 25 cm² cell culture flasks (Comet assay, 3 hours) or 6-well plates (apoptosis assay, 24 hours). All tests were treated with different concentrations of alkaloid. The experiments were carried out in triplicate and experimental data was submitted to statistical analysis of variance (ANOVA) followed by Dunnett's test ($\alpha=0.05$). The results indicated that sanguinarine significantly reduced cell viability at concentrations over 7.5 μM (24 hours) or 2.5 μM (48 hours), showing a dose-dependent effect, possibly by reducing mitochondrial activity, increasing oxidative stress and activating the intrinsic apoptosis pathway. Furthermore, analysis of the comet assay indicated that the plant alkaloid was genotoxic for the MRC-5 cell line, with a significant increase in the damage index at the concentration of 5 μM , when compared to the control. The genotoxic effect was confirmed by flow cytometry analysis with sanguinarine at 2.5 μM , which reduced the number of viable fibroblasts and increased the percentage of cells in early apoptosis or necrosis stage. Despite that sanguinarine presents chemotherapeutic effects, this study confirmed the toxicity of this alkaloid in normal human fibroblast cultures, suggesting that further studies should be conducted to bring evidences of other biological effects of this compound.

Financial Support: Fundação Araucária (277/14-Prot.39392).

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EFFECTS OF VITEXIN FLAVONOID ON CYTOTOXICITY AND GENOTOXICITY IN HUMAN BREAST ADENOCARCINOMA CELLS (MCF-7) IN VITRO*Fernandes LM¹; Lucio FT¹; Almeida IV¹; Yoshimoto, M¹ and Vicentini VEP¹*

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Key-words: C-glycosylated, herbal medicine, human breast adenocarcinoma, MTT assay, comet assay.

Flavonoids are polyphenolic secondary metabolites of natural origin, which have as part of their molecular structure a variable number of hydroxyl/phenolic groups that are responsible for their high antioxidant capacity. Within the group of flavonoids, many studies have been conducted on vitexin, a flavone C-glycoside present in several medicinal and other plants, for example, *Crataegus oxyacantha*, *Passiflora alata*, *Passiflora edulis*, *Aloysia citriodora* and *Saccharum officinarum* L.. Although Vitexin has various biological functions like anti-aging, antinociceptive, anti-inflammatory and antioxidant activities, a few studies have investigated the cytotoxicity and genotoxicity effects of Vitexin in tumor cell lines. So this study aimed to evaluate the possible cytotoxic and genotoxic potentials of the flavonoid Vitexin (CAS 3681-93-4 Sigma-Aldrich) in human breast cells (MCF-7) using the MTT cytotoxicity assay to determine mitochondrial activity and cell viability, in addition to the comet assay for DNA fragmentation analysis. For the MTT assay, MCF-7 cells were treated with ten different concentrations of the drug [2, 3, 23, 46, 93, 138, 185, 231, 278, 324, 370 μ M], and also for the Comet assay three non-cytotoxic concentrations were evaluated [46, 96 and 185 μ M]. In the MTT assay, MCF-7 cells were plated in a 96-well plate at a density of 10^5 cells/well whereas in the Comet assay cell culture flasks (25 cm²) were used. The results were confirmed by three independent experiments and then experimental data was submitted to statistical analysis of variance (ANOVA) followed by Turkey's test (MTT) and Dunnett's test (Comet assay). The MTT assay indicated that at the level of mitochondrial activity, Vitexin did not show cytotoxic activity at the available time of exposure (24, 48 and 72 hours). In addition to this, the comet assay statistical analysis demonstrated that the concentrations of the vitexin treatment did not show a genotoxicity effect with scores similar to that of the control. In conclusion, the presented results of this study showed that vitexin showed no cytotoxic and genotoxic effects on breast tumor cells. Further studies should be conducted in order to bring evidences of other effects of this compound, such as antigenotoxic activity being used in drug development with chemo protective potential.

Financial Support: Capes.

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IN VITRO STUDY OF THE CYTOTOXICITY AND GENOTOXICITY OF THE CHELERYTHRINE ALKALOID IN NORMAL HUMAN LUNG FIBROBLASTS (MRC-5)*Almeida IV¹, Syritiuk PHS¹, Yoshimoto M¹, Almeida ACC¹, and Vicentini VEP¹.*

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Keywords: benzo [c] phenanthridine alkaloid, non-tumor cells, DNA fragmentation, cell viability.

Plants produce a large number of secondary metabolites with a high biological potency, among which are the benzo [c] phenanthridine alkaloids. Chelerythrine is the main active component of the medicinal plant *Macleaya cordata*, also found in other plants of the Papaveraceae family, such as *Chelidonium majus* and *Sanguinaria canadensis*, whose pharmacological properties include antimicrobial, antifungal, anti-inflammatory and apoptotic activities. Chelerythrine inhibits protein kinase C, which plays a crucial role in cell growth, differentiation and apoptosis; inhibit antiapoptotic proteins, promoting programmed cell death; and inhibits mitochondrial succinate dehydrogenase enzyme in rats, favoring the production of reactive oxygen species. In order to analyze the cytotoxic and genotoxic effects of chelerythrine in non-metabolizing MRC-5 cells (non-tumoral human lung fibroblasts), the MTT test were performed to assess mitochondrial activity and cell viability, and the DNA fragmentation by the comet assay. The cells (10^5 /mL) were seeded in 96-well plates (MTT assay, for 24 and 48 hours) or 25 cm² flasks (comet assay, 3 hours), evaluating different alkaloid concentrations. Three independent experiments were performed and the results were submitted to analysis of variance followed by Dunnett's test ($\alpha=0.05$). The results indicated that chelerythrine significantly reduced cell viability at concentrations of 10 μ M, at 24 and 48 hours, with dose-dependent effect, possibly reducing mitochondrial activity, increasing oxidative stress and activating the intrinsic apoptosis pathway. Furthermore, analysis of the comet assay indicated that the plant alkaloid was genotoxic for MRC-5 cells, with a significant increase in the damage index at a concentration of 5 and 10 μ M, when compared to the control. In this case, the genotoxic effect might be related to the DNA-binding ability of the compound, which could cause DNA fragmentation. Therefore, despite the chemotherapeutic effects of chelerythrine, the results of this study confirm the toxicity of this alkaloid in non-metabolizing human lung cells, suggesting that further studies should be conducted to bring evidences of other biological effects of this compound.

Financial Support: Fundação Araucária (277/14-Prot.39392).

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ALTERATIONS IN DNA REPAIR GENE EXPRESSION MAY HAVE PROGNOSTIC VALUE IN THYROID CANCER*Lutz BS¹, Cabral NK¹, Meirelles NL¹, Zanella V², Meyer ELS² and Saffi J¹*¹Laboratory of Genetic Toxicology, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, Rio Grande do Sul, 90050-170, Brazil²Thyroid Section, Endocrine Division, Santa Casa de Misericórdia de Porto Alegre (ISCOMPA), Porto Alegre, Rio Grande do Sul, 90020-090, Brazil

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Key-words: thyroid cancer, DNA repair, BRAF, prognosis.

Papillary thyroid carcinoma (PTC) is responsible for up to 80% of malignant thyroid tumors and the majority of patients have a favorable outcome. However, 5%–20% will develop tumor recurrence while 10% will have distant metastasis. Despite the etiology of PTC is still unknown, exposure to ionizing radiation is reported as a risk factor. Some polymorphisms have been described in genes involved in DNA repair systems, such as XRCC1 (base excision repair - BER), XPD (nucleotide excision repair - NER) and XRCC3 (homologous recombination - HR). In addition, hypermethylation of the hMLH1 (mismatch repair - MMR) is associated with BRAF-promoted thyroid tumorigenesis. Thus, alterations in gene expression levels of DNA repair mechanisms can be associated with development and progression of thyroid tumors. The purpose of this study was to investigate the mRNA expression of DNA repair genes and its association with clinicopathological features. Twenty consecutive PTC and matched normal thyroid specimens were obtained from patients undergoing total thyroidectomy. The gene expression of MGMT (direct repair); MLH1 and MSH2 (MMR); OGG1, APE1 and XRCC1 (BER); XPD (NER); XRCC2, XRCC3, RAD51 (HR); KU86 (non-homologous end joining - NHEJ) was evaluated by qPCR Array system. Gene expression was calculated through $\Delta\Delta CT$ method and normalized by two housekeeping genes, ACTB and B2M. For statistical analyses, Wilcoxon matched-pair and Spearman correlation tests were performed. Differences with a p value of 0.05 or less were considered to be statistically significant. Mean age was 41.1 ± 14.8 years and the proportion of females was 85.0%. The median (range) of tumor size was 2.3cm (0.7-5.5). Gene expression fold changes (tumor/normal tissues) varied from 1.069 to 1.761. Only XRCC2 was increased in mRNA expression in tumor in comparison to normal tissue (1.761, $p=0.030$). Increased XRCC2 gene expression was associated with presence of metastatic cervical lymph nodes ($p=0.040$). Reduction of MLH1 and MSH2 gene expression were related with patients with more than 45 years old ($p=0.028$). Elevated mRNA levels of XRCC1 ($p=0.042$) and Ku86 ($p=0.028$) were related with larger tumors. These preliminary results suggest that different levels in DNA repair gene expression was associated with advanced local disease and may have a potential prognostic

value in thyroid cancer. Further investigations to comprehend DNA repair role in thyroid carcinogenesis and prognosis are required.

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EVALUATION OF WATER QUALITY OF THE SANTA MARIA DA VITÓRIA RIVER BY ASSAYS *in vitro* OF CELL CULTURE*Martins IO1, Duarte ID1, Galter IN2, FariaPR1, Malini M1 and Matsumoto ST1,2*

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Key-words: CHO-k1, comet assay and micronucleus test.

The Santa Maria da Vitória river is located in the central region of the state of Espírito Santo. It constitutes one of the main sources for water supply in the metropolitan region of Grande Vitória, besides the water is also used in industries and agricultural regions. Despite its great importance, the river is constantly impacted by erosion, silting and effluent and industrial waste dumping. Our objective was to evaluate the mutagenic and genotoxic potentials in the water of the Santa Maria da Vitória river by doing assays *in vitro* with the lineage CHO-k1. Water collects were performed in six spots along the river after periods of rain. The comet test and the micronucleus test were performed to evaluate the mutagenicity and genotoxicity, respectively, of the collected water samples. Approximately 1×10^6 of the lineage CHO-k1 cells were exposed to 12 hours of the following treatments: a) PBS – Phosphate buffer (negative control); b) Methyl methanesulfonate ($10^{-4}M$); c) water samples collected from the 6 spots along the Santa Maria da Vitória river. For the mutagenicity evaluation, after treatment, cells were subjected to 18 hours of exposure to cytochalasin B in order to induce binucleation. For the analysis of genotoxicity, cells were submitted to stages of cell lysis, electrophoresis, neutralization and fixation. The results obtained from the comet test established that all spots – where samples were collected – analyzed showed little difference to the negative control, therefore there is no evidence of any genotoxic effect. In the other hand, results from the micronucleus test revealed that the collecting spots 3 (hydroelectric reservoir), 4 (dam reservoir) and 5 (City of Santa Leopoldina) showed significant difference when compared to the negative control, therefore the water from these collecting spots presents compounds with mutagenic potential, possibly derived from anthropic actions such as effluent dumping and agricultural activities.

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SANTA MARIA DA VITÓRIA RIVER: ECOTOXICOLOGICAL ASSESSMENT IN *Allium cepa* L. and *Lactuca sativa* L.

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Key-words: toxicity, cytotoxicity, genotoxicity, mutagenicity.

The watershed of Santa Maria da Vitória river measuring 1,876 km², is located in the state of Espírito Santo and has a great ecological and socioeconomic importance with several activities such as water collection, establishment of hydroelectric and fishing. However, this watershed suffers different impacts such as degradation of the riparian forests with effluent discharging. Biological monitoring is essential, since it helps identify the combined effects of substances and evaluate deleterious effects and their influences on organisms caused by contaminants which complement physical and chemical evaluations. In this scenario, plants like *Allium cepa* L. and *Lactuca sativa* L. stand out for providing sensitive ecotoxicological tests to environmental pollutants and are used as an important tool in the evaluation of aquatic environments. Therefore the aim of this study was to evaluate the water quality of Santa Maria river through both toxicity tests: *Allium cepa* and *Lactuca sativa*. Six sampling stations were established along the river to collect water and sediment. The *A. cepa* test was conducted through bulbs exposed to water samples and elutriate solutions prepared from the sediment. Slides of meristems and F1 region of roots were made using Feulgen method and analyzed to calculate three important indexes such as mitotic, chromosomal aberrations and micronuclei frequency. The tests performed in *L. sativa* root used the same aforementioned samples and after root growth was measured the germination rate and root elongation. Statistical analysis was performed using ANOVA followed by Tukey or Kruskal Wallis test ($p < 0.05$). The results of *A. cepa* demonstrated that water and elutriate samples exhibit cytotoxicity. Such cytotoxicity could have influenced the low genotoxicity and mutagenicity observed in some stations. However, other stations have significant potentials of genotoxicity and mutagenicity. Results of the assay with *L. sativa* showed toxic effect of some samples on the seed germination and root elongation, indicating low to moderate toxicity. Additionally physical and chemical analyses will be carried out to elucidate the probable agents of the observed damage. However, it suggests the presence of complex pollutant mixtures as the potential cause.

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CYTOTOXICITY OF NATURAL PHENOLIC COMPOUND ZINGERONE (*Zingiber officinale* Roscoe) IN HUMAN HEPATOMA CELLS (HepG2/C3A)Yoshimoto M¹, Fernandes LM¹, Buzo MG¹, Almeida IV¹, and Vicentini VEP¹¹Department of Biotechnology, Cell Biology and Genetics, State University of Maringá – UEM, Maringá, PR, Brazil.

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Key-words: Vanillyl Acetone, cell viability, colorimetric assays, tumor cell.

The Zingerone (Vanillyl Acetone or [4-(3-methoxy-4-hydroxyphenyl) butan-2-one]), a phenolic compound derived from ferulic acid and present in dried or boiled ginger (*Zingiber officinale* Roscoe), has several pharmacological activities, such as antioxidant potential, anticancer, radioprotective, anti-inflammatory, anti-diabetic, anti-lipolytic, among other benefits, assisting in the prevention and maintenance of health of people who consume it. Therefore, the aim of this study was to investigate the cytotoxicity of Zingerone using the assays of Cytotoxicity (MTT) and Trypan Blue Cell Viability in human hepatoma cells (HepG2/C3A). In the MTT assay, were seeded and treated 10⁵/mL HepG2/C3A cells in 96-well plates for 24 and 48 hours. The treatments were: Control (100µL of DMEM culture medium supplemented with 10% of fetal bovine serum and absolute alcohol [0.01%]), Methyl methanesulfonate (MMS, 150µM) and Zingerone (50, 75, 100, 125, 150, 175, 200, 250, 300 and 500 µg/mL). The data were submitted to analysis of variance (ANOVA), followed by the Tukey's test ($\alpha=0.05$, $p<0.05$, $n=3$). In the Trypan Blue Cell Viability Assay, the cells (5mL, $\approx 10^5$ /mL HepG2/C3A cells) were seeded and treated in 25cm² flasks for 24 and 48 hours. Concentrations of 50, 100, 150, 200 and 250 µg/mL of Zingerone were tested. The Control and the cytotoxic agent were similar in both experiments. The data were subjected to analysis of variance (ANOVA) followed by Dunnet's test ($\alpha=0.05$, $p<0.05$, $n=3$). All analyzes were performed using the GraphPad INSTAT software. The results indicated that only dosages between 250-500 µg/mL of Zingerone in 48 hours showed cytotoxic activity in HepG2/C3A by the MTT assay, and these data were confirmed by the results of cell viability in this test. In the Trypan Blue Cell Viability Assay, none of the concentrations of Zingerone evaluated in 24 and 48 hours interfered on cell viability. Thus, under the experimental conditions this work was conducted, it is possible to infer that the Zingerone is cytotoxic at higher concentrations and longer exposure time, by decreasing the mitochondria activity; however, this phenolic compound did not affect the cell viability in level of integrity of the plasma membrane of HepG2/C3A cells.

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INVESTIGATION OF THE CYTOTOXIC POTENTIAL OF NATURAL PHENOLIC COMPOUNDS VANILLIC ACID AND GENTISIC ACID IN HUMAN HEPATOMA CELLS (HepG2/C3A)*Yoshimoto M¹, Almeida IV¹, Heck MC¹, Silva JS¹, and Vicentini VEP¹*

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Key-words: phenolic compounds, toxicity, MTT assay, tumor cell, *in vitro* assay.

The phenolic acids are widely distributed in plants and are an inexhaustible source of compounds with promising biological activity. The Vanillic Acid is a phenol derivative found naturally in various fruits and edible plants. This compound is widely used in industry as flavoring, preservative or food additive and perfumery, also has various biological functions, such as anti-inflammatory, chemopreventive, antioxidant, among others functions. The Gentisic Acid is a biosynthetic derivative of salicylic acid found in citric fruits, grape, sesame and other foods. Studies showed that the Gentisic Acid has antioxidant potential, anti-inflammatory, anti-rheumatic, analgesic effects, among others biological activities. Considering the therapeutic potential described for these two phenolic compounds, this study aimed to evaluate the cytotoxic effect of these substances on human hepatoma cells (HepG2/C3A), using the MTT assay. Therefore, 10^5 /mL HepG2/C3A cells were seeded and treated in 96-well plates for 24 and 48 hours. The treatments were: Control (100 μ L of DMEM culture medium supplemented with 10% of fetal bovine serum); Methyl methanesulfonate (MMS, 150 μ M); Vanillic Acid (100, 300 and 500 μ M); and Gentisic Acid (100, 300 and 500 μ M). The results were submitted to analysis of variance (ANOVA), followed by Dunnett's test ($\alpha=0.05$, $p<0.05$, $n=3$). All analyzes were performed using the GraphPad INSTAT software. The results indicated, in the level of mitochondrial activity, that the Vanillic Acid was cytotoxic only at the concentration of 500 μ M in 24 hours. However, the Gentisic Acid showed cytotoxic activity in all evaluated concentrations (100, 300 and 500 μ M) in the time of exposure of 24 hours and at the concentration of 500 μ M in 48 hours. These results suggest that further studies should be developed to determine the safe amount of Vanillic Acid and Gentisic Acid that can be consumed by the people, minimizing the risks and bringing beneficial and therapeutic effects for health, such as reducing the risk of developing diseases and improved quality of life.

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DUAL INHIBITION OF ATR AND ATM POTENTIATES THE ACTIVITY OF TRABECTEDIN AND LURBINECTEDIN BY PERTURBING THE DDR AND HOMOLOGOUS RECOMBINATION REPAIR

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Keywords: DNA double strand breaks, DNA alkylators, DNA replication, Homologous recombination, Checkpoint abrogators

Trabectedin (Yondelis®, ecteinascidin-743, ET-743) is a marine-derived natural product approved for treatment of advanced soft tissue sarcoma and relapsed platinum-sensitive ovarian cancer. Lurbinectedin is a novel DNA minor groove binder structurally related to trabectedin. The structural variation of lurbinectedin is accompanied by important modifications of the pharmacokinetic and pharmacodynamic properties in cancer patients although the preclinical activities of this drug remain close to those observed for trabectedin. Both ecteinascidins generate DNA double-strand breaks that are processed through homologous recombination repair (HRR), thereby rendering HRR-deficient cells particularly sensitive. Until now, no strategy has been evaluated to inhibit or to perturb this repair pathway although this approach is likely to improve the activity of ecteinascidins by mimicking HRR deficiency. In this study, we characterize the DNA damage response to trabectedin and lurbinectedin in human carcinoma cell lines. Our results show that trabectedin and lurbinectedin activate the ATM/Chk2 (ataxia-telangiectasia mutated/checkpoint kinase 2) and ATR/Chk1 (ATM and RAD3-related/checkpoint kinase 1) pathways. Pharmacological inhibition of Chk1/2, ATR or ATM is not accompanied by any significant improvement of the cytotoxic activity of the ecteinascidins. Interestingly, simultaneous inhibition of both ATM and ATR strongly potentiates the activity of both ETs against human cervical and ovarian carcinoma cells by efficiently blocking the

foci formation of HRR proteins following exposure to ecteinascidins, resulting in extensive chromosome damage. Together, our data identify ATR and ATM as central coordinators of the DDR to ecteinascidins and provide a mechanistic rationale for combining these compounds with ATR and ATM inhibitors.

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EVALUATION OF THE EFFECT OF *Citrullus lanatus* ON TOXICITY INDUCED BY CISPLATIN IN C57BL/6 MICE*Rinaldi Neto F, Cruz RCR, Furtado RA, Tavares DC and Oliveira PF.*

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Key-words: *Citrullus lanatus*, C57BL/6 mice, cisplatin, melanoma, toxicity.

The cisplatin is between the most widely used antineoplastic drugs for cancer treatment that, in spite of improving patient's lifetime, it also favors the occurrence of side effects. An alternative to decrease the toxic effects have been studied combinations that work in reducing the systemic toxicity without diminishing the antitumor activity of the drugs already used. In this sense, the natural products have been investigated. *Citrullus lanatus*, popularly known as watermelon, has been a promising alternative since it is effective in the treatment of cardiovascular disease, loss weight, urinary infections, insulin resistance, metabolic syndrome, hypertension and especially its antimutagenic effects. The present study purpose to analyze the capacity of watermelon to reduce toxicity induced by the treatment of cisplatin in C57BL/6 mice with melanoma. The syngeneic murine B16/F10 tumor model was employed. Approximately 700,000 viable cells suspended in 100 uL were implanted subcutaneously in the dorsal region. At the time the mice showed a tumor with a size of 100 mm³, these were treated subcutaneously with cisplatin at dose of 10 mg/kg and with watermelon at dose of 500 mg/kg by gavage for five days. The antitumor activity was performed by measuring the survival rate, body weight, organ weights (liver, kidney, spleen, heart and lungs) and tumor. The assessment of renal toxicity was carried out by the quantification of serum blood levels of creatinine and urea. The results showed no significant reductions in the weight of the organs of the treated groups when compared to the control. Biochemical analysis revealed that the group treated with cisplatin showed increased levels of creatinine and urea, whereas these biomarkers were significantly reduced in the animals treated with cisplatin plus watermelon. The results contribute to the search for alternative therapies to cisplatin chemotherapy.

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EFFECT OF L-TYROSINE CHRONIC ADMINISTRATION ON DNA DAMAGE IN RATS BRAIN TREATED WITH ANTIOXIDANTS

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Introduction: Tyrosinemia type II is an inborn error of metabolism (IEM) caused by deficiency of the enzyme tyrosine aminotransferase, leading to an increased levels of tyrosine in the plasma, causing damage to eyes, skin and neurological disorders. Studies have shown that excessive production of reactive oxygen species can cause damage to macromolecules, such as DNA, lipids and proteins. Whereas the tyrosine accumulation can promote oxidative stress, the aim of this study was to investigate the effects of chronic administration of tyrosine on DNA damage in brain of rats treated with antioxidants (N-acetylcysteine (NAC) + Deferoxamine (DFX)). **Materials and Methods:** Male Wistar rats 7 days old were divided into 3 groups: (1) control; (2) tyrosine; (3) tyrosine + antioxidants. L-tyrosine (i.p.) and NAC (subcutaneous route) were given at intervals of 12/12 hours and DFX (subcutaneous) every 48 hours until the animals are 28 days. Twelve hours after the last administration the animals were euthanized and the structures cerebellum, hippocampus, striatum and cortex were separated for further analysis. **Results:** In the structures cerebellum, hippocampus and cortex was observed an increase in the DNA damage index and damage frequency (comet assay parameters) in tyrosine group, and the administration of antioxidants was able to reverse these damage. Already striatal tyrosine group also showed an increase in the DNA damage index and damage frequency, but the group treated with antioxidants showed significant difference just in relation to DNA damage index, not in damage frequency. **Conclusion:** Our results suggest that DNA damage can be found in the brain of animals submitted to tyrosinemia model and that these alterations on DNA may represent another means to explain the neurologic dysfunction in patients with Type II tyrosinemia. Moreover, it was possible to observe that the administration of NAC and DFX can be a potent adjuvant for this inborn error of metabolism.

Keywords: tyrosinemia type II; DNA; N-acetilcisteína; Deferoxamina.

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DUSP3 SILENCING AFFECTS DNA REPAIR AND PROLIFERATION IN NER-DEFICIENT HUMAN CELL LINES TREATED WITH UV RADIATION*Lilian C. Russo¹ and Fabio L. Forti¹*

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Key-words: DUSP3, DNA repair, ultraviolet radiation, nucleolar proteins

The dual specificity phosphatases represent one subclass of protein tyrosine phosphatases (PTPs) with large diversity of substrates. The atypical dual specificity phosphatase 3 (DUSP3), one of the smallest DUSPs known for its role in cell cycle progression of human cancers, is a negative regulator of mitogen-activated kinases (MAPK), which in turn are well known regulators of cellular proliferation and stress conditions. DUSP3 knocking down, through siRNA, arrests human cervix cancer cells and leads to senescence. We hypothesized if this enzyme could be involved in other nuclear processes such as DNA damage and repair. Previous results from protein-protein interactions by proteomic and interactome approaches of HeLa cells submitted to UV revealed intriguingly proteins with nuclear localization physically interacting with DUSP3 and entirely related to processes involving nucleic acids stability, processing, and expression (Panico K & Forti FL, *J. Proteome Res*, 2013). Also, preliminar data showed the new partners of DUSP3, the nucleolar proteins Nucleolin and Nucleophosmin, had their expression profile altered under silencing of DUSP3 in HeLa and XPA (deficient in nucleotide excision repair – NER – pathway) cells. Thus, here we used MRC5 (human fibroblasts) and XPA cells, with or without stable DUSP3 silencing (through shRNA) and submitted to low doses of ultraviolet radiation B or C. Through comet assay, we identified that DNA repair is delayed after UVB or UVC radiation exposure, and it does not occur within 6 hours in DUSP3 silenced cells, results that are more prominent in XPA cell lines. In addition, in these cell lines, an almost complete silencing of DUSP3 provoked a very slow rate growth, while the silencing in DUSP3 in about 50% provoked an increase in growth. Altogether our data are pointing up to the new roles of the PTP DUSP3 in the repair of genotoxic damages promoted by UV.

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OXIDATIVE DNA DAMAGE AND IL-6 SERUM LEVELS IN PATIENTS WITH DIFFERENT STAGES OF CORONARY OBSTRUCTION

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Key-words: inflammatory cytokine, SNP, cardiovascular

Coronary artery disease (CAD) is a serious public health problem and the leading cause of morbidity and mortality in the world. Characterized by inflammatory process, atherosclerosis can result in blood vessel lumen obstruction. Currently, the most widely used procedure for coronary atherosclerosis treatment is the stent-implantation angioplasty. However, despite the improvements in such technique, restenosis remain as the major limiting factor. Therefore, the present study aimed to determine the relationship between serum level of IL-6 (pro-inflammatory cytokine) and the SNP *IL-6 -174 G / C - rs1800795* (Taqman real time PCR); the serum levels of IL-8, IL-1 β , IL-6, IL-10, TNF, IL-12p70 (flow cytometry), and oxidative DNA damage in mononuclear cells (comet assay with ENDOIII and FPG enzymes). A total of 60 patients with different clinical features were enrolled in the study: control patients, with coronary obstruction $\leq 20\%$ and no stent implantation (n=20); patients without in-stent restenosis (coronary obstruction $\leq 50\%$; n =20); patients with in-stent restenosis (coronary obstruction $\geq 50\%$; n = 20). The results showed an increase IL-6 serum levels in patients without restenosis (obstruction $\leq 50\%$) when compared to control and to patients with in-stent restenosis. A significant increase of oxidative DNA damage was detected in patients with in-stent restenosis (ENDOIII sensitive sites) and in those patients without in-stent restenosis (FPG and ENDOIII sensitive sites), both compared to the control group. Furthermore, patients without in-stent restenosis presented increased levels of FPG sensitive sites compared to patients with in-stent restenosis. Taken together, these results point out the role of oxidative DNA damage in early and late steps of restenosis, with more prominent effect at the early stage (obstruction $< 50\%$), with an additional increase of serum levels of IL-6. Therefore, reduction of inflammation and oxidative stress may improve the disease outcome in patients with stent implantation.

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Investigating the contribution of the DNA mismatch repair (MMR) components to the oxidative stress response in TrypanosomatidsGrazielle-Silva V^{1,2}, Zeb TF², Machado CR¹, McCulloch R² and Teixeira SMR¹¹Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil²The Wellcome Trust Center for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, Scotland, United KingdomEmail: santuzat@ufmg.br and/or Richard.mcculloch@glasgow.ac.uk

Key words: DNA mismatch repair, Trypanosomatids, oxidative stress response.

DNA repair mechanisms are crucial for maintenance of the genome in all organisms, including parasites, where successful infection is dependent both on genomic stability and sequence variation. MSH2 is an early acting, central component of the Mismatch Repair (MMR) pathway and together with MSH6 or MSH3 is responsible for the recognition and correction of base mismatches that occur during DNA replication and recombination. In addition, we have recently shown evidence suggesting that MSH2 might also play an important but poorly understood role in responding to oxidative damage in both African and American trypanosomes. Unexpectedly, *Trypanosoma brucei* procyclic form cells *MSH2* null mutants showed increased resistance to H₂O₂ exposure when compared with wild type cells, a phenotype distinct from the previously observed increased sensitivity of *T. brucei* bloodstream forms *MSH2* mutants. Complementation studies indicated that the increased oxidative resistance of procyclic *T. brucei* was due to adaptation to *MSH2* loss. Here we aim to show if *MSH2* is the only protein of the complex involved in the oxidative stress response in these parasites. To answer that question one allele knockout of *MSH6* was generated in *T. cruzi* and null mutants in *T. brucei*. Analysis of MMR efficiency in the mutants show that MMR is not impaired in *Tcmsh6*^{+/-} cells. Treatment with the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in *T. cruzi Tcmsh6*^{+/-} does not show any difference in tolerance/ susceptibility when compared with wild type cells. However, in *T. brucei Tbmsh6*^{-/-} cells have increased tolerance to MNNG treatment, a phenotype characteristic of MMR – impaired cells, but no difference is observed in microsatellite instability (MSI) analysis. This suggests that *MSH2* together with *MSH3* can take the role in microsatellite maintenance. In *T. cruzi* we also observe an increased susceptibility of *Tcmsh6*^{+/-} cells to H₂O₂, but No difference was observed after treatment with H₂O₂ in *T. brucei Tbmsh6*^{+/-} or *Tbmsh6*^{-/-} cell lines. Suggesting that at least in *T. cruzi* *MSH6* can also play a role in the signalling response followed oxidative stress. By immunoprecipitation we can also show that *MSH2* interacts with *MSH3* and *MSH6* in both bloodstream form and procyclic forms in *T. brucei*.

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ARE ZEBRAFISH EMBRYOS GOOD MODELS FOR EMBRYOTOXICOLOGICAL ASSESSMENT OF ANTIDEPRESSANTS? THE CASE OF STUDY OF AMITRIPTYLINE*Lisboa CA¹, Oliveira R¹ and Grisolia CK¹*

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Keywords: model organisms, *Danio rerio*, teratology, psychiatric compounds.

A trend of increase in the use of psychiatric drugs has been suggested by governmental authorities worldwide. The psychiatric drugs are a diverse group of chemicals (i.e. antidepressants, anxiolytics, mood stabilizers, antiepileptic) which acts on central nervous system. The antidepressants are one of the most prescribed pharmaceuticals being administered for the treatment of different depressive disorders. Amitriptyline is a tricyclic antidepressant which has an inhibitory effect on the serotonin and norepinephrine uptake in the presynaptic nerve endings, thereby reducing the hyperactivity of the hypothalamus–pituitary–adrenocortical axis. In this study, the Fish Embryo Toxicity (FET) Test OECD No. 236 is proposed as a tool to evaluate the effects of an antidepressant drug – Amitriptyline. Test started with newly fertilized eggs exposed to amitriptyline concentrations of 0; 0.1; 0.28; 0.79; 2.23; 6.3; 17.5 and 50 mg/L. Sixteen eggs per treatment, divided in 03 replicates, were selected and distributed in 24-wells microplates, one per well. The tests were carried out at 26 ± 1 °C conditioned in a climatic chamber during 168 h. Embryos were daily observed under a stereomicroscope (Stemi 2000-C, Zeiss, Germany). In the embryo phase, were evaluated: egg coagulation, otolith formation, eye and body pigmentation, somite formation, tail circulation and hatching; after hatching: oedema, equilibrium, undersize, spine deformation and mortality. At 48 h, the results showed that amitriptyline significantly reduced the hatching time of embryos. Regarding mortality, at 72 h of exposure a LC50 of 17.2 mg/L was obtained. Several teratologies were observed in concentrations higher than 17.5 mg/L, including abnormal development of the tail. Behavior alterations were also observed including loss of equilibrium, paralysis and abnormal posture (embryos side-lying in the bottom of the microplate well). In summary, the present study aimed to evaluate the usefulness of zebrafish embryos for the embryotoxicity assessment of antidepressants, namely amitriptyline. Effects on behavior might be explained by the modulation of target neurotransmitters of amitriptyline, serotonin and norepinephrine, or indirect effects on cholinergic pathways. Considering the high sensitivity of embryos and the wide range of responses (teratologies, behaviour and mortality) triggered by amitriptyline exposure FET test seems to be a promising tool for the toxicity assessment of psychiatric drugs.

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Allyl isothiocyanate (mustard essential oil) modulated gene expression and protein profiling in bladder cancer cell lines according to *TP53* genotypeALV Sávio¹, GN da Silva², AC Sato³, SA Andrade³, DMF Salvadori¹.¹ UNESP - São Paulo State University, Botucatu, SP, Brazil; ² UFOP - Federal University of Ouro Preto, Ouro Preto, MG, Brazil; ³ Instituto Butantan, São Paulo, Brazil.

Bladder cancer is one of the tumors with highest cost for health systems due to its high recurrence rate and routine cytology. Chemotherapeutical protocols for this neoplasia include drugs that cause molecular damage and disturbances in cell cycle and DNA repair. The high toxicity of these protocols has stimulated studies on alternative treatments. Allyl isothiocyanate (AITC), found in the seed of mustard and cruciferous vegetables, has attracted special attention, since it can interfere on cell cycle and apoptosis rates in tumor cells. Its high bioavailability and rapid absorption in bladder make AITC a promising agent for treating urothelial cancer. Previous studies demonstrated that AITC induced primary DNA damage, but not micronuclei, in bladder cancer cell lines carrying the wild type (RT4 cells) and a mutated (T24 cells) *TP53* gene. Furthermore, cell cycle changes, increased apoptosis rate and modulation of mRNA levels of apoptosis-related genes were also observed after AITC treatment. Therefore, since AITC induced primary DNA damage without raising the MN frequency, the present study aimed to investigate endpoints related to DNA repair. Expression of DNA repair genes and protein profile, assessed through PCR-array (PAHS-042C - Qiagen), were evaluated 24 hours after treatment with AITC (62.5 μ M; concentration defined by the clonogenic survival assay). Results showed significant decrease in the number of colonies in both cell lines. Additionally, data demonstrated increased expression of *LIG4*, *RAD54L* and *RFC1*, and decreased expression of *UNG*, *APEX1*, *MRE11A* and *RPL13A* in RT4 cells. These genes belong to the network of the *TP53* gene, and could explain the lower sensitivity of RT4 cells, as observed in a previous study using the comet assay. In T24 cells, it was only observed increased *XRCC2* expression, suggesting lower activity of the DNA repair system. These findings corroborate the high sensitivity of T24 cells to AITC-induced DNA damage. Proteins related to cell proliferation (K167 and 1-DACHI), DNA binding (TEAN2), mRNA transports (RBP2) and cytoskeleton (MOES) in RT4 cells, and CCNT1 and HTRA1 in T24 cells were identified after treatment with AITC. In conclusion, data demonstrated an anti-proliferative potential of AITC, dependent on the *TP53* genotype.

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TRANSPOSITION MECHANISM OF *mariner-mos1* UNDER STRESS CONDITIONSJardim SS¹, Schuch AP^{1,2}, Pereira CM¹ and Loreto ELS^{1,2}

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Key-words: stress, *mariner*, *Mos1*, transposition somatic, cell cycle.

The mobility is a principal key feature of transposon elements (TEs). This capacity of moving within a host genome gives to TEs the role of contributing to mutagenesis and genetic variability. However, the mechanisms that trigger the activation of TEs under stress condition are not well understood. The *mariner-Mos1* DNA transposon mobilizes both somatic as well as in germline cells in the *Drosophila simulans white-peach* genome. This mutant lineage allows the phenotypic study of this transposon activity through the formation of mosaic eyes. First, this mutant lineage was exposed to different stresses: ultraviolet radiation (UVC, 25J/m²), mild heat stress (28°C) and oxidative stress (Paraquat, 1mM and 2mM). Then, the *mariner-Mos1* and positive control *Hsp70* and superoxide dismutase gene expression profiles were evaluating by RT-qPCR. The *mariner-Mos1* mobilization activity was determined based on the number of red spots in the eyes of flies submitted to the same stresses, and the impact of each stress on cell cycle was also evaluated by flow cytometry. The UVC treatment had no effect in the *mariner-Mos1* gene expression, as well as in the formation of mosaic eyes. In contrast, the expression of *Hsp70* increased after UVC stress suggesting that *mariner-Mos1* expression is not directly shaped in response to this heat shock gene. Furthermore, the treatment with 28°C increased the expression of both genes and the number of red spots in the eyes of flies. After the UVC exposure, it was observed a long delay in the development of flies, as well a transient arrest in the cell cycle progression with an accumulation of cells in the G1 phase. On the other hand, the flies treated with 28°C showed a reduced time of development and a faster cell cycle progression. These results indicate that heat induces the increase of transcription and mobilization of *mariner-Mos1*, but UVC only induces the expression of *Hsp70* gene, suggesting that the mechanism of activation of *mariner-Mos1* transposition must be coupled to conditions that promote DNA replication and cell cycle progression. The effects of oxidative stress in *mariner-Mos1* transposase gene expression are in progress, although the exposures to Paraquat does not induced the formation of red spots in the eyes of flies, which indicates that genotoxic agents does not induce somatic transposition.

Financial support: CAPES, FAPERGS.

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HIGH INCIDENCE OF MUTATORS IN CLINICAL ISOLATES OF *P. mirabilis**Fonseca MRB1, Fernández-Silva FS1, Noronha MAL1 and Galhardo RS1.*

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Key-words: Mutation frequency, mutator, *Proteus mirabilis*.

Proteus mirabilis causes a large number of nosocomial infections in the world, especially for its capacity to grow in urine, finding in urinary catheters appropriate environments to establish infection and reach the patient's bladder. Mutator strains are found in bacterial populations in a frequency of 1%, and the high mutation rate of a strain is usually attributed to defects in DNA repair systems. A high incidence of mutators was previously reported for *P. mirabilis* clinical isolates, but the molecular basis of this phenomenon remains unclear. In this study, 77 clinical isolates of *P. mirabilis* and a reference strain ATCC 25933 were screened for mutator phenotypes through the analysis of squatter colonies in antibiotic disk diffusion tests with fosfomicin and rifampicin disks. 5 isolates had at least 15 squatter colonies by cm² in fosfomicin halo, while the population had between 1 and 2 colonies. 8 had at least 20 squatter colonies by cm² in rifampicin halo, while the population had about 3 colonies for this antibiotic. The 13 selected isolates were then tested for spontaneous mutation frequencies with selection of mutants in the same antibiotics in concentrations that are approximately 4 times the MIC. All 5 isolates selected in fosfomicin disk showed emergence of resistant mutants in frequencies lower than 10⁻⁶ in this antibiotic, while only 1 of the isolates selected in rifampicin had emergence of resistant mutants with a frequency of 10⁻⁷. The other 7 isolates selected in rifampicin and the reference strain had mutation frequencies of 10⁻⁸ for both antibiotics. 2 of the 5 isolates selected in fosfomicin disk, when tested with selection in rifampicin, had emergence of resistant mutants in frequencies of 10⁻⁷, a 10 fold higher mutation frequency than observed in the reference strain. 1 extra isolate was identified with mutation frequencies of 10⁻⁷ in rifampicin and 10⁻⁵ in fosfomicin. We will perform the same mutation frequency tests on all isolates to confirm the efficiency of the fosfomicin disk diffusion method on selecting mutators. With at least 4 mutators out of 77 isolates, we confirm the higher occurrence of mutators in a population of *P. mirabilis* clinical isolates, approximately 5.2% of mutators. We will continue the research using ERIC-PCR to distinguish whether there are clones among the isolates and we will investigate the reason for the increased mutation frequency and the possible involvement of the ICE family SXT/R391 in these events.

Financial support: São Paulo Research Foundation - FAPESP. Process: 2015/11348-3.

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CHARACTERIZATION AND ASSESSMENT OF THE ROLE OF XPF PRODUCT GENE OF *TRYPANOSOMA CRUZI* IN CELL RESPONSE TO DNA DAMAGE.*Oliveira KA¹, Resende BC¹, Franco GR¹, Macedo AM¹, Pena SD^{1,2} and Machado CR¹*

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Key-words: DNA Repair, *Trypanosoma cruzi*, DNA replication stress, XPF

Trypanosoma cruzi, the agent of Chagas disease, affects 10 to 20 million people, primarily in the Americas and its importance for public health highlights the need of studies for a better understanding of the parasite biology. DNA repair mechanisms are crucial for maintenance of the genome and account for the success of parasitic infections since the parasite is exposed to DNA damaging agents derived from its host, besides the endogenous sources from metabolic processes. Genome analyses of trypanosomatids have identified differences in the DNA maintenance mechanisms between these organisms and other eukaryotes, mainly due to the peculiar transcriptional process, since *T. cruzi* transcribes constitutively all the genome in polycistronic transcription units. The understanding on how *T. cruzi* genome is affected during its lifecycle and how the cells respond to DNA damage could provide new and more effective therapy for Chagas disease. The XPF gene codify an endonuclease that has a conserved role in nucleotide excision repair (NER) and seems to be involved in repair of interstrand crosslinks (ICLs). The aim of this work is the analysis of the roles of XPF in maintenance of genomic stability upon induced DNA replication stress by DNA damaging agents and its importance for cell cycle of *T. cruzi*. For that, XPF-deficient cells were produced. The modified cells showed an impaired proliferation ability characterized by a lower cell growth. Besides, these cells reached stationary phase with a reduced number of cells, compared to the wild type control. Response of XPF modified strain to UV-induced damage in DNA was assayed in three different doses (500, 1000 and 1500 J/m²) and no clear result was observed. Analyses of responses of the modified strains to others DNA-damaging agents are in progress. Thus far, our results suggest that XPF is important to cellular growth and it could be due to its involvement in ICL repair since no clear involvement was detected after UV irradiation.

Financial Support: FAPEMIG, CNPq, Newton Fund.

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Study of bone marrow cells of mice exposed to methamidophos, in vivo: Micronucleus assay*Oliveira, LBC and Aranha, IP*

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Keywords: Methamidophos, bone marrow, mice, in vivo, micronucleus

The use of pesticides is still the main strategy to fight plagues in agriculture. Methamidophos is an organophosphorous pesticide largely used in the world crop due to its efficiency. The purpose of the present work was to study the effect of methamidophos on chromosomes of mice bone marrow cells in vivo, using the micronucleus assay. Animals (ICR mice) were separated into four groups. In the first group, 6 animals received methamidophos intraperitoneally during five consecutive days in a concentration equivalent to 25% of the LD₅₀. In the 6th day, animals were sacrificed, their femurs removed, the bone marrows collected and smears were made for slides preparation. After 24h cells were stained with Giemsa Gurr (2%) and analyzed under optical microscope. As positive control, 6 animals received cyclophosphamide (50mg/mL), once and 6 animals not exposed to any drug served as negative control for the experiment. In the test group, 13353 cells were observed and 183 showed micronuclei. In the positive control group, 12037 cells were observed and 238 had micronuclei and in the negative control group, of 12156 cells observed, none had micronucleus. The chi-square test for independence showed that our results were extremely significant (P<0.0001). They suggest that methamidophos is responsible for the micronuclei observed.

Financial Support: UERJ.

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HIGH DOSES OF ALCOHOL DURING PREGNANCY CAUSES DNA DAMAGES IN OSTEOBLASTS OF NEWBORNS RATS

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Key-words: ethanol, pregnancy, osteoblasts, genotoxicity, comet assay, micronucleus assay

Alcohol exerts teratogenic effects and its consumption during pregnancy can cause deficit of bone development. The aim of the current study was to evaluate the genotoxic effects of prenatal exposure to ethanol on newborn rat osteoblasts. Wistar rats were initially divided into two groups: Ethanol group which received Ethanol 20% V/V in liquid diet and solid diet *ad libitum*, and Control group, which received solid diet and water *ad libitum*. Each group received a specific diet for eight weeks prior to breeding and throughout three weeks of gestation and the treatment was finished on the day the pups were euthanized. On the fifth day of life the pups from each group were euthanized for removal of the calvaria and isolation of osteogenic cells by sequential enzymatic digestion. The cells were cultured for a maximum period of 14 days. The detection of genotoxic effects of alcohol was investigated by the comet and the micronucleus assay. Micronucleus and comet assay showed significant increases in DNA damage at 7 days in Ethanol group ($p=0.0302$, $p=0.0446$, respectively). However, at 14 days both assay showed no significant difference between the groups ($p=0.6194$, $p=0.8326$, respectively). Our results showed that prenatal exposure to ethanol induced DNA damage in osteoblasts, as shown by micronucleus formation and higher percentage of DNA in the comet tail. It can be concluded that prenatal exposure to ethanol damages osteoblast DNA in newborns exposed to high doses of ethanol during pregnancy, suggesting that prenatal ethanol consumption has a direct effect on fetal osteoblasts.

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GENOTOXICITY ASSESSMENT OF ENVIRONMENTAL SAMPLES CONTAINING DISPERSE DYES AND AROMATIC AMINES*Vacchi FI¹, Vendemiatti JAS², Brosselin V³, Bony S³, Devaux A³, Umbuzeiro GA^{1,2}*¹ Faculty of Pharmaceutical Sciences, USP, São Paulo, SP, Brazil² School of Technology, UNICAMP, Limeira, SP, Brazil³ Université de Lyon, INRA, LEHNA, ENTPE, Vaulx-en-Velin, France

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Key-words: disperse dyes, aromatic amines, genotoxicity

Surface water and effluents under the influence of textile discharges can exhibit mutagenic activity. In ecotoxicology, *in vitro* genotoxicity testing often relies on prokaryotic organisms such as in the well standardized Salmonella/microsome assay. Many studies have demonstrated that primary DNA damage measurement with the comet assay represents a very early and sensitive genotoxicity biomarker in aquatic species. For *in vitro* studies, fish cell lines are considered an interesting eukaryotic model retaining specific fish physiological characteristics. The aim of this study was to identify dye and aromatic amines in samples under the influence of textile discharges; and evaluate the genotoxic responses of those samples in Salmonella/microsome assay using TA98 and YG1041 and in Fpg-modified comet assay using RTL-W1. Samples were collected in Piracicaba River (upstream and downstream), Wastewater Treatment Plant treated effluent and Quilombo River at Americana city, São Paulo State, Brazil. We identified 9 disperse dyes and 11 aromatic amines, including ones prohibited in European Community. Genotoxicity comet assay shows the presence of compounds producing reactive oxygen species in all the sites (similar response among them). Standard comet assay performed without Fpg better indicates a contribution of the discharges in the genotoxicity of the river downstream. But this is much more evident with the Salmonella/microsome assay results. Mutagenicity was not detected with TA98 with and without S9, except for the Quilombo River. But when YG1041 was included, mutagenicity was detected in all sites with a very different profile comparing upstream and the other sites. The response of the Salmonella/microsome assay strongly indicates that aromatic amines or other compounds that require S9 metabolism to become active, as some azo disperse dyes, are contributing to the observed mutagenic activity downstream, which was corroborated by the chemical analysis. It is possible to conclude that without the use of the diagnostic strain YG1041 this would not be revealed. The influence of the textile discharges was also confirmed by chemical analysis, because most part of dyes and aromatic amines were found in the river downstream. As a conclusion it is important to use assays based on complementary endpoints to better characterize the mutagenicity of environmental samples with the advantage of the indication of what classes of compounds are responsible for the effect.

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STRESS OXIDATIVE AND GENOTOXIC DAMAGE INDUCED BY SILICA CRYSTALS IN MICE*Santos TOC1, Caiado MP1, Gonçalves NL1, Murata MM1, Martins PMRS2, Caldeira-de-Araujo A1, and De Mattos JCP1*

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Key words: silica, comet assay, genotoxicity, catalase.

Silicosis is a high widespread pneumoconiosis attributed to inhalation of crystalline silica (SiO_2), one of the most abundant minerals on Earth. Silicosis is still a worldwide problem despite efforts have been made for many decades to its prevention. In most cases, the silicosis occurs due to intense occupational exposure to silica particles. Its occurrence outside of the workplace is restricted to specific climatic and geological situations, so silicosis is considered an occupational disease. This disease is characterized by an inflammatory process with intense production of ROS, and subsequent lung tissue fibrosis. Once initiated, it is irreversible and progressive. Moreover, diagnosis is almost late and the disease has no cure or treatment. Because of these characteristics, there is great interest and practical consequence the evaluation of physiological responses as prospective biomarkers that could indicate initial exposure to crystalline silica or early silicosis development. This way, the present study aimed to determine both the genotoxic effects and oxidative stress induced in Swiss-Webster mice exposed to silica crystals. The animals were instilled with SiO_2 and the parameters cited above were measured in mice peripheral whole blood cells, 3 and 7 days after silica exposure. The comet assay was used to evaluate DNA strand breaks and the activity of catalase enzyme to oxidative stress analysis. The first one showed a significant difference ($p < 0.05$) between exposed and control groups after 7 days. The comparison between exposed animals, after 3 and 7 days, also showed significant difference. On the other hand, the statistical analysis pointed to no difference between the control animals and after 3 days of exposure to the agent. Concerning to catalase enzyme activity, the obtained results showed no statistical difference between control and exposed samples. Together, obtained data suggest that silica exposition could be responsible for DNA damage and the comet assay could be a hopeful tool in the evaluation of initial silica exposition. In the opposite direction the evaluation of the catalase activity indicate that this enzyme could not be effective as a biomarker of initial exposure to the agent.

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DNA-BINDING AND TOPOISOMERASE INHIBITION BY RUTHENIUM(II)-BASED COMPOUNDS WITH MUTAGENIC ACTIVITY

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key-words: Ruthenium(II) complexes, DNA binding, genotoxicity

Medicinal inorganic chemistry remains a field of great promise with many challenges. The potential for a major expansion of chemical diversity into new structural and reactivity motifs of high therapeutic impact is unquestionable. Especially, Ruthenium complexes have received worldwide emphasis in the treatment of various diseases. However, even with promising effects, it is essential to assess the safety of their use. In this context, this study aimed to evaluate the genotoxic effects and antitumor activity of three Ruthenium(II) SCAR complexes. The genotoxic effects of the complexes were evaluated with the *Salmonella*/microsome reverse gene mutation test and the Cytokinesis-block micronucleus cytome assay (CBMN-cyt). The antitumor activity was investigated for cytotoxicity against five cancer cell lines and inhibition of human topoisomerase I (Top1). The interaction of these complexes with calf thymus DNA (CT-DNA) was investigated by circular dichroism (CD) and viscosity measurements. The results showed that only the SCAR6 complex presented mutagenicity after undergoing metabolization. The cytotoxic evaluation on tumor cell lines revealed that all complexes have markedly high cytotoxicity against prostate and breast cancer cell lines, SCAR5 also showed strong cytotoxic effect against Caco-2 and HepG2 cells. The SCAR5 complex was highly active in inhibiting the ability of Top1 to cleave the phosphodiester backbone of supercoiled DNA. The decrease in the DNA solution viscosity indicates that SCAR6 may form bonds with DNA. Considering the results, it is clear that the SCAR6 complex can interact with DNA, causing gene and chromosomal mutations. The complexes also showed to be inhibitors of Top1, an important antitumor mechanism. The research contribution arising out of this work cannot be overstated in that it unravels promising results seen to be crucial in the search for new anticancer candidates. Other studies on the mechanisms of action of these complexes are underway and preliminary results have suggested mechanisms of covalent interaction with DNA.

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Use of zebrafish model in the teratogenic assessment of antiparkinsonism compounds: the case of biperiden*Nunes NA¹, Moura DS¹, Farias NO¹, Oliveira R¹ and Grisolia CK¹*

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Keywords: embryotoxicity, *Danio rerio*, toxicology, psychiatric compounds.

Millions of people use psychiatric pharmaceuticals daily to treat different neurological disorders (i.e. depression, epilepsy, anxiety, and bipolar disorder). After administration parent compounds, metabolites and conjugates are excreted, mainly in the urine and faeces. Currently, the pollution rising from domestic effluent is a major problem in the management of water resources. Due to the insufficient removal rate in the wastewater treatment plants, several psychiatric pharmaceuticals have been detected aquatic ecosystems compartments including sediments, surface and ground waters. Thus, pharmaceuticals have been called as emerging pollutants. In spite of the increasing use of psychiatric drugs few data are found on their possible effects on aquatic biota and on their fate in the compartments aquatic systems. To evaluate the environmental risk of pharmaceuticals new ecotoxicological tools are needed and others need to be refined. In this study, the Fish Embryo Toxicity Test OECD No. 236 is proposed as a tool to evaluate the effects of an antiparkinsonism drug – Biperiden. Test started with newly fertilized eggs exposed to biperiden concentrations of 0; 1; 2, 4; 8.6, 17.5; 35.8 and 73.3 mg/L. Sixteen eggs per treatment (divided in 03 replicates) were selected and distributed in 24-wells microplates. The tests were carried out at 26 ± 1 °C and run for 96 hours. Embryos were daily observed under a stereomicroscope (Stemi 2000-C, Zeiss, Germany), using 70 x magnification for embryos and 40 x for hatched embryos. In the embryo phase, the following parameters were evaluated: egg coagulation, otolith formation, eye and body pigmentation, somite formation, tail circulation, detachment of the tail-bud from yolk sac, absorption of the yolk sac, alterations of the amniotic fluid and hatching. After hatching: oedema, equilibrium, undersize, spine deformation and mortality were observed and reported. After 96 h of exposure a LC50 of 15,8 mg/L was obtained. Moreover, were observed several anomalies in the development including abnormal pigmentation of eyes and body of embryos and general developmental delay in concentrations higher than 17.5 mg/L. For concentration from 4 mg/L were observed behaviour alterations characterized by the loss of equilibrium, paralysis and abnormal posture of the embryos, lying in the bottom of the plate. In summary, biperiden was toxic for zebrafish embryos with effects on survival, development, and behaviour. Since biperiden is an antiparkinsonian agent of the anticholinergic type further studies including neurological biomarkers (i.e. acetylcholinesterase) might be useful to explain the effects observed in the present study.

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Evaluation of the cytotoxic, genotoxic and mutagenic potential of jucá (*Libidibia ferrea*) using the *Allium cepa* test system.*Ferreira TAA¹, Azevedo LFC¹, Melo KM¹, Dias CLP¹, Nagamachi CY^{1,2} and Pieczarka JC^{1,2}*

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Key-words: Jucá, *Libidibia ferrea*, *Allium cepa*, Mitotic Index, Mitotic alterations, Nuclear alterations.

The jucá (*Libidibia ferrea*) is a plant widely used in folk medicine. However, it has not been sufficiently studied on its DNA damaging potential. Thus, this study evaluated the cytotoxic, genotoxic and mutagenic potential of the infusion made from the jucá fruit on the *Allium cepa* test system. The seeds of *Allium cepa* were germinated and measured before and after exposure to agents. Five treatments were made, where four were made on a period of 24 hours: T1, negative control; T2, positive control with Doxorubicin; T3, positive control with colchicine; T4, infusion. The main treatment was made on a period of 48 hours: T5, reversion (24 hours infusion + 24 hours water infusion) to test the recovering of possible damage made by the infusion. All seeds were kept in fixative for 48 hours prior to slides preparation and meristematic root cells analysis. The results demonstrated that the size of the roots was not significantly different in any of the five treatments. The Mitotic Index in T2 was significantly different when compared to the other treatments, but this was an expected result, since Doxorubicin is a drug that generates reactive oxygen species able to cause damage to various molecules, including DNA, a process that can lead the death of cells by apoptosis. On mitotic alteration, T3 treatment showed a significant higher amount with predominance of C-metaphases, also expected since literature indicates that colchicine affects the formation of mitotic fuse and the higher amount of polyploidy cells. The T4 treatment showed a higher frequency of nuclear alterations, especially an increase in the frequency of nuclear sprouts. Many authors suggest that sprouts may be the result of genic DNA amplification. This increase was also observed in T5 reversion treatment. We can infer that there was recovery of nuclear damage after reversion treatment. In addition, there was no statistical difference for Micronuclei in any of the treatments. It is concluded that infusion treatment resulted in significant presence of nuclear sprouts. The continuity of the cytotoxic, mutagenic and genotoxic studies on jucá it is necessary to ensure their use in folk medicine.

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ANTIROLIFERATIVE EFFECT OF THE DIPHENYL DITELLURIDE IN HUMAN CANCER COLON CELLS

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Diphenyl ditelluride (DPDT) is an organotellurium (OT) compound with pharmacological effects, such as potential antioxidant, antigenotoxic and antimutagenic in low concentrations. On the other hand, higher concentrations of DPDT showed cytotoxic action in mammalian V79 cells by induced oxidative damages, DNA strand breaks, cell cycle arrest and topoisomerase I inhibition. In this sense, the cytotoxicity of other OT compounds has been reported, and their employment in anticancer therapy was suggested. Thus, the objective of this study is to investigate the antiproliferative potential of this molecule in human colon cancer cells (HCT116) and human fibroblast cells (MRC5). For this, the cells lines were exposed to DPDT in concentrations range from 0.1 to 10 μ M. To evaluate cell viability we perform the MTT and clonogenic assay, for the period of 3 (with 24h cellular recovery), 24, 48 or 72 h. In 3, 24 and 48h of exposure to DPDT showed similar cytotoxicity to both cell lines. In contrast, DPDT was able to induce decrease in the cell viability to 72h of exposure in MTT and clonogenic assay. The IC₅₀ values are 13 and 3 μ M to MRC5 and HCT116, respectively in MTT assay. Consistently, clonogenic assay showed similar values of IC₅₀ (MRC5 = 8 μ M and HCT116 = 3 μ M) in 72 h of exposure in relation to MTT. To evaluate genotoxicity we perform the alkaline comet assay with cell exposure of 3 and 24 h. For 3h, 10 μ M of DPDT induced similar DNA damage index (DI) increase to both cell lines. However, concentrations >5 μ M of DPDT increase the DNA strand breaks occurrence only to HCT116 cells, in 24 h of exposure. The results showed that HCT116 cells are more sensitive than MRC5 to DPDT and this difference in cell viability between both lines may be due to genotoxic effects induced by this compound. Therefore, DPDT is an interesting molecule for colon cancer cells antiproliferative approach with selective cytotoxic potential, but more studies must be done.

Support: CNPq, FAPERGS, CAPES

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CYTOTOXICITY AND MUTAGENESIS INDUCED BY STANNOUS CHLORIDE IN *Escherichia coli**Sarcinelli JM, Maciel VB; De Lima PVS;; Murata MM; Caldeira-de-Araujo A e De Mattos JCP**Radio and Photobiology Laboratory, Department of Biophysics and Biometry, Roberto Alcantara Gomes Biology Institute, Rio de Janeiro State University - UERJ, Rio de Janeiro, RJ*

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Keywords: SnCl₂, *E. coli*, prokaryote, Survival fraction, Resistance to Rifampicin.

Stannous chloride (SnCl₂) is used in various sectors of human activity, besides its potentiality of producing reactive oxygen species and induce cell death in *E. coli*. In addition, single and double breaks in DNA *in vivo* as *in vitro*. With the aim of increasing knowledge about the action of SnCl₂ in *E. coli*, the present study evaluated the potential cytotoxic and mutagenic of this agent in the wild strain AB1157 cells. The data obtained in bacterial survival tests were consistent with results already described in the literature, confirming the cytotoxic potential of stannous ion in two concentrations of SnCl₂, 20 µg/mL and 25 µg/mL, during 40 minutes of exposure. The sample treated with SnCl₂ - 25 µg/mL - showed a significantly higher mortality (p<0.05) at the end of 40 minutes of incubation, compared to the other one exposed to 20 µg/mL concentration, suggesting that the SnCl₂ promotes inactivation in a dose dependent manner. The data obtained in mutagenesis assay for resistance to the antibiotic rifampin showed that SnCl₂, at a concentration of 20 µg/mL and after 20 minutes of incubation, was able to increase the rate of mutagenesis in 2.2 times than that one observed in control sample (p<0.05). At 25 µg/mL, SnCl₂ induced 4.3 times more cells resistant to the antibiotic than spontaneous mutants (p<0.05). Moreover, at the end of 40 minutes of incubation at concentrations of 20 µg/mL and 25 µg/mL, there were, respectively, 7.4 and 8.0 times more mutant cells than the control sample (p<0.05). Hydrogen peroxide, H₂O₂, used as positive control, induced an increasing in the number of cell mutants only 1.2 times after 40 minutes. The mutation frequency after 40 minutes of incubation of the cells with SnCl₂ was greater for the concentration of 25 µg/mL of 20 µg/mL, even with mortality being higher for bacteria incubated with 25 µg/mL of SnCl₂ for 40 minutes. Through the experimental model carried out, the results suggest that stannous chloride is able to promote bacterial inactivation, showing an increasing death rate as a function of concentration and incubation time. Besides, SnCl₂ is also able to induce an increase in mutagenesis, therefore it could be considered a mutagen.

Financial support: FAPERJ, CNPq, CAPES e UERJ.

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IN VITRO EVALUATION OF CYTOTOXIC AND MUTAGENIC OF THE MARUPAZINHO INFUSION EFFECT (*Eleutherine plicata*)Azevedo LFC¹, Cordeiro APB¹, Nagamachi CY^{1,2} and Pieczarka JC^{1,2}

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Key-words: Marupazinho, *Eleutherine plicata*, cell viability, necrosis, micronucleus.

Eleutherine plicata (marupazinho) is a plant widely used in Brazil for the treatment of various gastrointestinal diseases. However, there are few studies investigating its toxic potential. *In vitro* toxicity tests are commonly used to analyze the mutagenicity and cytotoxicity of herbs. This study evaluated the *in vitro* toxic potential of marupazinho bulb infusion with the MTT test, apoptosis / necrosis and micronucleus. In all assays, squamous carcinoma cells of the larynx human cells (HEp-2) were seeded in plates and exposed in triplicate at different concentrations of marupazinho infusion. In the MTT assay was used seven concentrations (62,5µg/ml, 125µg/ml; 250µg/ml; 500µg/ml; 750µg/ml; 1000 µg/ml e 1125 µg/ml). From this data it was calculated the IC⁵⁰ and the doses used in the remaining tests corresponding to 15%, 20%, 25% and 30% of the IC⁵⁰. On the positive control we used cyclophosphamide and in negative control DMEM medium of culture. The analysis of cell viability from the MTT assay showed reduced proliferative activity in a dose dependent manner. The apoptosis / necrosis test reiterated that the infusion reduces the cell viability, while the number of cells in necrosis increases. It is possible that the cytotoxicity observed in experiments occurs because of the presence of naphthoquinone as a phytochemical constituent of the bulb, which can induce cell death and, depending of the intensity of the stimulation, lead to cell necrosis. There was no significant presence of micronuclei (not mutagenic effect), but there was significant reduction in the mitotic index in the highest concentrations tested. Together, our data indicate that the marupazinho causes cell death (cytotoxic effect) with consequent reduction of the mitotic index in a dose-dependent manner. The reduced cell viability affected by infusion may reflect the absence of structural and / or numerical aberrations in the micronucleus test and not necessarily the absence of genotoxic effects. Considering our results, we do not recommend the *in vivo* use of *Eleutherine plicata* in the treatment of diseases. Further, more studies are required to indicate or not its safely use.

Financial Support: CNPq (Brazil).

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ASSESSMENT OF ANDIROBA (*Carapa guianensis*) OIL POTENTIAL MUTAGENIC EFFECT in *Allium cepa* ROOTS

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Key-words: *Carapa guianensis*, *allium cepa*, mitotic index, apoptosis

Carapa guianensis (andiroba) it is a plant widely used by Amazonian population as herbal medicine and repellent. However there are still few studies evaluating its potential mutagenicity. This study used the *Allium cepa* test in order to assess the mutagenic potential of the oil extracted from the andiroba seeds and its effect on root growth (RG) and mitotic index (MI). The sample was characterized on its the lipid profile. The seeds were exposed to distilled water (negative control-NC) and oil in four different periods (24, 48, 72 and 96h); doxorubicin and colchicine were used as positive controls. After exposure, the seeds were fixed, stained with orcein and observed under a light microscope. Five individuals were used per treatment, being analyzed 1,000 cells on each. The parameters evaluated were: RG, MI, and mitotic abnormalities (MAf), nuclear abnormality (NAf), micronucleus (MNf) and nuclear fragmentation frequencies (NFf). The standard normal distribution of the results was valued by Kolmogorov-Smirnov test and possible differences investigated or by ANOVA or the nonparametric Kruskal-Wallis when the data were not normally distributed. Our results showed a statistically significant reduction in RG treated with the oil when compared to the NC in all periods. This can be explained by interruption of the cell cycle caused by the oil of andiroba since there was also a statistical reduction in the MI. MAf and NAf showed no statistically significant difference. However, a significant increase in the numerical values in the MNf and NFf was observed, but without statistical difference, probably due to the samples high standard deviation. Regarding MN, previous studies have not identified genotoxicity after treatment with andiroba oil. On the other hand, it was already described that a high concentration of fatty acids (oleic, palmitic, linoleic and stearic acids) can cause apoptosis. In our analyzes we observed many cells with fragmented nuclei, giving evidence of apoptosis or cell death. Lipid characterization of the sample used in this study contains as major components oleic acid (55.23%) and palmitic acid (37.95%). A more refined analysis could assist in a better understanding of the andiroba effect on the formation of MN and apoptosis.

Financial support: CAPES

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ULTRAVIOLET-INDUCED DNA DAMAGE: PHOTOREPAIR IN THE SKIN OF DNA REPAIR DEFICIENT MICE ON CELL PROLIFERATION AND INFLAMMATION*Kajitani, GS1, Quayle, C1,2, Hoeijmakers, J2, van der Horst, G2 and Menck, CFM1*

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Keywords: Ultraviolet irradiation, photolyases, DNA repair, inflammation.

Ultraviolet (UV) irradiation is considered one of the most genotoxic agents present in our environment. It damages DNA molecules, inducing mainly cyclobutane pyrimidine dimers (CPD) and pyrimidine 6-4 pyrimidone photoproducts (6-4PP). These lesions interfere in essential cellular processes, such as transcription and replication, promoting severe effects in the skin, such as inflammation, dysplasia and cancer. In placental mammals, UV-induced lesions are repaired by the Nucleotide Excision Repair (NER) pathway. This pathway is subdivided into two recognition pathways, the Transcription Coupled Repair (TCR) and the Global Genome Repair (GGR). In this work, we used XPA knockout (KO), NER deficient and CSA KO, TCR deficient mice. Both mice strains transgenically expressed either CPD or 6-4PP photolyases (enzymes that repair specifically CPD or 6-4PPs through a light dependent mechanism) in order to assess the *in vivo* effects of the photoremoval of each of these lesions after low, chronic UVB exposure. In CSA KO mice, the removal of CPD resulted in a reduction of hyperplasia and cell proliferation, while 6-4PP removal did not change these effects. In the XPA KO mice, the removal of CPD completely prevented the UV hyperplasia effect, while the 6-4PP removal promoted only partial reduction. These data suggest that CPD is the main lesion triggering hyperplastic processes, both in TCR and NER deficient mice, with 6-4PP having a minor role in NER deficient mice. We also studied the effect of CPD or 6-4PP removal in XPA KO mice in the induction of the inflammatory process by *in vivo* imaging of ICAM-1 and MPO expression after a single, high UVB dose. The removal of either type of lesion was able to prevent ICAM-1 and MPO expression 6 hours after UVB irradiation and reduce the expression of MPO after 24 hours. These results indicate that both lesions have a major role in UVB induced skin inflammation.

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Assessment of genotoxic effects of Copper (II) complex of 1,10-Phenanthroline and Doxycycline (CuDoxPhen) in somatic cells of *Drosophila melanogaster*.Lopes JC^{1,2}, Guimarães LMM¹, Polloni L¹, Júnior RJO¹ and Nepomuceno JC^{1,3}

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Key-words: CuDoxPhen, free radicals, direct mutagenic

Metal synthetic nucleases can be useful in the treatment of cancer because they can block genetic expression. An inconvenience is that usually the cleavage of DNA requires the concomitant addition of an external reagent, such as thiols and hydrogen peroxide. Certain copper complexes that break DNA strands by an oxidative pathway were reported to be cytotoxic against tumor cells, many studies have shown that copper (II) complex of 1,10-phenanthroline and doxycycline (CuDoxPhen) cleave the DNA strands by an oxidative mechanism involving the generation of ROS, suggesting mechanism of cytotoxic action. This work aims to evaluate the genotoxic effects of a copper (II) complex CuDoxPhen, *in vivo* using the *Drosophila melanogaster* as organism model. In the present study, the Somatic Mutation And Recombination Test in *Drosophila melanogaster* (SMART) was employed to determine the genotoxic effects of CuDoxPhen. Chronic treatments with CuDoxPhen were performed with 3-day-old larvae of the standard (ST) cross of the wing spot test at concentrations of 6,25; 12,5 and 25mg/L. In addition, the carcinogen doxorubicin was administered at 0,04 mM, as a positive control, as negative control was employed osmosis reverse water. Somatic spots on normal wings from marker heterozygous (MH) flies were scored to determine mutational events in somatic cells for each compound. The results showed mutagenic effects of CuDoxPhen at the 6,25 and 25mg/L concentrations in the ST cross, when compared with the negative control. In addition, at the concentration of 12,5mg/L the CuDoxPhen showed non-mutagenic effect, when compared with the negative control. However, when associated with DXR, CuDoxPhen enhanced the doxorubicin effects at all concentrations. In view of this experimental conditions and results it was concluded that CuDoxPhen was associated with direct mutagenic effects and had a synergic action with doxorubicin, once CuDoxPhen and doxorubicin promote the formation of free radicals, explaining the enhanced mutagenic effects, if used during chemotherapy it could enhance the effects of the chemotherapeutic used. However, Further studies can improve the comprehension about the mechanisms of CuDoxPhen action.

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EVALUATION OF DNA DAMAGE IN A ANIMAL MODEL OF METASTATIC MELANOMA

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Introduction: Melanoma is considered the worst prognosis skin cancer due to its high possibility of metastasis. This cancer originates in the melanocytes, the melanin producing cells, and tumor development is the result of multiple and progressive changes in DNA, which may be caused by activation of proto-oncogenes by mutations of tumor suppressor genes or structural alterations of chromosomes. The objective of this study was to evaluate DNA damage in an animal model of metastatic melanoma, in order to use it in future studies to test chemicals or functional foods antigenotoxic or antimutagenic activity. **Materials and Methods:** Were used 16 mice C56BL/6 male and female divided in two groups: control (PBS) and melanoma (B16–BL6 - 10⁵ cells/mL). The induction of melanoma was carried out by melanoma cells inoculation in the right hind paw and 14 days after this, peripheral blood, lung, and spinal cord were collected for the Comet assay, and bone marrow for the micronucleus test. **Results:** The results of this study demonstrated that the animals in the melanoma group had significantly higher genetic damage in all the evaluated structures when compared to the control group for both parameters of the comet assay (damage index and damage frequency). In the micronucleus test, the animals in the melanoma group showed a significantly higher incidence of micronucleated cells compared to the control group both in polychromatic erythrocytes and in normochromatic erythrocytes. **Conclusion:** From these results it can be concluded that this is a suitable cancer model to test the potential antigenotoxic and / or antimutagenic different substances.

Keywords: cancer; melanoma; genotoxicity; mutagenicity; Comet Assay; Micronucleus Test.

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ASSESSMENT OF GENOTOXIC AND ANTIGENOTOXIC EFFECTS OF ANDIROBA (*Carapa guianensis* Aublet) OIL AND NANOEMULSION IN SWISS STRAIN MICE.

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Key-words: andirobeira, micronuclei, comet.

Known as andirobeira, *Carapa guianensis* is a plant whose oil is extracted from its seeds and widely used by the Amazonian population for medicinal purposes. The objective of this study was to assess the genotoxic and antigenotoxic potential of oil (AO) and its nanoemulsion (AN). Seven groups of Swiss strain mice were used to this study: AO (2000mg / kg) IN (2000mg / kg) Doxorubicin DOX (40 mg / kg), AO + DOX, AN + DOX and as a control was used corn oil and the nanoemulsion surfactant. In order to administer the proposed doses, AO was diluted in corn oil. AO has been characterized by its lipid profile and tests were conducted to determine the best formulation to be used in the composition of the nanoemulsion. The substances were administered to mice by gavage for 14 consecutive days and DOX was applied intraperitoneally 48 hours before euthanasia. Comet assay were performed on blood cells and the micronucleus test in bone marrow cells. The three compounds were rich in AO oleic acid (55.23%), palmitic acid (37.95%) and linoleic acid (4.21%). The composition of IN followed the oil / surfactant ratio of 0.69 and Tween 80 / Span 80 was maintained at 0.9. In the comet assay there was no statistical difference for any of the treatment groups. The micronucleus test showed that the percentage of polychromatic erythrocytes (MNEPC) in control groups and treated with AO and AN remained close to 50%, indicating that the AO and AN alone are not cytotoxic. However, in all doxorubicin treated groups, including those pretreated with AO and AN, there was a statistically significant reduction in the number of cells. Therefore, the DOX is cytotoxic and the tested samples did not protect cells from this factor. On the other hand, with regard to the formation of MN, our results showed a great increase in their formation when the mice were treated with DOX and these numbers were reduced when animals were pretreated with TO and AN. This reduction was more significant in the group AO + DOX (89% reduction of MNEPC and 45% of MNENC) than in AN + DOX (62% reduction of MNEPC and 42% of MNENC). As it was used corn oil as a vehicle for dilution, it may be that this effect has is

strengthened by their joint action. A more detailed study about this interaction may help clarify our results.

Financial support: CAPES.

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Possible effect of *Luffa operculata* infusion on cell polarity in the *Allium cepa* test system with association of metanalysis data.*Cordeiro, APB¹, Azevedo, LFC¹, Nagamachi CY^{1,2} and Pieczarka JC^{1,2}*

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The *Luffa operculata* (cabacinha) infusion é commonly used in the treatment of rhinosinusitis, and as an abortifacient, and it may cause death after ingestion. Two different studies already tested its toxic potential in the *Allium cepa* test system. However the evaluated concentrations were too high and different between the studies, and the results do not match. This study aimed to investigate the toxic effect of cabacinha infusion in *Allium cepa* test system using the concentrations popularly applied in the airways. We also associated our results with the previously published ones to determine the toxicological evolution of the herb in higher concentrations. The *A. cepa* seeds were cultivated in distilled water and the roots with 2 cm were separately exposed to three concentrations of the infusion. The negative control was performed with distilled water and the positive control with paracetamol. All testes were made in triplicate. For each treatment, the Mitotic Index, the presence of micronuclei and nuclear aberrations were determined from the analysis of 1000 cells. Additionally the number of cells in each mitosis phase and the occurrence of chromosomal aberrations were recorded in 100 cells. For statistical analysis we used the oneway ANOVA test or the Kruskal-Wallis test, both with 5% of significance level. Our results show that the infusion has dose-dependent cytotoxic effect and aneugenic genotoxic effect even on the lower evaluated concentrations. The progressive shortening the cell cycle with increasing concentration of the infusion, although the tissue remains alive, can indicate endomitosis from the metaphase, leading to the polyploid cells found. The high occurrence of c-metaphases and polyploid anaphasic bridges indicate that the infusion can cause multipolar spindles. Studies suggest a possible relationship between change in cell polarity, aneuploidy and polyploidy with tumors emergence and progression. Because of the recognized high correlation between the results of toxicology tests in *Allium cepa* and animals, including mammals, an extrapolation of our findings allows us to suggest that the infusion of *Luffa operculata* has carcinogenic potential. Other studies are being conducted with the herb to confirm its toxicological potential.

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PTEN*-DEFICIENT GLIOBLASTOMA CELLS ARE MORE SENSITIVE TO TEMOZOLOMIDE COMBINED TO PARP-1 INHIBITORMontaldi AP¹, Lima SC¹, Oliveira AC², Godoy PRDV¹ and Sakamoto-Hojo ET^{1,2}.*¹ Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo - USP; Ribeirão Preto, SP, Brazil.² Department of Genetics, Ribeirão Preto Medical School, University of São Paulo – USP; Ribeirão Preto, SP, Brazil.E-mail: apmontaldi@usp.brKeywords: Glioblastoma, *PTEN* gene, PAPP inhibitor, Temozolomide, cell resistance.

Pharmacological inhibition can also be applied to test alternative strategies of DNA repair inhibition in cancer. Based on the concept of synthetic lethality, PARP inhibitors (PARPi) have emerged as a promising tool, especially for those tumors that are defective in homologous recombination repair (HRR), such as those carrying mutations in the *PTEN* gene, which participate in HRR, among other functions. Alterations in *PTEN* have been identified in at least 60% of GBMs, emphasizing the importance of investigating its contribution for synthetic lethality promoted by PARPi. To test the hypothesis that PARP inhibition might increase TMZ cytotoxicity in *PTEN* deficient cells, we used the NU1025 agent (PARP inhibitor) combined to TMZ treatment in T98G and LN18 GBM cell lines, which are *PTEN* deficient and proficient, respectively; both cell lines are mutant for *TP53*. The results showed that while NU1025 (100 and 200 μ M) tested alone did not significantly reduce cell viability of both cell lines, the drug combination (NU1025 added 20 minutes prior to 200 μ M TMZ) was more efficient in reducing the viability of T98G cells (80.4% reduction) compared to LN18 cells (68,2%). In addition, TMZ plus NU1025 elevated the G2/M arrest in T98G cells caused by TMZ after 24 and 72 h, as well as gamma-H2AX induction (6, 24, and 120 h), which is a marker of double strand breaks (DSBs); accordingly, apoptosis induction was also increased in T98G cells as analyzed by the sub-G1 content and the annexin assay (33.3 and 44.2% of apoptotic cells, for 3 and 5 days, respectively) for 200 μ M NU1025 plus TMZ, while TMZ alone induced 18.3% of apoptotic cells (5 days). LN18 cells also showed a G2/M blockage, gamma-H2AX and apoptosis induction (200 μ M NU1025 plus TMZ), although at lower levels relative to T98G cells. Therefore, we showed the efficiency of PARP inhibition towards potentiating cell death, compatible with DSB induction in TMZ-treated cells. Furthermore, the results indicated that *PTEN* deficient cells were more sensitive to combination of PARPi and TMZ than the wild-type cells, which did not occur when PARPi was tested as monotherapy, suggesting that *PTEN* status is relevant for drug responses. Financial Support: FAPESP – São Paulo Research Foundation (Proc. N^o 2013/12033-0; 2013/13253-4; 2013/09352-7) & CNPq – Research National Council, Brazil.

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THE COMET ASSAY IN ASSESSING THE GENOTOXICITY INDUCED BY SILICA CRYSTALS IN A549 CELL LINE

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Key-words: genotoxic assay, silicosis, biomarker, diagnostic.

Silica (SiO_2) is an abundant mineral in the earth formed from the elements silicon and oxygen. Continuous inhalation of crystalline form could lead to a serious lung disease known as silicosis, characterized by lung fibrosis. Silicosis is one of the most important occupational diseases in the world since the release of dust containing silica particles occurs in activities such as excavation, construction, mining and ceramic industries. The size-intermediate silica particles are not removed by the mucociliary and lymphatic clearance and they are deposited mainly in the respiratory bronchioles and alveoli. Thus, it induces the formation of ROS and inflammatory process, contributing to the induction of fibrotic nodules and interstitial fibrosis. Silica exposure has been associated with other diseases, such as tuberculosis, lung cancer and COPD. Diagnosis is made by radiography and CT scan, but only in later disease stages. Because of this, it is of great interest the possibility of using physiological responses as prospective biomarker to indicate the initial exposure to crystalline silica. The study aimed to standardize the comet assay with silver salts staining in the cell line A549 and to evaluate the genotoxic risk of silica exposure to cells. Different volumes of *Low Melting Point* agarose (80, 90, and 100 μL) and cell concentrations (25, 50, 75, 100, 300 and 500 $\times 10^3$ cells / well) were tested to assess the extent of DNA damage in function of incubation time. Cultures of A549 cells were exposed to 300 mg/ml silica for different periods of time (30 minutes, 1, 2, 3 and 4 hours). It was established as optimal for the experiments the concentration of 100 $\times 10^3$ cells as well as LMP agarose volume of 80 μL . After 30 minutes incubation, the silica was capable to promote a significant increase in DNA lesions ($p < 0.05$) and this damage increased after 4 hours of exposure ($p < 0.05$), without, however, significant variation between 1 and 3 hours incubation with silica. The staining by silver salts seems to be a viable alternative to fluorescent dyes to evaluate the results of the comet assay. The early silica exposure promotes DNA damage in cells, which increases in a time dependent way. Data collected suggests that the comet assay may be, along with other biomarkers, an interesting method to diagnose early silica exposure.

Financial support: FAPERJ, CNPq, CAPES and UERJ.

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INFLUENCE OF DNA DAMAGE RESPONSE MECHANISMS IN LEUKEMIA CELLS RESISTANT TO THE ANTINEOPLASTIC MITOXANTRONE*Viero VP¹, Busatto FF^{1,2}, Rocha JC^{1,2}, and Saffi J^{1,2}.*

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Keywords: *leukemia, DNA repair, mitoxantrone, tumor resistance.*

Chemotherapy is one of the main cancer treatment strategies; however, tumors can show resistance, which makes the treatment partial or totally inefficient. Among the mechanisms that may be related to the resistant profile, the most studied is increased drug efflux through ABC transporter proteins. On the other hand, altered DNA repair pathways may contribute to cancer resistance, since the lesions are removed before they become toxic to cells, which reduces chemotherapy effectiveness. Among DNA repair pathways, Nucleotide Excision Repair (NER) is one of the most versatile, and there are studies showing its involvement in removal of anthracyclines-induced lesions. Mitoxantrone (MXT) is a structural analogue of anthracyclines drugs, widely used in the treatment of different types of cancer. These compounds act by inhibiting Topoisomerase II (TopoII) enzyme, causing lesions as single and double strand breaks, DNA adducts, interstrand cross-links and also free radical release. Therefore, our aim was to evaluate the contribution of DNA damage response (DDR) mechanisms, focusing on NER, to the resistance to MXT using the mitoxantrone-resistant leukemia cell line HL-60/MX2 as a model. After treatment with MXT and etoposide (ETO), another TopoII inhibitor, in different times and concentrations, cell survival was assessed by trypan blue exclusion method; cell cycle profile and H2AX histone phosphorylation (γ H2AX) were evaluated by flow cytometry; and gene expression levels of NER and efflux proteins were determined by RT-qPCR. Results indicate a different treatment response, mainly time-dependent, between the resistant HL-60/MX2 and the sensitive HL-60 cells, as observed in the survival assay, cell cycle, and H2AX phosphorylation profile. Furthermore, in the resistant cells, RT-qPCR analysis showed an increase in the expression of NER genes, with ERCC1 expression increased before and after treatments with TopoII inhibitors, and XPA mainly after the treatments, while the expression of efflux proteins did not demonstrate any difference in both line cells. In conclusion, our results show that ABC proteins do not mediate the resistance of HL-60/MX2, and indicate the contribution of NER machinery in the resistance of these cells to MXT and ETO.

Financial support: CAPES, CNPq and FAPERGS.

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CITOTOXICITY AND GENOTOXICITY OF THE FLAVONOID VITEXIN IN HepG2/C3A CELL LINE, *IN VITRO**FERNANDES LM¹, MARIUCCI RG¹, ALMEIDA IV¹, BUZO MG¹, and VICENTINI VEP¹*¹Department of Biotechnology, Genetics and Cell Biology, State University of Maringá - UEM, Maringá, PR.

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Keywords: C-glycosylated; compounds of the diet; human hepatoma, comet assay.

Vitexin is a flavone C-glycosylated found in various medicinal plants and has many proven biological properties such as anti-inflammatory, spasmolytic, antimicrobial, anticytotoxic and antioxidant. Thus, it is important to evaluate the effects caused by the compound, because the people are exposed to many foods containing this flavonoid. Therefore, the aim of this study was to evaluate the cytotoxic and genotoxic activity in human hepatoma cell line (HepG2/C3A), *in vitro*, by the MTT Cytotoxicity Assay and Comet Assay. In the cytotoxicity assay, different concentrations of Vitexin (2.3, 23, 46, 93, 138, 185, 231, 278, 324, 370 μ M) were evaluated. For the Comet Assay, three non-cytotoxic concentrations of Vitexin (46, 93 and 185 μ M) were evaluated. The cells (10⁵/ml) were seeded in 96-well plates and 25 cm² flasks, for the MTT and the Comet assay, respectively. Three independent experiments were performed and the results were submitted to analysis of variance followed by Tukey's test (MTT) and Dunnett's test (Comet) ($\alpha=0.05$). The results indicated that the Vitexin, in the cytotoxicity test, was not cytotoxic to HepG2/C3A cells, as significant differences were not observed relative to control at 24, 48 and 72 hours. The Comet Assay analysis showed that Vitexin 185 μ M was statistically genotoxic, with a predominance of class 1 comets, compared to control. Thus, it was observed that Vitexin, present in small concentrations in various plants, herbal medicines and drugs used frequently by the population, has no cytotoxic activity and has genotoxic activity only at the highest concentration evaluated. These results can be attributed to the antioxidant action of this flavonoid, but using in moderation can help prevent various diseases and for improving the quality of life of those who consume it.

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CYTOTOXICITY AND MUTAGENICITY INVESTIGATION OF THE LEACHATE LANDFILL FROM MARINGÁ-PR, IN PLANT AND MICROORGANISM TEST SYSTEMS*Almeida ACC¹, Heck MC¹, Mariucci RG¹, Pedroso K², Scandelai APJ², Vicentini VEP¹.*

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Keywords: Environmental monitoring; *Allium cepa*; Ames test; toxicity; environmental effluents.

Leachate is a substance produced from the decomposition of solid waste present in dumps and landfills, making it a substance with variable chemical and biological characteristics. It is important to conduct biological assessments and monitoring to prevent future environmental impacts that the slurry will cause, since in many dumps and landfills there is no treatment for this pollutant effluent. This study aimed to evaluate the cytotoxicity and mutagenicity of raw leachates and treated by ozonation method, obtained in the landfill of the city of Maringá-PR, in two biological test systems. In the *Allium cepa* L. bioassay, three groups were performed, each one with five onions: 1) negative control (water), 2) crude leachate, and 3) treated leachate. The roots were collected at different times: control of the bulb itself (Co-0h); treatment with effluent, for 24 hours (24-Tr); and recovery with filtered water for 24 hours. Statistical analysis was performed using the Chi-square test ($\alpha=0.05$). For the Ames test, two strains of *Salmonella typhimurium* (TA98 and TA100) were evaluated in the presence and absence of S9 microsomal fraction. In the Ames test, different solutions were analyzed: raw leachate, raw leachate 50% and 25% diluted, 30min and 120min ozonation treated leachate. The results were submitted to analysis of variance (ANOVA) followed by Tukey's test ($\alpha=0.05$). The plant test system *Allium cepa* showed cytotoxicity to the raw leachates and treated in time of 24 hours of treatment. In this treatment was total inhibition of the mitotic index of meristematic cells, but after the recovery period the meristematic cells showed the same percentage of cell division of the negative control. For the Ames test strains TA98 and TA100 used without microsomal fraction (S9) and the TA98 with S9 presented mutagenicity only for the raw slurry samples. These results may be associated with the presence of heavy metals and high ammonia nitrogen content, a substance that increases the toxicity of the medium, and without treatment can contaminate water bodies causing eutrophication. Leachates have a high risk of contamination and constitutes a threat to the environment and human health, thus biological tests in a combined manner, as in this work should be performed periodically and can contribute to the prevention and environmental contamination detection.

Financial Support: CNPq.

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TAURINE CAUSES A REDUCTION IN THE NUMBER OF MICRONUCLEI*Godoi BH¹, Carvalho ICS¹, da Silva NS² and Pacheco-Soares C¹.*

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Key-words: taurine, genotoxicity, cytotoxicity, micronucleus assay, crystal violet

Taurine is a product of the metabolism of amino acids existing in the human body, such substance become popular with the consumer supplements and beverages with stimulating character. This substance is found in specific regions of the body such as the brain, heart muscle, liver and pancreas. Several studies shows that taurine acts in processes such as: participation in the formation of bile salts, anti-inflammatory action, inhibition of oxidative stress process, among others, however its physiological action is not fully understood. The aim of this study is to evaluate the cytotoxicity and genotoxicity of cells exposed to taurine. L929 cells cultured in DMEM medium were used (Dulbecco`s Medium® Modified Eagle) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic in an oven at 37 °C in atmosphere of 5% CO₂. For the cellular assays, the cells were treated with taurine dissolved in phosphate buffered saline (PBS) at a concentration of 0.4 mg / ml and incubated for 24h and 48h. The detection of the cytotoxicity and genotoxic effects was investigated by the crystal violet assay and micronucleus, respectively. Crystal violet assay showed that taurine was not cytotoxic in periods of 24h (p = 0.99) and 48h (p = 0:40). Micronucleus assay showed significant decreases in micronucleus formation at 48h (p = 0.0002) in taurine group. In addition when compared number of micronucleus the control group showed difference between the periods of 24h and 48h (p = 0.0004). It can be concluded that taurine is not cytotoxic and showed a reduction in the number of micronuclei.

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A CRUCIAL AND CONSERVED ROLE FOR Dpb11^{TopBP1} IN DNA END RESECTION*Cussiol JR¹, Liu Y¹, Dibitetto D², Freire R³, Pellicoli A² and Smolka MB¹*¹Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA²Department of Biosciences, University of Milan, Milano, Italy³Unidad de Investigación, Hospital Universitario de Canarias, Instituto de Tecnologías Biomédicas Tenerife, SpainE-mail: jr867@cornell.eduKeywords: DNA end resection, DNA repair, Dpb11^{TopBP1}, Rad9^{53BP1}

During DNA replication, cells are particularly susceptible to accumulate genomic instability, as replication forks often stall and collapse, leading to the generation of DNA double strand breaks (DSBs) and gross chromosomal rearrangements. Proper repair of DSBs in S-phase relies on homologous recombination (HR)-based mechanisms, which use the information from the undamaged sister chromatid as a template. In the absence of a functional HR-machinery, such as in cancer-prone *BRCA1* mutations, cells repair DSBs via non-homologous end joining (NHEJ), which is a highly mutagenic repair mechanism, especially during DNA replication. Despite the importance of properly controlling the use of HR and NHEJ to prevent genomic instability and cancer, how cells regulate repair pathway choice during the cell cycle is not well understood. A critical step in triggering NHEJ is the recruitment of the 53BP1 protein (Rad9 in *Saccharomyces cerevisiae*) to sites of lesions and the consequent block of DNA end resection, which otherwise commits to HR repair. Here, using a synthetic biology approach, we characterize in budding yeast a central role for the multi-BRCT domain protein Dpb11 in the modulation of DNA end resection. Dpb11 licenses the recruitment of pro-resection (Slx4-Rtt107) and anti-resection (Rad9) factors for DNA lesion. Targeting of Rad9 to the 9-1-1 complex by fusing Rad9 to a BRCT domain of Dpb11 that interacts with 9-1-1 results in a severe block of DNA end resection and reduction in Rad52 foci, indicative of a strong defect in HR repair. Moreover, cells expressing the Dpb11 BRCT-Rad9 chimera show enhanced sensitivity to genotoxins and Rad53 hyperactivation, consistent with the notion that Rad53 signaling has a major role in regulation of DNA end resection in yeast. Importantly, a point mutation that disrupts the BRCT domain from the chimera restores all the phenotypes described above to wild type levels, implying that recruitment of Rad9 to the 5' recessed end of a ssDNA::dsDNA junction where the 9-1-1 complex is loaded is a key step for resection control. Finally, we present evidence that this mechanism is evolutionary conserved as TopBP1, the human ortholog of Dpb11, interacts with pro-resection (CTIP and BRCA1) and anti-resection factors (53BP1) in different stages of the cell cycle. Our results, place Dpb11^{TopBP1} as a regulator of the DNA repair pathway choice of the cell with implications for anticancer therapies.

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EVALUATION OF CYTOTOXICITY OF CRUDE EXTRACT OF *Acmella oleracea* IN L929 CELLS CULTURE AND HEp-2*Moraes, A.V.¹, Joaquim, W.M.², Da Silva, N.S.³ and Pacheco-Soares, C¹*

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Key-words: MTT assay, Neutral Red, popular medicine

The spilanthol and the components extracted from *Acmella oleracea* (Jambu) are employed in the pharmaceutical and cosmetic industries, but popular medicine uses, for the treatment of ailments of the mouth and throat, as well as tuberculosis, pulmonary lithiasis, appetite stimulant and toothache due to its local anesthetic and anti-inflammatory action. Various bioactive components of this plant have been isolated, but little is known about and their cytotoxicity and their potential on tumor cells. According to information from the National Cancer Institute (INCA), cancer is the second most common cause of deaths in the country, with growth trend over the next few years, cancer is a public health issue, especially when you take into consideration your percentage of prevention: about one third of new cases of cancer worldwide could be avoided. Therefore, the evaluation of cytotoxicity via mitochondrial and lysosomal, by techniques of neutral Red and MTT (3-(4,5dimethylthiazol-2yl) -2,5-diphenyl tetrazolina bromide), carried out in line L929 (mouse fibroblasts), in concentrations of 2 mg, 4 mg, and 6 mg of extracts obtained the root, stem, leaf and inflorescence of *A. oleracea* in the period of 24 hours and 48 hours demonstrated a reduction of viable cells, but with little significance, however in the period 48 hours in tumor line HEp-2, the root extract in different concentrations proved to be very significant as its cytotoxicity in lysosomal assay. In the evaluation of the mitochondrial activity at concentrations of 2 mg, 4 mg, and 6 mg of extracts of the root, stem, flower clusters and leaf on period of 24h and 48h, a reduction of viable cells, very significant was observed with respect to control.

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GRAPE SKIN EXTRACT MITIGATES TISSUE DEGENERATION, GENOTOXICITY AND OXIDATIVE STATUS IN MULTIPLE ORGANS OF RATS EXPOSED TO CADMIUM

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The aim of this study was to investigate if grape skin extract is able to mitigate the noxious activities induced by cadmium exposure in multiple organs of rats. For this purpose, histopathological analysis genotoxicity and oxidative status in blood and liver were investigated in this setting. A total of twenty Wistar rats weighing 250g on the average, and 8 weeks age were distributed into three groups (n=5), as follows: Control group (non-treated group, CTRL); Cadmium group (Cd) and Grape skin extract groups (Cd+GS) at 175 mg/L or 350 mg/L. Histopathological analysis in liver revealed that animals treated with grape skin extract improved tissue degeneration induced by cadmium intoxication. Genetic damage was reduced in blood and hepatocytes as depicted by comet and micronucleus assays in animals treated with grape skin extract. SOD-CuZn and cytochrome C gene expression increased in groups treated with grape skin extract in liver cells. Grape skin extract also reduced the 8OHdG levels when compared to cadmium group in liver. Taken together, our results demonstrate that grape skin extract is able to mitigate tissue degeneration, genotoxicity and oxidative stress induced by cadmium exposure in multiple organs of Wistar rats.

Key words: grape skin extract; cadmium; mutagenicity; genotoxicity; oxidative stress

Financial support: CAPES

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EFFECTS OF E2F PHARMACOLOGICAL INHIBITION IN IRRADIATED GLIOBLASTOMA AND ASTROCYTE CELL LINES.*Godoy, PRDV¹, Montaldi, AP¹, Santana, VS² and Sakamoto-Hojo, ET^{1,2*}*

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Keywords: glioblastoma, HLM006474, pharmacological inhibition, E2F transcription factors

Glioblastoma multiforme (GBM) is a lethal tumor and radiation remains one of the main treatments. New strategies are needed to overcome the resistance to treatment and E2F family members may be promising targets. The functional overlapping of E2F proteins can be overcome by treatment with the small molecule HLM006474 (HLM), which is an inhibitor of E2F protein family members (E2F1-8); the inhibitor is an 8-hydroxyquinoline compound, whose antiproliferative and pro-apoptosis properties were demonstrated in tumor cells. Our objective was to reduce GBM cell viability through E2F pharmacological inhibition combined with irradiation. We used four GBM cell lines (U343MG-a, U87MG, M059K and M059J) and primary astrocyte cell line as control (ACBRI-371). E2F family members were inhibited using different HLM concentrations (3.125 to 50 μ M for cell proliferation and 6.25 to 25 μ M for apoptosis and cell cycle analyses) in a continuous treatment until collection time; cells were irradiated with 0.25 to 4 Gy (X-rays) in association or not with the HLM. HLM inhibition was detected by western blot at 24, 72 and 144h after treatment. All cell lines showed a significant decrease in cell proliferation (XTT method) after irradiation. M059K and U87MG were more resistant to treatment, while U343 (except the dose of 2 Gy), ACBRI and M059J were more sensitive. The combination treatment (radiation plus HLM) in U87MG, U343MG-a, ACBRI 371, M059K and M059J caused an additive inhibitory effect in cell proliferation (120h). When analyzed the effects caused by the HLM inhibitor itself, we observed approximately 90% reduction in proliferation (120h, 50 μ M HLM) in experiments with all cell lines, although changes in proliferation were not found following 24 h. Comparing the proliferation curves in the intermediate HLM concentrations (12.5 and 25 μ M), we found that U343MG was the most resistant, followed by astrocytes. Still, U343MG did not show apoptosis induction and cell cycle block after treatments. The primary astrocytes showed induction of apoptosis (120h, 25 μ M), but without changes in cell cycle progression. U87MG cells presented apoptosis in 72 (25 μ M) and 120h (12.5 and 25 μ M), and G2 (72 and 120 hours) and S block (120h) at HLM concentrations of 12.5 and 25 μ M. E2F inhibition seemed to be efficient to control cell proliferation for all cell lines tested. However, only U87MG cell line presented apoptosis and cell cycle arrest after treatment, at different harvesting times.

Financial Support: FAPESP (Proc. N^o 2013/09352-7 e o 2013/13253) and CNPq (Brazil).

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EVALUATION OF ANTIMUTAGENIC POTENCIAL OF LEAF EXTRACT OF *Anadenanthera colubrina* var. Cebil (Griseb) Altschul USING *Allium cepa* L. TEST SYSTEM*Correia DS¹, Siqueira EA¹, Silva CMA², Silva MV² and Brasileiro-Vidal AC¹*

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Key words: *Allium cepa*, angico, antimutagenicity

Anadenanthera colubrina var. Cebil (Griseb) Altschul (Fabaceae family), also known as angico-branco, is one of the typical woody species of the Caatinga biome. In Brazil, it occurs in a band ranging from Maranhão to São Paulo and has been used in popular medicine due to its antiseptic, healing and expectorant action. However, its possible adverse or beneficial effects to human health are not known. Thereby, this study aimed to evaluate the toxicity, genotoxicity, mutagenicity, antigenotoxicity and antimutagenicity of different concentrations of cyclohexanic leaf extract of *A. colubrina* through *Allium cepa* L. test system. In the experiment, germinated seeds of *A. cepa* were exposed to three extract concentrations: 0.78 mg/ml; 1.56 mg/ml; 3.12 mg/mL for 24 h. Then, part of the roots was fixed in Carnoy (ethanol: acetic acid; 3:1; v:v), and the other part was transferred to Methyl Methane Sulfonate (MMS, 4.10⁻⁴ Mv) to check the protective potential of the extract (antimutagenicity/antigenotoxicity assay). After 24 h exposure to MMS, the remaining roots were fixed and stained with Schiff's Reagent. As a negative control ultrapure water was used and as positive controls MMS (4.10⁻⁴ Mv) and Trifluralin (0.84 ppm) were used. Statistical analysis was performed using the Kruskal-Wallis test ($p < 0.05$). Toxicity was verified by the average length of the roots compared with negative control; all extract concentrations showed toxic activity. For genotoxicity and mutagenicity index, it was analyzed the frequency of chromosomal aberrations and micronuclei, respectively, in 5000 meristematic cells of onion in each treatment. In the three concentrations studied, *A. colubrina* showed no genotoxic and no mutagenic activity. In antimutagenicity assay, the frequency of micronuclei in the extracts was compared with positive control (MMS); all extract concentrations showed antimutagenic activity. However, it was not seen antigenotoxic potential. In conclusion, it is presumable that these concentrations of cyclohexanic extract do not cause adverse effects to human health. In addition, protective action was observed, so, more studies are needed to provide better information about this action and how it could be better utilized in the production of new medicines.

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ALTERED BASE EXCISION REPAIR ACTIVITIES AND LOWER MITOCHONDRIAL GENOME CONTENT IN BRAINS FROM ALZHEIMER'S DISEASE PATIENTS*Soltys DT¹, Pereira CPM¹, Farfel JM², Ericson NG³, Bielas JH³ and Souza-Pinto NC¹*

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Keywords: Base Excision Repair, Alzheimer's disease, mitochondrial DNA.

Alzheimer's disease (AD) is characterized by a progressive cognitive decline, which affects the individual's social and occupational roles. Several lines of evidence suggest that accumulation of DNA lesions and changes in the pathways that remove these may have a role in the progression of AD. **Base Excision Repair (BER)** is the main repair pathway for small base modifications, abasic sites and single strand breaks, which are quantitatively the most relevant types of DNA lesions in AD patients. We investigated whether alterations in BER activities in the brain play a causative role during the development of AD. Nuclear and mitochondrial fractions were prepared from autopsy brain samples (cerebellum and temporal cortex) from cognitively normal, AD subjects and individuals who show neuropathological AD features, but remained cognitively normal (asymptomatic AD - asAD). BER activities were measured using a fluorescence-based *in vitro* assay. Mitochondrial and nuclear UDG activities from cerebellum were significantly reduced in both AD and asAD, when compared with age-matched controls, while in temporal cortex only AD subjects showed statistically significant decrease. Nuclear APE1 activities were similar in all groups, while asAD subjects displayed higher mitochondrial APE1 activity in the cerebellum. As BER activities contribute to maintaining mtDNA integrity, we measured mtDNA mutation rate using the Random Mutation Capture Assay, a single-molecule sequencing approach that allows the detection of even rare and low frequency mutations. No difference was observed in mtDNA mutation frequencies between the three groups included in this study. However, we detected a significant decrease in mtDNA copy number in brains from AD individuals in the temporal cortex, which is one of the most affected brain regions in AD pathology. Even with lower BER activities, AD individuals do not accumulate mutations but rather seem to lose mitochondrial genomes in the most stressed brain environment. The observation of reduced BER activities in AD brains - even in the cerebellum, the last brain region to show AD-associated pathological features - suggests that BER capacity may be an underlying variable that modulates the cellular responses to the insults that result in the AD neuropathology.

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Characterization of two translesion DNA polymerase genes of *Trypanosoma cruzi* POLK and XPV and their involvement in replicative stress*Resende BC, Oliveira KA, Franco GR, Pena SDJ and Machado CR.*

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Key-words: DNA Repair, *Trypanosoma cruzi*, replicative stress

Chagas disease, caused by *Trypanosoma cruzi*, affects 10 to 20 million people worldwide and is endemic in Latin America. Some aspects of the parasite's molecular biology remains unknown and this understanding could unveil new ways to improve treatment and lead to disease eradication. It includes the mechanisms used by *T. cruzi* to circumvent DNA replication stress and their potential relation to parasite quiescence, observed between acute and chronic phase of infection. It was recently shown that after the parasite treatment with camptothecin, a topoisomerase I inhibitor, some of the cells restored their DNA, whereas others entered early apoptosis but with no progress to late apoptosis, indicating that the protozoa stay alive in a "senescence-like" state. The genes POLK and XPV codify DNA polymerase κ and DNA polymerase η respectively, both translesion DNA polymerases able to reduce replicative stress during events of damage accumulation, as described in yeast. The aim of this work is the study of the roles of POLK and XPV gene products during replication stress induced by DNA damage in *T. cruzi*. For that, strains overexpressing Polk and under expressing Pol η were produced. The full-length ORF of POLK was cloned into pROCK vector and the construction was used for transfection of *T. cruzi* CL Brenner cells. For production of XPV mutants, neomycin resistance gene was cloned flanked by XPV 5' e 3'UTR into pCR- 2.1 TOPO vector and used for transfection of *T. cruzi* CL Brenner cells. The impact of these modifications in parasite proliferation was assessed and initial results showed an impairment in cell growth characterized by a reduced growth rate in both mutants cells compared to Wild Type strain. Preliminary result showed a no clear response of these modified cells to UV irradiation. New experiments are being done to confirm the involvement of these DNA polymerases in replicative stress response.

Financial Support: CNPq, FAPEMIG and Newton Fund

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HYDROGEN PEROXIDE SENSITIVITY IN LYMPHOCYTES OF PATIENTS CHRONICALLY INFECTED WITH HEPATITIS C VIRUS (HCV).*Braga LN1, Rocha YP1, Passos MB1, Souto FJD1 and Bassi-Branco CL1.*

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Key-words: HCV, oxidative DNA damage, micronucleus.

The mutagen sensitivity test in lymphocytes has been used to investigate individual mutagen sensitivity and DNA repair capacity. Although mutagen sensitivity is determined by individual genetic composition, it also may be influenced by the chronic exposition to mutagens that could compromise components of the mechanisms of DNA repair. Considering that the infection HCV has been related with increased DNA damage in peripheral blood mononuclear cells and with impaired DNA repair, it is possible to hypothesize that chronically infected patients have higher sensitivity to mutagen. The aim of this study was to investigate if patients chronically infected by HCV (HCV-patients) are sensitive to hydrogen peroxide (H₂O₂). H₂O₂ sensitivity was measured by the micronucleus (MN) test in 16 chronically HCV-infected patients and 23 healthy controls. The net increase of ‰ MN (‰ of MN in cells treated with H₂O₂ - spontaneous ‰ of MN) was similar in cells of HCV-infected patients (6.69±5.31) and controls (4.95±3.31, $p=0.2$, t test). When individuals were separated into H₂O₂ sensitive and non-sensitive (considering as cut-off point the 50 percentile of values of the net increase of ‰ MN), the percentage of HBV-infected patients (53%) and controls (47%) was similar among sensitive individuals as compared with the non-sensitive group (32% and 68%, respectively; $p=0.2$, Fisher's exact test). Therefore, our preliminary data suggest that the chronic infection by HCV do not increases H₂O₂ sensitivity.

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ANTIGENOTOXIC AND CYTOTOXIC EFFECT OF *PUNICA GRANATUM* LINN*Araújo AC1, Menezes BAA1, Silva DGKC1 and Tavares JCM1.*

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Key-words: Pomegranate, Cytotoxicity, Antigenotoxicity.

The phytotherapy is considered a practice largely used into low development index regions. The pomegranate (*Punica granatum* Linn) is useful in the treatment of infections, due to the presence of phenolic compounds in its bark. The use of the pomegranate bark as well as the other medicinal plants often occurs of form indiscriminate without knowledge about its effects at the genetic material. Thus, the objective of this study was to evaluate the activity cytotoxic, genotoxic and antigenotoxic of the aqueous extract of pomegranate bark. This was realized through the analysis of cell cycle phases and chromosomal abnormalities identified in the meristem cells of *Allium cepa* roots. 1000 cells/slide in duplicate for each experiment triplicates were counted. The tested concentrations of the lyophilizate extract were: 0.1mg/ml, 1.0mg/ml, 10mg/ml. Distilled water was used as negative control and copper sulphate as positive control. The doses of 0.1mg/ml and 1.0 mg/ml were tested with the positive control. It was found that all of the extract doses promote reduction of the mitotic index compared to the negative control, setting a dose-dependent relationship. This pattern was also observed for concentrations tested with the positive control, in which the cytotoxic action of copper sulfate was higher. The alterations genetics as micronucleus, anafásica bridge, lost chromosome in anaphase and metaphase were identified in the positive, negative control and other doses of the extract. Telophase bridge and nuclear bud occurred in high and low dose of extract, respectively. All doses of the extract were able to decrease the frequency of alterations present in the *A. cepa* roots. No dose was genotoxic, only copper sulphate, validating their genotoxicity. All doses tested with the positive control, pomegranate protective action exerted, reducing the frequency of DNA damage, being more effective at lower concentration. Therefore, it is concluded that the pomegranate extract is cytotoxic, particularly in high concentrations, but not genotoxic. Thus, the use of this phytoterapic becomes a good alternative therapy, which is especially antigenotoxic. However, should worry with its uncontrolled use, which result in harmful effect to the health.

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EVALUATION OF THE CYTOTOXIC, GENOTOXIC AND ANTIGENOTOXIC EFFECT OF *ANACARDIUM OCCIDENTALE* LINN*Menezes BAA1, Araújo AC1, Silva DGKC1 and Tavares JCM1.*

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Key-words: Cytotoxicity, Antigenotoxicity, *Anacardium occidentale* Linn

Recent studies have related biological activity of medicinal plants and their effects in treatment of some diseases due to their effect in cell cycle progression and induction of apoptosis. The cashew (*Anacardium occidentale* Linn) has a therapeutic potential against infectious diseases. Furthermore, various studies show the presence of antioxidants in its extracts. However, little is known about its genetics and cytotoxic effects. Thus, this study aims to evaluate possible cytotoxic, genotoxic and antigenotoxic effects the lyophilized aqueous extract of bark *Anacardium occidentale* L. through the *Allium cepa* test, analyzing the stages of cell division and chromosome abnormalities. To this end, 2000 cells were counted for each triple of treatments. Three extract doses were tested: 0.1 mg/ml, 1.0mg/ml, 10.0mg/ml. It was used as positive control copper sulphate and distilled water as negative control. Treatment uniting the dose 1.0 mg / ml copper sulphate were tested. It was observed that all of the extract had dose cytotoxic action, wherein the mitotic index reduced as the dose increased. For the dose tested with the positive control, it was seen that the bit extract presence interfered in frequency of cells that enter in cell division. With regard to alterations, micronucleus, nuclear bud, anaphasic bridge and metaphase with lost chromosome were seen in all treatments. The relation between the frequency abnormalities and treatments cashew extract, has been demonstrated dose-dependent, decreasing with increasing extract concentration. Copper sulfate was genotoxic, whereas none dose of the extract exercised this activity. The solution that united the copper sulfate to extract indicates protective action performed by cashew, due to the reduction in chromosomal and nuclear alterations. It was observed that the two largest doses they had antigenotoxic action. Thus, the results of the study showed that the shell cashew is cytotoxic even at low concentrations and has antigenotoxic activity at higher doses. So, it is necessary to monitor the use of the bark of the cashew tree, for obtain a favorable outcome to health.

Financial support: FACISA-UFRN/PROPESQ.

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PHOTOACTIVATED DMMB INDUCES SPECIFIC MITOCHONDRIAL DNA DAMAGE*Abrantes ABP, Souza-Pinto NC and Baptista MS*

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Keywords: Photodynamic Therapy; 1,9-dimethylmethylene blue; XL-PCR

Photodynamic Therapy (PDT) takes advantage of the interaction of a photosensitizer with visible light in the presence of molecular oxygen. This interaction triggers photochemical reactions that result in the production of reactive species like singlet oxygen and hydroxyl radical. In a living cell, these species can react with biomolecules like lipids and DNA resulting in oxidative damage and cell death. Many photosensitizers preferentially accumulate in specific organelles leading to a localized damage. The photosensitizer 1,9-dimethylmethylene blue (DMMB) accumulates in lysosome and mitochondria, and DMMB-induced damage to these organelles promotes efficient cell death in HaCaT cells. Our research group has been interesting in testing whether mitochondrial DNA damage can be induced by exogenous agents without simultaneously inducing nuclear DNA damage. Induction of specific lesions in mtDNA in absence of nDNA damage will constitute an important model in studies of mtDNA damage response. For this purpose, we evaluated the capacity of photoactivated DMMB to induce DNA lesions by gene-specific quantitative PCR-based assay – XL-PCR (Kovalenko and Santos, 2009) in HEK293T cells. The treatment of cells was made through incubation of the cells with DMMB 10nM in phosphate-buffered saline (PBS) by 1 hour, followed by washing in PBS and irradiation with 9,8 J/cm² red light using a LED platform. Immediately after irradiation total DNA was extracted, XL-PCR was performed, the amplicons were resolved in agarose or polyacrylamide gels and quantified by densitometry. We also investigated the DMMB phototoxicity by clonogenic assay. As expected, we found that mitochondrial DNA is damaged following DMMB plus irradiation treatment. On the other hand, the treatment did not induce detectable nuclear DNA lesions, at least as assessed by XL-PCR. Mitochondrial DNA damage persisted for 6 hours after irradiation, suggesting that the lesions induced were not completely repaired in this time frame. The survival curve indicated that the DMMB exhibited high phototoxicity with no dark toxicity. The ability of DMMB to induce specific mitochondrial DNA damage makes it a useful model in mechanistic studies of DNA repair particular to mitochondria.

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CHEMICAL INVESTIGATION AND EVALUATION OF CITOTOXICITY AND GENOTOXICITY OF THE AQUEOUS EXTRACT OF *Rhizophora mangle* L. IN CHO-K1 CULTURES

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Rhizophora mangle L. is a native mangrove species popularly known as the red mangrove. The extract of its bark is widely used in folk medicine for its antiseptic, antifungal and healing purposes. To evaluate the safety of the use of this extract in the production of clay pots used for food preparation, this study evaluated its effect on cell viability and genomic instability in vitro using CHO-K1 cells, as well as characterize the chemical composition of this extract. Fingerprinting of the aqueous extract of the bark of *R. mangle* by FIA-ESI-IT-MSⁿ was performed to provide chemical characterization. The MTT assay was performed with 2×10^4 cells exposed to different concentrations of the extract (4.37 to 140 µg / ml) for 12 hours. The same concentrations of the extract were evaluated for their potential genotoxicity by comet assay. The fingerprint analysis of the extract of *R. mangle* obtained by FIA-ESI-IT-MSⁿ confirmed the presence of various polyphenolic compounds previously demonstrated in a pharmacognostic screening study by our research group. The previous study identified flavonoids (rutin, quercetin-O-hexose), trimer proanthocyanidins (condensed tannin) and precursors of the group of hydrolyzable tannins (quinic acid and caffeoylquinic acid). The results showed no cytotoxicity among nine concentrations of the extract of *R. mangle* when evaluated after 12 h of treatment. For the assessment of genotoxicity were analyzed three concentrations (17.5, 35.0 and 70.0 µg / mL) to *R. mangle* extract by means of comet assay. No genotoxic effect of the three concentrations of the extract evaluated was observed in the CHO-K1 cells. In conclusion, the present findings are consistent with the literature described for this extract and for various polyphenolic compounds and highlight the safe use of the aqueous extract of *R. mangle* bark at the concentrations evaluated.

Key Words: FIA-ESI-IT-MSⁿ, comet assay, MTT.

(FAPES – 69980497 and CAPES)

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ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS OF BRAZILIAN RED PROPOLIS EXTRACT IN BREAST CARCINOMA CELLS MDA-MB-231*Brandalize APC¹, Brum ES¹, Ashton-Prolla P², Roesch-Ely M¹ and Henriques, JAP¹*

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Key words: Brazilian red propolis, cancer therapy, breast carcinoma.

For hundreds of years, natural compounds have been used in the treatment of various diseases, including cancer. Several studies with propolis extracts demonstrated the induction of apoptosis in different cell types. However, there is no report on the effect of ethanol extract of Brazilian red propolis (RP) in breast carcinoma cell line, MDA-MB 231. The objective of this study was to assess the anti-proliferative and pro-apoptotic effects of the crude extract of RP in breast cancer cells. The MTT assay confirmed the cytotoxicity of RP extract in these cells. The RP extract inhibited the proliferation of MDA-MB-231 in a dose-time-dependent manner, showing an IC₅₀ of approximately 73% / mL at 24 h of treatment and 42 ug / ml after 48 h. Induction of apoptosis was confirmed by acridine orange / ethidium bromide and Giemsa staining, where the increase in RP extract concentration was proportional to the rise in the number of apoptotic cells. Western blot analysis showed that RP triggers Bax expression in high concentrations. The increased level of apoptosis induced by RP treatment was also associated with inhibition of caspase-3 without inducing DNA fragmentation. Taken together, our results demonstrated that RP ethanolic extract induces apoptosis in MDA-MB 231 cells, indicating that the apoptosis signaling may be related mainly to the extrinsic pathway.

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INFLUENCE OF *PTEN* DEFICIENCY ASSOCIATED WITH *RAD51* EXPRESSION IN GLIOBLASTOMA CELL LINES TREATED WITH ETOPOSIDE*Oliveira, AC¹, Montaldi, AP¹, Godoy, PRDV¹, and Sakamoto-Hojo ET^{1,2}*

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Key words: Glioblastoma-derived cell lines, *PTEN*, *RAD51*, homologous recombination.

Glioblastoma multiforme (GBM) is the most common malignant brain tumor. Loss of *PTEN* (Phosphatase and tensin homolog deleted on chromosome 10) gene is the most common alteration associated with GBM. *PTEN* encodes a phosphatase enzyme that antagonizes the PI3K, by inhibiting AKT phosphorylation thereby regulating signaling pathways related to cell survival and proliferation. *PTEN* deficiency has been associated with genomic instability and increased endogenous DSBs, as reduced expression of *RAD51*, which is a key gene with role in HR. We aimed to evaluate the influence of *PTEN* status in GBM cell lines and how this affects *RAD51* expression and HR efficiency under drug treatments. We used two cells lines: T98G (*PTEN* mutated) and LN18 (*PTEN* wild-type). Cells were treated with etoposide, which is a drug capable of inducing DSBs, and several assays were carried out: cell proliferation, quantification of necrosis and apoptosis (Annexin V), cell cycle kinetics, immunofluorescence staining and Western blot. For cell proliferation, the treatment with etoposide led to a significant difference ($p < 0.001$), but LN18 cells showed a greater reduction in cell proliferation, compared to T98G. Both cell lines showed a significant increase ($p < 0.001$) in cell death induction, but LN18 presented a greater percentage of apoptotic and necrotic cells than T98G (24, 72 and 120h). The induction of DSB was analyzed by immunofluorescence staining with gamma-H2AX antibody; for all concentrations (50 and 75 μ M) LN18 showed higher number of gamma-H2AX positive cells than that observed for T98G ($p < 0.001$). Analysis of cell cycle kinetics performed for cells treated with etoposide (50 and 75 μ M) showed significant differences ($p < 0.001$): LN18 presented a greater G2-blockage, as compared to T98G. We analyzed the expression of *RAD51* and *RAD51B/C* proteins; *RAD51B/C* expression was higher in LN18 compared to T98G treated samples and controls. However, the expression of *RAD51* protein was not different between the two cell lines. Our results showed that under etoposide treatment, LN18 (*PTEN* wild-type) displayed a low cell proliferation kinetics, with a marked G2-blockage and higher induction of gamma-H2AX, and higher expression of *RAD51B/C* proteins compared to T98G (*PTEN* mutated). Our results provide information about the mechanisms of DNA damage responses in GBM cells by focusing the influence of *PTEN* status in the repair of etoposide-induced DSBs.

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DEVELOPMENT OF A MODEL FOR SILENCING OF XPF AND ERCC1 GENES TO INCREASE CISPLATIN TOXICITY FOR HUMAN TUMOR CELLS.*Pelegri AL¹, Ribeiro PF¹, Silva MM¹, Menck CF¹*¹Institute of Biomedical Sciences, University of São Paulo – USP, São Paulo S.P.E-mail: aleepelegri@gmail.com

Keywords: cisplatin, lung cancer, XPF-ERCC1

Cisplatin is a powerful agent used clinically to treat a wide variety of tumors such as ovarian, testis and lung. However, the efficacy of the treatment can be reduced due to the resistance development. There are multiple suggested mechanisms for cisplatin resistance in tumors and the increase of DNA repair is proposed to be one of the most relevant. Cisplatin acts by forming DNA adducts, which include monoadducts, intra- and interstrand DNA crosslinks (ICLs). The ICLs are highly toxic lesions that can inhibit and block DNA replication and transcription, which may lead to cell death. ERCC1–XPF is a structure-specific endonuclease that is required for the repair of these lesions through the Nucleotide Excision Repair and Interstrand Crosslink Repair pathways. It has been suggested that expression of ERCC1 correlates with cisplatin drug resistance in non-small cell lung cancer (NSCLC) and other kinds of tumors and the silencing of these proteins can alter the expression levels of other. In this work, different strategies were used to obtain silenced strains for these two genes to test the effect on the cytotoxicity of cisplatin in lung cancer cells (A549) and lung fibroblasts (MRC5 and IMR90). For this purpose, RNA interference techniques have been applied, with transient and permanent silencing by short RNA sequences (siRNA) and lentiviral transduction (shRNA), respectively. We also used the CRISPR/Cas9 technique to inactivate these genes. These cells, silenced for XPF, ERCC1 or both, were analyzed by different tests and compared with fibroblasts extracted from patients deficient in XPF for their sensitivity to cisplatin and other ICLs inducing agents. Interestingly, XPF silencing appears to also reduce the expression of ERCC1. In general, it has been demonstrated that silenced cells present higher sensitivity to these agents. Moreover, glutathione (GSH) inside the cells functions as barrier to cisplatin cytotoxicity. Our results show that addition of a GSH synthesis inhibitor (BSO) to the cisplatin treatment induced a strong increase in cisplatin sensitivity. Thus, XPF-ERCC1 silencing combined with cisplatin and BSO appears to be an interesting therapeutic strategy for improving the clinical protocol against lung cancer.

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CYTOTOXIC POTENTIAL ASSESSMENT OF THE INDUSTRIAL LAUNDRY WASTEWATER AND SUPERFICIAL WATERS OF THE CLEOPATRA RIVER, FROM MARINGÁ - PR*Silva JS1, Heck MC1, Yoshimoto M1, Vieira CAM1, and Vicentini VEP1.*

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Keywords: environmental monitoring, *Allium cepa* L., water contamination.

The accelerated process of Brazil's industrialization triggered a significant increase in the production of wastewater containing toxic substances, daily released indiscriminately into rivers, water streams and lakes. One of the high environmental impact sectors of activity is the textile industry, and in this segment the industrial laundries emerged as assessoriais activities for the processing of the tissues. The textile and laundry rooms have water as one of its main raw materials, and release effluent with high pollution load in large quantities, which are usually discarded in the environment *in natura* or are ineffectively treated. Because of the requirement for biological monitoring of these effluents and disposal sites thereof, the present study aimed to investigate the cytotoxic potential of raw sewage and treatment lagoons of an industrial laundry, placed in the urban area of Maringá - PR, and superficial stream waters from Cleopatra river, where the effluent is discarded, using the *Allium cepa* L. meristematic root cells. The experiments were divided into five groups, 5 bulbs each: 1) negative control; 2) Gross Effluent; 3) Effluent from the activated sludge pond; 4) Treated effluent; 5) surface stream waters at the dump site. The roots of the bulbs were collected in three sampling periods: the bulb own control (Co-0h); Treatment with the effluent for 24 hours (24-Tr); and recovering filtered water for 24 hours (Re-24), to analyze reverse 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 statistical analysis performed using the Chi-square test ($\alpha=0.05$). According to the results, all wastewater treatment for 24 hours decreased the mitotic index, however only the raw effluent showed cytotoxic effect when compared to himself control bulb, with recovery of cell division after 24 hours in water. Treatment with treated wastewater results showed cytotoxic effect only for the recovery period for 24 hours. The other evaluated samples showed no significant damage compared to the control. Considering the above data, it is noteworthy that the constant evaluation of industrial wastewater and water from streams and rivers receiving these evictions, aiming to preserve and maintain water quality, organisms and consequently, the ecosystem.

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CHEMOPREVENTIVE EFFECT AND LACK OF GENOTOXICITY AND MUTAGENICITY OF THE EXOPOLYSACCHARIDE BOTRYOSPHAERAN ON HUMAN LYMPHOCYTES

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β -D-Glucans of the (1→3)-, (1→3)(1→6)- and (1→6)- linked types such as produced by certain bacterial, yeast and fungal species and strains have been the subject of intense investigation because of their biological response modifying (BRM) activities in the host, and this attribute favors their application in medical therapy. Identifying and studying the biological effects of macromolecules that include polysaccharides and polysaccharide–protein complexes, have helped in introducing effective cancer therapeutic agents onto the pharmaceutical market. Although much of the literature associated with these biopolymers is of a protective nature, and usually bears little or no toxic activity, there are literature reports involving the mutagenic potential of molecules found in fungal extracts that may contain biopolymers. In the present study the objectives were to investigate (i) the effects of the treatment with botryosphaeran alone, or in combination with the mutagen, methyl methanesulfonate (MMS), on normal and tumor (Jurkat cells) human T lymphocytes in order to evaluate its genotoxic and chemoprotective effects on these cell types; (ii) the possible mutagenicity of botryosphaeran assessed by the Ames test on different *Salmonella typhimurium* strains; and (iii) the antioxidant effect against H₂O₂-induced production of reactive species on normal and tumor lymphocytes. This study revealed that botryosphaeran was not mutagenic and genotoxic in normal and tumor human T lymphocytes as evaluated by the Ames and Comet assays, respectively. This biopolymer exhibited antigenotoxic activity against damage induced by the alkylating agent MMS in normal and tumorigenic lymphocytes. Although there are reports in the literature on the antioxidant capacity of fungal exopolysaccharides, botryosphaeran

did not appear to reduce H₂O₂-induced reactive species production in the human lymphocyte cells studied herein. The findings of this study reinforce the absence of genotoxic effects of botryosphaeran and helps validating the use of this β -glucan for commercial applications. Moreover, the findings reported here will encourage further investigation to foster better understanding of the protective mechanisms of botryosphaeran as triggered in human lymphocytes following their exposure to alkylating agents.

Key-words: Ames test; Comet assay; ((1→3)(1→6)- β -D-glucana)

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INFLUENCE OF EXPOSURE TO PESTICIDES ON TELOMERE LENGTH MAINTENANCE IN TOBACCO FARMERS: A BIOLOGY SYSTEM APPROACH*Da Silva FR1, Kahl VF2, and Da Silva J2*

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Keywords: pesticide, nicotine, tobacco farmers, system biology, telomere length

Several pesticides must be used to keep tobacco crops pest-free, and they are used in the form of mixtures. Recent studies have shown that occupational exposure to pesticides in tobacco fields is related to increased damage to the DNA, micronucleus, nuclear buds and binucleated cells in buccal cells as well as micronucleus in lymphocyte. Furthermore, pesticides used specifically for tobacco crops shorten telomere length (TL) significantly. However, the molecular mechanism of pesticide action on telomere length is not fully understood. Our study evaluated the interaction between a complex mixture of chemical compounds (tobacco cultivation pesticides plus nicotine) and proteins associated with TL maintenance, as well as the biological processes related to this exposure by system biology tools to provide insight regarding the influence of pesticide exposure on TL maintenance in tobacco farmers. Our analysis showed that one cluster was associated with TL proteins that act in bioprocesses such as (i) telomere maintenance via telomere lengthening; (ii) senescence; (iii) age-dependent telomere shortening; (iv) DNA repair (v) cellular response to stress and (vi) regulation of proteasomal ubiquitin-dependent protein catabolic process. We also describe how pesticides and nicotine regulate telomere length. In addition, pesticides inhibit the ubiquitin proteasome system (UPS) and consequently increase proteins of the shelterin complex, avoiding the access of telomerase in telomere and, nicotine activates UPS mechanisms and promotes the degradation of human telomerase reverse transcriptase (hTERT), decreasing telomerase activity.

(CAPES, CNPq)

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ECOTOXICGENETIC EVALUATION OF WATER BODIES OF THE LIMEIRA/SP-BRAZIL REGION, BEFORE AND AFTER DISCHARGE FROM THE SEWAGE TREATMENT STATIONS.*Pamplona-Silva MT¹ and Marin-Morales MA¹*

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Email: maria.pamplona@gmail.comKey words: *Allium cepa*, chromosomal aberration, cytotoxicity, genotoxicity, micronucleus, mutagenicity.

The populational growth of recent years has resulted in an increased use of natural resources of domestic, industrial, agricultural and livestock activities, and in an intensified residues generation. Therefore, anthropic activities had caused impacts on the environment, especially compromising the quality of aquatic ecosystems and, consequently, the health of related organisms. The aim of the present study was the evaluation of the cytotoxic and genotoxic potential of water bodies in the region of Limeira, which receives effluents of sewage treatment stations (STS). They were evaluated, by chromosomal aberrations (CA) and micronucleus induction (MN) tests in *Allium cepa*, 8 points distributed in the Ribeirão Tatu (Tatu creek) (P1, P2 and P3), Ribeirão Água da Serra (P4 and P5), Taboinha stream (P6) and Ribeirão Graminha (P7 and P8). The investigated parameters were mitotic index (MI), cell death index (CDI), chromosomal aberrations index (CAI) and MN frequencies. The MIs were statically significant on for P4 and P5, located, respectively, upstream and downstream from the Água da Serra STS. Alterations were only found on the CDI on the P1, indicating high toxicity of the water in this site. The water collected in P3, P4 and P6 proved genotoxic and a high number of MN were registered for P3, situated downstream from Tatu STS. By the found results, we can conclude that the treatment of the Água da Serra STS and Graminha STS are being highly efficient because the sites downstream of the discharge did not show abnormalities. However, the obtained results with the water of brook Tatu, downstream of the discharge of Tatu STS, alert for the compromising of water quality of this point due to cytotoxic and genotoxic effects registered on the exposed organisms. Given the results it was also possible to infer the extreme importance of realizing a constant monitoring in rivers which receive STS effluents, because these studies can indicate the environmental compromising level as a result of the discharges without suitable treatment on hydric resources.

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CYTOTOXIC AND GENOTOXIC EFFECT OF NARINGIN FLAVONOID IN HUMAN HEPATOMA CELLS (HepG2/C3A), *IN VITRO**BUZO MG1, SILVA VRC1, FERNANDES LM1, YOSHIMOTO M1, and VICENTINI VEP1*

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Keywords: HepG2/C3A, MTT test, comet assay, cell viability, natural compound.

Naringin is a natural compound of the flavonoid class, which is considered the main responsible for the bitter taste of citric fruits. It is traditionally used in the Chinese medicine and also in the industry as a colorant, flavorant and flavoring agent. Several researches have demonstrated many activities of this compound, such as antioxidant, anti-inflammatory and antitumoral. Therefore, the aim of this study was to evaluate the cytotoxic potential, by the MTT cytotoxicity test, and genotoxic activity, by the comet assay in metabolizing human hepatoma cell line (HepG2/C3A). The cells (10^5 /ml) were seeded in 96-well plates and 25 cm² flasks, for the MTT and the Comet assay, respectively. Three independent experiments were performed and the results were submitted to analysis of variance followed by Tukey's test (MTT) and Dunnett's test (Comet) ($\alpha=0.05$). For the cytotoxicity assay, cells were treated with different concentrations of Naringin [5, 50, 100, 150, 200 and 250 μ M]; a cytotoxic agent, MMS [150 μ M] and the control group. For the comet assay, it was used three concentrations of Naringin [50, 100 and 150 μ M]; a genotoxic agent, Benzo[a]pyrene [80 μ M] and the control group. The results of the MTT test indicated that none of the tested concentrations of this flavonoid showed cytotoxic activity at 24 and 48 hours, that is, the cell viability was greater than 80% at all concentrations of Naringin. The comet assay also showed no statistically significant damage to any of the tested concentrations, when compared with the control group. Therefore, within the trials it was observed that Naringin was not able to induce cytotoxicity and genotoxicity in metabolizing human hepatoma cells. Thus, the results of this study indicated that Naringin consumed by the population does not cause harm to health and its use can contribute to an improved quality of life of those who use it.

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CYTOTOXIC AND GENOTOXIC EFFECTS OF NARINGIN FLAVONOID IN ADENOCARCINOMA HUMAN BREAST CELLS (MCF-7), *IN VITRO**BUZO MG1, SILVA VRC1, FERNANDES LM1, HECK MC1, and VICENTINI VEP1*

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Keywords: MCF-7; MTT test; comet assay; cell viability; natural compound.

Naringin is a glycosylated flavone with a bitter taste, found mainly in orange and other citric fruits, but also can be found in the pulp, leaves, flowers and seeds of these plants. This flavonoid is traditionally used in Chinese medicine as anti-oxidant and anti-inflammatory, and because of it many researches have been presented, demonstrating the biological activities of this compound. Therefore, the aim of this study was to evaluate the cytotoxic potential, by the MTT cytotoxicity test, and genotoxic activity, by the comet assay in non-metabolizing human breast adenocarcinoma cells (MCF-7). The cells (10^5 /ml) were seeded in 96-well plates and 25 cm² flasks, for the MTT and the Comet assay, respectively. Three independent experiments were performed and the results were submitted to analysis of variance followed by Tukey's test (MTT) and Dunnett's test (Comet) ($\alpha=0.05$). For the cytotoxicity assay, cells were treated with five concentrations of Naringin [50, 100, 150, 200 e 250 μ M]; a cytotoxic agent, MMS [150 μ M], and control group. As for the comet assay, three concentrations of Naringin [50, 100 e 150 μ M] were evaluated; a genotoxic agent, Benzo[a]pyrene [80 μ M], and the control group. The results of the MTT test indicated that none of the tested concentrations of flavonoid showed cytotoxic activity at 24 and 48 hours, with cell viability greater than 95% at all concentrations of Naringin. Similarly, the comet assay induced no statistically significant damage to any of the tested concentrations, when compared with the Control. Then, in the performed tests it was noted that Naringin was not able to induce cytotoxicity and genotoxicity in non-metabolizing human breast adenocarcinoma cells. Probably, this compound did not interfere in the mitochondrion activity or induced DNA damage. Thus, taking into account that this flavonoid has therapeutic properties such as antioxidant and anti-inflammatory, as well as being a component which is present in much of the population diet, food containing Naringin can be consumed by the population without prejudice by the presence of this compound.

Financial Support: CNPq.

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ASSESSMENT OF THE CYTOTOXIC AND GENOTOXIC POTENTIAL OF *Eugenia brejoensis* MAZINE ESSENTIAL OIL, A POSSIBLE BIOPESTICIDE, USING *Allium cepa* L. SYSTEM.*Siqueira EA*¹, *Pereira IFM*¹, *Malta FQ*¹, *Souza VC*¹, *Silva AG*²; *Silva MV*³ and *Brasileiro-Vidal AC*^{1,4}¹ Laboratório de Genética e Biotecnologia Vegetal, Departamento de Genética, UFPE, Recife, PE² Instituto Nacional do Semiárido/Ministério da Ciência, Tecnologia e Inovação – INSA/MCTI, Recife, PE³ Departamento de Bioquímica/Centro de Ciências Biológicas, UFPE, Recife, PE⁴ brasileirovidal.ac@gmail.comKeywords: *Allium cepa*, Antigenotoxicity, Antimutagenicity, *Eugenia brejoensis*.

Eugenia brejoensis Mazine is an endemic plant of Brazil, found in altitude forests in the northeastern semi-arid region, called "Caatinga enclaves moist forests". The locals use their leaves as a natural wound healer, and its essential oil has, as its major components, δ -cadinene, caryophyllene oxide and (E)-caryophyllene. These compounds have antimicrobial potential, plus larvicidal action against the 4th instar of the *Aedes aegypti* mosquito, a vector for Dengue fever and the Zika virus. This study aimed to evaluate the potential cytotoxic and genotoxic effect at the chromosomal level and test the antigenotoxicity and antimutagenicity of the essential oil of *E. brejoensis* (a possible biopesticide), at different concentrations, using the *Allium cepa* L. (onion) test system. For the experiments, *A. cepa* seeds were germinated in ultrapure water and their roots were transferred to different essential oil concentrations: 1.25; 2.5 and 5 mg/ml for a period of 24h. Subsequently, part of the roots was fixated in Carnoy (ethanol : acetic acid; 3 : 1; v : v) and another part was transferred to the MMS for 24h, except the negative control, which was transferred to ultrapure water. Then the roots of the MMS positive control were also fixated similarly. The Methyl Methane Sulfonate (MMS, 4.10⁻⁴ Mv) and Trifluralin herbicide (0.84 ppm) were used as positive controls, and the ultrapure water as negative control. Then, the roots were fixed and stained with Schiff reagent. Statistical analysis was performed using the Kruskal-Wallis test, at a 5% level of significance. For the toxicity analysis, we compared the average length of the roots and to assess the mitotic index and cell aberrations, 5,000 meristematic cells were analyzed per treatment. All concentrations showed significant toxic activity compared to the negative control. However, no concentrations showed cytotoxic, genotoxic or mutagenic activity. In addition, the concentration of 2.5 mg/mL showed a protective effect, evidenced by its antimutagenic and antigenotoxic effect, since no significant presence of micronuclei and chromosomal abnormalities were found, respectively, after treatment with MMS. Thus, we can infer that the use of essential oil of *E. brejoensis* as a natural insecticide against *A. aegypti* seems to be safe for the environment and, in a particular concentration, can have a protective effect.

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EVALUATION OF THE GENOTOXIC POTENCIAL OF AN L-AMINO ACID OXIDASE ISOLATED FROM CALLOSELASMA RHODOSTOMA VENOM (CR-LAAO) OVER HUMAN CELL LINES*Amstalden MK¹, Costa TR¹, Ribeiro DL², Ghisla S³, Antunes LMG¹, Sampaio SV¹*

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Keywords: Snake venoms; L-amino acid oxidase; genotoxicity.

Introduction: Snake venoms are rich in proteins, enzymes and biologically active peptides. Many of these biomolecules have a great biotechnological potential to be developed as new drugs with variable applications, such as L-amino acid oxidases, which are enzymes that present great microbicidal and antiparasitic effects. Moreover, it was observed that it has a great antitumor potential, as the enzyme promoted more cytotoxicity over tumor than normal cells. Thus, the genotoxic potential of the L-amino acid oxidase from *Calloselasma rhodostoma* venom (CR-LAAO) over those cell lines should be investigated so it could be developed as a new model of antitumor drugs. **Objectives:** The objective of this study was to determine the genotoxic potential of an L-amino acid oxidase over normal and tumor cell lines. **Methods and Results:** The CR-LAAO was isolated according to Ponnudurai et al. (1994) and gently given by prof. Dr. Sandro Ghisla, University of Konstanz, Germany. HepG₂ tumor cell line was obtained from the American Type Culture Collection (ATCC) and human peripheral blood mononuclear cells (PBMC) were extracted using Histopaque-1077. The genotoxicity of CR-LAAO was evaluated by Comet assay with a fluorescence microscope (Carl Zeiss, AxioStar Plus, Thornwood, NY, United States) using the Comet Assay IV software, 4.3 version. The analyses were made according to the *Tail Intensity* parameter, which gives the percentage of DNA present at the nucleoid's tail. The cells were treated for 4 hours with enzyme concentrations of 0.2; 1 and 5 µg/mL. Non-treated cells were used as negative control and the positive control were made applying Benzopyrene at HepG₂ cell lines and MMS (Methyl methanesulfonate) at HepG₂ and PBMC. CR-LAAO induced DNA damage at the concentrations of 1 and 5 µg/mL in HepG₂ while in PBMC the damage was only observed at the concentration of 5 µg/mL. **Conclusion:** Our results showed that the L-amino acid oxidase of *Calloselasma rhodostoma* snake venom induces significant genotoxicity on tumor cells and low genotoxicity on human normal cells. More studies with the enzyme are necessary so that it could serve as a model for the development of new drugs with potential applications in the treatment of diseases such as cancer.

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CHARACTERIZATION OF LUNG CANCER CELL LINES FOR RESISTANCE TO CISPLATIN*Silva MM, Pelegrini AL and Menck CF*

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Keywords: DNA repair, lung cancer, cisplatin

Cancer is one of the most important causes of deaths worldwide, with lung cancer being one of the most deadly and invasive types, with more than one million cases diagnosed every year. The high rate of mortality is mainly due to the lack of treatment that provide permanent cure or even guarantee the patient a longer survival rate. One of these treatments is the chemotherapy, in most cases based on the cytotoxicity caused by the induction of DNA damage on tumor cells by therapeutic agents. However, changes in the protection systems of the genome may arise, causing resistance to chemotherapy and consequently reducing the success of therapy. Among these changes, the increased expression of XPF and ERCC1 proteins in tumor cells stand out, these proteins being components of the nucleotide excision repair pathway (NER). Thus, this project aims to characterize three lines of lung cancer cells (A549, NCI H23 and NCI H1155) and one line of lung fibroblast (IMR-90) for their sensitivity to cisplatin, a chemotherapeutic drug widely used clinically, and to understand the relation between sensitivity, mRNA and protein expression levels of XPF and ERCC1, and the repair of the caused injuries. Thus, we hope this project will provide the basis for potential interference studies in the activity of these genes and some other mechanisms related to cisplatin resistance, enhancing the action of chemotherapy. Initially, the cells were treated with cisplatin and sensitivity was evaluated by XTT colorimetric assay, alamarBlue cell viability assay and the determination of the Sub-G1 cell population by Flow Cytometry. The data obtained in these experiments were correlated with the levels of the two proteins of interest, XPF and ERCC1, using the western blot assay. We also measured the levels of glutathione with the method of determination of thiol groups by Ellman's reagent. The viability experiments showed that the most sensitive cell lines are NCI H23 and NCI H1155, while the western blot experiments indicate that these two cell lines have higher levels of the proteins of interest. On the other hand, the Ellman's reagent assay indicate these cell lines also have the lower levels of glutathione. This allow us to conclude that the resistance to cisplatin in these cell lines are not linked to the levels of ERCC1 and XPF proteins, although glutathione levels seem to be very important for the resistance to treatment with cisplatin.

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BIOSYNTHESIS OF SILVER NANOPARTICLES BY *TRICHODERMA HARZIANUM*, EVALUATION OF ITS CYTOTOXICITY AND GENOTOXICITY, AND APPLICATION IN WHITE MOLD CONTROL

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Key-words: biogenic silver nanoparticles, citotoxicity, genotoxicity, white mold control

Introduction: Silver nanoparticles (AgNPs) have been widely applied in different fields due to its antimicrobial potential. In agriculture, its use is recent but there are high expectations about its application for crop pathogens control. Silver nanoparticles can be synthesized biogenically through the reduction of silver ions by extract of fungi, plants or bacteria. This work aims to synthesize silver nanoparticles using the biocontrol fungus *Trichoderma harzianum* and evaluate its cytotoxicity, genotoxicity and potential in the control of white mold, a soybean disease caused by the plant pathogenic fungus *Sclerotinia sclerotiorum*. **Material and Methods:** AgNPs were synthesized through the addition of AgNO₃ (10⁻³ molxL⁻¹, 25°C) to the fungus filtrate and then characterized by Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA) to determine size distribution, polydispersity index (PDI), zeta potential and concentration. Cytotoxicity and genotoxicity evaluations were performed through the assays *Allium cepa*, MTT, Comet and MIC. MTT and Comet assays were performed with the cells 3T3, V79, HaCat and Hela. MIC was performed with the microorganisms *E. coli*, *S. aureus*, *C. albicans* and a pool of soil bacteria. To evaluate AgNPs potential in the control of white mold the fungus sclerotia were plated in agar amended with AgNPs in different concentrations and incubated at room temperature for 14 days. **Results and Discussion:** Biogenic synthesis of AgNPs was confirmed by the change in fungus filtrate colour from light yellow to dark brown. By DLS AgNPs showed a size distribution of 100.73 nm, PDI 0.273 and zeta potential -6.85 mV. By NTA the average size was 58.00 nm and the concentration 3.16x10¹² NPs/mL. *Allium cepa* results indicate that the biogenic AgNPs cause significant increase in mitotic and chromosomal aberration index in comparison with negative control. In MTT assay cells 3T3 showed the lowest viability and Hela the highest. In comet assay there was a dose dependent increase in DNA damage in all the cells. MIC results indicate that soil bacteria are more resistant to AgNPs than pathogenic bacteria and fungus. Lastly, with respect to white mold control, the AgNPs showed efficiency in the inhibition of mycelium and sclerotia

development. **Conclusions:** Biogenic AgNPs synthesized through *T. harzianum* can inhibit *S. sclerotiorum* micelium and sclerotia development in a way that it can be one of the first steps to white mold control.

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EVALUATION OF THE GENOTOXIC POTENTIAL OF THE DIAMINE CADAVERIN, PRESENT IN THE NECROCHORUME BY USING THE CELL LINE SPEEDY (*Xenopus tropicalis*)HARA, RV¹; CAMPOS-PEREIRA, FD¹; MARIN-MORALES, MA¹

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Key words: comet assay, cell culture, amphibian cell, cemeteries, environmental contamination.

The pollution caused by cemeteries due to the production of an organic residue called necrochorume, besides compromise the environment it can cause serious problems to the human health. During the putrefaction process of dead bodies is produced an amine called cadaverin ($C_5H_{14}N_2$), which is a highly toxic substance found on this liquid. Even facing signs of toxicity, there is no studies that evaluate the genotoxic potential of this diamine. Thus, this study aimed to evaluate the genotoxic potential of different concentrations of this diamine cadaverin (307.5 mg/L, 184.5 mg/L e 61.5 mg/L) by using the comet assay in Speedy cells (*Xenopus tropicalis* cells), maintained in culture. PBS solution was used as negative control and MMS sterile aqueous solution ($4 \times 10^{-4}M$) as positive control. The cells were exposed to the established concentrations for 24 hours. After the exposition, the cells were collected and the cell viability test with Trypan Blue and the comet assay were performed. Per treatment were recorded three hundred nucleoids and they were analyzed on the comet assay software IV. The significance analysis of the data passed through the normality test Shapiro-Wilk that showed a normal distribution of the data and, thus the ANOVA parametric test was performed with the criterion Tukey ($p < 0.5$). It was observed by the cellular viability test that all samples had viability higher than 80%. The genotoxicity data obtained by the comet assay indicate that none of the studied concentrations showed genotoxic potential when compared with the negative control. These are preliminary data, which must be further investigated for the certification of the effect of the cadaverin over the genetic material of the organism eventually exposed.

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SYNTHESIS, CHARACTERIZATION AND TOXICITY OF BIOGENIC SILVER NANOPARTICLES*Rheder DT¹⁻², Pasquoto T¹⁻², Bilesky-José N¹, Guilger, M¹⁻², Fraceto LF³, Lima R¹.*

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Key-words: biogenic silver nanoparticles, antibacterial, toxicity

Nanomaterials are widely used in industry and silver nanoparticles, which are known for their bactericidal power, are the most used ones. In this way its long term disposal in environment is inevitable, which raises concerns about its toxicity. Thus the analysis of cytotoxicity and genotoxicity are a way to evaluate toxic effects, either the environment or organisms. The aim of this study was to synthesize biogenic silver nanoparticles using extract of *Althaea officinalis*, perform physical-chemical analysis, access their antibacterial potential, cellular viability and genotoxicity. To evaluate the MIC the organisms *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* were used. For cell viability evaluation the v79, HaCat and A549 cell lines were used. The evaluation of genotoxicity was performed through comet assay with human lymphocytes and *Allium cepa* assay. As a result the synthesized nanoparticles had a mean size of 168 nm, zeta potential of 27 mV and polydispersity of 0.2. In MIC evaluation microorganisms showed death in concentrations near 5×10^7 NPs / mL. In connection viability in all tests remained viable until the concentration of 5×10^8 NPs / mL. In comet assay analysis it was observed that the number of damaged cells was approximately 3 times higher than the control when measured at a concentration of 5×10^8 NPs / mL. The *Allium cepa* test performed in concentration 5×10^8 showed that the number of chromosomal aberrations increased 2 fold compared to control. Developed biogenic silver nanoparticles have potential as antibacterial agent, especially considering that the necessary concentrations for use as a bactericide do not present cytotoxicity, but further studies are necessary due to the fact that the results likely present genotoxic potential.

Acknowledgement: Uniso, Fapesp, CNPQ, UFSCar, Unesp.

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Exposure period of *Lithobates catesbeianus* embryos as alternative organism teratogenic tests - FETAX (Frog Embryo Teratogenesis Assay – Xenopus)França FM¹, Teixeira PC², Marcantônio AS¹, Rocha Filho C², Schalch SHC¹, Ferreira CM²¹ Pólo APTA Vale do Paraíba – SAA, Pindamonhangaba/SP - Brazil² Fisheries Institute, APTA – SAA, São Paulo/SP – Brazil

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Key-words: amphibian; developmental toxicity; teratogenicity

The FETAX (Frog Embryo Teratogenesis Assay – Xenopus) regulated by ASTM E 1439-98 (2012) is a bioassay used to determine the teratogenic potential of chemicals in *Xenopus laevis* embryos. Although it was designed for this kind of species, the standard allows the use of alternative species. The aim of this study was to determinate the exposure period of *Lithobates catesbeianus* to FETAX test. After obtaining eggs through hormonal induction of three adult couples, previously selected, 25 embryos of each couple were distributed in 130-mm glass Petri dishes, filled with 100 ml of FETAX solution. The embryos were daily photomicrographs, for tracking the embryonic development of the species, until they reach Gosner stage 24, equivalent to Nieuwkoop and Faber stage 46. The room was held in controlled temperature of 25 ± 1 °C in a photoperiod of 12-h day/12-h nigh. The necessary exposure time for 90% of embryos to reach the Gosner stage 24 was 168 hours. For *X. laevis* embryos, the necessary exposure time to ending the test is 96 hours at 23 ± 1 °C. With the standardization of specific protocol to the species, the test can be used in other laboratories and researchers institutes. This assay can be an excellent tool to evaluate products for regulatory purpose and for monitoring impacted environments.

Economical support: FAPESP (Process: 2014/07293-6)

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CHEMOPREVENTIVE EFFECT OF ATORVASTATIN AND ANALOGUES AGAINST ALKYLATING AGENTS*Araujo-Lima CF^{1,2}, Christoni LSA¹, Bastos MM³, Boechat N³, Felzenszwalb I² and Aiub CAF¹*

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Statins are widely used as drugs to reduce plasmatic levels of cholesterol and lipoproteins by the inhibition of the enzyme hydroxi-3-methyl-glutaryl-coenzyme A reductase. Atorvastatin is a synthetic drug from the statin family, introduced in the market at 1996. Some other effects, independent of cholesterolaemia reduction, can be presented in statins, named pleiotropic effects. Among these pleiotropic effects it's been included regulation of endothelial function, reduction of oxidative stress and inflammation and chemopreventive effects. The atorvastatin analogues PCS09.001 and PCSR08.008 were developed in order to present more selective and safe compounds. Our objective was to investigate the antimutagenic and chemopreventive effect of atorvastatin and its analogues against alkylating agents (4-nitroquinoline-n oxide, methyl methane sulfonate and cyclophosphamide). It was proceedthe modified Salmonella/Microsome assay using TA1535, TA100, TA102 and TA104 strains. We also exposed hepatoma cell cultures (HepG2) to alkylating agents and treated with the statins to observe their chemopreventive effects in eukaryotic cell viability. All statins were capable to reduce the mutation ratio by a dose-response manner, for all Salmonella enterica serovar Typhimurium strains in co-treatment with alkylating agents by direct action or under exogenous metabolism. The results suggested that statins were capable to enhance TA1535 bacterial survival, an error prone repair deficient strain, more expressively. In HepG2 model, we observed the same phenomenon, with a dose-response increase in the viability after 24h or exposure. Although the results indicates promising chemopreventive and antimutagenic effects even so, it is recommended to proceed the analysis of these effects in other experimental models.

Key-words : Atorvastatin, alkylating agents, antimutagenicity, chemoprevention.

Financial support: CNPq, CAPES, FAPERJ

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MOLECULAR ANALYSIS OF PHOTODYNAMIC THERAPY CELL DEATH INDUCED IN *Tritrichomonas foetus*Margraf-Ferreira A¹, Galvão CW³, Etto RM⁴, Pacheco-Soares C^{1,2} and da Silva NS^{1,2}¹Laboratory of Cell Biology and Tissue, IP&D, UNIVAP, Sao Jose dos Campos SP, Brazil.²Laboratory of Dynamic of Cell Compartments, IP&D, UNIVAP, Sao Jose dos Campos, SP, Brazil³ Department of Structural Biology, Molecular and Genetic, UEPG, Ponta Grossa, PR, Brazil.⁴ Department of Chemistry, UEPG, Ponta Grossa, PR, Brazil.aline.margraf@gmail.com cpsoares@univap.br nsoares@univap.br

Bovine trichomoniasis is a sexually transmitted disease caused by the flagellate protozoan *Tritrichomonas foetus*. This causes considerable economic losses in several countries. There are few studies related to chemotherapy and molecular bases of the disease despite the economic importance attributed to it, leaving therefore to be eradicated. According to numerous studies it is noted that, depending on stimulating factor *T. foetus* has the ability to choose from within a set of multiple cell death pathways, which path will follow. However, the Photodynamic Therapy promoting changes in nuclear morphology of this parasite, with apoptotic characteristics. Studies of the physical, chemical and physiological interaction are of extreme importance since it can be directly related to the infection of the host, as well as their pathogenicity to host cells. In this context, considering the possibilities offered and the epidemiological importance and health of trichomoniasis, we are looking for analyze and understand the mechanisms of cell death of the parasite after treatment with PDT in order to elucidate the cell survival and death of *T. foetus*. It was noted by the present work that this parasite can adopt different mechanisms of cell death according to the treatment used, especially after the use of PDT associated with AIPcS4, they showed cell death characteristics by apoptosis in the presence of a "ladder pattern" characteristic of DNA fragmentation, visible on agarose gel electrophoresis. Studies and experiments in progress are essential for the finalization of this work, which will be of great importance for elucidating the mechanism of cell death played by the parasite. These studies will form the basis for the development of future treatments alternative, been less harmful to the host and effectively; as will be of great importance for studies of cell death in organisms lacking mitochondria.

Key-words: *Tritrichomonas foetus*, Photodynamic Therapy, Cell Death, Apoptosis

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The roles of XPC and CSB genes in DNA repair and cell cycle in *Trypanosoma cruzi*.

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Nucleotide Excision Repair (NER) is a versatile DNA repair pathway, responsible for detecting and removing distorting lesions in the DNA double helix. The pathway is divided into two subpathways, global genome repair (GGR) and the transcription coupled repair (TCR). In most eukaryotes, XPC performs distortion detection throughout the genome in GGR, while CSB is recruited by stalled RNA polymerase II in actively transcribed genes during TCR. *Trypanosoma cruzi* is the etiological agent of Chagas' disease, a tropical infirmity that affects 10 million people in tropical regions of the globe. Like all kinetoplastids, *T. cruzi* displays highly unusual gene expression, with virtually all its nuclear genes transcribed in multigenic gene clusters and little evidence for control of gene expression at the small number of poorly defined promoters. In this study, we show that NER in *T. cruzi*, similar to *T. brucei*, is organized in a different way from most characterized eukaryotes. Depletion and overexpression of *T. cruzi* XPC and CSB proteins revealed no evidence that XPC is involved in UV- or cisplatin- induced damage, but depletion of XPC results in delayed cell cycle progression and multinucleated cells. Depletion of CSB causes increased sensitivity to UV, even though the lesions induced are slowly repaired. In addition, depletion of CSB increases sensitivity to cisplatin and MMS, which are rapidly repaired. Overexpression of CSB caused elevated mortality at high levels of UV in an ATM/ATR-dependent manner, since that death can be abolished in presence of caffeine. These results indicate the predominant use of TCR in *T. cruzi*, perhaps due to the transcriptional processes in the parasite.

Financial support: CNPq, CAPES, Fundo Newton.

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EVALUATION OF TOXICOLOGICAL POTENTIAL OF FLUCONAZOLE DERIVED TRIAZOLES

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Fungal infection pharmacotherapy changed significantly after the introduction of new drugs, from “azoles” class, relatively nontoxic, with oral and parenteral formulation and more direct therapy. Fluconazole and Posaconazole are wide spectrum azole to treat fungal infections. There is no available drug to treat Chagas’ disease in chronic phase and, in addition of the expressive side effects of the chemotherapy used in acute phase, it is necessary to develop safe and efficient drugs in both disease phases. In this sense, it was synthesized a novel triazolic derived serial, PFCT (10 compounds), with potential antichagasic activity whose proposed mechanism of action is the inhibition of ergosterol synthesis by the blockage of 14 α -demetilase, the same of fluconazole. So, our objective was to investigate the toxicity of fluconazole and its derivated compounds using *in vitro* experimental models. To determine the selectivity of the compounds we obtained the efficacy outcome, EC₅₀ against Tulahuen strain of *Trypanosoma cruzi* and the safety outcome, DL₅₀ against human hepatoma cells (HepG2). The more promising compounds PFCT 011/14 (SI=656,25) e PFCT 017/14 (SI=540) were followed to mutagenicity assays, using Ames Test, jointly to fluconazole. To fluconazol, it was observed cytotoxicity in all tested strains, TA97 ($\geq 0,5\mu\text{M}$), TA98 and TA102 ($\geq 5\mu\text{M}$) and TA100 ($\geq 50\mu\text{M}$), and no evidence of mutagenicity. PFCT 011/14 presented citotoxicity for TA97 ($\geq 50\mu\text{M}$), TA 100 and TA102 ($\geq 5\mu\text{M}$), without mutagenic responses. To PFCT 017/14, there were no mutagenic and cytotoxic responses in the tested concentrations. The two compounds presented a safety profile more promising than fluconazole in the used models. However, it is necessary more toxicological tests to define the safety profile of these triazoles.

Palavras-chave: Fluconazole, antifungic, mutagenicity, experimental chemotherapy, Chagas’ disease.

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IN VITRO AND IN VIVO ANTITUMOR ACTIVITY AND TOXICITY OF RUTHENIUM(II)/AMINO ACID/DIPHOSPHINE COMPLEX AGAINST SARCOMA 180 TUMOR-BEARING MICE*Mello FMS₁, Delmond KA₁, Lima AP₁, Batista AA₂ and Silveira-Lacerda EP₁*

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Key-words: ruthenium, toxicity, damage DNA, micronucleus.

Cancer is a public health problem, considered one of the most common and serious diseases in the world. Most of current anticancer treatments show non-specificity and side-effects. Ruthenium complexes represent a new alternative anticancer chemotherapeutics, with different mechanisms of action, lower toxicity and higher selectivity for tumor cells. The antitumor effectiveness of the [Ru(L-Met)(bipy)(dppb)]PF₆ (RuMet) complex was evaluated *in vitro* and *in vivo* against Sarcoma 180 (S180) tumor cells. *In vitro* activity: RuMet is cytotoxic to S180 cells after 48 hours of treatment by MTT assay with IC₅₀ value of 22.53±3.40 µM. The RuMet was tested at concentrations of 2, 20 and 50 µM in S180 tumor cells and L929 normal cells for 24 and 48 hours of exposure by Comet Assay. RuMet did not induced DNA damage of the L929 cells by none of the concentrations or time tested. However, RuMet is genotoxic to S180 cells at 24 and 48 hours in all concentrations tested. *In vivo* activity: The toxicological profile of RuMet was determined in healthy Swiss mice by Oral Acute Toxicity following the Guideline 423, genotoxicity by Comet Assay in peripheral blood cells, and mutagenicity by Micronucleus assay in bone marrow cells. According to the Guideline 423, RuMet would be classified as a low toxicity substance, since the animals did not show any sign of toxicity, no deaths occurred and no behavioral change were observed. Moreover, gross necropsy did not reveal any alteration in the main organs either. The administration of RuMet solutions intraperitoneally (ip.) in healthy female and male Swiss mice, demonstrated after 24 and 48 hours low DNA damage in peripheral blood cells and no mutagenicity in bone marrow cells. Within the cisplatin group, genotoxicity was induced in peripheral blood cells and mutagenicity in bone marrow cells of the Swiss mice (p>0.001, vs. vehicle group). The antitumor effectiveness of RuMet was established by the percentage of S180 solid tumor regression. S180-bearing mice were treated with RuMet solutions in the doses of 2 and 4 mg.kg⁻¹/body weight through ip. injection for 8 days, after 13 days of subcutaneous implantation of S180. S180 tumor was reduced in 72±11% (n=6, p<0.001) and 70±4% (n=6, p<0.001) at the RuMet treatments of 2 and 4 mg/kg/day, respectively. RuMet is cytotoxic *in vitro*, induce DNA damage of S180 cells, but not in L929 normal cells. Therefore, this complex presented antitumor effectiveness, with less toxicity than cisplatin.

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VITAMIN D TREATMENT IMPROVES GLUCOSE HOMEOSTASIS AND REDUCES DNA DAMAGE OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Keywords: Vitamin D, type 2 diabetes mellitus, glucose, Comet assay., DNA damage.

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. In Brazil, at the end of the 80s, a multicenter study showed that the prevalence of DM2 occurred at about 8% of the population, data confirmed by WHO. There is a strong link between type 2 diabetes and the presence of DNA damage, mainly related to the persistence of hyperglycemic state and excessive production of free radicals. Knowing that vitamin D has beneficial effects on glucose homeostasis, the aim of this study was to evaluate the influence of vitamin D supplementation in the modulation of genomic instability and other parameters evaluated in type 2 diabetes mellitus patients. We evaluated 79 patients with type 2 diabetes mellitus, registered in the Integrated Clinic of University of Southern Santa Catarina. Participants received 4000UI vitamin D3 (25(OH)D) supplementation daily for eight weeks. The blood was collected at the beginning, at the end of supplementation, and after 4 weeks at the end of supplementation for analysis of Comet assay, 25(OH)D, fasting glucose and insulin and glycosylated hemoglobin (HbA1c). Vitamin D3 supplementation for eight weeks proved to be enough to significantly increase blood levels of 25(OH)D ($P < 0,0001$ / Wilcoxon), reduce fasting blood glucose ($P < 0,001$ / Wilcoxon) and HbA1c levels ($P \leq 0,03$ / Wilcoxon). In addition, an increase on fasting blood insulin was observed ($P \leq 0,0001$ / Wilcoxon) after treatment. Furthermore, there was a significant reduction in the amount of 25(OH)D 4 weeks after the end of supplementation, but values still remained higher than before the start of supplementation. The increased plasma levels of 25(OH)D associated with the glycemic control has shown to be able to significantly reduce DNA damage in patients with DM2 ($P < 0,0001$ / Wilcoxon). Vitamin D3 supplementation showed influence in blood glucose homeostasis and reduce the DNA damage. Use of vitamin D supplementation can be an ally in the health modulation type 2 diabetic patients.

Financial support: UNESC.

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BLOOD MONONUCLEAR CELLS OF TYPE-2 DIABETES MELLITUS AND ALZHEIMER DISEASE PATIENTS SHOWS ALTERATIONS IN COMMON RELATED TO INFLAMMATION AND DNA DAMAGE RESPONSE

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Key-words: Type 2 Diabetes Mellitus, Hyperglycemia, Alzheimer's disease, Microarrays, MicroRNA prediction

Both type 2 Diabetes Mellitus (T2D) and Alzheimer's disease (AD) cases are increasing worldwide, causing a great impact on public health. The first is characterized by hyperglycemia, related to several metabolic complications, comorbidities and increased DNA damage, while the last is a type of senior dementia, whose development has also been associated with accumulation of DNA damage. Evidences in the literature shows that T2D patients have increased risk to develop AD and *vice-versa*, supporting a connection between AD and T2D, manly related to inflammation, impaired glucose metabolism and increased oxidative stress. In the present work, we aimed to compare the differentially expressed genes in both diseases, expecting to find altered gene functions in common between them, including pathways related to DNA damage and stress responses. Firstly, we compared the transcriptional expression (mRNA - Microarray technique) displayed by peripheral blood mononuclear cells (PBMCs) from hyperglycemic T2D patients (T2D-H, n=14), non-hyperglycemic T2D patients (T2D-N, n=15), and healthy non-diabetic individuals (n=16). The same comparison was carried out between a group of AD patients (n=25) and age-matched healthy individuals (n=15). After bioinformatics analysis, the differentially expressed genes from each comparison were crossed, to search for common differentially expressed genes. 41 differentially

expressed genes were found in common between the two comparisons. When submitted to DAVID functional enrichment tool, inflammatory response was the enriched term indicated by this analysis. Accordingly, *IL8*, *CCL3L3* and *CXCL1* genes were all significantly upregulated in T2D and AD patients. Comparing the gene set enrichment and gene set analyses in both data sets, altered pathways such as regulation of DNA repair and superoxide response were found in DM2, while DNA damage response pathways were found in AD. In addition, after using the MirWalk 2.0 tool to predict the MicroRNAs that may regulate those 41 genes, apart from MicroRNAs that were already implicated in T2D and AD (such as miR-29b) we found microRNAs (hsa-miR-224 and hsa-miR-148a), which were not yet described for both diseases including. The present results provide support for the involvement of inflammation process in the pathogenesis of AD and T2D, and point out new MicroRNAs that might be altered in this context. We also demonstrate evidence that DNA damage responses may be compromised in T2D and AD.

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AZADIRACTIN (NEEM OIL) NANOSTRUCTURED CARRIER FOR POTENTIAL APPLICATION IN PEST CONTROL.

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Key-words: Neem oil, pcl nanocapsules, nanotoxicity.

Organic agricultural systems are methods which pursue productivity and economic sustainability, preserving products nutritional quality without environmental changes. One of the requirements of this model is the prohibition of the synthetic substances use on pest control, promoting the use of botanical biocides such as Neem oil, whose main secondary metabolite is Azadirachtin, which is employed against insects. The disadvantage of using this compound is its photosensitivity, which demands several applications for the desired effect. The use of carrier systems for controlled release is an alternative that appears to promote the biocidal persistence, resulting in fewer applications. Due to the stated, this paper proposed the synthesis, characterization and analysis of *in vitro* toxicity of poly(ϵ -caprolactone) nanocapsules as Neem oil carriers for potential use in agriculture. Formulations with different amounts of Neem oil (100, 150 and 200 mg) were prepared by the emulsion/solvent evaporation technique, and its characterization was made from size distribution by the Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA) and morphology by Transmission Electron Microscopy (TEM). From these analyzes it was possible to characterize the morphology, size, polydispersity and zeta potential of the formulations. Toxicity analyzes were developed from the reduction of the tetrazolium dye (MTT assay), comet assay, the *Allium cepa* assay and Minimum Inhibitory Concentration (MIC) assay, resulting in data of cell proliferation and viability, genotoxicity and antimicrobial action. The results showed that the different amounts of neem oil employed in the synthesis did not affect the final size of the nanocapsules, which remained stable size and PDI for a period of 120 days, but had a strong influence on toxicity, with IC₅₀ increased directly proportional to the quantity of Neem oil used in the synthesis in fibroblast cell line (1.4 mg / ml, 3.43 mg / ml and 3.82 mg / mL for NC_10, and NC_15 NC_20, respectively) The genotoxicity assays showed low development by the nanoparticles. These initial results showed

that the development of nanostructured systems to applications in organic farming should be subject to toxicity studies in order to get more reliable information on the use of these systems.

Acknowledgement: CAPES, CNPQ, FAPESP, UNISO, UNESP and UFSCar.

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PROTEOMIC ANALYSIS OF RAT EPIDIDYMIS IN RESPONSE TO METHYLMERCURY EXPOSURE*Sanches ML¹, Almeida MFS¹, Leite AL², Buzalaf MAR², Perobelli JE³, and Peres-Buzalaf C¹*

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Key-words: proteomics analysis, environmental contaminants, reproductive toxicology.

The advances of modern life have been accompanied by various environmental impacts with important implications to the human health. Among these, the rise in male infertility cases is a common problem worldwide. In most cases, the etiology of infertility remains unknown. Although exposure to methylmercury (MeHg) can affect transport, storage and maturation of sperm, which occur in the epididymis, little is known about the molecular mechanisms involved. The label-free proteomics approach was used to identify and evaluate changes in expression of proteins in the epididymis of rats exposed to MeHg. Male *Wistar* rats were divided into two groups: (1) control non-exposed and (2) receiving 0.5 mg/kg/day MeHg. Epididymis proteins were extracted and analyzed by two-dimensional electrophoresis and liquid chromatography, coupled to mass spectrometry, and followed by semi-quantitative label-free differential expression analysis. The results demonstrated that exposure to MeHg significantly altered the qualitative and quantitative expression of proteins involved in DNA repair, gene information processing and signal transduction. In conclusion, the data show that exposure to MeHg impacts gene transcription related to gene expression control in the epididymis. This work opens prospects in order to extrapolate their functional biological significance and discovery of biomarkers of reproductive toxicity and male infertility.

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DEATH'S PROBLEM AND CURRENT CONCERN: STUDYING THE EFFECTS OF POLYAMINE PUTRESCINE ON GENETIC MATERIAL OF HEPG2 CELLS.*Campos-Pereira FD¹, Cagnoni LB¹, Hara RV¹ and Marin-Morales MA¹**¹Laboratório de Mutagênese Ambiental (LMA), Universidade Estadual Paulista – UNESP, Avenida 24-A, nº 1515, Bela Vista, CEP 13506-900, Rio Claro, São Paulo, Brazil.*

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Key-words: necrochurume, DNA damage, cell culture, cytotoxicity, genotoxicity

Among the consequences of population growth is the necessity of increasing areas for burying human bodies. The destined areas for the implementation of cemeteries are usually inadequate by its hydrogeological characteristics, which can cause serious health and environmental problems. The main causes of environmental contamination is the liquid called necrochurume released by bodies decomposition process, which can percolate into the soil and contaminate the groundwater supplies. This liquid is formed by different organic substances among which is putrescine ($C_4H_{12}N_2$), a polyamine which acts as an intracellular messenger and it is related to cell growth and differentiation processes. The aim of this study was to evaluate the cytotoxicity and genotoxicity of the polyamine putrescine in cell culture of HepG2 through the MTT test and comet assay. For determination of the cytotoxicity, HepG2 cells were plated on 96 wells microplates and exposed for 24 h to twelve different concentration of putrescine defined from the LD50 (463 mg/kg) already established for rats. Four non-cytotoxic concentration in this test were used to perform the genotoxicity test. The comet assay was performed with approximately 5×10^6 cells in 25 cm² flasks exposed for 24 h to concentrations of 70%, 50%, 30% e 10 % of LD50. The comets were visually evaluated by analyzing around 100 nucleoids per slide, non-overlapped and randomly chosen, totaling approximately 600 nucleoids per treatment. The tests were performed in triplicate, and the obtained results were statistically analyzed by the parametric ANOVA (one way), followed by Dunnett's comparison test ($p < 0.05$). The results obtained with the MTT assay showed cytotoxicity at the concentrations of 100, 95, 90, 85 and 80% of LD50 putrescine LD50. Among the concentrations tested on the comet assay, the lower concentrations (10 and 30%) were statistically significant for genotoxicity. The data showed genotoxic potential of polyamine putrescine for lower concentrations in these experimental conditions. The results will be of great importance for understanding the mechanisms of action of this substance and relevant to define its supposed potential as an environmental pollutant.

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NITROFURANTOIN – EVALUATION OF THE EFFECTS ON ZEBRAFISH EARLY LIFE-STAGES*Oliveira RCS1, Rodrigues MAC1, Farias NO1, Oliveira R1 and Grisolia CK1*

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Keywords: model organisms, *Danio rerio*, teratology, antimicrobial compounds

Antibiotics are among the most extensively used pharmaceuticals worldwide being used in hospitals, agricultural fields, and livestock production facilities. Nitrofurantoin is a broad-spectrum antibiotic of the nitrofurans family introduced into clinical practice in 1953 being used in the treatment of urinary infections. However, NTF has being suggested as a genotoxic compound causing DNA damage on different cell types and fish embryos. Zebrafish (*Danio rerio*) have been used as model organism for ecotoxicological and toxicological studies due to its genetic similarity with humans. The objective of this study was to evaluate the embryotoxic effects of nitrofurantoin using zebrafish embryos. The toxicity tests were an extended version of the OECD protocol No. 236 - Fish Embryo Toxicity (FET) test. Embryos were exposed, in 24-well microplates, to seven concentrations: 0, 4; 9; 44; 100; 223; 500 mg/L of NTF. The test was performed in a climatic chamber at 26 °C during seven days. For biomarkers analysis a second test was performed where embryos were exposed to 500 ml NTF (0; 0,001; 0.02; 0.32; 5.62; 100 mg/L). After 07 days, the embryos were sampled and activity of cholinesterases (ChE), lactate dehydrogenase (LDH), glutathione S-transferase (GST) and catalase (CAT) were measured. In summary, the results of nitrofurantoin test indicate low toxicity of this compound to zebrafish embryos with a 168 h – LC50 value of 129.2 mg/L. The major effects on development were the loss of equilibrium due to swim bladder not inflated in exposed organisms (168 h – EC50 = 96.72 mg/L). No effect on hatching was noticed. In general, biomarkers activity was induced in concentrations as low as 0.02 mg/L (LOEC for LDH, GST and ChE). The results of this study showed that a short-term exposure of zebrafish to nitrofurantoin can provoke alterations in the normal development at high concentrations (mg/L range) but at lower concentrations, few µg/L, were observed biochemical changes. To the best of our knowledge is unclear the link between the DNA damage, previously reported in zebrafish embryos in other studies, and the teratologies found in the present study. Further studies gene expression changes might help to elucidate the embryotoxic effects of NTF. In summary, acute effects of NTF are not expected to occur but physiological changes might be triggered after long-term exposure.

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EVALUATION OF THE GENOTOXICITY AND MUTAGENIC POTENTIAL OF ANTILEISHMANIAL MILTEFOSINE (HEXADECYLPHOSPHOCHOLINE)*Castelo Branco PV, SOARES, REP, Alves HJ, Pereira SRF*

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The miltefosine (hexadecylphosphocholine), recorded as Impavido® emerged in United States in the 80s, for treatment of skin cancer, however, proved to be quite toxic. Later studies showed that this drug also showed activity against Leishmania, being introduced as a treatment in 2002 in India. The identification of this antileishmanial drug is considered as an important therapeutic advance because it is the first effective oral agent for the treatment of leishmaniasis, facilitating the maintenance of the treatment and may be a therapeutic alternative in the case of infections resistant to conventional therapy. The objective of this study was to evaluate the ability of miltefosine drug to induce DNA damage, to better understand the drug's action and its ability antileishmanial. In trials *in vivo* with mice *Mus musculus* Swiss males, the Comet assays were performed with peripheral blood, and micronucleus with bone marrow. *In vitro* tests were conducted with human leukocytes, being made Cytotoxic Test, Comet assay conventional and a modified comet, such as the addition of the FPG enzyme, besides Apoptosis Test. *In vitro* assays with human leukocytes, it was observed that miltefosine is not a cytotoxic drug, but is genotoxic and causes cell death by apoptosis and necrosis at the same concentrations (1.0, 0.5 and 0.25 ug / ml) with the same exposure time (24 h). In trials acute *in vivo* (24), the miltefosine was genotoxic and mutagenic in the doses 35, 70 and 140 mg / kg. Compared damage score between the conventional and modified comet assays, we observed a significant increase damage in doses 70 mg / kg e140 mg / kg, suggesting that miltefosine cause DNA damage by oxidative stress. This was the first study to assess the genotoxic and mutagenic effect of miltefosine. Although the use of this drug in treating leishmaniasis is considered advantageous, since it has lower toxicity and be administered orally, their use should be controlled, as this drug has broad potential genotoxic and mutagenic. Additional studies will be conducted to evaluate its action *in vivo* in models treated with *Leishmania*.

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EMBRYOTOXIC EFFECTS OF PSYCHOACTIVE COMPOUNDS CAFFEINE AND FLUOXETINE ON ZEBRAFISH EARLY LIFE-STAGES

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Keywords: *Danio rerio*; embryotoxicity; model organisms; psychoactive compounds

The occurrence of psychoactive compounds in natural waters has drawn attention of researchers worldwide. Caffeine (CAF) and fluoxetine (FLX) residues are found in aquatic environments, including surface waters, sediments and groundwater. For these reasons, there is an immediate concern to understand how these compounds may cause adverse effects the biota in natural ecosystems acting as toxic agents. The aim of this study was to evaluate the embryotoxic effects of caffeine and fluoxetine using zebrafish embryos as model organisms. To assess the lethal and sub lethal effects of both psychoactive compounds was used an extend version of the OECD Protocol nº 236 "Fish Embryos Toxicity (FET) test". Newly fertilized eggs were exposed to seven concentrations of CAF (30.1; 45.3; 68.2; 102.6; 154.5; 232.5 mg/L) and FLX (0; 0.01; 0.27; 0.74; 2.02; 5.51; 15.0 mg/L). All tests were performed during 168 h in a climatic chamber at 26 °C. Additionally, for cholinesterase activity analysis, embryos were exposed to lower doses of both compounds ranging from 0.001 to 5.6 mg/L for CAF and from 0.001 – 1.0 mg/L for FLX. After 168 h of exposure the activity of acetylcholinesterase (AChE) was measured using two substrates, propionylcholine (PCh) and acetylcholine (ACh). The results of this study showed that caffeine is not embryotoxic to zebrafish with a 168 h-LC50 value of 148.6 mg/L. On the other hand, fluoxetine was approximately 125 times more toxic than caffeine with a 168 h-LC50 = of 1.2 mg/L. The main teratologies found for caffeine were tail deformities (168 h-EC50 = 75.9 mg/L). For fluoxetine were observed behavioural changes, as loss of equilibrium (168 h-EC50 = 1.02 mg/L). A slight inhibition of AChE was observed in embryos exposed to caffeine in all tested concentrations. A dose-response inhibition of AChE was observed for fluoxetine with significant effects concentrations as low as 0.006 mg/L. Both, caffeine and fluoxetine are emerging pollutants widely spread in aquatic ecosystems. Fluoxetine was embryotoxic for zebrafish. Low acute toxicity was observed for zebrafish embryos exposed to caffeine; however, adverse effects on developmental and biochemical parameters caused by both compounds were induced at low concentrations levels (0.001 µg/L for CAF and 0.006 µg/L for FLX).

Altogether the data obtained in present study suggest effects of FLX and CAF on aquatic fish species exposed to low doses of both psychoactive compounds.

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CYRTOPODIUM PUNCTATUM AQUEOUS EXTRACT TOXICOLOGICAL EVALUATION USING IN VITRO MODELS

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Cyrtopodium punctatum is a typical orchid from Latin American rainforest region. These orchids have several chemical compounds as alkaloids, flavonoids and terpenoids phenanthrenes and these compounds can be used as a diuretic, anti-inflammatory, anticarcinogenic, anti-convulsant, antiviral, neuroprotective, hypoglycemic, antirheumatic and antimicrobial agents. Our objective was to evaluate the mutagenicity and toxicity of the aqueous extract of *Cyrtopodium punctatum*. It was proceeded the *Salmonella*/Microsome assay (Ames Test) using TA97, T98, TA100, TA102 and TA104 strains of *Salmonella enterica* serovar Typhimurium in presence or absence of exogenous metabolism. We also proceeded the *in vitro* micronucleous assay using RAW264.7 macrophage cell lineage after 3 and 24h of exposure. There is no mutagenic response of *Cyrtopodium punctatum* extract in the presence or absence of S9. In absence of S9, the extract was cytotoxic since 50µg/plate. The reduction of cell viability was not observed in the presence of S9. In micronucleous assay it was a significant dose-response increase in mitotic index and a reduction on cell viability, besides there was no clastogenic effect. We can conclude that *Cyrtopodium punctatum* aqueous extract is nor mutagenic or clastogenic in these experimental conditions but it is capable to induce cytotoxic effects in bacterial and eukaryotic cell cultures.

Key-words : *Cyrtopodium punctatum*, ethnopharmacology, mutagenicity, clastogenicity.

Financial support: CNPq, CAPES, FAPERJ, UNIRIO.

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EMBRYOTOXICITY OF THE ANXIOLYTIC DIAZEPAM ON ZEBRAFISH EARLY LIFE-STAGES*Neto PGDS1, Moura DS1, Farias NO1, Oliveira R1 and Grisolia CK1*

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Keywords: model organisms, *Danio rerio*, teratology, psychiatric compounds

In the last decades, the use of psychiatric drugs has grown. The anxiolytics are the biggest class of psychiatric drugs in the Brazilian market being fundamental in the treatment of various neuropsychiatric disorders. Diazepam is an anxiolytic commonly used in the treatment of anxiety syndrome and epilepsy. Its mechanism of action involves the modulation neurotransmitter γ -aminobutyric acid (GABA) inducing sedation, reducing anxiety and causing anterograde amnesia. This study aimed to assess the embryotoxicity of diazepam using zebrafish embryos (*Danio rerio*) as model organisms. In order to archive this objective the Fish Embryo Toxicity Test OECD No. 236 was used. The test was performed with newly fertilized eggs exposed to diazepam concentrations of 0; 1.53; 3.84; 9.6; 24; 60 and 150 mg/L. Sixteen eggs per treatment (divided in 03 replicates) were selected and distributed in 24-wells microplates. The tests were carried out at 26 ± 1 °C in a climatic chamber during 168 h. Embryos were observed daily using a stereomicroscope (Stemi 2000-C, Zeiss, Germany) with a 70 x magnification (for embryos) or 40 x (for hatched embryos). In the embryo phase, the following parameters were evaluated: egg coagulation, otolith formation, eye and body pigmentation, somite formation, tail circulation, detachment of the tail-bud from yolk sac, absorption of the yolk sac, alterations of the amniotic fluid and hatching. After hatching: oedema, equilibrium, undersize, spine deformation and mortality were observed and reported. After 96 h of exposure a LC50 of 28.6 mg/L was obtained. Moreover, at 48 h, was observed a delay on hatching in concentrations from 24 mg/L. For concentrations from 60 mg/L several teratologies were observed including abnormal pigmentation, delay in the yolk-sac absorption, oedemas and spine deformations. Overall, diazepam was toxic affecting the survival and development of zebrafish embryos in concentrations above 24 mg/L. Altogether, the results of the present study suggest the Fish Embryo Toxicity test as an effective tool for embryotoxicity assessment of psychiatric drugs. However, further studies including analysis of specific biomarkers (neurotransmitters levels) and gene expression would contribute for a better understanding of the mechanisms of embryotoxicity of diazepam.

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MUTAGENICITY ASSESSMENT OF TYROSINE KINASE INHIBITORS , ANALOGUES OF IMATINIB

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keywords: mutagenicity, chronic myeloid leukemia

Chronic Myeloid Leukemia (CML) is a disease with intense proliferation of hematopoietic tissue and stem cells in bone marrow, released into the blood with immature red blood cells. In an attempt to reduce the anormal proliferation caused by disease, some drugs with inhibitory capacity tyrosine kinase BCR-Abl-1 are used for being able to inhibit proliferation and induce apoptosis in Bcr-Abl + cell lines. However, for a chemical entity to be released for use in toxicological safety it's necessary, starting mutagenicity tests, called the Ames Test. Our objective was to investigate the mutagenicity and cytotoxicity of anticancer compounds, imatinib analogues capable of selectively inhibiting the tyrosine kinase activity of the BCR-Abl-1. TMINA and TMFAP molecules were synthesized in Organic Synthesis Department FarManguinhos/ FIOCRUZ and evaluated for antileukemic activity against cell lines K562 and LUCENA. It was proceed the modified *Salmonella*/Microsome assay using TA1535, TA100, TA102 and TA104 strains. The cytotoxicity assay was used to limit the Ames test concentration (0, 0.001, 0.01, 0.1, 1, 10 µM) .The TMINA molecule showed cytotoxicity to TA98, TA97, TA102, and in all TMFAP strains and concentrations. Both molecules show mutagenicity TA102 strain, which has the pair of AT base on the critical reverse site thats detecting a wide variety of mutagens and oxidative crosslinks agents that preferentially attack these base pairs. Being this, the secure concentrations without toxicity and mutagenicity for the drug are TMINA 0.001 and 0.01 uM, while for TMFAP any concentration was safe for efficacy tests. It is recommended to proceed the analysis of these effects in other experimental models.

FAPERJ; CNPQ;

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EXPOSURE TO COMBINATION OF ENVIRONMENTAL TOXICANTS AFFECTS PROTEIN PROFILE IN THE EPIDIDYMIS*Almeida MFS¹, Sanches ML¹, Leite AL², Buzalaf MAR², Perobelli JE³, and Peres-Buzalaf C¹*

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Key words: proteomics analysis, environmental contaminants, reproductive toxicology.

Methylmercury (MeHg) and bisphenol polychlorinated (PCB) are contaminants found in various environmental compartments with high capacity of bioaccumulation and biomagnification, affecting the reproductive system. After synthesis in the seminiferous tubules, sperms are directed to the epididymis, where they undergo the process of maturation that gives them the acquisition of cell motility and fertilization capacity. This process is dependent on the interaction of proteins secreted by the epididymal epithelium with sperm proteins, which undergo various post-translational modifications during their transit through the epididymis. However, little is known about the protein profile of rat epididymis in response to MeHg in combination of PCBs exposure. For this, male *Wistar* rats were divided into three groups: (1) control non-exposed; (2) receiving 0.05 mg/kg/day MeHg + 0.01mg/kg/day Aroclor (PCBs mixture) and (3) 0.5mg/kg/day MeHg + 0.1mg/kg/day Aroclor. Proteins were extracted and analyzed by two-dimensional electrophoresis and liquid chromatography, coupled to mass spectrometry, and followed by semi-quantitative label-free differential expression analysis. Changes in various epididymal proteins were found between control and exposed groups, including elongation factor 1-alpha, interferon-induced transmembrane protein 1, Ras-related protein Rab-2, ubiquitin and annexin. In conclusion, the results demonstrate that exposure of rats to MeHg and Aroclor, in combination, induces qualitative and quantitative changes in protein expression in the epididymis, regardless the dose. This study highlights the molecular mechanisms involved in the environmental contaminants-induced toxicity in the male reproductive system.

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CYTOTOXICITY OF RED ONION EXTRACTS (*Allium cepa*) and QUERCETIN FLAVONOID IN HEP-2 CELLS*Tini IRP¹, Rodrigues RP¹, Pacheco-Soares C^{1,2} and Da Silva NS^{1,2}*¹Laboratory of Cell Biology and Tissue, IP&D, UNIVAP, Sao Jose dos Campos SP, Brazil.²Laboratory of Dynamic of Cell Compartments, IP&D, UNIVAP, Sao Jose dos Campos, SP, Brazilitalotini@hotmail.com rafaelp.rodrigues@ig.com.br cpsoares@univap.br nsoares@univap.br

Fruits and vegetables contain a large variety of antioxidant compounds, among them quercetin (QCT), which are polyphenols that may help protect cellular systems from oxidative damage. The red onion (*Allium cepa*) is a vegetable that is a major source of quercetin in the human diet, contributing around 30% of consumed flavonoids, which act as anticarcinogenic, anticancer, antibacterial, antiviral, anti-allergic and antimutagenic, displaying also, activities on the cardiovascular system. The Photodynamic Therapy (PDT) consists in a photochemical reaction used in order to cause selective destruction of tissue. The non-thermal photochemical reaction leads to a cascade of biological events that involve the production of reactive oxygen species (ROS), especially singlet oxygen inside the cells resulting in photodamage and subsequent cell death. Based on this evidence, this study aimed to evaluate the influence of red onion extracts and QCT (standard), with and without PDT, on human larynx carcinoma cells (Hep-2). Different solvents were used for red onions extractions, (chloroform, 70% methanol and 70% ethanol) using a Soxhlet extractor. Samples were subjected to gas chromatography test (GC), evaluation of mitochondrial metabolic activity (MTT) and fluorescence microscopy (MitoTracker and DAPI). According to the analysis carried out by the GC test, specific peaks that characterize the extracts as flavonoid quercetin can be observed. The results obtained from the MTT assay without PDT showed that at a concentration of 5µm extracts and QCT there is no loss of mitochondrial activity, while at concentrations of 50 and 100µM there was a significant reduction in mitochondrial activity. In the MTT assay, in the presence of PDT, there was only a reduction of mitochondrial activity in the concentration of 100µM. In the analysis by fluorescence microscopy, mitochondrial damage were confirmed. There were no morphological changes in the nucleus. Based on these results, the extracts and QCT at and above 50µM, with or without the presence of PDT demonstrated to be cytotoxic in HEP-2 cells, causing a decrease in cell viability.

Key-words: *Allium cepa*. Quercetin. Cell Culture. Cytotoxicity.

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THE TERATOGENIC EFFECT OF BUPROPION HYDROCHLORIDE ON *DANIO RERIO* EMBRYOS*Silva ML1, Oliveira R1 and Grisolia CK1*

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Keywords: embryotoxicity, zebrafish, model organism, antidepressants, NDRI

Bupropion is an atypical antidepressant that is mainly used in tobacco cessation treatment. Its mode of action includes the inhibition of dopamine and norepinephrine reuptake interfering in the reward systems triggered by nicotine. Furthermore, bupropion has an unspecific effect as a non-competitive cholinergic nicotinic receptor antagonist. The aim of this study was to evaluate the potential use of zebrafish embryos as model organism for teratogenic assessment of bupropion hydrochloride. The test started with newly fertilized eggs exposed to bupropion hydrochloride in concentrations of 0; 1; 18.8; 37.5; 75; 150 and 300 mg/L. For exposition, sixteen eggs per treatment (divided in 03 replicates) were selected and distributed in 24-wells microplates. The tests were carried out in a climatic chamber at 26 ± 1 °C with a total duration of 168 h. Exposed organisms were daily observed under a stereomicroscope (Stemi 2000-C, Zeiss, Germany), using 70 x magnification for embryos and 40 x for hatched embryos. In the embryo phase, the following parameters were evaluated: egg coagulation, otolith formation, eye and body pigmentation, somite formation, tail circulation, detachment of the tail-bud from yolk sac, absorption of the yolk sac, alterations of the amniotic fluid and hatching. In the end of the test, at 168 h, an LC50 value of 103 ± 34.2 was obtained for bupropion hydrochloride. No effect on the hatching was found. However, at 96 h, hatched embryos exposed to ≥ 18.7 mg/L showed loss of the equilibrium (side laying in the bottom of the microplate well). At 48 h of exposure the main effect on development observed was partial hatching, where affected embryos had part of the body (head or tail) inside of the egg (75, 150 e 300 mg/L); at 168 h of exposure were observed abnormal curvature of the tail and edema (150 and 300 mg/L). The effects on multiple endpoints (mortality, teratologies and behaviour) might indicate a chronic effect of bupropion in organisms exposed to lower concentrations of this drug. Moreover, the high responsiveness of zebrafish embryos to bupropion exposure corroborates the use Fish Embryo Toxicity test as a sensitive tool for the toxicological assessment of psychiatric drugs.

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WATER CYTOTOXICITY INVESTIGATION OF THE CARIOCA STREAM, OURIZONA-PR, IN MERISTEM CELLS OF *Allium cepa* L.*Vieira CAM¹, Silva JS¹, Heck MC¹, Almeida ACC¹, and Vicentini VEP¹*

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Key-words: environmental monitoring, surface water, pesticides

The increase in population and consequently the production of waste from various sources, contributes to the improper deposition of pollutants in ecosystems, such as sewage, anthropogenic activity waste and pesticides. Environmental monitoring studies are extremely important to evaluate the effects of these activities on water resources, contributing to the prevention of environmental impacts. The Carioca stream suffers from the improper deposition of sewage, little riparian vegetation, proximity to urban areas, disposal of domestic waste and intensive farming and for these reasons, the aim of this study was to evaluate the cytotoxic potential of surface waters of the Carioca stream, in Ourizona - PR, in vegetal test system using meristematic cells of *Allium cepa* L. root. The bulbs of *A. cepa* L., obtained from commercial source, were divided into five groups: four groups were treated with the waters of the Carioca stream, - Headspring, Supply Station, Sewer and Lagoon - collected on August 2015, and a negative control group, with filtered water. The roots were collected in three sampling times: the bulb control (Co-0h), treatment with water of the stream for 24 hours (Tr-24h), and recovery after 24 hours (Re-24h) on filtered water. The negative control group remained on filtered water throughout the experiment. After each sampling, the roots were fixed in methanol/acetic acid (3:1) and subjected to Feulgen reaction and stained with Schiff reagent. To calculate the mitotic index were analyzed 5.000 cells per group and sampling time, in blind test and light microscope (100X). The statistical analysis by the Chi-square test ($\alpha=0.05$) showed that none of the groups evaluated was statistically different from the negative control, *i.e.*, the waters of four points evaluated showed no cytotoxic potential in this test system. However, it is important that environmental monitoring studies still performed, because this stream is used as a source of supply, for fishing and as recreation spot for the population. Furthermore, it is important that people have awareness of the impacts that inappropriate disposal of sewage and other pollutants can cause to humans health and to the environment.

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PSO2 INTERACTIONS WITH DNA DAMAGE RESPONSE GENES AFTER EXPOSURE TO INTERSTRAND CROSSLINK-INDUCING AGENTS IN SACCHAROMYCES CEREVISIAE

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Keywords: DNA repair, PSO2/SNM1, DNA double strand breaks, 8-methoxypsoralen, bifunctional chemotherapeutic drugs, MRX complex

Bifunctional mutagenic agents are largely used as chemotherapeutics and produce interstrand crosslinks (ICLs), which covalently link both DNA strands, effectively blocking replication and transcription. ICL processing may cause DNA double strand-breaks (DSBs). Pso2 protein (mammalian orthologs SNM1A, SNM1B/Apollo and SNM1C/Artemis), a member of the highly conserved metallo- β -lactamase super family of nucleases, plays a central role in ICL repair in yeast. In this study, we aimed to extend the characterization of Pso2 function in ICL repair through the identification of interacting proteins, using the two-hybrid system (THS) in yeast. In addition, the genetic interaction of *PSO2* with genes involved in early stages of ICL repair was investigated. Nine fusion protein products were isolated for Pso2p using THS, among them the Sak1 kinase, which interacted with the C-terminal β -CASP domain of Pso2p. Comparison of mutagen-sensitivity phenotypes of *pso2* Δ , *sak1* Δ and *pso2* Δ *sak1* Δ disruptants revealed that SAK1 is necessary for complete WT-like repair. The epistatic interaction of both mutant alleles suggests that Sak1p and Pso2p act in the same pathway of controlling sensitivity to DNA-damaging agents. We also observed that Pso2p is phosphorylated by Sak1 kinase *in vitro* and co-immunoprecipitates with Sak1p after photoactivated 8-methoxypsoralen (8-MOP+UVA) treatment. Survival data after treatment of *pso2* Δ , *yku70* Δ and *yku70* Δ *pso2* Δ with nitrogen mustard, *PSO2* and *SAK1* with *YKU70* or *DNL4* single-, double- and triple mutants with 8-MOP+UVA indicated that ICL repair is independent of YKu70p and DNL4p in *S. cerevisiae*. Furthermore, a non-epistatic interaction was observed between *MRE11*, *PSO2* and *SAK1* genes after ICL induction, indicating that their encoded

proteins act on the same substrate, but in distinct repair pathways. In contrast, an epistatic interaction was observed for *PSO2* and *RAD52*, *PSO2* and *RAD50*, *PSO2* and *XRS2* genes in 8-MOP+UVA treated exponentially growing cells. Taken together, these results showed that Sak1 kinase plays an important role in contribution to Pso2 nuclease in the repair of ICL-induced DSBs. According to the proposed model in this work, Xrs2p could be directing the DSB to Pso2p or to MRX complex for further processing as *PSO2* showed non-epistatic interaction with *MRE11* in repair of 8-MOP+UVA-induced ICL, and epistatic interaction with *XRS2*.

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EVALUATION OF THE GENOTOXICITY OF WATER EXTRACTS FROM SALTO GRANDE RESERVOIR (AMERICANA-SP) IN PERIOD OF CYANOBACTERIA BLOOM BY THE COMET ASSAY*PAMPLONA-SILVA MT¹; GONÇALVES LC¹ and MARIN-MORALES MA¹*

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Key words: cyanotoxins, solid phase extraction, dam water, cell culture, hepG2.

Currently the degradation of water resources is one of the major global worries because it can cause direct or indirect damages to ecosystems as well to the health and survivor or the exposed organisms. The alteration of the water quality can result in great blooms of cyanobacteria that produce toxins and can cause serious damages when in great amount. Thus, this work aimed to evaluate, by using the comet assay, the genotoxicity of the solid phase extracts (SPE) obtained from the water reservoir of Salto Grande (Americana-SP) collected on a period of great cyanobacteria bloom, over the HepG2 cells. The sterile PBS solution was used as negative control and the sterile MMS aqueous solution ($4 \times 10^{-4} \text{M}$) as positive control. The cells were exposed to the extracts for 24 hours. After the exposition, the cells were collected for performing the cell viability test with Trypan Blue e and the comet assay. Were recorded 600 nucleoids per treatment and analyzed on the software comet assay IV. The significance analyses of the results passed through the normality test Shapiro-Wilk that showed a normal distribution of the data and, thus, the one-way ANOVA parametric test was performed with the criterion Tukey ($p < 0.5$). Among the tested samples, significant results were found for the P1, point near to Atibaia river, the most affected region by the drought on the period of October 2014, and therefore, the point with the higher concentration of algae and, consequently, with the higher possibility to have toxins. The results found here show the importance of performing the monitoring in flood and drought periods on the region and the need of new studies to removing these cyanobacteria.

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BLOOD MONONUCLEAR CELLS FROM ALZHEIMER'S PATIENTS DISPLAYED TRANSCRIPTIONAL ALTERATIONS ON CELL CYCLE REGULATION AND DNA REPAIR*Leandro GS¹, Lobo RR², Evangelista AF³, Xavier DJ¹, Moriguti JC² and Sakamoto-Hojo ET^{1,4}*

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Key-words: Alzheimer's disease, cell cycle, DNA repair

Alzheimer's disease (AD) is an aging related neurodegenerative pathology associated with accumulation of DNA damage. DNA repair processes and cell cycle alterations seem to be implicated in the pathogenesis of AD, although the molecular mechanisms are still uncertain. The aim of the present study was to evaluate whether peripheral blood mononuclear cells (PBMCs) of AD patients display alterations in gene expression profiles, mainly focusing on processes related to cell cycle and DNA damage responses. Blood samples were collected from 25 AD patients and 15 age-matched healthy individuals in order to perform genome-wide mRNA expression (microarray). Bioinformatics analysis indicated 593 differentially expressed genes in AD compared to controls, 428 upregulated and 165 downregulated. By performing the gene set enrichment analysis, we observed the enrichment of gene sets involved in pathways such as base excision repair, homologous recombination, DNA damage response, cell cycle regulation, and apoptosis. Moreover, functional annotation analyses identified that the differentially expressed genes are strongly related to pathways associated with cell cycle regulation. The results of transcriptional analyses indicated that PBMCs of AD might be proliferative, despite the well-known non-proliferative status of most PBMCs; however, PCNA protein expression (a marker of cell proliferation) analyzed by Western blot did not show difference in proliferative status between AD cells and controls. In addition, protein expression analyses of polymerase β (Pol β), a base excision repair enzyme, whose expression was found increased in non-proliferative cells, was found decreased in AD, and especially in individuals with severe AD. Thus, the present results showed transcriptional alterations in genes with role in cell cycle progression in PBMCs of AD patients, although we could not find difference in PCNA status. As a whole, the results support the hypothesis that DNA damage response may be altered in AD, since the amount of Pol β is decreased, and furthermore, it was observed the

enrichment of pathways related to DNA repair in AD. Our results demonstrated that PBMCs present alterations in cell cycle and repair pathways as reported in the literature for AD neurons, reinforcing the idea that blood peripheral cells can provide information about the status of AD disease, and may contribute to the understanding of AD etiopathogenesis.

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ITAPEMIRIM/ES RIO: CYTOGENETICS EVALUATION AND MOLECULAR BY Oreochromis niloticus MEDIA IN POST-SEASON DROUGHT

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Key-words: genotoxic, mutagenic e bioassay

Water can be classified as an essential natural resource for the survival of most living things, especially for humans. In recent years the water has been treated with neglect, however, the water quality is of paramount importance. Given the environmental issues surrounding the Itapemirim-ES river, which is characterized mainly by population growth and consequently, the increase in inappropriate discharges of industrial and domestic waste, this study aimed to evaluate the water quality of the Itapemirim river / ES post-dry period by testing with *Oreochromis niloticus*. Water samples were collected at four points along the Itapemirim-ES river route. The test genotoxicity and mutagenicity were performed through the comet assay and micronucleus, respectively. To assess the genotoxicity and mutagenicity, the fish were submitted to 96 hours of exposure to treatment with the water sample collected in the river Itapemirim / ES. As a negative control we used the exposure of fish to dechlorinated water and as positive control exposure to water dechlorinated followed by intraperitoneal injection of cyclophosphamide 0.1 mL of solution, 2 mg / mL. All procedures followed the recommendations of the Ethics Committee for the Use of Animals of the Federal University of Espírito Santo, where this project was approved. Statistical analysis was performed Shapiro-Wilk normality test, followed by analysis of variance (ANOVA) and Kruskal-Wallis test at $p = 0:05$ of significance. The results in the micronucleus test revealed que todos evaluated sampling points showed no mutagenic potential, as there was no significant difference in induction of micronuclei compared to the negative control. However, the number of nuclear alterations, featuring a genotoxic effect, showed a significant increase in all sampling points compared to the negative control. The information obtained in the comet assay showed a significant increase in induction of DNA damage to the molecule compared to the negative control group. Thus, the results of genotoxicity found in the comet assay corroborate those obtained in the analysis of genotoxic index micronucleus test. In both tests, the PRI point 03 foi the one with the most changes. It can be inferred that the waters of the assessed points showed no mutagenic effect, but a genotoxic effect, particularly in Section 3, which is closest to the urban area of one of the largest cities in the Holy Spirit.

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ANALYSIS *in vitro* cytotoxic and genotoxic POTENTIAL OF RIVER WATER SAMPLES ITAPEMIRIM / ES IN DROUGHT PERIOD*Galter IN¹, Malini M², Martins, IO², Duarte ID², David JAO¹, and Matsumoto ST²*¹Departamento of Biological Sciences, Federal University of Espírito Santo - UFES, Alegre, ES²Department of Biology, Federal University of Espírito Santo, Vitória - UFES, Vitoria, ES

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Key-words: cell culture, viability, comet assay.

The river Itapemirim / ES is considered in capixaba southern extension, as the main source of water for the development of living things. However, it has suffered a deterioration over the years as a result of human actions, due to population growth and the release of solid waste. This work aimed to evaluate the cytotoxic and genotoxic potential *in vitro* of water samples collected from the river Itapemirim / ES, in the dry season. For the tests we used wild type cells of Chinese hamster ovary (CHO-K1). Cytotoxicity was assessed by the MTT test performed with 1×10^4 cells exposed to the sample water for 12 hours, with subsequent reading absorbance at 550 nm. The comet assay was performed in culture bottles containing 1×10^6 cells subjected to treatment with the samples of the water of the collected points. The negative control was performed with cells treated with saline (PBS) and the positive control with mutagen agent Methyl Methane Sulfonate (0.8mM). All treatments were submitted to cellular viability test, by coloring with blue of trypan, viable being considered for genotoxicity analysis only treatment with cellular viability above 80%. To statistical analysis was performed Shapiro-Wilk normality test, followed by analysis of variance (ANOVA) and Kruskal-Wallis test at $p = 0:05$ of significance. The results in cytotoxicity tests, both via the blue of trypan test and by the MTT assay showed that no sample points *avaliados* apresentaram cytotoxic potential compared with the negative control. While the genotoxicity results revealed that significant lesions were induced in the DNA molecule in CHO-K1 cells treated with the water samples from two sampling points and the three-river Itapemirim ES compared to the negative control. Thus, the data suggest that the two and three points, which are close to the urban center of one of the largest cities in the southern state of Espírito Santo showed genotoxic potential, but that damage from water samples in the DNA molecule, in dry period, did not influence the viability of the CHO-K1 cells, both the MTT assay and trypan blue to test.

Financial support: Higher Education Personnel Improvement Coordination.

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INVESTIGATION OF THE CYTOTOXIC AND GENOTOXIC ACTIVITY OF THE COCOA IN HUMAN HEPATOMA CELL - HEPG2/C3A*Heck MC¹, Syritiuk PHS, Yoshimoto M¹, Silva JS¹ and Vicentini VEP¹.*

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Key-words: *Theobroma cacao* L., phytochemicals, antioxidant.

Cocoa, *Theobroma cacao* L. (Malvaceae), is widely consumed in many countries and cultures, is a rich source of fiber, protein, carbohydrates, lipids, minerals and vitamins and has received much attention in recent years due its content of polyphenols. It is recognized as an important source of phytochemicals with beneficial effects on health and is a very rich source of dietary flavonoids, procyanidins, catechin and epicatechin. The aim of the present study was to investigate the cytotoxicity and genotoxicity of cacao, *Theobroma cacao* L., in human hepatoma cells HepG2/C3A through of the assays Trypan Blue Viability and Comet. For the Trypan Blue Viability Test a cellular suspension of 10^4 cells was cultivated in 500 μ L DMEM culture medium supplemented with 10% fetal bovine serum, in 24-well plates, and treated with: 1) Negative Control (0,2% DMSO in DMEM); 2) Doxorubicin (18 μ M); 3) 1 μ g/mL; 4) 10 μ g/mL; 5) 50 μ g/mL and 6) 100 μ g/mL of cocoa powder, for 24, 48 and 72 hours. For the Comet Assay 10^6 cells were seeded into 25cm² culture flask and after 24 hours received treatments for 3 hours: 1) Negative Control (0,2% DMSO in DMEM); 2) Benzo[a]pireno (80 μ M); 3) 1 μ g/mL; 4) 50 μ g/mL and 6) 100 μ g/mL of cocoa powder. Three independent experiments were performed and the experimental data were submitted to statistical analysis of variance (ANOVA) followed by Tukey's test ($\alpha = 0.05$). According to the results obtained with treatments 1; 10; 50 and 100 μ g/mL cocoa no were observed significant differences from the control, in the three sampling periods, therefore, cocoa was not cytotoxic. Concerning the Comet Assay, any of the tested concentrations demonstrated significant differences compared to the negative control, so the cocoa in the evaluated conditions showed no genotoxic effects. The cocoa is a rich source of flavonoids widely consumed and accessible for the population. Products containing cocoa exhibit enhanced antioxidant capacity and higher total flavanol content than many other plant-based foods such teas and red wine. Despite the cocoa processing reduce flavonoid content, it is a rich source of antioxidants and according to the results of the present study did not showed cytotoxic and genotoxic action of the manner how is consumed by the population.

Financial Support: Capes

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THE RECOMBINATION PROCCESSED COULD BE INVOLVED IN GENERATION OF HYBRID STRAINS OF *TRYPANOSOMA CRUZI*Repoles, BM¹; Santos-Silva. S¹; Alves. C. L¹; Franco, GR¹; Macedo, AM¹; Pena, SDJ¹; Machado, CR¹

Trypanosoma cruzi is the etiological agent of Chagas Disease. The variety of symptoms of Chagas disease can be associated with the genetic variability of different strains of *Trypanosoma cruzi*. Studies subdivided *T. cruzi* into six discrete taxonomic units (*T. cruzi* I to *T. cruzi* VI). The occurrence of recombination events are rare in the literature, but it is known that they occur between populations, creating hybrids populations of the parasite. In others organisms, the recombination process is associated with the generation of hybrid cells. The homologous recombination repair (HRR) is also involved in the repair of the DNA double strand break. While other organisms have other pathways, such as non-homologous end joining to deal with double strand breaks, *Trypanosoma cruzi* relies only on HRR to deal with this kind of damage. We studied the response to ionizing radiation, an agent capable of causing double strand breaks, in different strains of *Trypanosoma cruzi*. Although the parasite does not face high doses of radiation on his life cycle, it can resist to doses as high as 500Gy. It was observed that non-hybrid lineages have a distinct phenotype when compared with a hybrid strain in response to this kind of damage, i.e, the hybrid cell respond faster to this lesion. The analysis of genes involved in the repair revealed differences in the sequence of certain proteins between strains, and the prediction of the impact of these changes showed that the interaction between the proteins of each strain could be different. In addition, the expression of some HRR essential genes are high in the hybrid strain. These data suggest that a more efficient recombination process is important to the generation of hybrid strains in *Trypanosoma cruzi*.

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RADIOPROTECTIVE EFFECT OF EPICATECHIN FLAVONOID AGAINST RADIOPHARMACEUTICAL IODINE-131 IN HEPG2/C3A CELLS*Heck MC¹, Fernandes LM¹, Yoshimoto M¹, Almeida IV¹, Lopes NB² and Vicentini VEP¹*

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Key-words: *Theobroma cacao* L., antioxidant, cytoprotection, ionizing radiation.

The radioisotope Iodine-131 (I-131) is used for the diagnosis and treatment of thyroidal disorders such as hyperthyroidism and cancer, for evaluation of hematologic disorders and as a treatment option for hepatocellular carcinoma. However, the radiopharmaceuticals emit ionizing radiation which may cause serious damage to non-target cells, mainly through the interacting with the environment where cellular constituents are leading to production of free radicals. The radioprotective agents are generally antioxidant compounds that have the ability of protect the living tissues against ionizing radiation action. The Cocoa, *Theobroma cacao* L., recognized as significant source of phytochemicals, with healthful effects and is among the most concentrated sources of the flavonoids such as epicatechin. The aim of the present study was to evaluate the protective effects of epicatechin against cytotoxicity and genotoxicity induced by I-131 in human hepatoma HepG2/C3A cells. The cells 10^4 cells to the MTT assay and $3,5 \times 10^4$ cells for assays Viability Trypan Blue and Cell Proliferation Kinetics were plated in 96 and 12-well plate respectively, and treated with: 1) Negative Control (0,2% DMSO in DMEM); 2) 10 μ Ci of I-131; 3) 10 μ M of Epicatechin, and 4) Association of I-131 and 10 μ M of Epicatechin, for 24, 48 and 72 hours. Three independent experiments were performed and the experimental data were submitted to statistical analysis of variance (ANOVA) followed by Tukey's test ($\alpha = 0.05$). According to the results obtained, treatment with 10 μ Ci of I-131 significantly decreased MTT activity (mitochondrial activity), reduced the cell viability and proliferation kinetics compared with control. The cytoprotection treatments, combining epicatechin and I-131, showed protective effects against the damage induced by the radioisotope, was observed increased mitochondrial activity and cell viability compared to treatment accomplished only with I-131. In addition, the epicatechin treatment associated with the radiopharmaceutical after 48 hours was effective in preventing reduction cell proliferation caused by I-131. Therefore, epicatechin was effective in protecting cells HepG2/C3A against the radiopharmaceutical, possibly for its strong antioxidant action.

Financial Support: Capes.

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CYTOTOXIC AND GENOTOXIC ACTIVITY OF THE FLAVONOID EPICATECHIN IN HUMAN HEPATOMA CELLS HEPG2/C3A*Heck MC¹, Fernandes LM¹, Yoshimoto M¹, Almeida IV¹, and Vicentini VEP¹*

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Key-words: *Theobroma cacao* L., antioxidant, flavanol.

The epicatechin is a non-glycosylated flavonoid abundant in cocoa, *Theobroma cacao* L., and has proved to be bioactive in humans. It is extensively metabolised during absorption in the small intestine and the liver. This flavonoid has strong antioxidant activity and so it has been suggested that epicatechin may exert beneficial and /or cytotoxic actions, stimulating the defense metabolism. Therefore, this study aimed to investigate the cytotoxicity and genotoxicity of flavonoid epicatechin in human hepatoma cells HepG2/C3A, using MTT and Comet assays. A cellular suspension of 10⁴ cells was cultivated in 100µL DMEM culture medium supplemented with 10% fetal bovine serum, in 96-well plates, for 24, 48 and 72 hours in the MTT assay and treated with: 1) Negative Control (0,2% DMSO in DMEM); 2) Doxorubicin (18µM); 3) 1µM; 4) 5µM; 5) 10µM; 6) 25µM; 7) 50µM; 8) 100µM; 9) 250µM; 10) 500µM; 11) 750µM; 12) 1000µM of epicatechin. For the Comet Assay 10⁶ cells were seeded into 25cm² culture flask and after 24 hours received treatments for 3 hours: 1) Negative Control (0,2% DMSO in DMEM); 2) Benzo[a]pireno (80µM); 3) 10µM; 4) 50µM; 5) 250µM of epicatechin. Three independent experiments were performed and the experimental data were submitted to statistical analysis of variance (ANOVA) followed by Dunnett's test ($\alpha=0,05$) for MTT and Tukey's test ($\alpha = 0.05$) for Cometa assay. According to the results, the treatments with 1, 5, 10, 25, 50, 100, 250 µM of epicatechin showed no reduction in mitochondrial activity compared to control, for the three sampling periods, so did not have cytotoxic activity. With respect to the Comet assay, the concentrations of 10 and 50 µM showed no genotoxic activity for HepG2/C3A cells, however, it can be seen that the highest concentration evaluated in this assay, 250µM, induced damage, and was significantly different that the observed in control, so this treatment showed genotoxic activity. In the present study we found that epicatechin, an antioxidant, can stimulate damage in HepG2 / C3A cells at high concentrations.

Financial Support: Capes

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CYTOTOXICITY OF RUTHENIUM(II)/BIPYRIDINE COMPLEXES AGAINST EHRlich TUMOR CELLS*Silveira-Lacerda EP¹, Magalhães LF¹, Pereira FC¹, Mello FMS¹, Pires WC¹, Batista AA²*

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Key-words: ruthenium, DNA damage, apoptosis.

Among the anticancer agents derived from metals, the most promising are ruthenium compounds for demonstrating antimetastatic properties, low toxicity to normal cells and high selectivity for tumor cells. This study aimed to determine the potential cytotoxic, genotoxic and elucidate the signaling pathway involved in cell death process of Ru(II) complexes bipyridine, Ru8, Ru21 and Ru26, *in vitro*, in cell line murine breast carcinoma, Ehrlich tumor. To assess the cytotoxicity of the complexes was performed MTT assay. IC₅₀ values were: for Ru8 of 21µM, Ru21 and Ru26 of the 8.52µM and 14.93µM. The complex with better cytotoxic activity and potential selective for tumor cells was selected for further study. The complex with better cytotoxic activity and potential selective for tumor cells was selected for further study. To assess the genotoxicity of Ru21 was held the comet test. After 24 hours, the Ru21 at concentrations of 4, 8 and 16 µM induced DNA damage of 44, 53 and 44, respectively. After 48 hours, Ru21 induced DNA damage in the concentrations of 4µM of ID=48 in 8µM concentration of ID=45 and 16µM ID=42.5% compared to the negative control 16.5. Digite um texto ou endereço de um site ou traduza um documento.

To evaluate the effect of Ru21 complex on the cell cycle kinetics was performed testing the cell cycle by flow cytometry. After 24 hours of treatment, an increase in the percentage of cells in G0/G1 phase and a considerable reduction of cells in S phase and G2/M was observed, considering a cycle arrest. In addition, an increased number of cells in sub-G1 was observed, featuring a DNA fragmentation. To evaluate the type of induced death Ru21 from morphology, apoptosis detection test/necrosis by fluorescent microscopy was conducted. After 24 hours of treatment with Ru21 at concentrations of 4, 8 and 16µM were observed 33%, 42% and 30% respectively of the initial cells in apoptosis compared to negative control 11%. In the treatment of 48 hours at concentrations of 4, 8 and 16µM were observed 50%, 64% and 60% of cells in early apoptosis respectively compared to the negative control. When assessed the expression of the Tp53 genes Bax and Caspase 9 by qPCR, we observed a significant increase in the rate of expression of the Tp53 gene and Bax. The new complex of ruthenium (II), tested is promising because it has low IC₅₀, selectivity for tumor cells, and induce DNA damage, cell cycle arrest and cell death by apoptosis.

Financial Support: CAPES and FAPEG.

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THE RUTHENIUM (II) COMPLEX *CIS*-[RUCL(BZCN)(PHEN)(DPPB)]PF₆ INDUCES CELL CYCLE ARREST AND CELL DEATH TRIGGERED BY EXTRINSIC APOPTOSIS IN S180 CELL LINE

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Key-words: Cancer, cell cycle, cell death, Sarcoma 180, Ruthenium.

The pharmaceutical industry has intensified the search for new metal-based drugs that offer the possibility of oral administration, reduction of serious side effects and reduced clinical costs. For this purpose, we assessed the cytotoxic and genotoxic interference on the cell cycle kinetics and mechanism of cell death of a new complex of ruthenium (II). Cytotoxicity was assessed by the MTT assay and it has shown that the compound tested showed inhibition of tumor cell viability in S180 in lower concentrations and cytotoxicity at higher concentrations for normal lymphocytes. Cis-[RuCl(BzCN)(phen)(dppb)]PF₆ also showed a significant lethality to brine shrimp nauplii *Artemia salina*. The comet assay indicated that the compound is not genotoxic for normal cells, and exhibited low genotoxicity to tumor cells. The cis-[RuCl(BzCN)(phen)(dppb)]PF₆ complex induced changes in cell cycle identified by flow cytometric test, increasing the number of cells in the G₀/G₁ phase and lowering synthesis phase. The complex induced an increase of cells positive for Annexin-V for flow cytometric test, suggesting cell death by apoptosis. By the Western Blot assay, it was possible to infer that the mechanism of death cell triggered by the complex in S180 cell line is extrinsic apoptosis, but we can't exclude that intrinsic pathway is also been activated. However, others specifics markers are needed to understand the cascade of events in which the extrinsic apoptosis pathway are affecting this process of cell death, and if only this pathway is being activated in apoptosis, thus elucidating the mechanism of action in the cells S180.

Financial Support: CAPES, CNPq and FAPEG.

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Characterization of two different ASF1 (*Anti Silencing Factor 1*) in *L. major*Juliana BF Garcia¹, Tiago R Ferreira¹, Lucas Lorenzon¹, Felipe F Castro¹, Angela K Cruz¹.¹ Faculty of Medicine of Ribeirão Preto, São Paulo University – USP, Ribeirão Preto, SP.Email for contact: julianaborio@usp.brKeywords: *Anti Silencing Factor 1*, DNA repair, cell cycle, chromatin, *Leishmania*.

Anti Silencing Factor 1 (ASF1) is involved in chromatin remodeling, cellular response to DNA damage and transcriptional silencing. This chaperone forms a complex with histones H3 and H4 facilitating this histone dimer deposition (or removal) from chromatin. Currently, there are few studies on the function of chaperone ASF1 in trypanosomatids. Similarly to mammals and other eukaryotes, these organisms have two genes encoding for ASF1a and ASF1b isoforms and their specific functions are still unknown. We have been investigating the involvement of ASF1a in the cellular response to DNA damage in *Leishmania major*. We conducted a comparative analysis of the response of transfectants overexpressing ASF1a and control line to hydrogen peroxide (H₂O₂). Overexpression of ASF1a in *L. major* contributes significantly to the resistance of the cells to the oxidative stress. We observed that ASF1a is distributed in the cytosol and, as described for other organisms, histone H3 co-immunoprecipitates with in ASF1a. Overexpression of ASF1a does not modify the levels of histone H3 and H3 acetylation. For a comparative study between the isoforms – ASF1a and ASF1b in *L. major* – we overexpressed ASF1b tagged with HA. We observed that ASF1b overexpressor submitted to the oxidative stress has a resistance profile even more pronounced than that exhibited by the ASF1a overexpressor. Our data indicate that *L. major* ASF1a interferes with the parasite response to DNA damage by halting cell cycle progression. This study is a contribution to improve the understanding of the two ASF1 chaperones in *Leishmania*.

Financial Support: FAPESP

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SAFETY ASSAYS WITH THE ZNX PLANT TO DETERMINE MUTAGENICITY AND CYTOTOXICITY IN SWISS MICE*Abreu DC¹; Delmond KA¹; Sousa MAM²; Reis PRM²; Silveira-Lacerda, EP¹*

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Key-words: Cerrado plant, Apocynaceae family, ZNX.

The plant of this study will be referred as ZNX due to patent issues. It is a plant from Brazilian Cerrado vegetation and it belongs to the Apocynaceae family. There is no available scientific data about this specie but it has already been cited as having antitumor potential by popular culture. Moreover, cytotoxicity activity of other species from Apocynaceae family has been shown in the literature. Thus, the respective study aims to evaluate the safety through micronucleus assay of an acetonic fraction of the ethanolic crude extract obtained from the stem bark of ZNX. The study was performed with 24 males and 24 females of Swiss animals. The micronucleus test (Schmid, 1975) was performed on bone marrow red blood cells. The mice tumor free were subjected to intraperitoneal injections in doses of 15 and 25 mg / kg for periods of 24 and 48 hours. Negative control groups were treated with saline solution and positive control group were treated with Doxorubicin 1.2 mg / kg. After the treatment, cells obtained from bone marrow cells were stained with Panótipo® Kit. For the analysis of micronuclei, 2000 polychromatic erythrocytes (EPC) were counted. A relation between EPC and normochromatic erythrocytes (ENM) was established to determine cytotoxicity. For both treatment periods and in both doses tested, none significant difference was observed compared to the negative control. In 24 hours treatment, doses of 15 and 25 mg / kg showed a micronucleated EPC average frequency of 3.0 and 3.66 for male mice and 2.3 and 3.33 for females, respectively ($p > 0.05$). While in 48 hours treatment, it was obtained an average of 1.33 for both doses in males and 1.0 and 2.0, respectively, in females ($p > 0.05$), where the mean was 1. Similarly, there was no significant difference for the relation EPC / ENC ($p > 0.05$). In groups with 15 males treated with 25 mg / kg, the ratio was 0.60 and 0.58, respectively, in 24 hours and 0.58 and 0.51 in 48 hours. For females, the respective doses, the ratio was 0.63 and 0.43 in 24 hours and 0.67 and 0.48 in 48 hours. There was no statistical difference in response between the genders ($p > 0.05$). The results indicate that the fraction was considered not causing

mutagenicity and cytotoxicity. Thus, it suggests that acetonic fraction of ZNX plant should be safe for continued evaluation studies of antitumor activity in animal models.

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ZEBRAFISH EMBRYOTOXICITY TESTS USED IN THE ASSESSMENT OF THE TRICYCLIC ANTIDEPRESSANTS NORTRIPTYLINE*Ferraz IBM1, Moura DS1, Oliveira R1 and Grisolia CK1*

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Keywords: model organisms, *Danio rerio*, antidepressants, teratology

Tricyclic are class of antidepressant which acts mainly on the reuptake of serotonin and norepinephrine on the synaptic cleft. Nortriptyline (NTP) is one of the most used tricyclic antidepressant for depression treatment being also used for enuresis, neuralgia and nausea resulting from chemotherapy. In this study, zebrafish embryos were used as model organisms to evaluate the toxicity of NTP. An extended version of the OECD protocol "Fish embryo toxicity test" No. 232 was used. In a first step, a total of 20 embryos, per replicate, were exposed to 0.2 ml of five different concentrations of NTP (0; 1; 2.8; 7.9; 22.4; 63 e 177.5 mg/L) in order to determine its lethal concentration (LC50). The tests were performed during seven days in a 24 well microplates conditioned in a climatic chamber at 26 °C and 12h of light. Embryos were observed daily in a stereomicroscope (STEMI 2000, Zeiss Germany). Before hatching were evaluated: coagulation, eye/body pigmentation, edema, and tail malformations; after hatching: tail malformations, edema, and loss of equilibrium. Regarding mortality a lethal concentration values of 168 h-LC50 2.9 ± 0.4 was obtained. The main developmental alterations observed till 96 h the test were: alterations in pigmentation, edema, abnormal curvatures of the tail and delay in yolk-sac absorption of embryos exposed to concentration of 22.3 mg/L; at 144 h exposure were observed abnormal curvature of the tail and edema in embryos exposed to concentration of 7.9 mg/L; at 168 h exposure were observed abnormal curvature of the tail and edema in embryos exposed to the concentration of 2.8 mg/L. Our results suggest NTP as a teratogenic compound for fish in concentration above 2.8 mg/L. One could speculate that NTP exposure would lead to higher concentrations of norepinephrine in the synaptic cleft of embryos, stimulating the sympathetic system and, consequently, provoking a depression of the parasympathetic system. Thus, effects of loss of equilibrium and tail malformations could be related with alterations in the neurotransmitter levels (i.e. acetylcholine) produced in the nerves of the parasympathetic nervous system. However, additional studies focusing on the effects on neurotransmission and neuronal gene expression are necessary for better understanding toxicity mechanisms of NTP.

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ACUTE CRACK-COCAINE EXPOSURE INDUCES GENETIC DAMAGE IN MULTIPLE ORGANS OF RATS

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Key words: Crack cocaine, DNA damage, rats.

Context. It is well known that substances used to human consume might have genotoxic and/or mutagenic potential such as tobacco and alcohol. Crack cocaine is an illicit drug derived from cocaine, in which use and abuse have increased around the world, especially in developing countries. **Objectives.** The aim of this study was to evaluate genetic damage in multiple organs of rats following acute exposure to crack cocaine. **Material and Methods.** A total of twenty (20) Wistar rats were distributed into four groups (n=5), as follows: 0, 4.5, 9 and 18 mg/kg b.w. of crack-cocaine administered by intraperitoneal (i.p.). All animals were sacrificed 24h after i.p. injection. Peripheral blood, brain, liver cells and kidney cells were processed by a technique named assay Comet Assay or single cell gel electrophoresis. Bone marrow blood and liver cells were processed for micronucleus count. **Results.** The results showed that crack cocaine increased the number of micronucleated cells in bone marrow cells exposed to 18mg/kg crack-cocaine (p<0.05). Peripheral blood and liver cells presented genetic damage as depicted by single cell gel (comet) assay at 9mg/kg and 18mg/kg doses (p<0.05). Immunohistochemistry data revealed significant increase in 8OHdG immunoexpression in hepatocytes of animals exposed to crack cocaine at 9mg/kg and 18mg/kg (p<0.05) when compared to negative controls. **Conclusion.** Taken together, our results demonstrate that crack cocaine is able to induce genomic damage in multiple organs of Wistar rats.

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CYTOTOXICITY AND GENOTOXICITY EVALUATION OF MANGANESE FERRITE-BASED NANOPARTICLES*Pena RV₁, Ducas RN₁, Bakuzis AF₂ and Silveira-Lacerda EP₁*

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Keywords: Cytotoxicity, Genotoxicity, MnFe₂O₄, Nanoparticles.

Nanotechnology is currently considered as an emerging science and a global phenomenon which can contribute in a significant way to the technological evolution of 21st century. The progress of nanotechnology have expanded the types of biomaterias from traditional organic material-based nanoparticles to inorganic biomaterials. These nanostructured systems are currently applied in many fields, including drugs, biosensors, cancer treatment as diagnostic tools. Regarding that the nano-biointerface are also influenced by the composition of biological systems (fluids, proteins, membranes, phospholipides, organelles, DNA and others). Limitations of the techniques which are currently available and also the complexity involved in evaluating of the toxicity of nanoparticles (NPs) have also been widely reported. Evaluation of the cytotoxicity and genotoxicity potential of manganese ferrite-based nanoparticles and albumin-based nanoparticles were determinated in Sarcoma 180 (S180) tumor cells and L929 normal cells. Cytotoxicity of nanoparticles were evaluated by MTT 3-(4,5–dimethyl thiazol –2–yl)-2,5 diphenyl tetrazolium bromide) and Trypan blue assay in S180 and L929 cells at 48 hours. The Comet assay was utilized to evaluate the damage DNA (genotoxicity) in S180 and L929 cells. It was found through the set of results that nanoparticles and nanocarries promoted cytotoxicity responses beyond the leneage tumoral cell S180 and basal cell L929. In genotoxicity study however showed that the caused degradation of DNA in the tail regions. We can asses in this work that the nanoparticle and their nanocarries only showed cytotoxicity activity when they meet a biological molecule coupled to the nanocarrier.

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THE STILBENE (Z)-1,3-DIMETHOXY-5-(4-METHOXYSTYRYL)BENZENE INDUCES DNA DAMAGE AND CELL DEATH IN MCF-10A AND MCF-7 CELLS LINES*Santos RA1, Gonçalves NS1, Cintra PP1, Goulart MO1, Souza AR1, Mizuno CS2*

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Key-words: stilbenes, cytotoxicity, genotoxicity

Biologically active natural compounds have been extensively used as molecular leads to design new chemical structures. Stilbenes are a class of natural compounds with a wide range of biological effects, including vascular, antibacterial, antioxidant and antitumoral. In the present study the genotoxic and antiproliferative effects of the stilbene (Z)-1,3-dimethoxy-5-(4-methoxystyryl)benzene (ZMSB) was investigated in the breast tumoral MCF-7 cell line and its normal counterpart MCF-10A. Cytotoxicity and effective concentrations to genotoxicity and cell death assays were determined by XTT, BrdU (bromodeoxyuridine) and clonogenic survival assays. The genotoxicity was determined by comet assay and apoptotic cell death was evaluated by flow cytometry. For all experiments, after 24 h of plating, cells were treated for additional 24 h with different concentrations of ZMSB. Vehicle and positive controls were dimethyl-sulphoxyde 0.1% (DMSO) and doxorubicin 0.5 μM (DOX), respectively. The results of XTT showed that the inhibitory concentration (IC_{50}) of ZMSB was 16,2 and 42,2 μM for MCF-10A and MCF-7, respectively. Cellular proliferation evaluated by BrdU remonstrated that ZMSB reduced the fraction of proliferative cells in concentrations higher than 30 μM . The clonogenic survival assay demonstrated the fraction of survival significantly reduced by concentrations higher than 0.3 μM and 2.5 μM in MCF-10A and MCF-7, respectively. Thus, genotoxicity was evaluated at 0.3, 0.6, 1.25 and 2.5 μM by comet assay and revealed that ZMSB increased the score of DNA damage in both cell lines at 1.25 and 2.5 μM when compared to the negative control ($P < 0.05$). At 2.5 μM ZMSB increased the frequency of apoptotic cells (35.3%) compared to the negative control (4.5%) ($P < 0.05$) in MCF-10A. The frequency of apoptotic cells in MCF-7 was 32.7% against 3.5% in the negative control ($P < 0.05$) Although non-selective, the present results demonstrate that ZMSB induces DNA damage and apoptotic cell death in MCF-10A and MCF-7 cells.

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PROTECTIVE EFFECT OF *Persea americana* Mill PULP OIL ON CHROMOSOME DAMAGE INDUCED BY METHYL METHANESULFONATE AND DOXORUBICIN

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Persea americana Miller is also known as "abacate" or "avocado". This plant belongs to the Lauraceae family has oil extracted from its pulp, which has attracted the attention of the population due to its biological properties, such as lowering cholesterol, preventing prostatic hyperplasia and cardiovascular disease, wound healing, antioxidant, reducing blood glucose levels and hepatoprotection. Considering the pharmacological potential of this plant, the present study aimed to evaluate the influence of the *P. americana* pulp oil (PAO) on the genotoxicity induced by mutagens with different mechanisms of action, methyl methanesulfonate (MMS) and doxorubicin (DXR). It was employed the micronucleus test in Swiss mice bone marrow. The animals were simultaneously treated with different doses of PAO (250, 500 and 1000 mg/kg b.w.; *gavage*) and mutagen (MMS - 40 mg/kg b.w. or DXR - 25 mg/kg b.w.; intraperitoneally). The ratio of polychromatic erythrocytes (PCEs)/total red blood cells (RBC) was employed to evaluate the cytotoxicity of the treatments. The results obtained revealed that animals treated with PAO plus MMS or DXR showed significantly lower micronuclei frequencies compared to those treated only with the mutagens. The PCEs/total RBC ratio did not show significant statistically differences between the treatments and negative control groups. Thus, under these experimental conditions, PAO showed protective effect against genotoxicity induced by MMS and DXR.

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BIOMONITORING OF THE "AÇUDE GRANDE OF CAMPO MAIOR" (PIAUI) THROUGH GENOTOXIC AND MUTAGENIC TESTSPaz FAN¹, Cruz ER², Carvalho TM³, Carvalho LM⁴, Leite AS⁵, Cavalcante AACM⁶, and Silva J⁷

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The pollution of aquatic ecosystems can cause loss of biodiversity and damage to public health. A dam in the city Campo Maior (Piauí, Brazil) receives domestic, industrial and agricultural waste, and is also used as a water reservoir and fishing by the local population. The aim of this study was assess cytotoxicity, mutagenicity and genotoxicity effects of the water samples from the dam "Açude Grande de Campo Maior", by means of frequency analysis of the mitotic index, chromosomal aberrations and micronuclei in the stem cells of *Allium cepa* and in erythrocytes of *Geophagus brasiliensis* (fish). Water samples and fish were collected in the rainy and dry season at three points: (a) site 1, next to a restaurant (P1); (b) site 2, next to a marina (P2); and (c) site 3, next to a highway (P3). Dechlorinated water was used as a negative control and copper sulphate (CuSO₄), as a positive control in *Allium cepa* test. In the fish bioassay, the frequency of micronucleated cells was analyzed to measure the rate of mutagenesis. In *Allium cepa* test were analyzed for root length as a measure of toxicity, and the incidence of chromosomal aberrations and micronuclei to measure mutagenicity. In addition, analysis was carried out on physico-chemical parameters, and fecal coliforms. Results of water samples from the second point, (P2), drains, restaurant (P1), for the rainy and dry season, showed significantly higher number of chromosomal aberrations and micronuclei, on *Allium cepa* stem cells, as well as shorter root length, indicating toxicity and mutagenicity. Among the stations, there is difference in mutagenicity between the 3 points, although it highlighted the dry period. In accordance with the water samples, a significant

increase of micronuclei for fish collected from the second point, in both seasons was observed. Thus, both water and fish collected at point 2 (P2), had the greatest toxic and mutagenic effects, followed by Section 3, the location where flowing water current (P2> P3> P1). These results are consistent with those found in relation to the physic-chemical parameters. Data from this study show the need for constant monitoring along the dam where strong degradation caused by pollutants occurs.

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GENOPROTECTIVE POTENTIAL OF *LIPPIA* SPP ESSENTIAL OILS AND ITS MAJOR COMPOUNDS AGAINST DNA DAMAGE INDUCED BY ULTRAVIOLET RADIATIONQuintero N¹, Stashenko EE², and Fuentes JL^{1,2}¹Laboratorio de Microbiología y Mutagénesis Ambiental, Escuela de Biología, Facultad de Ciencias, Universidad Industrial de Santander, Bucaramanga, Colombia.²Centro de Investigación en Biomoléculas, Centro de Investigación de Excelencia, CENIVAM, Escuela de Química, Facultad de Ciencias, Universidad Industrial de Santander, Bucaramanga, Colombia.

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Keywords: antigenotoxicity, SOS chromotest, ultraviolet radiation, *Lippia* genus, essential oils, terpenes.

Different types of skin cancer are increasing its incidence rate worldwide, becoming a public health problem. Overexposure to solar radiation without proper protection has been well established as the main risk factor for its development, based on the fact that ultraviolet radiation (UVR), can cause structural changes in DNA; acting as an initiator and promotor of the carcinogenic process. As an alternative to this problem arise photoprotection strategies, aimed at mitigating the harmful effects of solar radiation in healthy human populations. The aim of this work was evaluate the protective effect against UVR induced genotoxicity of essential oils (EO) obtained from different species of the genus *Lippia*, as well as their main compounds and its mixtures. The EO major constituents were identified by gas chromatography–mass spectrometry. The assay SOS Chromotest was used as a study model to evaluate antigenotoxicity of samples against UVR, and the protective effect of testing samples was compared with standard genoprotective compounds. The EO major constituents were terpenes as carvacrol, 1,8-cineole, ρ -cymene, neral, genarial, limonene, α -terpinene, thymol and thymol methyl ether. Based on the minimum concentration that significantly inhibits genotoxicity values, the genoprotective potential of EO from the highest to the lowest is as follows: *L. alba*, citral-rich chemotype > *L. graveolens*, (thymol) \approx *L. organoides*, (thymol) \approx *L. organoides*, (carvacrol) > *L. micromera*, (carvacrol) \approx *L. citriodora*, (citral) > *L. alba*, (mircenone). EO from *L. alba*, (carvone), *L. dulcis*, (*trans*- β -caryophyllene) and *L. organoides*, (ρ -cymene/phellandrene) do not reduce the UVR- induced DNA damage in none of the doses tested. An analysis by gas chromatography coupled to a flame ionizing detector of the EO aqueous matrix showed that, at least partially, EO major constituents were water-soluble and therefore, they were related with the EO antigenotoxicity detected for EO. Among the EO constituents ρ -cimene, geraniol, carvacrol and thymol showed the higher genoprotective potency. Finally, the evaluation of paired mixtures of these compounds suggests that a synergistic effect can occur in some EO. Our results evidence that EO obtained

from plants of the genus *Lippia* spp, are a source of protector compounds against UVR damage induced on DNA. Additionally, it could be determined that the protective effect is closely related to the major compounds present in the extract.

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Genotoxic potential of Curbix® 200SC insecticide in several non-target organisms

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Key-words: phenylpyrazol, *Allium cepa*, *Oreochromis niloticus*,

The agrochemicals have as property the elimination or the control of target organisms for which are toxic. The insecticides have the function of controlling the insect pests of agriculture, one of the factors that reduce productivity, without injuring the crops. Among insecticides, the class of phenylpyrazoles has been highlighted in the agrochemical industry in the last ten years. The action of this insecticide is given by its binding to the chloride channel, blocking the activation of conduction of nerve stimuli, by gamma-aminobutyric acid (GABA), causing the hyperexcitation of nervous system and thus leading to the animal's death. Fipronil has been one of the main insecticides used in sugarcane cultivate; however, several studies have demonstrated its deleterious effects on many organisms. The ethiprole emerged as a less toxic alternative to fipronil and has gained ground in the agricultural field. Thus, this study aimed to investigate the toxic potential of Curbix®, commercial product ethiprole by means animal organisms (*Oreochromis niloticus*) and vegetable (*Allium Cepa*). The assessments were made by chromosome aberration test (AC) and micronuclei test (MN) in *A. cepa* (onion) and the MN test and comet assay in *O. niloticus* (Nile tilapia). The tests with *A. cepa* indicated genotoxicity for all concentrations of ethiprole. The tests with *O. niloticus* showed genotoxicity only for the lower tested concentration, showing less sensitivity of these organisms at concentrations tested of ethiprole in relation to onion.

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