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XVI CONGRESS OF MutaGen-Brazil

Disseminating knowledge for a sustainable world

Proceedings

October 4th to 7th, 2023 | Aurora Shopping, Londrina - Paraná - Brazil

Organizer

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PROCEEDINGS – XVI CONGRESS OF MUTAGEN-BRASIL
October 4th to 7th, 2023 | Londrina - Paraná - Brazil

Editors

Ilce Mara de Syllos Cólus; Denise Crispim Tavares; Fabio Vieira dos Santos

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MESSAGE FROM THE PRESIDENT

Ladies and gentlemen, good evening

The Mutagen-Brazil Congress is traditionally held every two years, and since the foundation of SBMCTA in 1989, 15 events have occurred.

As part of our mission, we are always seeking to help public policies aimed at assessing risk in human and environmental health, besides prevention and improvement in the quality of the environment and life. Our last Congress was held in 2021 in a 100% virtual format due to the Sars-Cov-2 pandemic, and today, we return to the face-to-face format under the theme **Disseminating Knowledge for a Sustainable World.**

In addition to the joy of the reunion, we will have access to high-level scientific content, addressing the different thematic areas of our association.

Today, we live in an innovative era in information technology, which allows multiple possibilities of information sharing among different people from different cultures. However, I believe that presential events, where we will discuss Mutagenesis in its different aspects, are more effective in developing and integrating research, education, and responsible application of knowledge.

I am convinced that MutaGen-2023 will be a great occasion for everyone to exchange ideas, get updated on the research of specialists in Mutagenesis and Environmental Genomics, exchange information and experiences, establish new partnerships and strengthen those already existing. To our dear students and young researchers in the field, I hope that this congress will contribute to your citizenship and scientific education.

A special reference and reverence to Dr Elza Tiemi Sakamoto-Hojo, honoured at this congress, who has dedicated herself, for years to improving teaching and research in Mutagenesis in Brazil.

I also thank each one of the speakers who honoured us by accepting to contribute to this event.

For the MutaGen-2023 activities we can count on the presence of renowned researchers from different regions of Brazil, different countries in Latin America and several other countries. They will share their experience and knowledge during the Hands-On Training on Comet Assay, at the Satellite event, at Mutagen Schools, at MutaGen Services, at Conferences and Symposiums. **All this is priceless.**

I thank also young doctors and PhD students that will be with us in Symposiums. I understand that joining experience with innovation will certainly contribute to the renewal of our association staff and will guarantee our constant evolution as a scientific society.

On behalf of our board, youth committee and other members of the MutaGen-2023 Organizing Committee, I welcome the participants and express our gratitude for the contributions and the opportunity to hold this Congress.

Our thanks to the support and sponsorship received.

Thank you very much for your presence.

Welcome to MutaGen-2023!!!

At this moment, I declare MutaGen-2023 open!

Ilce Mara de Syllos Cólus

Conference President

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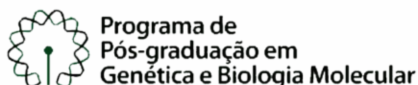

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PROGRAM - OCTOBER 2ND, 2023

OCTOBER 2ND, 2023

Schedule	Activity	Room
10:00 - 18:00	**HANDS-ON TRAINING SESSION**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
09:00 - 10:00	**Preparations of solutions - Only teachers**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
10:00 - 12:00	**TALK: Use of frozen blood in the comet assay;** *Speaker: Goran Gajski - Institute for Medical Research and Occupational Health - Croatia*	Classroom of the Graduate Program in Genetics and Molecular Biology - State University of Londrina
10:00 - 12:00	**TALK: Intro Comet assay and DNA repair assay** *Speaker: Victoria Claudino Bastos - Maastricht University, Netherlands*	Classroom of the Graduate Program in Genetics and Molecular Biology - State University of Londrina
10:00 - 12:00	**TALK: Traditional method and high throughput version** *Speaker: Sabine Langie - Maastricht University, Neederlands*	Classroom of the Graduate Program in Genetics and Molecular Biology - State University of Londrina
13:00 - 15:30	**Subgroup 1: Griding of tissues; Subgroup2: isolation of WBC from frozen blood**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
16:00 - 16:30	**Coffee break**	
16:30 - 18:00	**Subgroups 1 and 2: Preparation of substrate cells for NER and BER**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina

OCTOBER 3RD, 2023

Schedule	Activity	Room
09:00 - 17:00	**HANDS-ON TRAINING SESSION**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
09:00 - 10:30	**Subgroups 1 and 2: Embed substrate cells for NER and BER; Preparation of CometChip and embed frozen blood in 12 gels**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina

Schedule	Activity	Room
10:30 - 11:00	**Coffee break**	
11:00 - 12:00	**Joint session: Lysis and BB wash - Incubation with protein and/or enzymes - Loading of CometChip**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
12:00 - 13:00	**LUNCH BREAK**	
13:00 - 15:00	**Joint session: Denaturation & Electrophoresis - Difference between vertical and horizontal comet tank - Try out 12 gel systems or 48-96 gells on gel bond films**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
15:30 - 16:00	**COFFEE BREAK**	
16:00 - 16:30	**Comet staining and viewing in small groups**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina
17:00 - 18:00	**Theoretical session: Data analysis & General discussion: Data interpretation and calibration - Methods revisited: What went wrong, and why?**	Laboratory of Mutagenesis and Oncogenetics (CCB) - State University of Londrina

OCTOBER 4TH, 2023

Schedule	Activity	Room
08:30 - 20:30	**ACCREDITATION**	
09:15 - 12:00	**SATELLITE EVENT:** Genomic Instability and Cancer	Room 1
09:15 - 09:30	**Apresentação do Grupo e do Programa** *Palestrante: Enilze Maria Fonseca Ribeiro - UFPR, Curitiba, PR*	Room 1
09:30 - 10:00	**TALK: Implicações da oxidação e sanitização do "pool" de nucleotídeos em células tumorais** *Palestrante: Glaucia Regina Martinez - UFPR, Curitiba, PR*	Room 1
09:30 - 10:00	**TALK: Aspectos básicos da instabilidade genômica e câncer** *Palestrante: Daniela Fiori Gradia - UFPR, Curitiba, PR*	Room 1
10:30 - 11:00	**TALK: Micro-RNAs na modulação da instabilidade genômica** *Palestrante: Luciane Regina Cavalli - Research Institute Pelé Pequeno Príncipe, Curitiba, PR*	Room 1
11:00 - 11:30	**TALK: Exposição humana aos agrotóxicos e suas implicações no câncer de mama.** *Palestrante: Carolina Panis - UNIOESTE, Francisco Beltrão - PR*	Room 1
12:00 - 13:00	**LUNCH BREAK**	

Schedule	Activity	Room
13:30 - 15:20	**SATELLITE EVENT:** Genomic Instability and Cancer	Room 1
13:30 - 14:00	**TALK: O papel da instabilidade genômica na resposta antitumoral** *Palestrante: Patricia Araujo-Souza - UFPR, Curitiba, PR*	Room 1
14:00 - 14:30	**TALK: Instabilidade Genômica em Oncologia: Marcadores e Aplicações** *Palestrante: Vanessa Kozak - UFPR e Hospital Erasto Gaertner, Curitiba, PR*	Room 1
14:30 - 15:20	**Discussion**	Room 1
15:20 - 16:00	**COFFEE BREAK**	
16:00 - 17:00	**COUNCIL MEETING**	Room 1
16:00 - 17:00	**MUTAGEN SCHOOL 1 - From 2D to spheroids and organoids: the next generation of cell culture models applied to toxicogenomics** **:** ESCOLA MUTAGEN 1 - De 2D para esferóides e organoides: a próxima geração de modelos de cultivo celular aplicados à toxicogenômica *Palestrante: Mário Sérgio Mantovani - UEL* *Palestrante: Raul Ghiraldelli Miranda - FCFRP- USP, Ribeirão Preto, SP*	Room 2
16:00 - 17:00	**MUTAGEN SCHOOL 2 - Zebrafish model for genotoxic assessment of aquatic contaminants** **:** ESCOLA MUTAGEN 2 - O Modelo Zebrafish (*Danio rerio*) para avaliação genotóxica/embriotóxica de contaminantes aquáticos *Palestrante: Cesar K. Grisolia - UnB, Brasilia, DF* *Palestrante: Iara S. Squarisi - UNIFRAN, Franca, SP*	Room 3
16:00 - 17:00	**MUTAGEN SCHOOL 3 - Dietary natural products and bioactive compounds as candidates for chemoprevention** **:** ESCOLA MUTAGEN 3 - Produtos naturais e compostos bioativos da dieta como candidatos para a quimioprevenção *Palestrante: Mariane Aparecida Franco de Godoy - UEM, Maringá, PR* *Palestrante: Pollyanna Francielli de Oliveira - UNIFAL, Alfenas, MG*	Room 4
18:00 - 18:30	**OPENING SESSION**	Room 1
18:00 - 18:15	**Opening by the President of MutaGen-Brasil**	
18:15 - 18:45	**Tribute to Profª Elza Tiemi Sakamoto-Hojo**	
18:45 - 19:30	**CULTURAL ACTIVITY**	
19:30 - 20:45	**OPENING CONFERENCE:** Policies in Higher Education in Brazil *Speaker: Denise Pires de Carvalho - Secretária de Educação Superior do Ministério da Educação – Brasilia, DF; UFRJ, Rio de Janeiro, RJ*	Room 1
20:45 - 00:00	**COCKATAIL**	

OCTOBER 5TH, 2023

Schedule	Activity	Room
08:00 - 09:00	**MUTAGEN SCHOOL 1 - From 2D to spheroids and organoids: the next generation of cell culture models applied to toxicogenomics** **:** ESCOLA MUTAGEN 1 - Do 2D aos esferoides e organoides: a próxima geração de modelos de cultura de células aplicada à toxicogenômica. *Palestrante: Mário Sérgio Mantovani - UEL* *Palestrante: Raul Ghiraldelli Miranda - FCFRP- USP, Ribeirão Preto, SP*	Room 1
08:00 - 09:00	**MUTAGEN SCHOOL 2 - Zebrafish model for genotoxic assessment of aquatic contaminants** **:** ESCOLA MUTAGEN 2 - O Modelo Zebrafish (*Danio rerio*) para avaliação genotóxica/embriotóxica de contaminantes aquáticos. *Palestrante: Cesar K. Grisolia - UnB, Brasilia, DF* *Palestrante: Iara Squarisi - UNIFRAN, Franca, SP*	Room 2
08:00 - 09:00	**MUTAGEN SCHOOL 3- Dietary natural products and bioactive compounds as candidates for chemoprevention** **:** ESCOLA MUTAGEN 3 - Produtos naturais dietéticos e compostos bioativos como candidatos à quimioprevenção. *Palestrante: Mariane Aparecida Franco de Godoy - UEM, Maringá, PR* *Palestrante: Pollyanna Francielli de Oliveira - UNIFAL, Alfenas, MG*	Room 3
09:00 - 10:00	**CONFERENCE 2:** The Potential Causative Role of Acquired Mitochondrial DNA Damage in Diabetic Complications and common metabolic disease. *Speaker: Afshan Malik - Faculty of Life Sciences and Medicine - King's College London, UK*	Room 1
09:00 - 10:00	**CONFERENCE 3:** Application of In Vitro New Approach Methodologies to Determine Whole Mixture-Based Relative Cancer Potency Factors of Environmental Pollution *Speaker: Kristian Dreij - Karolinska Institutet - Stockholm, Sweden*	Room 3
10:00 - 10:15	**COFFEE BREAK**	
10:20 - 12:00	**SESSION 1:** DNA DAMAGE BY NATURAL AND SYNTHETIC AGENTS AND DNA REPAIR IN HEALTH AND DISEASE	Room 1
10:25 - 10:45	**Interconnection Between DNA Repair and Transcription in Response to Oxidative Stress** *Speaker: Lucymara Fassarella A. Lima - UFRN, Natal, RN*	Room 1
10:45 - 11:05	**NRF2 Modulates Ferroptosis in Temozolomide-Resistant Glioblastoma Cells** *Speaker: Clarissa Ribeiro Reily Rocha - UNIFESP, São Paulo, SP*	Room 1
11:05 - 11:25	**Genotoxicological Safety of Multifunctional Biopolymer Films** *Speaker: Flavia Aparecida Resende - UNIARA, Araraquara, SP*	Room 1
11:25 - 11:45	**Transcription-associated DNA Repair in *Trypanosoma cruzi*: Involvement in death and dormancy** *Speaker: Carlos Renato Machado - UFMG - Belo Horizonte, MG*	Room 1

Schedule	Activity	Room
11:45 - 12:00	**Discussion**	Room 1
10:20 - 12:00	**SESSION 2:** GENETIC EFFECTS OF IONIZING RADIATION: ENVIRONMENTAL AND HUMAN HEALTH	Room 3
10:25 - 10:45	**Genome Oxidative Damage in Humans Exposed to High Indoor Radon Levels in Northeast Brazil** *Speaker: Viviane Souza do Amaral - UFRN - Natal, RN*	Room 3
10:45 - 11:05	**Effects of Radiation Exposure on Offspring and Next Generations** *Speaker: Manoor Prakash Hande – National University of Singapore - Singapore*	Room 3
11:05 - 11:25	**Radiation Induced Mutations and Carcinogenesis** *Speaker: Marina Di Giorgio - Autoridad Regulatoria Nuclear - Buenos Aires, Argentina*	Room 3
11:25 - 11:45	**A New Cytomolecular Approach for Detecting Low Doses of Ionizing Radiation** *Speaker: Wilner Martínez-López - IIBCE, Montevideo, Uruguay*	Room 3
12:00 - 14:00	**LUNCH BREAK**	
14:00 - 15:45	**SESSION 3:** ECOGENOTOXICOLOGY	Room 1
14:05 - 14:25	**Risk Characterization of Human Exposure to Polycyclic Aromatic Hydrocarbons: Influence on DNA damage in vulnerable groups** *Speaker: Marília Cristina Souza – FCFRP-USP, Ribeirão Preto, SP*	Room 1
14:25 - 14:45	**Genotoxic Effects of Pesticides and Nanopesticides on Freshwater Fish: Searching for more sustainable agriculture** *Speaker: Cláudia B. Reis Martinez - UEL, Londrina, PR*	Room 1
14:45 - 15:05	**New Methodological Approaches (NAMs) in Genotoxicity Assays Using Fish** *Speaker: Taynah Vicari - Faculdades Pequeno Príncipe, Curitiba, PR*	Room 1
15:05 - 15:25	**Danger Is In The Air** *Speaker: Israel Felzenszwalb - UERJ, Rio de Janeiro, RJ*	Room 1
15:25 - 15:45	**Discussion**	Room 1
14:00 - 15:45	**SESSION 4:** ORAL PRESENTATION AWARD - THEMATIC AXES 1 AND 4 Thematic Axes 1 (Applied Toxicological Genetics; Toxicogenomics and Bioinformatics) and 4 (Genomic Instability; DNA Repair; Nutrigenomics)	Room 3
14:00 - 14:10	**1) Diosgenin induces multinucleation in NCI-H460 lung carcinoma cells by inhibition of cytokinesis:** *Matheus Felipe da Silva - UEL - Londrina, PR*	Room 3
14:10 - 14:20	**2) Differentiaiton capacity of the SH-SY5Y human neuroblastoma cell line by Y-27632 rock inhibitor:** *Larissa de Oliveira Piassi - USP - Ribeirão Preto, SP*	Room 3
14:20 - 14:30	**3) Genomic insights of marine bacteria in Antarctica's extreme environments:** *Rayana dos Santos Feltrin - USP - São Paulo, SP*	Room 3

Schedule	Activity	Room
14:30 - 14:40	**4) Phosphorylation dynamics of DNA damage response (DDR) pathway is orchestrated in a time-dependent manner during *T. cruzi* infection in non-phagocytic cells:** *Raul Alexander Gonzáles Córdova - USP - Ribeirão Preto, SP*	Room 3
14:40 - 14:50	**5) Influence of a beetroot peel flour extract over the clones formation of breast cancer cells, its toxicological safety and betalains profile description:** *Pedro Paulo Saldanha Coimbra - UNIRIO, UERJ - Rio de Janeiro, RJ*	Room 3
15:45 - 16:00	**COFFEE BREAK**	
16:00 - 17:40	**SESSION 5:** ORAL PRESENTATION AWARD - THEMATIC AXES 2 AND 3 Thematic Axes 2 (Environmental Mutagenesis; Genotoxic Risk Assessment and Public Health) and 3 (Carcinogenesis/Oncogenetics; Epigenomics; Germ Cells and Hereditary Effects)	Room 1
16:00 - 16:10	**1) Cytogenetic changes in oral mucosa cells from individuals submitted to oral HIV pre-exposure prophylaxis (PrEP) use:** *Thiago Guedes Pinto - UNIFESP - Santos, SP*	Room 1
16:10 - 16:20	**2) Impact of genetics, epigenetics and antioxidant response in occupational exposure to anesthetic gases:** *Mariane Aparecida Pereira Silva - UNESP, Botucatu, SP*	Room 1
16:20 - 16:30	**3) Assessment of embryotoxicity, visual motor response, and oxidative stress of nanostructured biomaterials based on hydroxyapatite in Zebrafish (*Danio rerio*):** *Augusto Monteiro de Souza - UFRN, Natal, RN*	Room 1
16:30 - 16:40	**4) Evaluation of the role of HMGA1 in the progression of esophageal adenocarcinoma:** *Maria Luísa Barambo Wagner - INCA, Rio de Janeiro, RJ*	Room 1
16:40 - 16:50	**5) Expression and functional analysis of plasma miRNAs in workers with occupational exposure to pesticides:** *Luiza Flavia Veiga Francisco – Hospital de Amor, Barretos, SP*	Room 1
16:00 - 17:40	**SESSION 6:** GENOMIC INSTABILITY, DNA DAMAGE REPAIR AND CANCER	Room 3
16:00 – 16:20	**The Hereditary Cancer-Associated N363K POLE Exonuclease Mutant Causes Replication Stress and DNA Damage in Addition to Enhanced Mutation Frequency** *Speaker: Jean Sébastien Hoffmann - Toulouse, France*	Room 3
16:20 - 17:00	**DNA Replication and Repair Defects in Rothmund Thomson Syndrome** *Speaker: Nicolas Hoch - IQ-USP- São Paulo, SP*	Room 3
17:00 - 17:20	**Clinical Relevance of DNA Repair Modulation in Colorectal Cancer** *Speaker: Jeniffer Saffi - UFCSPA, Porto Alegre, RS*	Room 3
17:20 - 17:40	**Discussion**	Room 3
17:40 - 18:40	**POSTER SESSION 1**	Room 1
19:00 - 20:45	**SESSION:** NIGHT WITH SCIENCE	Room 3

Schedule	Activity	Room
19:05 - 19:25	**Predatory Journals** *Speaker: Carlos F. Menck - USP, São Paulo, SP*	Room 3
19:25 - 19:45	**Impatient Science and Academic Quantophrenia: Reflections on modern scientific culture** *Speaker: Marcus F. Oliveira - UFRJ, Rio de Janeiro, RJ*	Room 3
19:45 - 20:05	**Science for the General Public** *Speaker: Marcelo Leite - Jornalista*	Room 3
20:05 - 20:25	**Responsible Conduct in Biomedical Research** *Speaker: Manoor Prakash Hande - National University of Singapore - Singapore*	Room 3
20:25 - 20:45	**Discussion**	Room 3

OCTOBER 6TH, 2023

Schedule	Activity	Room
08:00 - 09:00	**MUTAGEN SERVICE 1 - The Salmonella/Microssome Test - Results from the 4th Interlaboratory Proficiency Program** Serviço MutaGen 1 - O teste Salmonella/Microssomo - Resultados do 4º Programa Interlaboratorial *Speaker: Errol Zeiger - NIEHS – Consulting - USA* *Speaker: Deborah A. Roubicek - CETESB, São Paulo, SP* *Speaker: Gisela de Aragão Umbuzeiro – UNICAMP/Limeira, SP*	Room 1
08:00 - 09:00	**MUTAGEN SERVICE 2 - In Vitro Reconstructed Human Epidermal Model (RHE) as an Alternative Method to the Use of Animals and Its Applications** Serviço Mutagen 2 - O Modelo de Epiderme Humana Reconstruída in vitro (RHE) Como Método Alternativo ao Uso de Animais e Suas Aplicações *Speaker: Heloiza Diniz Nicolella - UFSCar, São Carlos, SP*	Room 3
09:00 - 10:00	**CONFERENCE 4:** MUTATIONAL PROFILE OF CANCER IN ADMIXTURE ANCESTRY BRAZILIAN PATIENTS *Speaker: Rui Manuel Reis - Escola de Medicina da Universidade do Minho, Braga, Portugal*	Room 1
09:00 - 10:00	**CONFERENCE 5:** GENOME INSTABILITY, DNA DAMAGE AND AGING *Speaker: Carlos F. Menck - USP, São Paulo, SP*	Room 3
10:00 - 10:20	**COFFEE BREAK**	
10:20 - 12:00	**SESSION 7:** MUTAGENESIS IN LATIN AMERICA	Room 1
10:25 – 10:50	**Elimination Metabolism and Genotoxic Damage from Exposure to Arsenic in Drinking Water in Populations of the Bolivian Highlands** *Speaker: Noemi Sandra Tirado Bustillos - Universidad Mayor de San Andres, La Paz, Bolivia - ALAMCTA President*	Room 1

Schedule	Activity	Room
10:50 - 11:15	**Situation of Mutagenesis Research in Mexico, Short History and Research Groups** *Speaker: Javier Spinosa - Univ. Nac. Autónoma do México - Ciudad do México - México*	Room 1
11:15 - 11:40	**More Than 3 Decades of MutaGen-Brasil** *Speaker: Silvia Regina Batistuzzo de Medeiros - UFRN, Natal, RN*	Room 1
11:40- 12:00	**Discussion**	Room 1
10:20 - 12:00	**SESSION 8:** GENOME PROTECTING THROUGH NATURAL PRODUCTS AND DIET	Room 3
10:25 - 10:45	**Inflammatory, Oxidative and DNA Damage Status in Healthy Subjects Based on Their Dietary Preferences** *Speaker: Goran Gajski - Institute for Medical Research and Occupational Health - Croatia*	Room 3
10:45 - 11:05	**Studying Nutritional Modulation of DNA Repair: The value of the comet based in vitro DNA repair assay** *Speaker: Sabine Langie - Maastricht University, The Neederlands*	Room 3
11:05 - 11:25	**Bioactivity and Protective Effects of Natural Products** *Speaker: Andréia Fernandes - UERJ, Rio de Janeiro, RJ*	Room 3
11:25 - 11:45	**Maternal Exercise During Pregnancy Modulates Genetic and Biochemical Damage Caused by High Consumption of Fructose in Offspring** *Speaker: Vanessa Moraes de Andrade - UNESC, Criciúma, SC*	Room 3
12:00 - 14:00	**LUNCH BREAK**	
12:00 - 13:00	**MUTAGEN-BRASIL ASSEMBLY**	Room 3
14:00 - 15:45	**SESSION 10:** GENOMIC INSTABILITY	Room 3
14:05 - 14:25	**The ATM Signaling Pathway as a Mediator of Human Papillomavirus Mediated Pathogenesis** *Speaker: Enrique Boccardo Pierulivo - USP, São Paulo, SP*	Room 3
14:25 - 14:45	**Transcription-induced Accumulation of DNA Lesions in XPD/ERCC2 Mutated Trichothiodystrophy Patients Cells** *Speaker: Giovana da Silva Leandro - USP, São Paulo, SP*	Room 3
14:45 - 15:05	**The Role of Bur1CDK9 kinase on DNA Replication Stress Response** *Speaker: Francisco Meirelles Bastos de Oliveira - UFRJ, Rio de Janeiro, RJ*	Room 3
15:05 - 15:25	**Chemical Repair of Etheno DNA Adducts by Singlet Molecular Oxygen** *Speaker: Glaucia Regina Martinez - UFPR, Curitiba, PR*	Room 3
14:00 - 15:45	**SESSION 9 -** INNOVATIONS IN MUTAGENESIS AND ENTREPRENEURSHIP: Regulatory Genotoxicity: Applying scientific knowledge to products safety	Room 1

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14:05 - 14:25	**How to Assess the Mutagenic Potential of Cosmetic Products Without Animal Tests?** *Speaker: Desiree Cigaran Schuck - Grupo Boticário, Curitiba, PR*	Room 1
14:25 - 14:45	**Regulatory Genotoxicity: Applying scientific knowledge to products safety.** *Speaker: Izabel Villela - InnVitro Suporte e Gestão em Toxicologia*	Room 1
14:45 - 15:05	**From Basic Research to Business in Bioremediation** *Speaker: Lucymara Fassarella A. Lima - UFRN, Natal, RN*	Room 1
15:05 - 15:25	**BioDos: Using UV Radiation for Innovation and Entrepreneurship** *Speaker: André Passaglia Schuch - UFSM, Santa Maria, RS*	Room 1
15:25 - 15:45	**Discussion**	Room 1
15:45 - 16:00	**COFFEE BREAK**	
16:00 - 17:00	**POSTER SESSION 2**	Room 1
19:00 - 00:00	**CONFRATERNIZATION DINNER**	

OCTOBER 7TH, 2023

Schedule	Activity	Room
09:00 - 10:00	**CONFERENCE 6:** UNCOVERING CELL-CELL COMMUNICATION IN HEALTH AND DISEASE THROUGH SINGLE-CELL AND SPATIAL TRANSCRIPTOMICS *Speaker: Robson Francisco Carvalho - UNESP-Botucatu, SP*	Room 1
09:00 - 10:00	**CONFERENCE 7:** TELOMERES, DNA REPAIR AND DIETARY INTERVENTIONS *Speaker: Manoor Prakash Hande – National University of Singapore, Singapore*	Room 3
10:00 - 10:20	**COFFEE BREAK**	
10:20 - 12:00	**SESSION 11:** CHRONIC-DEGENERATIVE DISEASES AND EPIGENOME	Room 1
10:25 - 10:45	**Expression of DNA Repair Genes is Modulated During Differentiation of Olfactory Sensory Neurons** *Speaker: Nadja Cristhina de Souza Pinto - USP, São Paulo, SP*	Room 1
10:45 - 11:05	**The Epigenetic Landscape of Infertility and Its Impact on Embryonic Development** *Speaker: Cristiana Libardi M. Furtado - UNIFOR - CE*	Room 1
11:05 - 11:25	**MicroRNA-mediated Modulation of Oncogenic Signaling Pathways in Breast Cancer** *Speaker: Luciane Regina Cavalli - Research Institute Pelé Pequeno Príncipe, Curitiba, PR*	Room 1

Schedule	Activity	Room
11:25 - 11:45	**Potential Therapeutic Strategies Based on the Induction of Neuronal Differentiation and Neuritogenesis Investigated in Human Neuronal Models for Alzheimer's Disease** *Speaker: Elza Tiemi Sakamoto-Hojo - FFCLRP-USP, Ribeirão Preto, SP*	Room 1
11:45 - 12:00	**Discussion**	Room 1
10:20 - 12:00	**SESSION 12 -** POPULATION RISK AND HUMAN HEALTH	Room 3
10:25 - 10:45	**Genetic Polymorphisms and Breast Cancer Prognosis in Women Exposed and Unexposed to Agrochemicals** *Speaker: Juliana Mara Serpeloni – UEL, Londrina, PR*	Room 3
10:45 - 11:05	**Genetic Polymorphisms Related to Se and Hg Toxicokinetics Alter Element Levels in Amazonian Riverside Populations Exposed to Hg via Diet** *Speaker: Flora Troina Maraslis - Doctoral student UNIFESP, Santos, SP*	Room 3
11:05 - 11:25	**Exploring the Link Between Type 2 Diabetes and Alzheimer's Disease: The role of hyperglycemia-induced oxidative and neurotoxic damage, DNA damage and mitochondrial dysfunction** *Speaker: Jéssica Ellen Barbosa de Freitas Lima - Doctoral student FMRP-USP, Ribeirão Preto, SP*	Room 3
11:25 - 11:45	**Biomonitoring of Human Genotoxicity Induced by Complex Occupational Exposures** *Speaker: Juliana da Silva - ULBRA & UniLaSalle - Canoas, RS*	Room 3
12:00 - 12:30	**AWARD FOR ORAL PRESENTATIONS AND POSTERS** **CLOSING SESSION**	Room 1

CONFERENCE NUMBERS

Registrations:

Professionals - 92
Postgraduate Students - 90
Undergraduation Students- 35

Abstracts Submited by Area:

Applied Toxicological Genetics - 27
Carcinogenesis / Oncogenetics - 26
Genotoxic Risk Assessment and Public Health - 25
Environmental Mutagenesis - 22
DNA Repair - 11
Nutrigenomic - 10
Epigenomic - 5
Genomic Instability - 5
Toxicogenomic and Bioinformatic - 4
Germ Cells and Hereditary Effects - 1

Registration by state:

São Paulo - 87
Paraná - 46
Rio de Janeiro - 23
Minas Gerais - 17
Rio Grande do Sul - 9
Mato Grosso do Sul - 9
Rio Grande do Norte - 7
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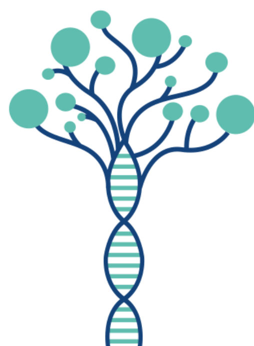
October 4th to 7th, 2023 | Londrina - PR



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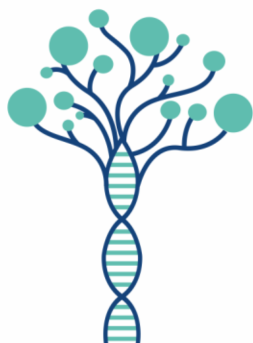
Abstracts - Speakers



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Satellite Event

SATELLITE EVENT: Genomic Instability and Cancer

Chair - Enilze Maria Fonseca Ribeiro - UFPR, Curitiba, PR

Talk: Basic aspects of genomic instability and cancer.
Speaker: Daniela Fiori Gradia - UFPR, Curitiba, PR

Genomic instability is a hallmark of cancer, contributing to tumor progression and heterogeneity through the accumulation of oncogenic and cancer suppressor gene mutations. Several surveillance mechanisms, including DNA damage checkpoint, DNA repair machinery, and mitotic checkpoint, fine-tune the genomic integrity. Dysregulation of any of these processes often results in genomic instability, predisposing the cell to malignant transformation. For example, mutations or other gene alterations that control cell growth and division can produce abnormal proteins that promote uncontrolled cell growth. Similarly, chromosomal rearrangements or changes in copy number can disrupt the normal functioning of genes involved in DNA repair, cell cycle regulation, or other critical cellular pathways. Other than mutations on key genes related to these processes, posttranslational modifications in histones and DNA methylation status is also involved in genomic stability maintenance. More recently, aberrantly expressed lncRNAs have been found to promote genomic instability. Although the exact mechanism is unknown, cancer cells have a tolerance limit to genomic instability, suggesting that a genomic destabilization is a therapeutic approach to cancer.

Talk: Chemical Repair of Etheno DNA Adducts by Singlet Molecular Oxygen
Speaker: Glaucia Regina Martinez - UFPR, Curitiba, PR

Hulyana Brum[1]; Ester Mazepa[1], Fernanda Prado[2]; Guilherme Lanzi Sassaki[1]; Paolo Di Mascio[2], Marisa Medeiros[2]; Glaucia Regina Martinez[1].
[1] ***UFPR, Curitiba, PR***
[2] **IQ/ USP, São Paulo, SP**

Etheno DNA adducts are deleterious DNA lesions with significant role in mutagenesis and carcinogenesis processes. They are induced by occupational and environmental carcinogens or by products of lipid peroxidation. Our aim was the elucidation of chemical mechanisms involved in the oxidation reactions of ethenoadducts, namely 1, N^2-etheno-2'-deoxyguanosine (1,N^2-εdGuo), 1, N^6-etheno-2'-deoxyadenosine (1,N^6-εdAdo) and 3, N^4-ethenocytosine (3,N^4-εCit), by singlet molecular oxygen ($^1O_2$) through analysis and characterization of generated products mainly by mass spectrometry. We demonstrated that $^1O_2$ can react with the 1,N^2-εdGuo through cycloaddition [2+2] resulting in 2'- deoxyguanosine (dGuo) as a final product. Oxidation of 1,N^6 -εdAdo resulted in the formation of *N*-formyl-dAdo and dAdo products. The structures were determined by LC-MS/MS. We proposed that $^1O_2$ reacts by cycloaddition [2+2] with the etheno group forming an unstable intermediate product, *N*-formyl-dAdo, which cleaves and losses a formyl group resulting in the dAdo as a final product when incubated at 37°C. Oxidation of 3,N^4-εCit resulted in the formation of cytosine-glycol, *N*-formyl-cytosine, and cytosine. Interestingly, cytosine-glycol and *N*-formyl cytosine decomposed to cytosine as final product when incubated at 37°C. Then, data obtained in this work suggest that the reaction of $^1O_2$ with 1,N^2-εdGuo; 1, N^6-εdAdo and 3, N^4-εCit resulted in the formation of dGuo, dAdo and cytosine as final products, respectively. Therefore, we suggest the possible role of $^1O_2$ in the cleanup of ethenoadducts by regenerating the normal nucleobase as chemical repair mechanism of these lesions.

Acknowledgments: CNPq – Conselho Nacional de Pesquisa e Desenvolvimento and CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Talk: MicroRNA-mediated Modulation of Oncogenic SignalingPathways in Breast Cancer
Speaker: Luciane Regina Cavalli- Research Institute Pelé Pequeno Príncipe, Curitiba, PR; Lombardi Comprenhensive Cancer Center, Georgetown University, Washington DC, USA.

Breast cancer is a heterogeneous disease marked by distinct molecular subtypes that impact the prognosis and clinical outcome of the patients. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and play critical roles in breast cancer development and progression. In this talk, it will be discussed the dysregulation of miRNAs in the context of the molecular breast cancer subtypes, their integration with copy number data, and their impact on oncogenic signaling pathways, such as the PIK3/AKT. It will be presented *in vitro* assays of selected breast cancer cell models using transfection systems to inhibit and ectopically induce miRNA expression demonstrating their role in modulating aggressive tumor phenotypes, including cell proliferation, invasion, and drug resistance. Finally, the therapeutic potential of the dysregulated miRNAs will be discussed based on the miRNA targets and downstream genes of the affected signaling pathways and corresponding tumor phenotypes.

Talk: Exposição Humana aos Agrotóxicos e suas Implicações no Câncer de Mama.
Speaker: Carolina Panis - UNIOESTE, Francisco Beltrão - PR

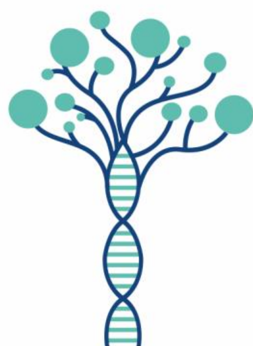
Serão apresentados dados dos últimos 9 anos sobre a pesquisa conduzida no Laboratório de Biologia de Tumores da Unioeste em parceria com diversas instituições nacionais e internacionais, demonstrando o impacto da exposição ocupacional aos agrotóxicos na epidemiologia do câncer de mama no Sudoeste do Paraná, bem como suas implicações clinicopatológicas referentes aos desfechos de pior prognóstico e os mecanismos moleculares associados.

Talk: The Role of Genomic Instability in Cancer Immune Response
Speaker: Patricia Araujo-Souza, Universidade Federal do Paraná, Curitiba, Brazil

Genome instability leads to genetic diversity in tumor cells, which can generate neoantigens that propitiate immune recognition and favor tumor destruction by T cells. However, the complexity of cellular composition and pathways activated in the tumor microenvironment can also contribute to tumor immune evasion. The knowledge of the molecular pathways involved in immune escape has enabled the development of cancer immunotherapies, such as the treatment with immune checkpoint inhibitors. This therapy is based on immune recognition of tumor antigens and has revolutionized the management of patients with immunogenic tumors, improving the clinical response and overall survival rate. We will discuss the crosstalk between genomic instability and cancer immune response, highlighting the advantages and challenges.

Talk: Genomic Instability in Human Cancer: Biomarkers and Applications
Speaker: Vanessa Kozak - UFPR & Hospital Erasto Gaertner, Curitiba, PR

Extensive research on the cancer hallmarks throughout the years have led to a better understanding of the mechanisms behind disease development and progression and allowed the exploitation of possible vulnerabilities of cancer cells as treatment targets. Molecular alterations underlying genomic instability can indicate susceptibility to certain therapies; examples include microsatellite instability as a biomarker to immune checkpoint inhibitor response; and loss of heterozygosity, large-scale state transition and telomeric allelic imbalance as indicators of homology repair deficiency and susceptibility to poly (ADP-ribose) polymerase (PARP) inhibitors. In this talk, we will discuss the main types of genomic instability in cancer, their clinical applications in targeted therapy and challenges in the identification of biomarkers to better guide patient selection.



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MutaGen Schools

MUTAGEN SCHOOL 1 - From 2D to spheroids and organoids: the next generation of cell culture models applied to toxicogenomics

Talk: **O uso de esferóides como modelo para estudos de genética toxicológica**
Speaker: Mário Sérgio Mantovani – UEL, Londrina, PR
Alguns dos avanços *in vitro* mais promissores têm propiciado analisar as células mimetizando as condições naturais. Em vez de olhar para imagens de células bidimensionais, por exemplo, os pesquisadores estão utilizando cada vez mais as culturas que crescem em três dimensões. Os pesquisadores já sabem que passar do sistema 2D para o 3D pode fazer a diferença na busca crescente por informações experimentais cada vez mais úteis para a saúde humana. As células podem apresentar comportamento diferente quando examinadas em placas que propiciam a formação de esferóides 3D, em relação às células estudadas em monocamadas 2D. Certamente, muitos outros sistemas celulares, possivelmente todos eles, funcionam de forma diferente no crescimento plano (monocamada) versus formas tridimensionais. O uso *in vitro* de modelos celulares avançados, incluindo esferoides, microtecidos, organoides e tecnologia *organ-on-a-chip*, estão se tornando cada vez mais amplamente adotado para investigar patologia de doenças, eficácia de medicamentos, segurança e toxicidade. Assim, comparações dos sistemas 2D e 3D são pertinentes na aplicação em genética toxicológica. Aqui vamos tratar dos sistema 3D-esferoide. Todos os clássicos ensaios de mutagênese têm sido adaptados para as condições de cultura 3D, com particularidades em cada sistems-teste. Os principais são os estudos de viabilidade celular (ex: resazurina), clonogênicos (formação de colônias), ensaio do cometa, estudos por citometria de fluxo das fases do ciclo celular, tipos de morte, espécies reativas de oxigênio (EROs) e expressão gênica. As boas práticas de cultura celular vão determinar o sucesso da transposição 2D para o 3D. Assim, a experiência no sistema 2D parece ser fundamental para a transposição para o sistema 3D. O sistema de 3D-esferoide *in vitro* é capaz de fornecer todos os *endpoints* atuais exigidos pelas agências reguladoras e podemos considerar um sistema simplificado, livre de animais e mais relevante para o ser humano do que os ensaios atuais. A análise de imagem e fotodocumentação experimental de células vivas tem um papel importante nesse contexto de acompanhamento e registro da informação 3D, juntando a análise morfológica aos métodos clássicos de genética toxicológica. O uso de marcadores fluorescêntes trazem contribuições importantes nessa análise toxicológica. Linhagens celulares clássicas, como as hepáticas (ex: HepG2) têm sido usadas com sucesso nos modelos 3D e são muito promissoras. A observação das zonas características do esferóide maduro (zona proliferativa, zona quiescente, núcleo necrótico) permitem avaliar o uso adequado desse sistema. Assim, culturas de células tridimensionais (3D) são ambientes criados artificialmente nos quais as células podem crescer ou interagir com seus arredores de forma 3D. As culturas de células 3D melhoram a função, diferenciação e viabilidade das células e recapitulam o microambiente *in vivo* em comparação com as culturas de células 2D convencionais. Os sistemas 3D fornecem uma plataforma de triagem fisiologicamente relevante, imitando as respostas *in vivo*, para muitos tipos de células, incluindo o câncer e células-tronco na morfogênese do desenvolvimento, farmacologia, metabolismo de drogas e estudos de toxicidade.

Speaker: Raul Ghiraldelli Miranda - FCFRP- USP, Ribeirão Preto, SP
Alternative methods to the use of animals in teaching and research have been gaining ground, in addition to being an international trend. Cell culture is one of the main methodologies used for toxicological evaluation, however, monolayer cell culture (2D) may be flawed in some respects, especially in terms of extrapolation of data from the *in vitro* to the *in vitro* environment. In this way, 3D cell culture emerges as an alternative to cover this space between monolayer cell culture and the *in*

vivo environment, being able to bring more reliable predictions to the effects that would occur in the animal and/or the human. In this way, we will address the versatility of three-dimensional (3D) culture applications, showing some examples of 3D culture methods, advantages and disadvantages and examples of applications within toxicological and toxicogenomic evaluation with a practical view of their use.

MUTAGEN SCHOOL 2 - Zebrafish model for genotoxic assessment of aquatic contaminants

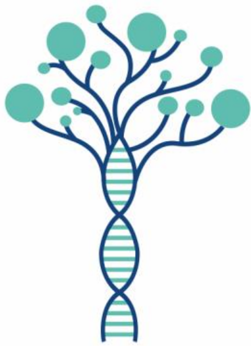
Talk: The zebrafish (Danio rerio) model for genotoxic/embriotoxic assessment of aquatic contaminants
Speaker: Iara Silva Squarisi – UNIFRAN, Franca, SP
Speaker: Cesar Koppe Grisolia – UnB, Brasília, DF
The zebrafish is a quite versatile *in vivo* experimental model, widely used in the different research area. It has been used in the assessment of the genotoxicity and embriotoxicity of pollutants in water. We will discuss the design, maintenance, and importance of studies on zebrafish embryos and larvae. Different types of research can be performed, and how they can be applied in studies of water contamination. Fish embryos represent an interesting model for assessing the environmental risk of chemicals, as they provide the opportunity for small-scale, high-throughput testing. The micronucleus (MN) test is a simple and fast method to study chromosome breaks or malsegregation in zebrafish peripheral erythrocytes. The slides stained by Acridine Orange allow more accurate analysis. The comet test (*Single Cell Gel Electrophoresis*) is also routinely performed on peripheral erythrocytes to study DNA breaks, following the conventional protocol. There is also a protocol for comet testing in zebrafish larvae, using a pool of homogenized larvae. As the zebrafish genome was already sequenced, different toxicogenomic studies can also be carried out, using the RT-PCR technique to analyze the expression of a single gene, or transcriptomics to have a wide view of gene expression changes. There are protocols for performing acute tests and chronic tests. It is easy to build and maintain a zebrafish facility. There are guidelines providing instruction for building zebrafish facilities at low-cost.

MUTAGEN SCHOOL 3 - Dietary natural products and bioactive compounds as candidates for chemoprevention

Talk: Evidências da Influência Quimiopreventiva de Compostos Bioativos, Derivados Semissintéticos e Dietas
Speaker: Pollyanna Francielli de Oliveira
O uso de agentes naturais, sintéticos e semissintéticos, desenvolvidos com base na estrutura de produtos naturais e até mesmo padrões dietéticos quando utilizados para minimizar a ocorrência de câncer em indivíduos é definido como quimioprevenção do câncer. De modo primário, os agentes quimiopreventivos inibem o desenvolvimento da doença impedindo o dano ao DNA que leva à malignidade, ou atuam revertendo ou, ainda, bloqueando a divisão de células pré-malignas geneticamente danificadas. No entanto, de maneira mais específica, os quimiopreventivos podem atuar nas etapas de progressão e promoção tumoral. O aumento contínuo dos casos de câncer, a falha das quimioterapias convencionais para controlar a doença e a toxicidade excessiva das quimioterapias clássicas exigem abordagens diversas. O sucesso do uso de agentes quimiopreventivos para proteger as populações de alto risco de câncer indica que a estratégia é racional e promissora. Logo, a capacidade quimiopreventiva desses bioativos representam uma alternativa importante para a modulação das vias moleculares envolvidas nas diferentes etapas da carcinogênese. Essas vias

representam respostas celulares coordenadas em decorrência da exposição celular ao estresse genotóxico e envolvem o controle da proliferação, crescimento e metabolismo, imunidade, identidade e controle do ciclo celular, resistência à apoptose, vida útil replicativa etc. Portanto, estratégias de quimioprevenção baseadas em química medicinal requerem um entendimento profundo dessas vias moleculares. Os mecanismos clássicos podem envolver a ativação de enzimas de eliminação de radicais livres, controle da inflamação crônica e regulação de vias de sinalização específicas. Mais recentemente, a epigenética permitiu uma maior compreensão do potencial quimiopreventivo de vários agentes, inclusive os dietéticos. No entanto, considerando que os tumores são geneticamente heterogêneos, diversos podem ser mecanismos de atuação. Ao longo da escola, discutiremos as quais são as evidências que permitem classificar um composto ou uma dieta como quimiopreventivo e quais são as evidências mecanicistas de sua atuação na iniciação, a promoção e progressão tumoral.

Talk: Bioprospecting of Natural Products for Chemoprevention
Speaker: Mariane Aparecida Franco de Godoy - UEM, Maringá, PR
O câncer é uma doença grave que acomete milhões de pessoas anualmente, sendo uma das principais causas de morte no mundo inteiro. Dessa forma, é de extrema importância não somente a investigação de substâncias que possam ser utilizadas no tratamento desta doença, mas também, a identificação de compostos que possam atuar na sua prevenção ou na redução do risco de seu desenvolvimento, bem como, na manutenção da saúde. Nesse contexto, produtos naturais têm grande destaque, pois são fontes de diversos compostos bioativos com ação quimiopreventiva comprovada e que, em sua grande maioria, estão presentes em alimentos utilizados na nossa dieta. Dessa forma, discutiremos como a genética toxicológica pode ser usada na investigação sistemática de compostos naturais presentes na dieta da população, provenientes de plantas, animais e microrganismos, para a identificação de agentes com potencial quimiopreventivo que possam atuar em diferentes etapas do processo de desenvolvimento do câncer, como a iniciação, a promoção e a progressão da doença. Assim, abordaremos os caminhos que devem ser seguidos durante a pesquisa para a obtenção de um composto quimiopreventivo. E trataremos da importância da avaliação da segurança de uso destes compostos, por meio de sistemas-teste *in vitro* como cultura celular, e *in vivo* com modelos animais, para identificarmos e desenvolvermos agentes quimiopreventivos mais seguros e eficazes.



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MUTAGEN SERVICE 1 - The Salmonella/Microssome Test - Results from the 4th Interlaboratory Proficiency Program

Speakers:
Gisela de Aragão Umbuzeiro – UNICAMP, Limeira, SP
Deborah A. Roubicek – CETESB, São Paulo, SP)

Interlaboratory comparisons are essential tools to demonstrate laboratory proficiency in conducting a test. Proficiency tests are compulsory to all laboratories accredited by ISO/IEC 17025, and optional to laboratories recognized as in conformity with GLP principles. Accreditation and Recognition in GLP are provided in Brazil by CGCRE/INMETRO. MutaGen Brasil promoted the 4th Interlaboratory Proficiency Program to assess the technical performance of several laboratories in the Salmonella/microsome assay, also known as Ames Test. All laboratories performing the test in Brazil were invited to participate, whether they were accredited or not. A total of nine laboratories from different parts of Brazil accepted our invitation. To maintain confidentiality, each lab received a code from the MutaGen Brasil secretary, not known to the study coordinators. Test materials were substances that are routinely used as positive controls, 4NQO (4 nitroquinoline) and 2AA (2-aminoanthracene). Each laboratory was responsible for the acquisition of the two substances. The laboratories were asked to provide dose response curves with *Salmonella* strains TA98 and TA100, with and without in vitro metabolic activation. Methodology and report should follow OECD Guideline 471 - Bacterial Reverse Mutation Test. In this session, we will have a discussion comprehending the importance of the participation in interlaboratory studies, all aspects of the methodology of the test, the results sent by the labs and their reports.

Speaker: Errol Zeiger – NIEHS Consulting - USA

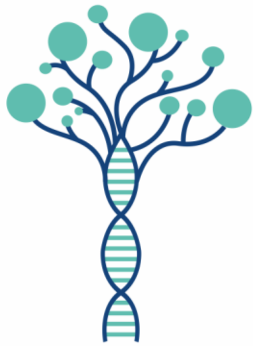
The Ames test for mutagenicity was developed in the early 1970's and has probably been the subject of some of the most introspective studies, including its inter- and intra-laboratory reproducibility. Fifty years after its development there still is interest in measuring its reproducibility primarily as a means to evaluate the performance of laboratories with limited demonstrated experience with the test, or to evaluate a proposed new or modified version of the test. This may be partly in response to the recent OECD test guidelines that require evidence of the laboratory's ability to perform and interpret genetic toxicity tests. Although the current OECD guideline for the Ames test (TG 471) does not require such evidence, there is a possibility that such a requirement will be inserted into future revisions of the guideline. This presentation will describe the uses and design of an inter-lab study, which includes record-keeping requirements.

MUTAGEN SERVICE 2 - In vitro Reconstructed Human Epidermal Model (RHE) as an Alternative Method to the Use of Animals and its Applications:

Speaker: Heloiza Diniz Nicolella - UFSCar, São Carlos, SP

A comunidade cientí5ica reforca cada vez mais seu compromisso em aplicar o princípio dos 3R's (*reduction*/redução; *replacement*/substituicão; *refinement*/refinamento) em relação ao uso de animais na pesquisa acadêmica e na indústria. Além disso, há um consenso entre esses setores de que existe uma lacuna na pesquisa translacional, uma vez que os resultados obtidos nos ensaios de fase pré-clínica não predizem com precisão as respostas nos testes clínicos. No Brasil, o Conselho Nacional de Controle de Experimentacão Animal (Concea) publicou uma resolucão, com vigência imediata, que proíbe o uso de animais em pesquisa, desenvolvimento e controle de cosméticos, produtos de higiene pessoal e perfumes, em atencão à demanda da sociedade e às prá ticas internacionais. Nesse sentido, cresce a busca por métodos alternativos para avaliar a seguranca e eficácia biológica de produtos, garantindo a qualidade e fidedignidade dos resultados. Assim, o modelo de pele humana

reconstruída *in vitro* (modelo RHE) é uma tecnologia que mimetiza a epiderme humana, com alta correlacão *in vitro-in vivo.* A sua estrutura abrange parâmetros cinético e metabólico, que são fundamentais para a avaliacă o de risco e a tomada de decisão dos órgãos reguladores. O modelo RHE é, portanto, um importante método alternativo ao uso de animais, capaz de atender as atuais exigências regulató rias e interesses das indústrias, uma vez que permite a sua aplicacão também para avaliacão de segurança toxicológica. Recentemente, ensaios para avaliacão da toxicidade genética de substâncias/produtos de exposicão dérmica em modelos RHE foram pré-validados e aceitos pelo programa de desenvolvimento de *guidelines* OECD, reforcando o potencial da tecnologia.



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OPENING CONFERENCE: ***Policies in Higher Education in Brazil***

Speaker: Denise Pires de Carvalho - Secretariat of Higher Education of the Ministry of Education – Brasilia, DF; UFRJ - Rio de Janeiro, RJ

CONFERENCE 2: The potential causative role of acquired Mitochondrial DNA damage in diabetic complications.
Chair: Elza Tiemi Sakamoto-Hojo - FFCLRP-USP - Ribeirão Preto, SP
Speaker: Afshan Malik: King's College London, London, United Kingdom

As diabetes reaches epidemic levels, the associated multi-morbidities have become a growing drain on health systems worldwide. Diabetes results in increased risk of diabetic complications which affect major organs, including kidneys (nephropathy), eyes (retinopathy), heart (diabetic cardiomyopathy), blood vessels (peripheral vascular disease) and brain (dementia). Numerous complex and overlapping biochemical pathways, including oxidative stress and chronic inflammation, are implicated, however strategies to target pathways, correct the redox balance and/or inflammation have been largely unsuccessful, suggesting there may be other factors contributing to progression. To date genetic studies have identified numerous loci with small and additive effects, but generally there has been little advancement in identifying genetic risk of diabetic complications. The multi-organ nature of diabetic complications resembles mitochondrial genetic disease. Mitochondria, cellular organelles in the cytosol of eukaryotic cells, harbour their own circular DNA genome (mtDNA). In patients with mitochondrial genetic disease, specific mtDNA mutations can lead to diabetes, as well as symptoms involving the kidney, heart, eyes, muscle, nerves and brain. These rare genetic mtDNA mutations that cause acute disease analogous to chronic diabetic complications could provide insight into the mechanisms of organ damage. This view is strengthened by our findings of mtDNA damage in experimental models of diabetes and in clinical samples from patients. The body of evidence linking diabetes and mitochondrial dysfunction is growing, however the specific mechanisms of damage to mitochondria, and the implications of this for the risk of diabetic complications and metabolic disease have not been elucidated. In this talk I will review data from my own group and from others to examine a novel hypothesis that diabetes-induced damage to mtDNA contributes to diabetic complications and may contribute to processes leading to common metabolic disease.

CONFERENCE 3: Application of In Vitro New Approach Methodologies to Determine Whole Mixture-Based Relative Cancer Potency Factors of Environmental Pollution
Chair: Silvia Regina Batistuzzo de Medeiros - UFRN - Natal, RN
Speaker: Kristian Dreij - Karolinska Institutet - Stockholm, Sweden

Air pollution and airborne particulate matter (PM) are classified as carcinogenic to humans, but their complex composition makes quantitative risk assessment a challenge. Current strategies for cancer risk assessment of air pollution are based on a pollutant-by-pollutant approach. This is clearly a simplification which excludes the possibility of interaction effects and may misestimate the actual cancer risk. Whole mixture-based testing using in vitro new approach methodologies (NAMs) has been suggested to facilitate the hazard and risk assessment of complex environmental mixtures. We have addressed this issue by developing a NAM for whole mixture-based cancer risk assessment of air pollution. The overall aim is to combine state-of-the-art methods for analysis of chemical

composition of urban, diesel and biomass burning PM with in vitro testing of PM samples in order to determine Mixture Potency Factors (MPFs) estimating the carcinogenic potency of whole mixtures. Our results so far show that MPFs based on whole mixtures better indicate cancer potency than looking at single pollutants. Moreover, that these MPFs are in good agreement with potency values based on published data from Salmonella mutagenicity and in vivo carcinogenicity studies. This research will develop an approach that can be used for assessment of total carcinogenic effects of air PM pollution both for larger city-wide and for smaller site-specific risk assessments. Ultimately, this in vitro NAM will improve the cancer risk assessment of environmental pollution by including the obtained knowledge about whole mixture potencies in already established models for estimation of cancer incidences in polluted environments.

CONFERENCE 4: Mutational Profile of Cancer in Admixture Ancestry Brazilian Patients
Chair: Diego Luis Ribeiro - USP, São Paulo, SP
Speaker: Rui Manuel Reis - University of Minho, Braga, Portugal

CONFERENCE 5: Genome Instability, DNA Damage and Aging
Chair: Lucymara Fassarella A. Lima - UFRN, Natal, RN
Speaker: Carlos F. M. Menck - USP, São Paulo, SP

Human genetic diseases defective in DNA damage repair have revealed many aspects causing the carcinogenic processes. Xeroderma pigmentosum (XP) patients, for example, have an increased frequency of skin tumors due to defective nucleotide excision repair (NER), which generally remove pyrimidine dimers induced by the ultraviolet component of sunlight. In cells unable to remove or correctly process those lesions, more mutations are induced, causing cell transformation and, thus, cancer. However, close to twenty to thirty percent of XP patients may also develop neurological developmental problems and premature aging phenotypes. Moreover, other human diseases defective in NER also show phenotypes related to neurological problems and premature aging, with examples of Cockayne's syndrome (CS) and trichothiodystrophy (TTD). In common, the cells from all these patients are defective in the transcription-coupled subpathway of NER (TC-NER), indicating that transcription blockage by accumulating DNA damage may cause their clinical phenotypes associated with aging. These concepts led to the hypothesis that aging could directly result from endogenous DNA damage accumulation during the lifetime, which would block transcription leading to stem cell death, decreased tissue regeneration, and increased degenerative processes. Although studies have been performed for almost four decades, the indication of which would be the endogenous DNA damage and how they would result in aging are still challenging questions. Our group investigates how fibroblasts and stem cells from CS and TTD patients respond to induced oxidative stress to simulate potential endogenous DNA damage. Comparing oxidative stress in stem cell lines from the same patients resulted in different sensitivity and differential genes expression in transcriptome analyses. However, cell cycle progression and RNA transcription blockage reveal little, if any, effect of oxidative stress treatment in CS or TTD cells. Interestingly, differentiating cortical organoids from CS-iPS cells revealed that the organoids lose their standard structure and the formation of TAU protein aggregates compared to normal organoids. Although these cell models have limitations, we are finding hints about possible events that link genetic instability, DNA damage, and aging processes.

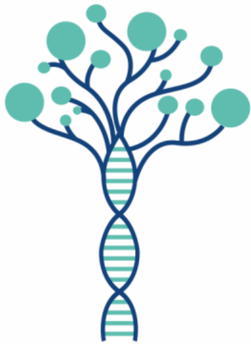
Financial Support: FAPESP (Proc.# 2019/19435-3), CNPq and CAPES, Brazil.

CONFERENCE 6: Uncovering Cell-Cell Communication in Health and Disease through Single-Cell and Spatial Transcriptomics
Chair: Enilze Maria Fonseca Ribeiro - UFPR, Curitiba, PR
Speaker: Robson Francisco Carvalho - UNESP-Botucatu, SP

Cell-to-cell communication is a fundamental process for the proper functioning of multicellular organisms. Aberrant communication between cells can lead to various diseases, such as cancer, autoimmune disorders, and metabolic diseases. Single-cell and spatial transcriptomics technologies offer a powerful tool to study cell-to-cell communication at the molecular level, providing insights into the underlying mechanisms of disease. In this presentation, we will discuss the latest advances in single-cell and spatial transcriptomics and how they can be used to uncover the intricate communication networks between cells in health and disease. We will highlight specific examples of how these technologies have been used to identify novel cell signaling pathways and interactions that play a critical role in disease progression. Additionally, we will demonstrate how single-cell and spatial transcriptomics can be used to identify potential therapeutic targets for various diseases. Our presentation will provide a comprehensive overview of the current state-of-the-art for single-cell and spatial transcriptomics and how these technologies can better understand cell-to-cell communication in health and disease.

CONFERENCE 7: Telomeres, DNA Repair and Dietary Interventions
Chair: Wilner Martinez-López - IIBCE, Montevideo, Uruguay
Speaker: Manoor Prakash Hande - National University of Singapore - Singapore.

Telomere dysfunction and DNA damage accumulation have been associated with replicative senescence and chronological ageing. The link between telomeres and ageing is highlighted when derangement in telomere dynamics often leads to premature ageing and age-associated diseases. Caloric restriction has consistently been proven to extend life span and improve age-related disease outcomes. Similarly, prophylactic intermittent fasting, a dietary regimen characterised by periodic restriction of energy intake, has also been shown to extend lifespan through exertion of protective effects against age-related diseases. Intermittent fasting was found to confer protective effects on genome stability by reducing spontaneous chromosomal damage in mice. In addition, intermittent fasting reduced the expression levels of proteins involved in DNA damage response and telomere function. Studies in mice have consistently shown disease-modifying efficacy of intermittent fasting on diseases including obesity, diabetes, cardiovascular disease, cancers, and neurodegenerative brain diseases. However, more focused prospective and follow-up studies with and without interventions are needed to unequivocally link dietary interventions with DNA repair and telomere maintenance in humans. The intricate relationship between dietary components and its potential to protect the integrity of telomeres may provide unprecedented health benefits and protection against age-related pathologies.



XVI CONGRESS OF MutaGen-Brazil

Disseminating knowledge for a sustainable world

Symposiums and Round Tables

SESSION 1: DNA Damage by Natural and Synthetic Agents and DNA Repair in Health and Disease
Chair: Carlos Renato Machado - UFMG - Belo Horizonte, MG

Talk: Interconnection between DNA repair and transcription in response to oxidative stress
Speaker: Lucymara Fassarella A. Lima - UFRN, Natal, RN

Oxidative stress is the primary endogenous source of DNA damage and is associated with several diseases. Despite these adverse effects, oxidative stress plays critical roles in several physiological processes, such as cellular signaling, cell growth, and differentiation. Furthermore, oxidized DNA damage as 8-oxo-7,8-dihydro guanine (8-oxoG) and 5-hydroxymethylcytosine have been described as epigenetic markers involved in transcriptional regulation. Besides, several DNA repair proteins have been described as members of the transcriptional complexes, controlling the expression of various genes. This talk will explore some aspects of the involvement of DNA repair proteins in transcription under oxidative stress.

Talk: NRF2 Modulates Ferroptosis in Temozolomide-Resistant Glioblastoma Cells
Speaker: Clarissa Ribeiro Reily Rocha - UNIFESP, São Paulo, SP

Glioblastoma patients tend to have a poor prognosis with a median survival rate of only 15 months due to temozolomide (TMZ) resistance. NRF2 is an important transcript factor involved in chemotherapy resistance due to its ability to regulate genes related to antioxidant response and to prevent cell death processes such as ferroptosis, an iron-dependent cell death recently described. Thus, this study aimed to analyze how NRF2 modulates ferroptosis in glioblastoma. It was analyzed two human glioblastoma cell lines (U251MG and T98G) after treatment with TMZ and ferroptosis inducers, and it was performed gene expression analysis of glioma patients from the GlioVis portal. Our results demonstrated that T98G compared to U251MG was more resistant to chemotherapy and showed elevated levels of NRF2 expression. Interestingly, T98G revealed higher sensitivity to ferroptosis. In the NRF2-silenced T98G cell line (T98G-shNRF2) there was a significant viability reduction after TMZ treatment. On the other hand, T98G-shNRF2 was resistant to ferroptosis, indicating that NRF2 plays a key role in the modulation of TMZ resistance and ferroptosis induction. Furthermore, it was observed that NRF2 has a positive correlation with its target genes associated with ferroptosis induction such as ABCC1 and HMOX1 in glioma patients, which are related to higher tumor aggressiveness and poor overall survival. In general, our data indicate that high levels of NRF2 may result in collateral sensitivity on glioblastoma through the expression of its pro-ferroptotic targets. Thus, combinatorial treatment between TMZ and ferroptosis inducers may be an important therapeutic strategy to reverse drug resistance.

Talk: Genotoxicological Safety of Multifunctional Biopolymer Films
Speaker: Flavia Aparecida Resende - UNIARA, Araraquara, SP

The exhibition proposes to discuss about the reuse of biomass residues to produce high added value bioproducts. Biopolymer films be explored for application in various segments of the industry, as edible packaging for fruits or other foods, coating of cosmetics, dressings, platforms for the controlled release of drugs at the wound site, as well as for the administration of drugs of systemic and local action, orally, buccal, sublingual, ocular and transdermal, among others. Besides being an ecologically viable alternative to minimize the negative impacts involving the problem of plastics derived from fossil hydrocarbons, also contribute to the reduction of food waste discarded in the

environment. Data on skin regenerative properties, antioxidant potential, and genotoxicological safety analysis will be presented.

Talk: Transcription-associated DNA repair in Trypanosoma cruzi: involvement in death and dormancy
Speaker: Carlos Renato Machado - UFMG - Belo Horizonte, MG
Trypanosoma cruzi is the etiological agent of Chagas disease. This disease is a neglected tropical disease that causes great economic and social impact. *T. cruzi* belongs to a group of organisms that have unique characteristics, such as: the presence of a single mitochondrion and polycistronic transcription. These features have led to adaptations of the nucleotide excision repair system. In this organism, the XPA gene is not present and the XPD gene is associated with transcription but does not participate in DNA repair. Furthermore, the XPB gene is duplicated and one of the copies is involved with DNA repair and the other with the transcription process. Trying to better understand the importance of nucleotide excision repair associated with transcription, we studied the CSB gene in this parasite. CSB is responsible for recognizing stalled RNA polymerase and initiating the DNA repair process. We generate cells deficient in the CSB gene and cells that overexpress this gene. Interestingly, cells overexpressing the CSB gene are more sensitive to cisplatin treatment and UV exposure. We found that death is signaled and dependent on the transcription process. Death was also reversed in the presence of ATR kinase inhibitors. In addition, we verified that the expression of the RNAse H1 gene that degrades the R-loops is also capable of reversing the death process. The data suggest that transcriptional inhibition is capable to generate R-loops that activate the ATR protein that signals for death. We also found that cells modified in the CSB gene have a smaller number of dormant cells than is seen in cells deficient in genes involved in the recombination process that activate ATM kinase. Finally, another relevant data that we verified in cells modified in the CSB gene is that there is a DNA repair associated with transcription in the parasite's mitochondria. This is the first time that this DNA repair pathway has been found in mitochondria and must be associated with the fact that this organism only has one mitochondrion. In this work, we show that the repair process associated with transcription is very important for T. cruzi both in the nucleus and in the mitochondria.

SESSION 2: Genetic Effects of Ionizing Radiation: Environmental and Human Health
Chair: Wilner Martínez-López - Biodosimetry Service and Academic Unit on Radiation Protection, IIBCE-FMED - Montevideo, Uruguay.

Talk: Genome Oxidative Damage in Humans Exposed to High Indoor Radon Levels in Northeast Brazil
Speaker: Viviane Souza do Amaral - UFRN - Natal, RN
The main source of exposure to ionizing radiation of human populations is still derived from the environment, and radon gas is the main element that contributes to natural terrestrial radiation emission. When inhaled, it continues the decay process inside the lungs, where it keeps emitting radiation until becoming stable as lead-210. The radioactivity released in this process can generate mutations in lung tissue, increasing the carcinogenic risk in this organ. In fact, this radioactive gas is the second cause of lung cancer worldwide. Therefore, natural exposure to Rn is a worldwide public concern. Rn concentrations inside dwellings (indoor radon) in some cities and communities could exceed the limits established by the World Health Organization of 100 Bq/m^3. A small city named Lajes Pintadas in Rio Grande do Norte state, Northeast Brazil, is an example of this scenario. The

mean indoor Rn levels are approximately 300 Bq/m^3, reaching maximum values of 4,000 Bq/m^3. Studies showed that chronic exposure to high levels of indoor radon significantly altered urinary 8-OHdG concentrations in inhabitants of Lajes Pintadas city. The Ser326Cys polymorphism in the hOGG1 gene significantly influenced the concentrations of the studied biomarker. Therefore, high levels of this radioactive gas may be associated with an increase in oxidative damage in the genome of exposed individuals.

Talk: Effects of Radiation Exposure on Offspring and Next Generations
Speaker: Manoor Prakash Hande - National University of Singapore, Singapore
Exposure to low dose radiation during pre- or post-conception result in both foetal and natal consequences. Cellular response to radiation, especially during organogenesis is manifold. Effects of pre-conceptional or in utero exposures to radiation may be transmitted to the next generation – the transgenerational effect, which is thought to result from epigenetic phenomenon. There is limited human-based evidence studying transgenerational effects of radiation which mainly focused on A-bomb data, Chernobyl and similar radiation exposures. Both human and animal data will be reviewed in the presentation. In this regard, it is imperative to appreciate the pathways of metabolic and endocrine intrauterine effects exaggerated by environmental factors such as smoking, alcoholism, obesity, diabetes mellitus, polycystic ovarian syndrome, pharmacological agents, etc. These environmental and life-style factors can increase the sensitivity of foetal cells to radiation-induced damage. Exposure to environmental toxicants, pesticides, herbicides, endocrine disruptors, prior to and/ or during conception generate an intrauterine environment of high oxidative stress. Moreover, while determining the effects of radiation, it is critical to discuss the (multiple) exposures to routine diagnostic ultrasound during pregnancy. Under such circumstances, effective mitigation of DNA damage and commencement of repair processes may be hindered. Individual genetic susceptibility varies depending on the race, dietary pattern, lifestyle, ethnicity, socio-economic conditions etc. Further research should focus on combined exposures of both environmental and physical agents. The presentation will review the literature and critically evaluate the potential influence of environmental confounding factors in radiation-induced changes in developing embryos and/or offspring.

Talk: Radiation Induced Mutations and Carcinogenesis
Speaker: Marina Di Giorgio - Autoridad Regulatoria Nuclear - Buenos Aires, Argentina
Cancer is best described as a multi-step process originating from single cells that have sustained mutations through DNA damage. Either directly or following the accumulation of additional mutations or epigenetic changes, such cells gain growth advantages or progress to a proliferative and ultimately malignant tumour. Radiation is judged to act most commonly by inducing initiating mutations in proto-oncogenes or in tumour suppressor genes; both proto-oncogenes and tumour suppressor genes have normal cellular functions in cell growth, development, and regulation. Radiation can also induce apoptosis and influence cell-cycle checkpoints, which together can affect the outcome of a radiation exposure. Most evidence suggests that DNA deletions are the major contributors to the mutations driving radiation carcinogenesis. Radiation can influence both initiation and promotion of cancer. DNA damage and repair, particularly double-strand breaks (DSB), genomic instability and epigenetic mechanisms have key roles in carcinogenesis. Cancer cells have to out-compete nearby cells for nutrients and other resources, avoid immune cell attack, and suppress apoptotic self-destruction. Due to the aberrant proliferation associated with cancer cells, there is an increased tendency of genomic changes and mutations that contribute to the damage of multiple genes regulating cell division and tumor suppression. This is known as genomic instability. Genomic instability tends to be exacerbated in cancer cells, as mutations that improve survival increase the

likelihood that those mutations will spread in future cells. Most mutations are not related to cancer. They can be spontaneous or the result of environmental insults such as chemicals and radiation. Despite the high probability that such mutations can occur, DNA remains relatively free of errors. Genome surveillance and maintenance systems, mitotic checkpoints, and DNA repair mechanisms mitigate common everyday factors that attempt to mutate the genetic code. A defect in any of these systems can increase the susceptibility of DNA to mutations, leading to genomic instability and increased risk of malignancy. One such mechanism is the G2/M DNA damage checkpoint, which serves to prevent the cell from entering mitosis (M-phase) with genomic DNA damage, facilitating genome surveillance and DNA repair. There are several key proteins involved: DNA-dependent protein kinase (DNA-PK), a serine/threonine kinase complex composed of a heterodimer of Ku proteins (Ku70/Ku80) and the catalytic subunit DNA-PKcs, is deployed to the site of double-stranded DNA breaks almost instantly to initiate repair via non-homologous end joining.• BRCA1 and BRCA2, two tumor suppressors that are found in breast and other tissue, contribute to DNA repair, chromosomal stability and transcriptional regulation in response to DNA damage. Studies have shown that, in response to DNA damage, BRCA1 is hyperphosphorylated and translocated to specific sites within the replication fork. BRCA1 has also been shown to regulate the expression levels of several genes activated in response to DNA damage. In addition, BRCA1 is required for the S-phase and G2/M-phase mitotic checkpoints. BRCA2 plays a slightly different role than BRCA1 and is predominantly active in maintaining chromosomal stability and mitotic recombination. Both BRCA1 and BRCA2 have been shown to repair double-stranded DNA breaks via homologous recombination. Chk1 and Chk2 are key signalling transducers that are part of a complex network of gene integrity checkpoints, damage detectors and tumour suppressors. p53 is also known as the “guardian of the genome” for its role in conserving genomic stability. p53 plays a central role in in a pathway that recognizes and mitigates oncogenic stress by halting proliferation and inducing apoptosis/senescence in an attempt to allay accumulating DNA damage that could lead to malignancy. Apart from genomic instability arising from compound DNA mutations, aberrant epigenetic modifications can also drastically change functional protein levels and affect genomic integrity. Two epigenetic mechanisms that play important roles in genomic instability are DNA methylation and histone modifications. Hypermethylation and/or hypomethylation of regulatory regions within genes can mimic DNA mutations and promote tumour progression. Furthermore, remodelling of chromatin structure through epigenetic histone modifications may allow chromosome rearrangements leading to chromosome instability. Together, these epigenetic changes may also affect cell cycle progression and checkpoint regulation, further contributing to genomic instability and cancer progression.

Talk: A New Cytomolecular Approach for Detecting Low Doses of Ionizing Radiation

Speaker: Wilner Martínez-López - IIBCE, Montevideo, Uruguay

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It is well known that high doses of ionizing radiation produce a number of adverse health effects in exposed populations. However, much less is understood about the effects of low doses, although there is increasing evidence of its links to cardiovascular disease, neurological disorders, immune dysfunction and cataracts, as well as cancer. New approaches to measure cellular and molecular changes resulting from exposure to low dose radiation will allow to establish causal links to radiation exposure. The coordinated response to radiation-induced DNA double strand break is initiated with epigenetic changes, such as the phosphorylation at Ser-139 of the modified histone H2AX (□H2AX). This phosphorylation expands ≈2Mb around the lesion allowing its visualization as nuclear foci by immunofluorescence. This reaches its maximum 15-60 min post-treatment, decreasing until it disappears (≈ 24h). In the presence of histone deacetylase inhibitors, such as valproic acid, an increase in the half-life of □H2AX has been described due to dephosphorylation to H2AX is delayed. In this

respect, we have been tested if valproic acid enhances the sensitivity of γH2AX to detect low doses of ionizing radiation. Human blood samples from four healthy donors were exposed to γ-rays (0, 0.1 or 0.5 Gy) and treated with valproic acid (0, 0.35 or 0.7 mM). The dynamics of radiation induced γH2AX foci yield was evaluated in lymphocytes at different recovery times after incubation at 37°C employing an automated microscope system named Metafer (MetaSystems, Germany). Thirty minutes after irradiation the lymphocytes exposed to γ-rays and treated with valproic acid did not present significant differences of γH2AX foci yields than the lymphocytes only exposed to γ- rays. On the other hand, from 30 minutes to 3 hours, after 0.5 Gy of γ-rays, the mean number of γH2AX foci per cell decreased 25% in the non-valproic acid treated lymphocytes whereas it increased 50% in the presence of valproic acid. Besides, we have analyzed the kinetics of □H2AX induction/removal after exposure of the human keratinocyte cell line called HaCaT to the radiomimetic agent bleomycin (40 ug/mL for 1h) alone or in combination with valproic acid (0.7 or 1.5 mM), either pre- (24h) or post-treatment, at different post-damage recovery times (1h, 3h, 6h and 24h). For all treatments, the average foci were compared using an automated fluorescence microscopy analysis system (Metasystems, GmbH). Preliminary results suggest a significant increase in the number of foci 3h post-treatment of bleomycin with valproic acid. These results reflect an accumulation of γH2AX foci. Therefore, a sensitization effect to ionizing radiation or radiomimetic agents, produced by valproic acid added even after treatments, would allow to use the □H2AX as a biodosimeter for determining primary damage induced by ionizing radiation in workers repeatedly exposed to low doses of ionizing radiation such as Interventional Cardiologists.

SESSION 3:Ecogenotoxicology
Chair: 1. Israel Felzenszwalb - UERJ, Rio de Janeiro, RJ.

Talk: Risk Characterization of Human Exposure to Polycyclic Aromatic Hydrocarbons: Influence on DNA Damage in Vulnerable Groups.
Speaker: Marília Cristina Souza – FCFRP-USP, Ribeirão Preto, SP

Nowadays, anthropogenic activities are a significant source of environmental pollutants at an alarming rate. Polycyclic aromatic hydrocarbons (PAHs) are widely spread and well-known mutagenic and carcinogenic legacy pollutants of public health concern. In underdeveloped countries like Brazil, limited data are available in the scientific literature on the risk assessment of exposure to PAHs, leading to a risk underestimation. In this context, the presentation will describe the concentrations of PAH metabolites measured in healthy vulnerable groups (n = 400), including pregnant and lactating women, newborns, and children. Besides, the risk characterization of this exposure also will be demonstrated, by calculating estimated daily intake, hazard quotient, hazard index, and cancer risk. Finally, the influence of this exposure on DNA damage will be discussed by the levels of 8-hydroxydeoxyguanosine (8-OHdG) quantified in urine samples, an important oxidative stress biomarker.

Talk: Genotoxic Effects of Pesticides and Nanopesticides on Freshwater Fish: Searching for More Sustainable Agriculture
Speaker: Cláudia B. Reis Martinez - UEL, Londrina, PR

Current agricultural models are strongly dependent on pesticides and chemical fertilizers making agricultural practices one of the main sources of contamination of aquatic environments around the world. These pesticides can produce a myriad of effects on exposed organisms, among them

genotoxic effects. In order to reduce the use of pesticides, the development of nanoparticles as nanocarrier system can promote a sustained release for pesticides and improve their efficacy and safety, reducing waste and the risks to non-target organisms and the environment. Nevertheless, for their safe use, it is essential to investigate the toxicity of nanopesticides to non-target organisms. In this sense, in this presentation we intend to show the genotoxic effects of some of the most applied pesticides in Brazil on freshwater fish, such as the native species *Prochilodus lineatus* as well as in zebrafish *Danio rerio*, as well as the results of some studies which analyzed the effects of nanopesticides on these same biological models.

Talk: New methodological approaches (NAMs) in genotoxicity assays using fish.
Speaker: Taynah Vicari - Faculdades Pequeno Príncipe, Curitiba, PR
Para regular novos produtos químicos, bem como a poluição consequente do processo industrial e agrícola, as agências governamentais regulatórias exigem testes de toxicidade para determinar como as substâncias afetam o meio ambiente e as mais diferentes espécies que o habitam. Atualmente, grande parte as organizações e das agências regulatórias estão priorizando o desenvolvimento e a validação de métodos que avaliem a toxicidade de substâncias sem a utilização de animais e que, por este motivo, seriam chamados de novas abordagens metodológicas (NAMs). NAMs refere-se então a qualquer tecnologia, metodologia ou abordagem que forneça informações sobre perigo químico, exposição ou avaliação de risco (por exemplo, "*organ-on-a-chip*", modelagens computacionais, ensaios químicos). Muitas das substâncias testadas em ensaios ecotoxicológicos acabam tendo como destino final o ambiente aquático – seja ele de água doce ou salgada – impactando a vida das espécies aquáticas. O grupo de interesse e mais representativo no ambiente aquático seriam os peixes, pois além de possuírem uma maior proximidade evolutiva com os seres humanos (por se tratarem de vertebrados), servem também como alimento e importante fonte de proteína na dieta. Desta forma verificaremos quais seriam os desfechos importantes na avaliação toxicológica, especificamente nas avaliações de genotoxicidade em peixes, com o intuito de desenvolver novas abordagens metodológicas que possibilitem a não utilização dos peixes para a realização deste ensaio.

Talk: Danger is in the air
Speaker: Israel Felzenszwalb - UERJ, Rio de Janeiro, RJ
"*Danger is in the air*!" This popular slang is often heard in various situations. But is it only in the air that danger can be found? Only in the air? The study of ecotoxicity is of importance given the need to know the effects that chemical products released into the environment can have on individuals, on populations and communities of organisms, in addition to knowing how humans can be affected. In recent years, a huge variety of chemicals have been produced either intentionally or as by-products. Some are artificial, others, despite having natural occurrence, had their concentration increased in the environment. Ecotoxicology appears precisely to show that the hazard is not only present in the air, but in water and on soil as well. Along with ecotoxicology, ecogenotoxicology appears and thus the active participation of Mutagen-Brasil in the theme with the involvement of many of its members. Ecogenotoxicology has also been used as a legal parameter for regulating the quality of water, effluents and sediment. Conama Resolution 344/04 instituted bioassays for cases of disposal of sediment to be dredged when the concentration of some substances may pose a risk. Conama Resolution 357/05 instituted the use of tests both as a water and effluent quality parameter. In addition to these laws at the federal level, several states have their own laws that regulate and provide guidelines for the use of these tests, such as the states of Rio Grande do Sul, Santa Catarina, Paraná, Rio de Janeiro, São Paulo. With that perspective and evaluating it now, I see that the work we have

been developing for a long time already had an ecogenotoxicological bias. Our presentation will be a reading of the work carried out in the Environmental Mutagenesis laboratory of the Roberto Alcantara Gomes Institute of Biology at the State University of Rio de Janeiro that permeates the air, water and soil, pointing out possible interferences in life.

SESSION 4: Oral Presentation Award - Thematic Axes 1 and 4
Thematic Axe 1: Applied Toxicological Genetics; Toxicogenomics and Bioinformatics;
Thematic Axe 4: Genomic Instability; DNA Repair; Nutrigenomics
Evaluators:
Kristian Dreij - Karolinska Institutet, Stockholm, Sweden
Nadja C. Souza Pinto - USP, São Paulo, SP, Brazil
Sabine Langie - Maastricht University, Maastricht, The Neederlands

SESSION 5: Oral Presentation Award - Thematic Axes 2 and 3
Thematic Axe 2: Environmental Mutagenesis; Genotoxic Risk Assessment and Public Health
Thematic Axe 3: Carcinogenesis/Oncogenetics; Epigenomics; Germ Cells and Hereditary Effects
Evaluators:
Noemi Sandra Tirado Bustillos - Universidad Mayor de San Andres, La Paz, Bolivia
Lusânia Maria Greggi Antunes – FCFRP-USP, Ribeirão Preto, SP, Brazil
Carlos Fernando Araújo Lima – UNIRIO, UERJ, Rio de Janeiro, RJ, Brazil

SESSION 6: Genomic Instability, DNA Damage Repair and Cancer
Chair: Jeniffer Saffi - UFCSPA, Porto Alegre, RS

Talk: The Hereditary Cancer-associated N363K POLE Exonuclease Mutant Causes Replication Stress and DNA Damage in Addition to Enhanced Mutation Frequency
Speaker: Jean Sébastien Hoffmann - Toulouse, France

It is well documented that mutations in the proofreading domain of the replicative DNA polymerase epsilon (Polε, encoded by the *POLE* gene), such the hereditary L424V mutation, cause colorectal- and endometrial cancers with an extreme burden of single nucleotide substitutions. We recently reported that the specific hereditary *POLE* exonuclease mutation N363K predisposes also to aggressive giant cell glioblastomas. To explain this additional aggressiveness of the N363K mutation, we knocked-in this mutation homozygously into human cell lines and compared its properties to knock-ins of the L424V mutation and to a complete proofreading-inactivating mutation (exo-null). We found that N363K cells have higher mutation rates as both L424V- or exo-null mutant cells. In contrast to L424V cells, N363K cells show a growth defect and generate replication stress and DNA damage. In non-transformed cells, these burdens lead to aneuploidy but macroscopically normal nuclei but in transformed cells, they phenocopy the enlarged and disorganized nuclei of giant cell glioblastomas. Taken together, our data characterize a POLE exonuclease domain mutant that not only causes single nucleotide hypermutation, but in addition DNA damage and chromosome instability, leading to an extended tumor spectrum. Our results expand the understanding of the polymerase exonuclease domain and suggest that an assessment of both the mutational potential and the genetic instability might refine classification and treatment of POLE-mutated tumors.

REFS: Labrousse et al., NAR 2023; Vande Perre et al., Familial Cancer 2018

Talk: DNA Replication and Repair Defects in Rothmund Thomson Syndrome
Speaker: Nicolas Hoch - IQ-USP, São Paulo, SP

Rothmund-Thomson Syndrome (RTS) is a rare autossomic human genetic disorder chracterized by poiquiloderma, skeletal abnormalities, sparse hair and nails, as well as cancer predisposition. The disease is currently classified into two sub-groups, with the majority of patients (~60%) classified at RTS type 2, caused by mutations in the *RECQL4* gene that encodes a DNA helicase involved in DNA replication and repair. RTS type 1 accounts for ~10% of patients ans is caused by mutations in *ANAPC1*, which encodes a component of the anaphase-promoting complex/cyclosome (APC/C) complex that is a crucial regulator of cell cycle progression, especially in mitosis. A further ~30% of RTS patients remain undiagnosed. Here we identified a cohort of brazilian patients diagnosed with an RTS-like disorder caused by mutations in DNA2, a helicase/nuclease with multiple functions in DNA replication and repair. Using patient fibroblast cell lines and RNA interference, we show that DNA2 protein levels are reduced in these patients, leading to a deficiency in DNA repair by the homologous recombination pathway, as well as DNA replication defects. We are currently characterizing a shared DNA replication defect observed in cells depleted of *DNA2*, *RECQL4* or *ANAPC1*, which may represent a shared patophysiological origin of the clinical presentation of Rothmund-Thomson Syndrome.

Talk: Clinical Relevance of DNA Repair Modulation in Colorectal Cancer
Speaker: Jeniffer Saffi - UFCSPA, Porto Alegre, RS, Brazil

Colorectal cancer (CRC) is a highly prevalent tumor globally and stands as the third leading cause of mortality worldwide. In Brazil, the southern states are classified as having high incidence rates. The strongest evidence of DNA repair system involvement in CRC pathogenesis lies in the malfunction of mismatch repair (MMR) found in hereditary versions of CRC and around 15% of sporadic cases. Despite advancements in CRC treatments and continuous efforts to optimize the effects of chemotherapy drugs, patient survival rates have not significantly improved in the past two decades. Our research group has been investigating the expression of genes and proteins involved in different DNA repair pathways. Our focus has centered on understanding their impact on prognosis and response to chemotherapy in patients with sporadic CRC, taking into account different MMR statuses. We have correlated these findings with clinicopathological data and current staging tools such as the tumor-node- metastasis (TNM) scale. Our data reveal that the gene and protein expression of base excision repair and double-strand break repair pathways, besides being elevated in tumor tissue, hold prognostic value in CRC. In particular, we found that the up-regulation of MRE11A, a key player in the resection step during homologous recombination, holds a strong clinical value in terms of tumor aggressiveness, sidedness, and the ability to predict patient survival.

SESSION 7: Mutagenesis in Latin America
Chair: Catarina Satie Takahashi - FFCLRP-USP, Ribeirão Preto, SP

Talk: Elimination metabolism and genotoxic damage from exposure to arsenic in drinking water in populations of the Bolivian highlands
Speaker: Noemi Sandra Tirado Bustillos - Universidad Mayor de San Andres, La Paz, Bolivia

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Exposure to arsenic (As) is a major problem in many parts of the world. In fact, it is estimated that more than 200 million people are exposed to arsenic, mainly through contamination of groundwater. Chronic arsenic exposure is associated with adverse effects on human health, such as cancer, cardiovascular diseases, neurological diseases and the rate of morbidity and mortality in the health of the exposed population is alarming. Arsenic has a strong genotoxic potential and is capable of causing DNA damage, such as aneuploidy; micronucleus formation, chromosomal aberrations, deletion mutations, sister chromatid exchange and DNA-protein crosslinks. We recruited 201 women from 10 villages around Lake Poopó. Arsenic exposure was determined as the sum concentration of arsenic metabolites (inorganic arsenic; monomethylarsonic acid; MMA); and dimethylarsinic acid, DMA) in urine (U-As), measured by HPLC-HG-ICP-MS. Efficiency of arsenic metabolism was assessed by the relative fractions of the urinary metabolites. The women had a wide variation in U-As (range 12–407 µg/L, median 65 µg/L) and a markedly efficient metabolism of arsenic with low %MMA (median 7.7%, range: 2.2–18%) and high %DMA (80%, range: 54–91%) in urine. In relation to genotoxic damage results showed no DNA damage in the population participants by the Comet assay.

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Talk: More than 3 Decades of MutaGen-Brazil

Speaker: Silvia Regina Batistuzzo de Medeiros – UFRN, Natal, RN

This presentation will focus on the history of the Brazilian Association of Environmental Mutagenesis and Genomics, as well as its objectives, areas of activity, and activities that it has carried out throughout its existence, alone or together with partnerships, namely ALAMCTA, IEMGS, SBPC and FesBe. The MutaGen-Brazil areas of interest were reviewed in the previous administration and expanded to include Evaluation of Genotoxic Risk and Public Health; Carcinogenesis / Oncogenetics; Germ cells and Hereditary Effects; Epigenomics; Applied Toxicological Genetics; Genetic Instability; Environmental Mutagenesis; Nutrigenomics; DNA Repair; Toxicogenomics and Bioinformatics. This new reformulation needs to be more publicized to attract new members. An exciting information search tool will be presented, such as members, their areas of activity, tools used, and association category, which is the map. After consulting the map, it was found that MutaGen-Brazil is a predominantly female association, with 70% women and 30% men, a percentage that also remains in the presidency or board of directors. Of the professional associates, around 26% receive a CNPq productivity grant, with a higher concentration for both men and women in Brazil's south and southeast regions. In the Northeast, women have more productivity grants than in other states, but no women were observed in the northern region with this type of grant. Each region of Brazil will be analyzed regarding its members, areas of activity, and categories to obtain a diagnosis to search for new members. Many of our members are also part of other associations, such as EcoTox-Brasil and SBTox, and work very well together in essential missions, such as writing documents concerning water quality to be sent to IBAMA and ANVISA to orientate regulations. Finally, the MutaGen tree will soon be released on the website, with information about the doctors trained by members, where they are, which Ph.D.s are formed, and so on.

SESSION 8: Genome Protecting Through Natural Products and Diet

Chair: Vanessa Moraes de Andrade - UNESC, Criciúma, SC

Talk: Inflammatory, Oxidative and DNA Damage Status in Healthy Subjects Based on their Dietary Preferences

Speaker: Goran Gajski - Institute for Medical Research and Occupational Health, Croatia

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The impact of a vegetarian diet on health is still under debate as there is evidence that it can lead to a higher intake of certain micronutrients, while potentially reducing others, thus influencing various metabolic pathways and health-related biomarkers. Therefore, we aimed to investigate the differences in those biomarkers by comparing individuals with different dietary preferences. Our study was conducted on a group of healthy adult vegetarians and matched non-vegetarians practicing a traditional mixed diet. Two groups were analyzed for various biomarkers related to DNA damage, oxidative stress, and inflammation. Additionally, we analyzed their hematological and biochemical profiles, telomere length, bone mineral density, nutrient levels, and toxic elements, as well as the presence of pesticides and mycotoxins. The results revealed different biomarker levels between the two groups, favoring the traditional mixed diet, particularly one rich in fruit and vegetables. This indicates that vegetarians have a lower nutritional status for some nutrients (Ca, Cu, Zn, vitamins B_{12} and D) accompanied by a lower antioxidant defense system (glutathione). They also displayed higher levels of homocysteine and genome damage in the form of micronuclei frequency and DNA strand breaks, along with shorter telomeres. When further dividing the participants into specific sub-groups, less DNA damage was observed amongst omnivorous subjects than vegetarians, with the lowest DNA damage found in females practicing a pescatarian diet. These findings suggest that incorporating animal-derived nutrients as supplements into the diet of this particular group would be beneficial for improving certain health-related biomarkers that were measured. In terms of inflammation, when relating the high-sensitivity C-reactive protein (hs-CRP) values between vegetarians and non-vegetarians, no significant difference was observed between the two groups. However, the level of certain toxic metals (As and Hg) was higher in non-vegetarians. This multi-biomarker approach offers a complex insight into the differences in selected biomarkers related to specific dietary preferences and health outcomes, which could directly benefit clinicians and nutritionists in patient counseling on nutrition and dietetics. Besides, further research in well-defined and sufficiently sized cohorts is needed to provide more exact evidence.

Talk: Studying Nutritional Modulation of DNA Repair: The Value of the Comet Based in vitro DNA Repair Assay

Speaker: Sabine Langie - Maastricht University, The Neederlands

School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, The Netherlands.

The comet assay is widely used as a biomarker assay in human population studies - primarily to measure DNA damage, but increasingly also to assess the capacity of cells for DNA repair. An *in vitro* comet-based DNA repair assay was developed, in which protein extracts from the cells or tissue of interest are incubated with agarose-embedded substrate nucleoids ('naked' supercoiled DNA), containing specifically induced DNA lesions – oxidised bases to measure base excision repair, or UV-induced pyrimidine dimers or bulky adducts to detect nucleotide excision repair activity. It has been applied in cell culture model systems, human biomonitoring and clinical investigations, and animal studies, using isolated blood cells and various solid tissues.DNA repair can be modified by various nutritional factors at different levels, affecting, for example, DNA methylation, gene expression or protein activity. I will briefly explain the use of the comet-based *in vitro* DNA repair assay for the phenotypic assessment of DNA repair activity. Subsequently, I will give an overview of my past and present studies into the nutritional modulation of DNA repair, with a focus on early-life exposures. To conclude I will present some recent results from our meta-analysis of DNA repair data within the hCOMET-COST Action (CA15132).
Acknowledgement: Part of the work was supported by the hCOMET project (COST Action, CA 15132).

Talk: Bioactivity and Protective Effects of Natural Products
Speaker: Andréia Fernandes - UERJ, Rio de Janeiro, RJ

Natural products and the substances derived from them are important for drug discovery and have been used as traditional remedies to treat several diseases. It is essential to know the genomic safety of these substances before their use. Studies related to genotoxicity are essential in characterizing the adverse effects of compounds present in cosmetics, drugs, food additives, natural toxins, nanomaterials, and others. Components present in natural products may protect against cellular damage caused by mutagens. Several studies have shown potential antigenotoxic agents in plants, vegetables, and fruits. Phytochemical activities of natural products and their derivates have been of broad interest. Our group have been investigating the bioactivity and protective effects of natural products, using genotoxicology techniques, including photoprotection, to investigate the efficacy and safety of chemical and biological substances. Generally, genoprotective effects of crude or fraction extracts have been detected using different test models such as *Salmonella*/microsome assay, antimutagenicity, photoames, cytokinesis-block micronucleus assay, photomicronucleus, phototoxicity, cytotoxicity assay and DNA electrophoresis applied through bacterial strains and cultured cells. Findings suggest that several extracts or fractions have antioxidant activities due to phenolic compounds such as quercetin, catechin, flavone, and isoflavone. Antioxidants, reduction of DNA strand breaks, DNA lesions, and micronucleus formation were the observed mechanisms of action of genoprotective substances. Considering the use of natural products for traditional or therapeutic purposes, it is vital to know their efficacy and safety, mainly if the substances derived from natural origin intend to develop as commercial products.

Talk: Maternal Exercise During Pregnancy Modulates Genetic and Biochemical Damage Caused by High Consumption of Fructose in Offspring
Speaker: Vanessa Moraes de Andrade - UNESC, Criciúma, SC

VM Andrade, AP Damiani, ML Magenis LM Longaretti, University of Southern Santa Catarina – UNESC. Criciúma, SC, Brazil.

Obesity have increased dramatically in recent years, as the population has been consuming more frequently high-calorie and high-fat foods, known as fast food. Excess weight is associated with the

development of low-grade chronic systemic inflammation in the adipose tissue, which contributes to the increased generation of reactive oxygen species, resulting in oxidative stress. In addition, obese individuals have decreased antioxidant defenses. This imbalance leads to DNA damage, as observed by several studies that have found that obesity causes damage to the genetic material of individuals, both in rodents and in humans. Thus, studies are being conducted in order to improve complications caused by obesity. Therefore, animal research is using the cafeteria diet, as a model for inducing obesity, since it is based on the consumption of ultra-processed foods. Complementary dietary strategies are currently being studied to prevent and/or improve complications caused by obesity, such as natural compounds with anti-obesity effects. Among these compounds, acerola fruit, melatonin and omega-3 have been standing out due to its antioxidant and anti-inflammatory properties. In an animal test series, groups of male mice were fed on a standard or a cafeteria diet (CAF) for 16 weeks. Subsequently, CAF mice were given additional diet supplements (acerola juice, melatonin and ômega 3) for one month. At the end of the experiment, the animals were euthanized and peripheral blood, liver and bone marrow were removed to perform the Comet Assay and Micronucleus Test. The results indicated that food supplementation with these compounds attenuate DNA damage present in obese mice.

SESSION 9 - Innovations in Mutagenesis and Entrepreneurship: Regulatory Genotoxicity: Applying scientific knowledge to products safety
Chair: André Passaglia Schuch – UFSM, Santa Maria, RS

Talk: How to Assess the Mutagenic Potential of Cosmetic Products without Animal Tests?
Speaker: Desiree Cigaran Schuck - Grupo Boticário, Curitiba, PR
The assessment of genotoxicity in cosmetics poses a challenge for the industry since it involves strategies that do not rely on animal testing. Nevertheless, the Seventh Amendment to the European Cosmetics Directive imposed a ban on the sale of cosmetic and personal care products within Europe that incorporated ingredients subjected to animal testing. There is a pressing demand for the development of enhanced in vitro genotoxicity assays and the adaptation of testing methodologies. These strategies must comprehensively incorporate all available data regarding the chemical characteristics of the test substance or chemical class, including aspects such as structure-activity relationships, metabolic activation pathways, and dermal absorption potential. When establishing in vitro genotoxicity testing protocols, it is paramount to ensure that they demonstrate both sensitivity and robustness, thus guaranteeing the reliability of negative test outcomes. The development and validation of novel or refined testing methodologies must consider the utilization of metabolically proficient primary skin cells, three-dimensional skin models, and cells possessing well-defined metabolic activation capacities, such as genetically engineered cell lines. This predicament presents a formidable challenge, complicating the assessment of the mutagenic potential of cosmetic ingredients without resorting to animal experimentation in numerous instances.

Talk: Regulatory Genotoxicity: Applying scientific knowledge to products safety.
Speaker: Izabel Villela - InnVitro Suporte e Gestão em Toxicologia, Porto Alegre, RS
Evaluate the mutagenic potential of chemicals has been a legal requirement for many years. The general feature of a standard mutagenicity test battery including a bacterial reverse gene mutation test and in vitro and/or in vivo mammalian mutagenicity test. In vitro alternatives to fully replace animal use for this endpoint are widely accepted. However, it’s important to understand the limitations of

these methodologies comparing to the in vivo alternatives and especially how to reduce this limitation and have more predictive results. The choice of a cell line, source and control of the cell line and sterility control are some of the important issues to be addressed during the assay. On the other hand, the standard test battery is devoted to somatic cell mutation, but germ cell mutagen is also a critical issue. The existence of germ cell mutagens is recognized, and a lot is discussed about its effects on reproduction and development.

Talk: From Basic Research to Business in Bioremediation
Speaker: Lucymara Fassarella A. Lima - UFRN, Natal, RN

Oxidative stress is the primary endogenous source of DNA damage and is associated with several diseases. Despite these adverse effects, oxidative stress plays critical roles in several physiological processes, such as cellular signaling, cell growth, and differentiation. Furthermore, oxidized DNA damage as 8-oxo-7,8-dihydro guanine (8-oxoG) and 5-hydroxymethylcytosine have been described as epigenetic markers involved in transcriptional regulation. Besides, several DNA repair proteins have been described as members of the transcriptional complexes, controlling the expression of various genes. This talk will explore some aspects of the involvement of DNA repair proteins in transcription under oxidative stress.

Talk: BioDos: using UV Radiation for Innovation and Entrepreneurship
Speaker: André Passaglia Schuch - UFSM, Santa Maria, RS

Bio Dos is a startup founded in the Laboratory of Photobiology (LabFotoBio) of the Federal University of Santa Maria (UFSM), and our mission is to develop solutions for UV radiation anywhere and at low cost. The founding partners are biologists who have large experience in researches related to the effects of ultraviolet (UV) radiation in organisms and biomolecules, mainly DNA. The business model for BioDos arose due to the difficulties associated with the use of equipment for measuring UV radiation and its acquisition on the market. Before the creation of our company, we developed and patented an equipment capable of measuring solar UVB and UVA radiation and air temperature. Despite our equipment (called RPD-01) measures UV radiation like the other sold in the market, it can measure the entire UV spectrum (UVC, UVB, and UVA) along with temperature and send all collected data to a smatphone app. On the other hand, other UV radiometers are expensive, require frequent calibration and are dependent on the electrical network and/or cable connection for use and data storage in computers, limiting its applications. Proudly, since March 2023, BioDos is one of the companies incubated at the UFSM Technological Park. The company's activities are now focused on concluding the final version for sale of the UV radiometer RPD-01, as well as on the development of a new UV radiation data collection station (called EUV) aimed at preventing the health of the population. Furthermore, we are also working on another UV radiation data collection station for the agricultural environment, which can calculate the efficiency of bioinputs applied to crops against the incidence of solar UV radiation doses in real time. We are also gathering efforts with the team of the Universitary Hospital of UFSM to create a new equipment for environment and instruments asepsis with UVC radiation. Through the established technical partnership with LabFotoBio, BioDos is developing portable UV sensors with low cost, making it possible to take data in the most varied environments and increase knowledge about the influence of UV radiation on health, agricultural and industrial production, as well as in the environment.

SESSION 10: Genomic Instability
Chair: Gláucia Regina Martinez – UFPR, Curitiba, PR

Talk: The ATM Signaling Pathway as a Mediator of Human papillomavirus Mediated Pathogenesis
Speaker: Enrique Boccardo Pierulivo – ICB/USP, São Paulo, SP
High-risk mucosal Human papillomaviruses (HR-HPVs) cause the totality of cervical cancers and a significant proportion of vulvar and vaginal carcinomas. These viruses are also associated with almost half of the cases of penile carcinomas in men, and with an increasing fraction of anal and head and neck tumors in both genders. Infection with these HPV types has been associated with genomic instability, a hallmark of most human malignancies. HR-HPV types express two oncoproteins, E6 and E7, which target specific cellular factors to promote cell proliferation. Furthermore, these proteins induce structural and numerical chromosome alterations and modulate cellular response to DNA damage. Interestingly, cervical cancer derived cell lines rely on the cell DNA damage repair pathways for survival. Synthetic lethality describes a cellular condition in which two (or more) non-allelic and non-essential mutations, which are not lethal on their own, become deadly when present within the same cell. HPV transformed cells represent interesting models for the study of synthetic lethality since E6 and E7 oncoproteins target several signal transduction pathways such as those regulated by p53 and pRb. Here, we systematically inhibited genes involved in DNA damage repair in cells expressing HPV oncogenes. We observed that these cells are highly dependent on the integrity of the ATM/CHK2 signaling axis since its inhibition selectively reduces the proliferation potential of cells expressing HPV oncogenes, with little effect on normal cells. We show that the observed effect depends on the expression of E6 oncogene and on its ability to induce p53 degradation. Our results indicate that inhibition of components of the ATM/CHK2 signaling axis reduces the proliferation potential of p53-deficient cells. This suggests the existence of a synthetic lethal association between CHK2 and p53. Altogether, we show ATM/CHK2 axis is critical for HPV-transformed cells survival and that its inhibition may constitute an alternative therapeutic strategy to treat HPV-associated pathologies.

Talk: Transcription-induced Accumulation of DNA Lesions in XPD/ERCC2 Mutated Trichothiodystrophy Patients Cells
Speaker: Giovana da Silva Leandro - USP, São Paulo, SP
Giovana da Silva Leandro[1,2], Davi Mendes [1], Andres Aguilera[2], Carlos FM Menck[1]
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Mutations in genes that encode nucleotide excision repair (NER) pathway-related proteins, as the helicase XPD, can cause some syndromes as xeroderma pigmentosum (XP) and trichothiodystrophy (TTD). XP patients present the sensitivity to sunlight as the main feature, resulting in a high frequency of skin cancer. However, mutations in XPD/ERCC2 that lead to TTD are associated with neurodegeneration and premature aging symptoms, not presenting susceptibility to cancer. Considering the hypothesis that the aging-related phenotype may be related to transcription-associated genomic instability, we aimed to understand the mechanisms involved in genomic instability in *XPD/ERCC2* mutant cell lines, addressing how transcription and DNA lesions can interfere with TTD and XP phenotypes. We assessed basal levels of DNA lesions by comet assay and immunofluorescence in fibroblasts from XPD mutated XP and TTD patients. In addition, we detected

the influence of some transcription-associated disturbs as the accumulation of DNA-RNA hybrids (R-loops). Moreover, we addressed the role of homologous recombination repair to lead with DNA lesions in those XPD/ERCC2-mutated cells and the influence of the NER nucleases XPG and XPF. Our results showed an accumulation of endogenous DNA breaks in TTD cells that can be decreased by the overexpression of RNAseH1 (responsible for solving DNA-RNA hybrids) and transcription inactivation. Those breaks also depend on the nucleases XPG and XPF. Meanwhile, XP cells presented a decreased number of DNA breaks in basal levels. Furthermore, despite all XPD/ERCC2 mutated cells being UV-sensitive, TTD cells are more able to lead to UV-induced lesions than XP. Curiously, the repair of UV-induced lesions and the repair of the endogenous DNA breaks depends on homologous recombination in TTD cells. Our data suggest that *XPD/ERCC2* might have a role in R-loops processing, and the lack of this protein culminates in the accumulation of those hybrids. Moreover, we suggest that the repair of UV-induced lesions and the R-loops processing is incomplete in TTD cells. Finally, the presence of DNA breaks and the absence of HR in post-mitotic cells may explain the neurodegeneration and the progeroid phenotype in TTD individuals.

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Talk: The Role of Bur1^{CDK9} kinase on DNA Replication Stress Response
Speaker: Francisco Meirelles Bastos de Oliveira - UFRJ, Rio de Janeiro, RJ

In eukaryotic cells, the cyclin-dependent kinase 9 (CDK9) is a positive regulator of transcription elongation. Although previous studies have implicated CDK9 in the control of DNA replication stress (DRS) response, the underlying mechanism remains poorly understood. In this study, we employed a combination of genetic and biochemical approaches to investigate the role of CDK9 in regulating the DRS response in *Saccharomyces cerevisiae*. In yeast, CDK9 is identified as an essential kinase known as Bur1. Our findings demonstrate that a hypomorphic mutant of *BUR1* (*bur1-107*) not only impairs transcriptional elongation but also renders cells sensitive to hydroxyurea (HU). We also showed that Bur1-mediated transcription of ribonucleotide reductase is critical for HU tolerance. Interestingly, our study has uncovered a surprising and novel role of Bur1 on checkpoint-deficient mutants. We found that in *mec1*Δ cells treated with low doses of HU, Bur1 elicits a highly toxic phenotype characterized by increased cell death, acceleration of S-phase progression and accumulation of double-strand DNA breaks. Our data suggests that, when subjected to DRS conditions, Bur1 may have a multifaceted impact on various cellular processes, leading to distinct phenotypic outcomes that depend on the genetic background of the cell.

Talk: Chemical Repair of Etheno DNA Adducts by Singlet Molecular Oxygen
Speaker: Glaucia Regina Martinez - UFPR, Curitiba, PR

Hulyana Brum[1]; Ester Mazepa[1], Fernanda Prado[2]; Guilherme Lanzi Sassaki[1]; Paolo Di Mascio[2], Marisa Medeiros[2]; Glaucia Regina Martinez[1].
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Etheno DNA adducts are deleterious DNA lesions with significant role in mutagenesis and carcinogenesis processes. They are induced by occupational and environmental carcinogens or by products of lipid peroxidation. Our aim was the elucidation of chemical mechanisms involved in the oxidation reactions of ethenoadducts, namely 1, N^2-etheno-2'-deoxyguanosine (1,N^2-εdGuo), 1, N^6-etheno-2'-deoxyadenosine (1,N^6-εdAdo) and 3, N^4-ethenocytosine (3,N^4-εCit), by singlet molecular oxygen ($^1O_2$) through analysis and characterization of generated products mainly by mass

spectrometry. We demonstrated that $^1O_2$ can react with the 1,N^2-εdGuo through cycloaddition [2+2] resulting in 2'- deoxyguanosine (dGuo) as a final product. Oxidation of 1,N^6 -εdAdo resulted in the formation of *N*-formyl-dAdo and dAdo products. The structures were determined by LC-MS/MS. We proposed that $^1O_2$ reacts by cycloaddition [2+2] with the etheno group forming an unstable intermediate product, *N*-formyl-dAdo, which cleaves and losses a formyl group resulting in the dAdo as a final product when incubated at 37°C. Oxidation of 3,N^4-εCit resulted in the formation of cytosine-glycol, *N*-formyl-cytosine, and cytosine. Interestingly, cytosine-glycol and *N*-formyl cytosine decomposed to cytosine as final product when incubated at 37°C. Then, data obtained in this work suggest that the reaction of $^1O_2$ with 1,N^2-εdGuo; 1, N^6-εdAdo and 3, N^4-εCit resulted in the formation of dGuo, dAdo and cytosine as final products, respectively. Therefore, we suggest the possible role of $^1O_2$ in the cleanup of ethenoadducts by regenerating the normal nucleobase as chemical repair mechanism of these lesions.

SESSION 11: Chronic-Degenerative Diseases and Epigenome
Chair: Elza Tiemi Sakamoto-Hojo - FFCLRP-USP, Ribeirão Preto, SP

Talk: Expression of DNA repair genes is modulated during differentiation of olfactory sensory neurons
Speaker: Nadja Souza Pinto - IQ-USP, São Paulo, SP

Fernanda T. Rowies, Caio M.P.F. Batalha, Thiago S. Nakahara, Bettina Malnic, Nadja C. de Souza-Pinto

Olfactory dysfunction is considered a biomarker of several pathological conditions, including age-associated neurodegenerations, glioblastoma and COVID-19. Olfactory sensory neurons (OSNs) are specialized neurons that detect odorants and send olfactory information to the brain through the olfactory bulb. To perform their function, they are in direct contact with the environment, where they are exposed to several environmental toxins such as atmospheric levels of O2 and volatile molecules. Nonetheless, very little is known about DNA damage levels and expression of DNA repair pathways in these cells. Here we measured nuclear and mitochondrial DNA damage in olfactory epithelium (OE) and compared with levels detected in olfactory bulb (OB) and temporal cortex (TC), as a non-olfactory related central nervous system region. Surprisingly, DNA damage was lower in OE and OB when compared with TC, both for nuclear and mitochondrial genomes. Accordingly, expression of representative genes for all excision repair pathways was detected in OSNs. Moreover, expression of most evaluated DNA repair genes was lower in mature versus OSN progenitors, suggesting that DNA repair is downregulated during differentiation. Analysis of single cell expression data confirmed that expression of the most differentially expressed DNA repair genes decreased from progenitor to mature OSNs. Finally, in situ hybridization data showed that APE1 mRNA levels are lower in the mature OSNs layer of the olfactory epithelium, closest to the nasal cavity lumen. Altogether, we show here that DNA repair pathways are relevant in protecting OSNs against DNA damage accumulation and that differentiation through the OE is accompanied by changes in the expression levels of DNA repair genes.

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Talk: The Epigenetic Landscape of Infertility and its Impact on Embryonic Development
Speaker: Cristiana Libardi M. Furtado – UNIFOR, Fortaleza, CE

Epigenetic mechanisms play an essential role in the modulation of the tissue-specific genomic architecture and the expression of genes. Disruption of the epigenetic programme is one of the main mechanisms underlying many human diseases, including neurodevelopmental disorders, cancer, cardiovascular and metabolic diseases, and infertility. Unlike genetic mutations, epigenetic changes are potentially reprogrammable, altering the cell landscape and cell fate in response to environmental changes. Global epigenetic reprogramming occurs during gametogenesis, with the establishment of sex-specific genomic imprinting, and after fertilizationin pre-implantation embryos. However, the genome is in constant interaction with the environment, and lifetime epigenetic reprogramming, whether intrauterine or postnatal inadulthood, contributes to the heterogeneous phenotype of disease. Aberrant DNA methylation,histone modifications and non-coding RNA expression in sperm and oocytes directly affect reproductive outcomes, resulting in reduced fertilization, impaired embryo development and miscarriage. In male infertility, global hypomyelination is associated with poor semen quality in men with varicocele. Additionally, the epigenetic component may be the main cause of anovulatory disorders such as polycystic ovary syndrome (PCOS). Environmentally induced epigenetic changes caused by an adverse intrauterine or postnatal environment may trigger PCOS-like symptoms or associated clinical changes such as endocrine and hormonal dysfunction. The adverse intrauterine environment during early embryonic development, such as androgen excess and metabolic syndrome, may trigger developmental PCOS or induce its associatedcomorbidities after birth. Given the reversible nature of epigenetic mechanisms, non- pharmacological interventions such as exercise and diet may improve metabolic and hormonal alterations in women with PCOS and thereby may impact infertility. The epigenetic changes underlying male and female infertility are important for understanding the physiopathology of the disease with implications for reproductive outcomes and treatment options.

Talk: MicroRNA-mediated Modulation of Oncogenic Signaling Pathways in Breast Cancer
***Speaker: Luciane Regina Cavalli - Research Institute Pelé Pequeno Príncipe, Curitiba, PR* Brazil; *Lombardi Comprenhensive Cancer Center, Georgetown University, Washington DC, USA*.**

Breast cancer is a heterogeneous disease marked by distinct molecular subtypes that impact the prognosis and clinical outcome of the patients. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally and play critical roles in breast cancer development and progression. In this talk, it will be discussed the dysregulation of miRNAs in the context of the molecular breast cancer subtypes, their integration with copy number data, and their impact on oncogenic signaling pathways, such as the PIK3/AKT. It will be presented *in vitro* assays of selected breast cancer cell models using transfection systems to inhibit and ectopically induce miRNA expression demonstrating their role in modulating aggressive tumor phenotypes, including cell proliferation, invasion, and drug resistance. Finally, the therapeutic potential of the dysregulated miRNAs will be discussed based on the miRNA targets and downstream genes of the affected signaling pathways and corresponding tumor phenotypes.

Talk: Potential Therapeutic Strategies Based on the Induction Of Neuronal Differentiation and Neuritogenesis Investigated in Human Neuronal Models for Alzheimer's Disease
Speaker: Elza Tiemi Sakamoto-Hojo - FFCLRP-USP, Ribeirão Preto, SP

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Currently, treatments available for Alzheimer's disease (AD) and other neurodegenerative diseases require the search for new therapeutic modalities that can be effective for patients with AD, aiming to achieve benefits in terms of memory and cognitive capacity. In this context, we have studied potential therapeutic approaches in human neuronal models, and found evidence that acetylcholinesterase (AChE) inhibitors can attenuate neuronal death induced byneurotoxic damage, and the PI3K/AKT pathway seemed to be implicated in the mechanisms. Since the protein encoded by the *PTEN* gene has a role linked to the downregulation of the PI3K/AKT pathway, the method of PTEN knockdown has been applied to look for possible neuroprotective effects against induced-neurotoxic damage and mitochondrial dysfunction. In the present work, we carried out studies to characterize the neuronal differentiation of NPCs (Neural Progenitor Cells) and SH-SY5Y cells (neuroblastoma cell line). Novel AChE inhibitor molecules (TA8Amino and TAHB3, donepezil-tacrine hybrid compounds) were tested under neurotoxic and oxidative stress stimuli, through experiments carried out in undifferentiated and neuron-differentiated SH-SY5Y cells. TA8Amino was capable of inhibiting AChE at non-cytotoxic concentrations after 24 h. Following neuronal differentiation for 7 days,TA8Amino and donepezil increased the percentage of neurodifferentiated cells and the length of neurites, as confirmed by β-III-tubulin and MAP2 protein expression. TA8Amino was also found to act in the activation of PTEN/AKT signaling. In addition, we studied the impact of *PTEN* inhibition (by siRNA), and 50% PTEN knockdown was found to induce morphological changes, such as an increase in neurite size and reduction of cytoplasmic mass in SH-SY5Y cells, following 3 and 7 days, but without changes in proliferation rates and cell cycle kinetics. Furthermore, similar experiments are under way, using NPCs differentiated from iPSCs (induced-Pluripotent Stem Cells), since NPCs can be differentiated in specific cell types, such as neurons and astrocytes. As a whole, the results obtained in neuronal models indicate that the hybrid tacrine-donepezil AChE inhibitors are capable of inducing neurodifferentiation andneuritogenesis, which are important characteristics of potential drugs, and PTEN seems to bea potential therapeutic target for the treatment of neurodegenerative diseases. (Financial support: FAPESP Proc. Nº 2018/21.709-1; CNPq-Proc. Nº 309854/2017-2; CNPq-Proc. Nº 311533/2021-3; CAPES-Finance Code 001).

SESSION 12 - Population Risk and Human Health
Chair: Juliana da Silva – ULBRA; UniLaSalle, Canoas, RS

Talk: Genetic Polymorphisms and Breast Cancer Prognosis in Women Exposed and Unexposed to Agrochemicals
Speaker: Juliana Mara Serpeloni – UEL, Londrina, PR

Juliana Mara Serpeloni[1], Beatriz Geovana Leite Vacario[1], Carolina Panis[2]
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According to the 2023 Estimate of the National Cancer Institute, female breast cancer has the most incidence globally, with 2.3 million (11.7%) new cases, followed by lung cancer, with 2.2 million (11.4%). In the southwest region of Paraná state, whose economy is centered on agriculture and agribusiness, a high incidence (40 to 60%) and mortality (20%) of patients with breast tumors were observed. Women occupationally exposed to agrochemicals had more aggressive tumors when compared to those with breast cancer not exposed. The prevalent agrochemicals in this region are glyphosate, atrazine, and 2,4-D, which are metabolized as xenobiotics by phase I enzymes (CYPs) and conjugated for elimination by phase II enzymes (mainly GSTs, UGTs, and SULTs). Thus, it is essential to investigate polymorphisms in genes encoding these enzymes (i.e., CYP3A4, CYP3A5, UGT2B7, and UGT2B15) as they may influence the incidence and prognosis of the disease and therapeutic response because they are involved in the metabolization of anticancer drugs. The oxidative stress (nitric oxide) measurement was evaluated using high-sensitivity chemiluminescence. The impact of polymorphisms on the occurrence of relapses, chemoresistance, and nitric oxide concentration was analyzed. The main objective of the present study is to favor, in the short and long-term, the implementation of preventive measures and the reduction of costs for the Brazilian Health System (SUS) with the treatment of aggressive relapses in exposed women.

Talk: Genetic Polymorphisms Related to Se and Hg Toxicokinetics Alter Element Levels in Amazonian Riverside Populations Exposed to Hg via Diet

Speaker: Flora Troina Maraslis – Doctoral student, UNIFESP/Santos, SP

Brazilian Amazon riverside villages are among the populations chronically exposed to the highest Hg levels in the world; mainly the organic form MeHg, present in fish, the main source of their animal protein intake. It is known that Se, another element quite present in their diet, can counteract the toxic effects of MeHg. Furthermore, Hg levels in biological fluids can be different despite similar amounts of MeHg intake. That can be related to individual differences such as the presence of polymorphisms in genes related to Hg toxicokinetic. The thioredoxin and glutathione/glutaredoxin systems are important cellular redox systems responsible for preventing oxidative damage, and the metallothionein (MTs) system plays an important role in the balance of the metallic state and the cellular redox. Thus, we studied the effect of *GLRX, GLRX2, TXNRD1, TXNRD2, MT1A, MT1M*, and *MT2A* polymorphisms on Hg and Se levels in a population exposed to high concentrations of mercury in the Tapajós River region, Brazilian Amazon. We observed that *TXNRD2* rs5748469 modulate bHg and pHg levels, and pSe/pHg ratio. *TXNRD1* rs11111979 modify bSe levels. *GLRX* and *TXNRD2* SNPs seem to influence Hg bioavailability, and *MT1M*, Se bioavailability. Hg levels and pSe/pHg ratio are modified according to the amount of fish intake by persons of different genotypes of *TXNRD1*, *TXNRD2* or *MT1M*. This study add evidence to a growing body of literature that shows how genetic polymorphism is capable to modulate Hg and Se body burden.

Acknowledgments: We thank the São Paulo Research Foundation (FAPESP, grants 2013/06033-8, 2018/24069-3, 2021/06695-7 and 2022/03476-5), the National Council for Technological and Scientific Development (CNPq, grant #406442/2022-3) and Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil for financial support.

Talk: Exploring the Link Between Type 2 Diabetes and Alzheimer's Disease: The Role of Hyperglycemia-Induced Oxidative and Neurotoxic Damage, DNA damage and Mitochondrial Dysfunction

Speaker: Jéssica Ellen Barbosa de Freitas Lima – Doctoral student, FMRP-USP, Ribeirão Preto, SP

Type 2 diabetes (T2D) and Alzheimer's disease (AD) are prevalent diseases among the elderly population. Epidemiological studies have shown that T2D patients are at a higher risk of developing AD, with a two to five-fold increased risk compared to those without T2D. T2D and AD share several pathophysiological mechanisms, including insulin resistance, oxidative stress, mitochondrial dysfunction, and inflammation, suggesting that the progression of these diseases occurs through common biochemical and molecular signaling pathways. Patients with T2D have been found to exhibit more DNA damage compared to controls, while patients with both T2D/AD tend to have increased DNA damage and reduced mitochondrial mass, possibly indicating mitochondrial dysfunction. Although the molecular mechanisms underlying the association between T2D and AD remain unknown, we explore the role of hyperglycemia in sensitizing neurons through the exacerbation of oxidative and neurotoxic damage, thus promoting neuronal death and potentially contributing to the observed neurodegeneration and development of AD later in life.

Talk: Biomonitoring of human genotoxicity induced by complex occupational exposures
Speaker: Juliana da Silva – ULBRA; UniLaSalle - Canoas, RS

There is a great concern for workers in their work environment due to the potential for many types of substances in this environment to cause negative health effects. Occupational exposure to different genotoxic agents causes DNA damage which may leads to genomic instability and susceptibility to diseases and cancer. Carcinogenic substances such as heavy metals, agrochemicals, dust and solvents can be found in different compounds, and higher concentrations of these carcinogens are found in the work environment. Cancer is the leading cause of work-related death in the world. Data from the World Health Organization (WHO) revealed that more than 500,000 deaths/year worldwide is caused by work-related cancer, twice as many as work-related accidents. DNA damage can be used as a reliable and accurate biomarker to quantify some exposure and can indicate its possible long-term effects and cancer risk. In this talk, some concepts related to indicators of damage to genetic material caused by occupational exposure agents will be discussed, as well as the effect on the health of rural workers in relation to exposure to different genotoxic agents in tobacco cultivation.

SESSION: NIGHT WITH SCIENCE
Chair: Carlos Renato Machado - UFMG, Belo Horizonte, MG

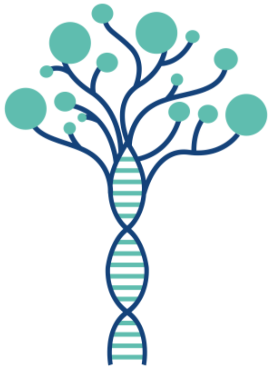
Talk: Predatory Journals
Speaker: Carlos F. Menck - USP, São Paulo, SP

Talk: Impatient Science and Academic Quantumphrenia: Reflections on modern scientific culture
Speaker: Marcus F. Oliveira - UFRJ, Rio de Janeiro, RJ

Talk: Science for the general public
Speaker: Marcelo Leite - Journalist, São Paulo, SP

Talk: Responsible Conduct in Biomedical Research
Speaker: Manoor Prakash Hande – National University of Singapore, Singapore

October 4th to 7th, 2023 | Londrina - Paraná



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Genotoxic Risk Assessment and Public Health

MITOCHONDRIAL DNA DAMAGE ACCUMULATION IN ENVIRONMENTALLY EXPOSED INDIVIDUALS FROM THE COMPLEXO LAGUNAR MUNDAÚ-MANGUABA (CELMM, MACEIÓ-AL)

Alice Helena Baldani Diatroptoff [1]; Maiara Ingrid Cavalcante Queiroz [3]; Helena Coutinho Franco de Oliveira [2]; Ana Catarina R. Leite [3]; Nadja Christina de Souza Pinto [1]

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Abstract:
High levels of contaminant metals, especially mercury (Hg) and lead (Pb) have been detected in the waters of the lagoon complex Mandaú-Manguaba, Maceio - AL. These metals were found to accumulate in the bivalve mollusk commonly known as sururu (Mytella strigata), which is an important foodstuff for the local populations. Previous studies have identified increased oxidative stress in blood cells from the exposed population when compared with non-exposed controls. Considering that redox imbalance is proposed to cause DNA damage, and that mitochondria is a main site of reactive oxygen species generation, the goal of this study is to determine whether the mitochondrial DNA (mtDNA) is a relevant target to the effects of exposure to the water contaminants. For that, total genomic DNA was isolated from white blood cells from 17 control and 22 exposed individuals and analyzed by polymerase chain reaction (PCR) for alterations in mtDNA copy number (mtDNAcn). MtDNAcn was calculated as the ratio between amplification efficiency of a mitochondrial (ND1) and a single-copy nuclear (β-globin) target. Our results show that environmental exposure to the lagoon waters and contaminated foodstuffs leads to decreased mtDNAcn, indicating that mtDNA is a relevant target for exposure-induced damage accumulation. We are now applying a long-extension PCR technique to directly detect DNA damage levels in mitochondrial and nuclear DNA from the samples.
Keywords: Toxic metals; Damage; PCR;

Support / Acknowledgment

Funding: FAPESP grants 2017/04372-0 to NCS-P and 2019/15320-7 to HCFO; AHBD is supported by a PUB/USP scholarship.

INITIAL CYTOGENOTOXIC ASSESSMENT OF N-TOSILINDOLE, LARVICIDE AGAINST THE TRANSMITTER OF DENGUE, CHICUNGUNYA AND ZIKA.

Aline Pereira da Silva [1]; Sócrates Cabral de Holanda Cavalcanti [3]; Edson Luis Maistro [1,2]

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Abstract:

Aedes aegypti is the mosquito that transmits Dengue, Chikungunya, Zika and yellow fever, diseases that affect millions of people around the world, killing thousands. Over the years, *A. aegypti* larvae have developed resistance to currently used larvicides, being the development of new larvicides of fundamental importance. In this context, N-tosilindole was developed, an indole derivative whose recent studies have shown great larvicidal activity against *A. aegypti* larvae. As this compound must be used in water tanks, it is essential to analyze its toxicity. The aim of this study was to initiate analyzes of the cellular and genetic toxicity of N-tosilindole in cultured human peripheral blood leukocytes. Cytotoxicity of N-tosilindole and its solubilizer was evaluated by trypan blue staining test, which assesses cell viability by evaluating the integrity of the cell membrane. The genotoxicity was assessed by comet assay and chromosome alterations by the cytokinesis-block micronucleus test. Statistical analysis was performed with analysis of variance (ANOVA) followed by Dunnett's and/or Tukey's post-test using GraphpadPrism® 5 software. At concentrations between 1 and 200 µg/ml, N-tosilindole and its respective solubilizing control did not cause a decrease in cell viability of PBMC cells. The genotoxicity analysis by the comet assay showed that, at concentrations of 10, 20 and 50 µg/ml, the larvicide did not produce DNA damage. Likewise, the micronucleus test did not show clastogenic/aneugenic effects. Considering that N-tosilindole produces a larvicidal effect at low concentrations (about 0.2 µg/ml), the preliminary data obtained, under the experimental conditions employed, reveal that the new larvicidal agent did not produce cytotoxic or genotoxic effects in human leukocytes. Additional studies with liver metabolizing cells are being carried out for a more complete determination of the cytogenotoxic potential of this promising larvicidal agent.

Keywords: Comet assay; Micronucleus test; Genotoxic evaluation; Mutagenic evaluation; N-tosilindole

Support / Acknowledgment

This study was partially financed by the Coordenaçao de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES), Finance code 001; and by the Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq; Grant: 303604/2021-2).

ASSESSMENT OF EMBRYOTOXICITY, VISUAL MOTOR RESPONSE, AND OXIDATIVE STRESS OF NANOSTRUCTURED BIOMATERIALS BASED ON HYDROXYAPATITE IN ZEBRAFISH (*DANIO RERIO*)

Augusto Monteiro de Souza [1]; Heloysa Araujo-silva [2]; Andréa Machado Costa [3]; Andre Linhares Rossi [3]; Alexandre Malta Rossi [3]; José Mauro Granjeiro [3]; Ana Carolina Luchiari [2]; Silvia Regina Batistuzzo de Medeiros [1]

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Abstract:
Hydroxyapatite (HA) is extensively utilized in clinical and pharmaceutical applications as a biomaterial. Researchers have investigated the incorporation of various ionic substitutes into HA to create a mineral composition that closely resembles natural bone tissue, thereby enhancing its biological suitability for bone regeneration purposes. However, before obtaining approval for use in humans, a comprehensive assessment of its biosafety is of paramount importance. Therefore, the objective of this study was to assess the embryotoxicity of ion-substituted HA biomaterials using zebrafish (Danio rerio) embryos as an alternative model, following OECD guideline 236. Zebrafish embryos were exposed to microspheres containing nanoparticles of HA and carbonate (cHA), strontium (SrHA), and zinc-substituted HA (ZnHA) from 4 to 120 hours post-fertilization (hpf). Larval behavior at 168 hpf was analyzed to determine whether the biomaterials adversely affected optomotor and avoidance responses, indicating potential neurotoxicity. The levels of expression of genes related to oxidative stress were measured by qPCR. After 120 hours of exposure, the embryos showed no mortality rates greater than 20%, indicating the non-embryotoxic character of the tested biomaterials. Moreover, all experimental groups displayed positive optomotor and avoidance responses, suggesting that embryo exposure to the ion-substituted HA biomaterials did not cause neurotoxic effects. Larvae exposed to SrHA microspheres exhibited a similar optomotor response compared to the negative control group. The mRNA levels of genes related to oxidative stress, including superoxide dismutase (sod1 and sod2), catalase (cat), and glutathione peroxidase 1 A (gpx1a), remained unchanged even after 120 hpf, indicating no significant impact on oxidative stress patterns. The ion-substituted HA biomaterials tested in this study demonstrated no toxicological effects, developmental and neuromotor impairments, or increased oxidative stress in zebrafish embryos/larvae. The results showed the biosafety of ion-substituted HA-based biomaterials using zebrafish embryos from 4 to 168 hpf. This study does not indicate toxic effects or developmental changes in response to zinc, strontium, and calcium carbonate-substituted HA microspheres.
Keywords: Zebrafish embryotoxicity test; Developmental toxicity; Hydroxyapatite microspheres; Visual-motor behavior;

EPICATECHIN HAS A PROTECTIVE EFFECT ON CYCLOPHOSPHAMIDE-INDUCED DAMAGE IN WISTAR RATS

Diane Marques Magnoni [1]; Michele Cristina Heck [1]; Mariane Aparecida Franco de Godoy [1]; Débora Elisa Antunes de Mendonça [1]; Pamela Altero Prado [1]; Gabriela Terue Rizzo Kodama [1]; Igor Vivian de Almeida [2]; Veronica Elisa Pimenta Vicentini [1]

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Abstract:
Epicatechin (EPI) is a flavonoid that possesses a bitter taste and astringency, usually found in cocoa, a typical Amazonian plant, and green tea. Research shows its anti-inflammatory, antiviral, antidiabetic, antioxidant, and antitumor potential. Thus, EPI has therapeutic effects against various cancers, considered an important bioactive for health protection. Thus, the present study evaluated the mutagenic and antimutagenic effects of EPI against the cyclophosphamide (CP) alkylating agent-induced DNA damage, in *Rattus norvegicus* bone marrow cells, by the Micronucleus Test. Five animals per group were treated with EPI at concentrations of 10, 50, and 250 µM by gavage for mutagenicity test. The control and cyclophosphamide groups received 1mL of water and 1.5mg/100g body weight of CP (intraperitoneally), respectively. The antimutagenicity of EPI 10µM was evaluated by the simultaneous exposition (SIM: EPI+CP), pre-treatment (PRE: EPI administered before CP application); and post-treatment (POST: EPI administered after CP application)., in bone marrow cells. wo thousand polychromatic erythrocytes (PCE) were analyzed to investigate the presence of micronuclei and 1,000 cells between PCE and normochromatic erythrocytes (NCE) for cytotoxicity test, per animal. The data obtained were submitted to analysis of variance (one-way ANOVA), followed by Tukey's Test (α=0.05, $p<0.05$, n=5), with the aid of the GrafPad Instat software. The results obtained showed that none of the evaluated concentrations of EPI showed a mutagenic or cytotoxic effect. For the investigation of the protective effect, it was observed that SIM, PRE, and POST treatments presented a reduction in the frequency of micronuclei induced by CP when compared to treatment with the alkylating agent. However, they showed a significant reduction in PCE/NCE frequency when compared to control, but were not statistically different from treatment with EPI 10µM. These results demonstrate the antimutagenic activity of EPI. The protective effect of EPI is possibly related to the antioxidant capacity of this flavonoid.
Keywords: *Theobroma cocoa*; antimutagenicity; antioxidante; micronucleus test; flavonoid

Support / Acknowledgment

CAPES, CNPq; Fundação Araucária, SETI-PR, UEM, PBA, LMMA.

ANTIMUTAGENIC EFFECT OF PYRIMIDINE DERIVATIVES FUNCTIONALIZED WITH THIOGLUCOSE AND THIOGALACTOSE IN *SWISS* MICE

Elvio Mora Junior [1]; Paola dos Santos da Rocha [2]; Valéria Dornelles Gindri Sinhorin [3]; Marina Mariko Sugui [3]; Kely Picolli de Souza [2]; Nelson Luís de Campos Domingues [2]

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Abstract:

The daily exposure to mutagenic agents comes from the use of cleaning and cosmetics products, medicines, UV irradiation, and pathogens, among others. The mutagenesis process can bring on the development of several diseases, for example, cancer. Thus, researchers search for new compounds, which can prevent damage to the genetic material that comes from exposure to mutagenic agents present in our lives. In this way, the development of new pyrimidine derivates compounds is promissory, once these compounds present antioxidant, antimicrobial and antineoplastic properties related in the literature, and also present an easy absorption by the organism. In this sense, the present work aimed to develop new pyrimidine derivates functionalized with 1-thioglucose and 1-thiogalactose, and evaluate their antimutagenic potential. For the synthesis of these compounds, 1.5 equivalents of 2,4-dichloropyrimidine, 1 equivalent of the thiosugar, 1 equivalent of triethylamine, and 30 mL of DMF were placed in a reaction vial with constant stirring, at 110 ºC under nitrogen atmosphere, and the reactions were performed in 6h. The compounds were purified in a chromatographic column, and after that, the acetyl protecting groups were removed from the compounds using a solution containing methanol and K_2CO_3. The obtained final compounds were characterized via RMN ^{1}H and ^{13}C to confirm their structure. To evaluate the antimutagenic potential of the compounds six groups of mice were analyzed, each group containing six animals, which were treated via gavage for 14 days, as follows: Group 1 and 2 - treated with NaCl 0,9%; groups 3 and 4 - treated with the pyrimidine derivate functionalized with thioglucose (DPSGlyOH); and the groups 5 and 6 - treated with the pyrimidine derivative functionalized with thiogalactose (DPSGalOH). On the 15º day, it was given 75 mg/kg of cyclophosphamide (CPA - mutagenic agent) for the animals in the groups 2,4, and 6 (via intraperitoneal). Euthanasia was performed after 15 days of experimentation. The presence of genetic material damage was evaluated by the presence of micronucleus in erythrocytes of the bone marrow, analyzing 500 cells by the animal in duplicate. In group 1, it was observed the presence of 156 micronuclei in the 6000 cells that were analyzed, an incidence of 2,6% of micronucleus formation. Group 2 (treated exclusively with CPA) presented 455 micronuclei (7.58%). Group 3 (treated with DPSGlyOH) and Group 4 (treated with DPSGlyOH and CPA) presented 107 (1,7%) and 145 (2,42%) respectively. Group 5 (treated with DPSGalOH) and group 6 (treated with DPSGalOH and CPA) presented 93(1,55%) and 122 (2,03%) cells with micronucleus respectively. Together, the data suggest that the pyrimidine derivates functionalized with thioglucose and thiogalactose presented antimutagenic effects against the clastogenic effects of cyclophosphamide, being promising compounds for investigation of the mechanism of action and a possible application in the pharmaceutical industry.

Keywords: Antimutagenic; Micronucleus; Cyclophosphamide; Thioglycosides; Pyrimidine Derivatives

Support / Acknowledgment

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NON-MUTAGENIC EFFECT AND PROTECTIVE ACTIVITY OF COCOA AND CHOCOLATE POWDER AGAINST THE MUTAGENICITY OF CYCLOPHOSPHAMIDE, BY THE MICRONUCLEUS TEST

Esperanca Edna Alexandre Chibite [1]; Michele Cristina Heck [2]; Mariana Yoshimoto-higaki [1]; Diane Marques Magnoni [1]; Gabriela Terue Rizzo Kodama [1]; João Arthur dos Santos de Oliveira [1]; Igor Vivian de Almeida [3]; Veronica Elisa Pimenta Vicentini [2]

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Abstract:
Theobroma cacao L., known as cacao, belongs to the Malvaceae family, is native to the Amazon basin, but is cultivated in many tropical and subtropical countries. It is an economically important crop and its beans are mainly used in the production of chocolate. In recent years, the benefits of cocoa and its derivatives for human nutrition have become more apparent as it is an abundant source of dietary polyphenols with many health benefits, such as antioxidant, anti-inflammatory, DNA-protective, and radioprotective action. It has been suggested that cocoa has bioactive compounds with the highest antioxidant activity among the foods ingested by individuals. Therefore, chocolate, one of the cocoa derivatives, constitutes a source of polyphenolic antioxidants for the population. Thus, the present study evaluated the mutagenic effect and the protective activity of cocoa and chocolate powder against cyclophosphamide (CP) DNA-induced damage on Wistar rats' bone marrow cells by the Micronucleus Test. Acute treatment was performed in three different ways: simultaneous (SIM: cocoa/chocolate+CP), pre-treatment (PRE: cocoa/chocolate administered before the application of CP); and post-treatment (POST: cocoa/chocolate administered after the application of CP). For this purpose, a cocoa and chocolate solution were prepared and administered via gavage (VG) to the animals at three concentrations: 1; 50, and 100 µg/mL to evaluate mutagenicity, and 50 µg/mL to investigate the protective effect on DNA. The control group and cyclophosphamide received 1 mL of water VG and 1.5mg/100g body weight of CP via intraperitoneal route, respectively. Five animals per group were used, and 2,000 polychromatic erythrocytes (PCE) were analyzed for the investigation of micronuclei and 1,000 cells between PCE and normochromatic erythrocytes (NCE) for cytotoxicity, per animal. The results were subjected to analysis of variance (one-way ANOVA), followed by Tukey's test ($\alpha=0.05$, $p<0.05$, $n=5$), with the aid of the GrafPad Instat software. The results showed that both cocoa and chocolate showed no mutagenic activity at the concentrations evaluated, and they were effective in reducing the cyclophosphamide-induced damage (SIM 79; 87, PRE 88; 68 and POST 85; 90% respectively for cacao and chocolate). For the PCE/NCE ratio, only in the POST treatment with cocoa was no reduction observed, but in the SIM and PRE groups the results did not differ from the treatment with the 50µg/mL concentration. For treatments with chocolate, the three groups (SIM, PRE and POST) showed a significant reduction in the PCE/NCE ratio. Thus, cytotoxicity was verified in the antimutagenicity treatments with chocolate under the study conditions. The data obtained through this research demonstrate that cocoa may have a protective effect against DNA damage, but further studies should be conducted to elucidate the mechanisms of action involved in the observed protection.
Keywords: Antimutagenicity; Antioxidants; Wistar rats; *Theobroma cacao* L;

Support / Acknowledgment

CAPES, CNPq; Araucária Fundation, SETI-PR, UEM, PBA, LMMA.

GENETIC POLYMORPHISMS RELATED TO GLUTAREDOXIN, THIOREDOXIN AND METALLOTHIONEIN ARE ASSOCIATED WITH ALTERATIONS ON SE AND ON HG LEVELS IN AMAZONIAN RIVERSIDE POPULATIONS EXPOSED TO HG, VIA DIET

Flora Troina Maraslis [1]; Emilene Arusievicz Nunes [1]; Fernando Barbosa Júnior [2]; Gustavo Rafael Mazzaron Barcelos [1]

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Abstract:
Riverside populations of the Brazilian Amazon are exposed to high Hg concentrations, mainly MeHg, which has great affinity for sulfhydryl (-SH) and selenohydryl (-SeH) groups present in low molecular weight molecules and/or proteins. The interaction of MeHg with these nucleophilic centers may impair the functioning of different compounds inducing disturbance of the cellular redox status. The thioredoxin and glutathione/glutaredoxin systems are important cellular redox systems responsible for preventing oxidative damage; and the metallothionein (MTs) system play an important role in the balance of the metallic state and the cellular redox. Most of the genetic polymorphisms assessed in Hg-exposed Amazonian populations, are related to the genes of GSH pathway, such as *GCLs, GSTs, GPx*, but no studies aimed to evaluate the impact of thioredoxin and glutathione/glutaredoxin system on Hg body burden. Thus, this study aimed to verify the effect of *GLRX* rs2007 (C/G)*, GLRX2* rs912071 (C/T)*, TXNRD1* rs11111979 (C/G)*, TXNRD2* rs5748469 (C/A)*, MT1A* rs11640851 (C/A)*, MT1M* rs2270837 (A/G) *e MT2A* rs10636 (G/C) polymorphisms on plasma Hg (pHg) and on Se (pSe), and blood Hg (bHg) and Se (bS), as well as on the pHg/bHg ratio, pSe/bSe ratio and pSe/pHg ratio in a population exposed to high concentrations of the metal, via diet, in the Tapajós River region, Brazil. Sociodemographic and food frequency questionnaires, and anthropometric measurements were obtained from 365 individuals, in addition to biological samples (blood). Metals concentrations were obtained using ICP-MS and SNPs were genotyped by Taqman® assays. Multiple linear regression models were applied, adjusted for potential variables that may impact the metal levels, age, sex, body mass index, fish intake, smoke, alcohol and element levels. Study participants age ranged between 15 and 87 years, with half being female and half male. Most of individuals are non-smokers (73.7%) and just over half (53.7%) reported consuming alcoholic beverages. The median of bHg was 39.5 µg/L, pHg was 5.80 µg/L, bSe was 228 µg/L and pSe was 134 µg/L. Individuals who are homozygous for *TXNRD2* (AA) had higher concentrations of bHg and pHg than those with non-polymorphic alleles (β=-0.121, *p*=0.015; β=-0.518, *p*=0.004; respectively). Participants that have the homozygous variant allele for *TXNRD1* (GG) showed higher concentrations of bSe than other ones (CC: β=-0.054, *p*=0.038; CG: β=-0.043, *p*=0.049). On the other hand, persons with the AA genotype for *TXNRD2* had lower values of pSe/pHg (CC: β=0.201, *p*=0.010; AC: β=0.157, *p*=0.012). Interestingly, individuals with the heterozygous *MT1M* (AG) genotype (β=0.049, *p*=0.042) tended to show higher pSe/bSe ratios than persons with variant genotype (GG). Regarding pHg/bHg ratio, individuals with heterozygous genotype (AC) (β=-0.036, *p*=0.027) for *TXNRD2* showed lower values than those with the homozygous variant genotype (AA); and lower values were also seen between CC genotype (β=-0.059, *p*=0.016) versus GG for *GLRX*. Taken together, this study added further evidence to a growing body of literature that shows how genetic polymorphism is able to modulate Hg and Se body burden.
Keywords: SNP; metabolism; toxic metal; essential element; distribution

Support / Acknowledgment

CAPES, FAPESP.

CYTOTOXICITY OF THE ANTIDEPRESSANT VORTIOXETINE IN HUMAN RENAL CARCINOMA CELL LINE (786-O)

Gabriela Terue Rizzo Kodama [1]; Mariane Aparecida Franco de Godoy [1]; Débora Elisa Antunes de Mendonça [1]; Michele Cristina Heck [2]; Diane Marques Magnoni [1]; Esperança Edna Alexandre Chibite [1]; Veronica Elisa Pimenta Vicentini [2]

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Abstract:
The current usage of antidepressants has been significantly increasing for the treatment of depression, with Vortioxetine being one of the most commonly used medications. This pharmacological agent acts on various neurotransmitter pathways to treat depression and improve associated issues with the disease, while causing fewer side effects. However, different drugs can be employed for purposes other than their typical use, demonstrating, for instance, potential antitumor effects. Therefore, investigating the action of this medication in different cell types is important. In vitro assays with tumor cell lines allow for a rapid and systematic investigation through various toxicological parameters. Among the tumor cell lines, renal cell carcinoma (786-O lineage) stands out, enabling studies on immunoreactivity and facilitating the isolation of specific antigens from renal tumor cells, as well as in vitro chemotherapy tests, leading to a better understanding of renal cell cancer in humans. Accordingly, based on the aforementioned information, the objective of this study was to evaluate the cytotoxic potential of the antidepressant Vortioxetine using the MTT Cytotoxicity Assay on the 786-O renal carcinoma cell line, in order to assess the possible antitumor action of this compound. The control and treatment groups received the DNA-damaging agent DOX [18µM] and Vortioxetine at concentrations of 1.5, 3, 6, 9, 12, 15, 18, and 21 µg/mL. The microplate reader (Labtech) was used to perform the readings at 550nm, and cell viability was estimated based on the absorbance of the control (Treatment A/Control A x 100). The MTT assay results indicated that all concentrations starting from 3µg/mL significantly reduced cell viability, with a percentage lower than 50%, indicating cytotoxicity within 24 hours. Therefore, it can be concluded that, at the tested concentrations, the antidepressant Vortioxetine exhibited cytotoxicity in the human renal carcinoma cell line (786-O).
Keywords: Brintellix®; Viability; MTT Assay; ;

Support / Acknowledgment

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INFLAMMATORY, OXIDATIVE, AND DNA DAMAGE STATUS IN HEALTHY SUBJECTS BASED ON THEIR DIETARY PREFERENCES

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Abstract:
The impact of a vegetarian diet on health is still under debate as there is evidence that it can lead to a higher intake of certain micronutrients, while potentially reducing others, thus influencing various metabolic pathways and health-related biomarkers. Therefore, we aimed to investigate the differences in those biomarkers by comparing individuals with different dietary preferences. Our study was conducted on a group of healthy adult vegetarians and matched non-vegetarians practicing a traditional mixed diet. Two groups were analyzed for various biomarkers related to DNA damage, oxidative stress, and inflammation. Additionally, we analyzed their hematological and biochemical profiles, telomere length, bone mineral density, nutrient levels, and toxic elements, as well as the presence of pesticides and mycotoxins. The results revealed different biomarker levels between the two groups, favoring the traditional mixed diet, particularly one rich in fruit and vegetables. This indicates that vegetarians have a lower nutritional status for some nutrients (Ca, Cu, Zn, vitamins B_{12} and D) accompanied by a lower antioxidant defense system (glutathione). They also displayed higher levels of homocysteine and genome damage in the form of micronuclei frequency and DNA strand breaks, along with shorter telomeres. When further dividing the participants into specific sub-groups, less DNA damage was observed amongst omnivorous subjects than vegetarians, with the lowest DNA damage found in females practicing a pescatarian diet. These findings suggest that incorporating animal-derived nutrients as supplements into the diet of this particular group would be beneficial for improving certain health-related biomarkers that were measured. In terms of inflammation, when relating the high-sensitivity C-reactive protein (hs-CRP) values between vegetarians and non-vegetarians, no significant difference was observed between the two groups. However, the level of certain toxic metals (As and Hg) was higher in non-vegetarians. This multi-biomarker approach offers a complex insight into the differences in selected biomarkers related to specific dietary preferences and health outcomes, which could directly benefit clinicians and nutritionists in patient counseling on nutrition and dietetics. Besides, further research in well-defined and sufficiently sized cohorts is needed to provide more exact evidence.
Keywords: vegetarians; pescatarians; omnivores; DNA damage; health effects

ASSESSMENT OF THE EFFECT OF ROSMARINIC ACID ON NON-ALCOHOLIC HEPATIC STEATOSIS INDUCED BY A HIGH-LIPID DIET IN WISTAR RATS

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Abstract:

Non-alcoholic fatty liver disease (NAFLD) initially is caused by the accumulation of triglycerides and free fatty acids inside the hepatocytes as a result of insulin resistance. Its progression to non-alcoholic steatohepatitis (NASH) is characterized by inflammation and hepatocytes injury. Disease progression occurs together with metabolic and inflammatory derangements that are accompanied by genetic and environmental factors, which promotes a continuous activation of the immune system. DHGNA represents a spectrum of diseases ranging from the benign status of non-alcoholic fatty liver until severe fibrosis and cirrhosis. The initial treatment of steatosis involves the elimination of environmental factors that resulted in the disease process. However, the search for new therapeutical approaches allied to changes in lifestyle can reduce or even eliminate the steatotic liver. Rosmarinic acid (RA) is a natural compound found in Lamiaceae e Boraginaceae to which rosemary, mint, thyme, basil, oregano and sage belong. RA is a dimer of caffeic acid, and among its biological activities are antioxidant, antiviral, antibacterial and anti-inflammatory. In the present study the effects of RA to a hyperlipidic fed diet rats were evaluated at histological, biochemical and genetic levels. Forty male Wistar rats were divided in four experimental groups: control fed with the standard diet of American Institute of Nutrition AIN-93 diet (C), control supplemented with 10 mg/kg b.w. of RA three times a week (C+RA), hyperlipidic fed group (HL) and hyperlipidic fed group supplemented with 10 mg/kg b.w. of RA three times a week (HL+RA). All animals were treated for 8 consecutive weeks. After this period, all animals were submitted to euthanasia to serum and hepatic tissue collection. 24 h before euthanasia, the fast glucose levels and glycemic curve were determined. The results demonstrated that hyperlipidic diet induced hepatic steatosis and increased the total lipid contend in the liver. Moreover, hepatic and serum levels of α-tocopherol were significantly reduced in high fatty diet animals and the hepatic levels of malondyaldehyde (MDA) was significantly reduced as well. Treatment with RA did not affect the occurrence of steatosis and did not influence total lipid contend, serum cholesterol, triglycerides, alanine transaminase, aspartate transaminase, α-tocopherol, MDA, reduced and oxidized glutathione, the DNA damage index obtained by Comet Assay and the frequency of micronucleus detected in bone marrow. Thus, considering the experimental design used in the present study, the induction of liver steatosis with a hyperlipidic diet was well succeed, but RA did not exert protective effects against this liver disease.

Keywords: liver steatosis; rosmarinic acid; hyperlipidic diet; oxidative stress;

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ROUNDUP® ORIGINAL DI HERBICIDE CAUSES GENOTOXIC EFFECTS ON NORMAL AND NEOPLASTIC THYROID CELL LINES

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Abstract:

The widespread use of glyphosate has been associated to thyroid cancer incidence rates increase by epidemiologic and experimental evidence. Glyphosate is an important endocrine disruptor that has been shown to produce disturbances in hormone regulation, including disorders of thyroid function such as hypothyroidism, as well as promoting the development of goiter and cancer. However, the mechanisms of action of glyphosate and of glyphosate-based herbicides (GBH) on thyroid cells are still poorly understood. More recently, the safety margins of glyphosate use have been brought into question by a series of studies suggesting health risks, especially when considered in combination with the surfactants it is usually applied with. The objective of this study was to evaluate whether an acute exposure (24 and 48 hours) to different doses of Roundup causes genotoxic effects in thyroid cell lines. We used 3 cell lines: Nthy-ori 3-1 (derived from thyroid follicular normal cells) which was used as a control for neoplastic cells; TPC-1 and BCPAP (derived from thyroid papillary carcinoma, harboring RET/PTC translocation and BRAF mutation, respectively). A comet assay was performed in duplicate with a negative and a positive control (H2O2 - 100µM), and the results were analyzed with the CometScore software. The concentrations employed ranged from 6.5 to 6500 µg/L, including the Acceptable Occupational Exposure Level (AOEL) and Acceptable Daily Intake (ADI) doses, present in ANVISA's Technical Note (Process No. 25351.056754/2013-17). The results were expressed as percentage of DNA damage compared to the negative control, and statistics were performed using the ANOVA test. We observed that Roundup caused injury to the DNA of all thyroid strains. Nthy-ori 3-1 suffered DNA damage both at 24 and 48h exposure and the lowest tested dose (6.5 µg/L) was the one that caused the greatest damage affecting 100% of the cells ($p<0.0001$). The doses considered acceptable by ANVISA (AOEL=160 µg/L and IDA=830 µg/L) caused damage in 67% and 80% of the cells in 24h. After 48h, they continued to cause important genotoxicity, affecting 55% and 85% of the cells, respectively. TPC-1 cells were also sensitive to Roundup effect, both after 24h and 48h exposures. AOEL and IDA caused DNA damage in 79% and 77% of the cells at 24h. After 48h exposure, 65 µg/L and 160 µg/L (AOEL) caused even greater damage (100% and 95%, respectively). BCPAP cells were more resistant than the other strains, however 65 µg/L injured 100% of the cells at 24h. After 48h exposure, the lowest employed dose of just 6.5 µg/L, caused 100% damage. We conclude that Roundup® Original DI acts as a potential genotoxicant in different thyroid cells and its effect are non-monotonic. Even low concentrations of the herbicide, considered acceptable, have important effects, especially in TPC-1 cells. Regulatory agencies should take into account that GBHs are more potent than glyphosate alone at activating cellular mechanisms, which may contribute to select mutated cells and drive carcinogenesis.

Keywords: Herbicide; genotoxicity; comet assay; thyroid;

Support / Acknowledgment

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INVESTIGATION OF THE CYTOTOXICITY OF AVOCADO AND SOY UNSAPONIFIABLES (ASU) ON BREAST (MCF-7) AND HEPATOCYTE (HEPG2/C3A) TUMOR CELL LINES

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Abstract:
The active compounds in plants, known as phytochemicals, have been used as medicines for thousands of years to treat and prevent various diseases, such as cancer. Avocado and soy are vegetables that contain numerous phytochemicals that may assist in carcinogenic processes. Avocado and soy unsaponifiables (ASU) are oily extracts composed in a 1:2 ratio, respectively. Their main constituents are phytosterols, which are similar to cholesterol in structure and function, contributing to the inhibition of intestinal cholesterol absorption. In addition, it has been demonstrated that a diet rich in phytosterols can reduce the risk of cancer by 20%. This combination of unsaponifiables is currently marketed as the drug Piascledine®300 (PSD) in many countries and is used for the treatment of painful osteoarthritis due to its anti-inflammatory activity. Given the therapeutic potential of phytosterols, this study investigated the cytotoxic potential of phytosterols present in avocado and soybean unsaponifiables (Piascledine®300) on human breast adenocarcinoma (MCF-7) and hepatocarcinoma (HepG2/C3A) cell lines using the MTT assay, evaluating concentrations of 0.5, 2.5, 5 and 10 µg/mL after 24, 48 and 72 hours of exposure. The readings were performed on a microplate reader at 550 nm. The data were subjected to analysis of variance (ANOVA), followed by Dunnett's test ($\alpha=0.05$, $p<0.05$). In the HepG2/C3A cell line, the concentration of 0.5 µg/mL exhibited statistically significant reduction in cell viability only within 24 hours. In addition, the concentrations of 2.5, 5 and 10 µg/mL showed statistically significant reduction at all exposure times. In the MCF-7 cell line, none of the concentrations evaluated promoted statistically significant reduction in cell viability. The result observed in the hepatocarcinoma line may be related to the metabolizing activity of liver cells, a characteristic that makes this line more susceptible to the action of compounds, and may also be associated with the hypocholesterolemic action of phytosterols, as they can replace cholesterol in the plasma membrane, causing changes that can lead to apoptosis. The result obtained in the breast line may be related to the well-known resistance of these cells to the action of different compounds. This resistance can be explained by the presence of some molecular pumps in the membranes of various tumor cells, including breast cells, which actively expel chemotherapeutic drugs from their interior. Thus, we can infer that Piascledine®300 acts on the cell viability of the HepG2/C3A cell line at the concentrations and times evaluated, making it a potential candidate for the treatment of this type of cancer. However, for the MCF-7 cell line, due to the resistance observed, it would be necessary to investigate whether higher concentrations of the drug would inhibit cell growth.
Keywords: Piascledine®300; Phytosterols; Cell viability; ;

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CAPES, CNPq, Araucária Foundation, SETI-PR, UEM, PBA, LMMA.

ASSOCIATION OF *SLC6A15* AND *SLC6A19* POLYMORPHISMS AND MERCURY (HG) CONCENTRATIONS IN A RIVERSIDE POPULATION OF THE BRAZILIAN AMAZON EXPOSED TO THE METAL, VIA DIET

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Abstract:
Amazonian soils constitute large natural reservoirs of Hg that are released into aquatic ecosystems through soil erosion and leaching and human activities. Previous studies demonstrate the occurrence of environmental exposure to mercury (Hg) in the Brazilian Amazon, mainly to methylmercury (MeHg). Due to its characteristic of bioaccumulation and biomagnification, humans are exposed by the consumption of contaminated fish, which is the main source of protein for these communities. In the body, MeHg linked to cysteine, would can be even more toxic and able to cross the blood-brain barrier since the MeHg-cysteine complex is able of mimic the neutral amino acid methionine. Composed of approximately 20 symporters, the human SLC6 family acts on transmembrane transport across the electrochemical gradient, facilitating the Na^+ and Cl^- dependent uptake of amino acids and derivatives and about 50% of these substrates are composed of neutral amino acids in epithelial cells present mainly in the central nervous system and intestinal cells. The S6A15 protein, coded by *SLC6A15* gene acts in the transport of neutral amino acids in the central nervous system, while the S6A19 (coded by *SLC6A19* gene) is present in intestinal cells, and both mediate the absorption of methionine into the cells. Thus, it is hypothesized that polymorphisms in these genes may modulate the relation between the metal exposure and cell uptake. Aim of the present study is to evaluate the effects of *SLC6A15* (C?T), *SLC6A19* (C?T) and *SLC6A19* (G?A) polymorphisms on the total hair mercury (tHg) concentrations, and its organic (oHg) and inorganic (iHg) forms) in the riverside population from Tapajós river exposed to the metal. In total, 365 participants who answered the sociodemographic and diet habits questionnaire were evaluated, then anthropometric data and hair samples were collected. Concentrations of Hg and its species were obtained using LC-ICP-MS and polymorphisms were genotyped by Taqman assays. Multiple linear regression models adjusted for confounders were used to evaluate the association between the polymorphisms and the elements studied; $p \leq 0.05$ was considered statistically significant. Individuals aged between 15 and 87 years, half female and half male. Most individuals are non-smokers (73.7%) and just over half (53.7%) reported consuming alcoholic beverages. Median of tHg was 13,8 µg/g, iHg was 2,02 µg/g and oHg was 11,7 µg/g. It can be seen that individuals with the heterozygous genotype (CT) for the *SLC6A15* rs11116642 had lower tHg and oHg levels than those with the homozygous genotype, either for the non-variant and variant ones. Interaction terms were observed between *SLC6A19* polymorphism and fish consumption, that is, individuals with the *SLC6A19* rs9418 (TT) polymorphism tended to have lower tHg concentrations, even at high fish consumption. On the other hand, opposite interactions terms were seen between individuals with the *SLC6A19* rs7732589 (GA) polymorphism and fish cinsumption. To the best of our knowledge, there is no study which assessed the effect of *SLC6A15* and *SLC6A19* polymorphisms on Hg levels in hair. Our findinds give further evidence that polymorphism in these genes may modify Hg toxicokinetics and, consequently, the adverse health effects related to the metal exposure.

Keywords: SNP; Metabolism; Mercury

ANTIMUTAGENIC AND TOXICOGENETIC EVALUATION OF FORMONONETIN AND ARBUTIN, FROM BRAZIL'S MEDICINAL ORCHID *CYRTOPODIUM GLUTINIFERUM* RADDI

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Abstract:

Cyrtopodium glutiniferum Raddi is an orchid from Brazil's traditional medicine employed to treat a plethora of diseases, as chest colds, tuberculosis, infectious abscesses and skin lesions. Previous studies on the chemical composition of *C. glutiniferum* Raddi showed that dihydroformononetin and arbutin are the secondary metabolites found in significant quantities in the aqueous extract of the plant's pseudobulbs. The abundance of phenolic compounds is thought to be related to plant's healing properties. Besides being a well-known anti-inflammatory to folk medicine, the extract posed no *in vitro* mutagenic or genotoxic harm. Nevertheless, the mechanisms underlying the medicinal features of the orchid have never been assessed. Thus, this work aimed to investigate the safety and efficacy of formononetin (FMN, the oxidized form of dihydroformononetin) and arbutin (ARB) through toxicogenetic alternative methodologies *in vitro* in both prokaryotic and eukaryotic models. Formononetin and arbutin were submitted to *Salmonella*/microsome assay (in the presence and in the absence of exogenous metabolic activation, to *Salmonella* Typhimurium strains TA97, TA98, TA100, TA102, TA1535; 0.001 to 10.0 µM). The *in vitro* cytokinesis-block micronucleus assay (CBMN assay) was conducted in HepG2 cells exposed to FMN and ARB for 24h (0.1 to 100.0 µM). The antimutagenicity assays were executed following pre, co, and post treatments. All methodologies cited were performed at least in three independent moments (n=3) and included experimental triplicates of each treatment and control groups. Results were analyzed in the GraphPad Prism software (version 8.02) and significant results of one-way ANOVA analysis (with Tukey's *post-hoc* analysis) were considered ($p< 0.05$). ARB showed no mutagenic response in the *Salmonella*/microsome assay in both metabolic conditions, although FMN exhibited cytotoxicity to TA97 and TA100 strains after metabolic activation. In this same condition, FMN was mutagenic to TA1535 (10 µM). In the antimutagenicity assay, ARB featured DNA damage protection against alkylating agent in all incubation conditions. Reduction levels of methyl methane sulfonate mutagenicity from 60% to 100% were detected for TA1535. For TA100, ARB performed 42% of damage reduction in post-incubation. FMN also presented an antimutagenic effect against alkylating damage in both strains, reaching a complete (100%) reduction of mutagenicity to TA1535 in pre-incubation, besides a 69% reduction in co-incubation to TA100. Results suggest that FMN and ARB play multi-targeted antimutagenic roles protecting DNA from mutagens, blocking alkylation action of MMS through chemical interactions before DNA is injured, as well as reverting mutation about to be fixed. In the CBMN assay of HepG2 cells, no increase in micronuclei formation was observed in any of the tested concentrations of both compounds and any genotoxic, cytotoxic, or cytostatic markers. Altogether, our findings reveal that phenolic molecules from aqueous extract of *Cyrtopodium glutiniferum* Raddi pseudobulbs feature remarkable antimutagenicity activities besides being not hazardous to chromosomes. Our data suggests that FMN and ARB show potential for further investigations aiming at chemopreventive approaches and treatment possibilities for diseases related to genomic instability and mutation.

Keywords: Phenolic compounds; Toxicogenetic assessment; Antimutagenicity; Chemoprevention;

Support / Acknowledgment

CNPq, CAPES, FAPERJ.

EVALUATION OF CYTOGENOTOXICITY OF WATER FROM VIENA STREAM (PORTO VELHO - RO, BRAZIL) IN BIOASSAYS WITH *ALLIUM CEPA*

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Abstract:
Porto Velho (RO) is crossed by several igarapés that drain the entire central region and flow into the Madeira River. The precarious treatment of urban waste and the lack of basic sanitation mainly affect urban and peri-urban streams. Faced with the significant problems of this city concerning urban waste treatment, this study aimed to evaluate the toxicity and citogenotoxicity potential of the Viena stream during the dry season (June to August 2022) using *Allium cepa as* a bioassay. The methodology was based on collecting water and basic water parameters such as pH, oxygenation, and temperature from the Viena stream, which is located on the banks of a residential condominium in the municipality of Porto Velho. *A. cepa* roots were processed through fixation, hydrolysis, pecking, and compression of the root meristem, followed by staining with Giemsa. Eighteen bulbs (six for each treatment) of *A. cepa* were cultivated (test) in water from the Viena stream, along with two other control treatments (Negative control - CN: distilled water, and positive control - CP: Paracetamol 800 mg/L), resulting in 54 slides (three slides for each bulb). These slides were analyzed under optical microscopy at 400X and 1000X magnification, with one thousand cells being analyzed for each slide. Two evaluations were conducted: one to measure the size of the three largest roots, and a genotoxicity test to analyze chromosomal alterations, such as bridges, abnormal metaphases, losses, micronuclei, and delayed chromosomes. After building the database in Excel, the records were subjected to the GraphPad Prism 8 software for the assessment of normality (Shapiro-Wilk normality test). Normality was found in the data of toxicity and mitotic index using the T - Student test, whereas data concerning Chromosomal Alterations and Micronuclei were treated non-parametrically (Mann - Whitney test). Viena stream presented oxygenation within the limits recommended by CONAMA (357/2005), a $pH > 6$, and a temperature of 25.4°C at the time of collection. The rainfall index for the region during the collection period was 19.3 mm according to INMET data from 2022. The results demonstrate that during the dry period, the Viena stream displays a remarkable genotoxic effect, being statistically significant ($p = 0.002$) compared to the negative control. However, it does not exhibit indications of significant toxicity ($p = 0.116$) or cytotoxicity ($p = 0.287$). As for the micronucleus index, it does not reveal statistical differences compared to the negative control, as the presence of micronuclei was not detected. While the impact is deemed low, the stream showed signs of genotoxicity in *A. cepa* cells. It is relevant to note that the condominiums might be exerting influence or contributing to the release of waste into this water. Based on this data, it is possible to observe a certain impact of anthropic activities on the stream's waters; however, measuring the exact level of impact requires further tests to confirm the influence of human occupation surrounding this body of water, as well as the necessary actions for its preservation.
Keywords: Environmental Mutagenesis; Water Resources; Amazon

Support / Acknowledgment

CAPES/FAPERO UNIR- UNIVERSIDADE FEDERAL DE RONDÔNIA PPGReN- PROGRAMA DE PÓS-GRADUAÇÃO EM CONSERAÇÃO E USO DE RECURSOS NATURAIS SUIG- (AÚDE ÚNICA NOS IGARAPÉS DO MUNICÍPIO DE PORTO VELHO-RO)

METALS BIOACCUMULATION AND GENOTOXICITY EFFECTS IN TWO NATIVE FRESHWATER FISH SPECIES FROM PANTANAL AND AMAZON BIOMES

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Abstract:
The Pantanal and Amazon biomes are under constant anthropic pressures that contribute to the entry of toxic metals into aquatic ecosystems. The presence of metals in water and sediments can pose serious risks to the biota and threaten the conservation of native fish species. The genotoxic damage caused by certain metals to fish is of great concern. Thus, our objective was to evaluate the bioaccumulation of Cd, Cu, Fe, Mn, Ni, Pb, and Zn in muscle tissue from the fish species *Prochilodus lineatus* (Pantanal biome) and *Curimata incompta* (Amazon biome), and compare the genotoxic damage frequencies observed in their erythrocytes. In the Amazon biome, we carried out two sampling campaigns during 2019 on the Araguari River (Amapá), and in the Pantanal biome we carried out only one sampling campaign during 2020 on the Aquidauana River (Mato Grosso do Sul). After identifying the fish species, we collected blood samples and then the fish were euthanized and muscle tissue samples collected. The metal concentrations determination was performed by Atomic Absorption Spectrometry. The slides for evaluation of genotoxic damage in erythrocytes were made in duplicate, fixed and subsequently stained with rapid panotype. Two thousand erythrocytes were analyzed by slides. We used the nonparametric Mann Whitney statistical test to compare metal concentrations and frequencies of genotoxic damage in both fish species. Seven specimens of *P. lineatus* were sampled in the Pantanal biome, and nine specimens of *C. incompta* in the Amazon biome. Both fish species are migratory, and present benthopelagic and detritivorous behaviors. Cd, Cu, Fe, Pb, and Zn concentrations bioaccumulated in the muscle tissue from *C. incompta* differed significantly ($p<0.05$) from the concentrations observed in *P. linetaus*. For Mn and Ni concentrations, there was no significant difference between the species ($p>0.05$). *C. incompta* showed higher concentrations of Cd, Cu, Fe, Pb, and Zn. These differences in the metal bioaccumulation may be related, at least in part, to the intense legal and illegal mining activity that exists throughout the Brazilian Amazon. Nuclear invagination, nuclear budding, binucleated cells, and lobulated nucleus were identified in the erythrocytes from both fish species. However, only in samples of *C. incompta* we observed micronuclei. The frequencies of nuclear invagination and binucleated cells did not differ among the two fish species ($p>0.05$). On the other hand, *C. incompta* showed higher frequencies of nuclear budding, lobulated nucleus, and micronuclei, differing statistically ($p<0.05$) when compared to *P. lineatus*. Our results demonstrate that both fish species collected in these two important Brazilian biomes present bioaccumulation of metals and genotoxic damage in their erythrocytes. In general, we could verify that the species *C. incompta*, collected in the Amazon biome, presented the highest concentrations of bioaccumulated metals and the highest frequencies of genotoxic damage, indicating greater contamination by metals and other genotoxic chemicals in the Amazon biome. It is important to emphasize that these two biomes are of great ecological importance because they harbor a vast biodiversity, including several endemic species, and the presence of metals and other genotoxic contaminants pose threats to their conservation.
Keywords: Araguari River; Aquidauana River; freshwater fish ; contaminants; genotoxic damage

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CONTRIBUTION OF PHYSICAL EXERCISE IN THE REGULATION OF GENOMIC INSTABILITY FOR NEUROPROTECTION IN MICE WITH EXPERIMENTAL GLIOBLASTOMA

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Abstract:
Among malignant neoplasms that affect the central nervous system, glioblastoma (GBM) has the highest incidence. Often fatal, even with pharmacological or radiotherapeutic treatment, GBM commonly leads to a poor quality of life associated with the disease. It is believed that regular physical exercise can be an important non-pharmacological agent because it has already shown significant changes in the pathophysiology of various other types of cancer. Therefore, the objective of this study was to evaluate the effects of physical exercise on genomic instability in animals exposed to an experimental model of GBM. Animal models are commonly used in tumor development studies and testing new therapeutic alternatives. For this investigation, 47 male C57BL/6J mice, 2 months old, randomly selected, were divided into two groups (GBM and SHAM), which were subsequently divided into four groups: sham untrained (Sut, n = 10), GBM untrained (Gut, n = 15), sham trained (Str, n = 10), and GBM trained (Gtr, n = 12). The exercise program consisted of aerobic activity on a treadmill, performed in 5 or 6 sessions per week, with one rest day every three sessions, for 4 weeks. The intensity was adjusted by treadmill speed progressively ranging from 50% to 60% of the pre-established maximum speed, with no inclination. Each session lasted up to 50 min/day. Three days after the last training session, the animals were euthanized, blood was collected, and tissues were removed and stored for analysis of genomic instability parameters. Blood and liver were used for the Comet Assay, and bone marrow from the mice's femur was used for the Micronucleus Test. As a result of the analyses, no alterations in tail intensity of the comet assay were identified in the blood. However, the GBM group showed a significant increase in tail intensity when compared to the sham group in the liver, and these values were significantly reduced by physical training. The micronucleus test in mouse bone marrow showed that animals with GBM had significant increase in the number of micronucleated polychromatic erythrocytes compared to the other groups. However, no significant differences were observed in the ratio of PCE/NCE among the groups. These findings suggest the therapeutic effects of physical exercise during the course of GBM. However, further studies are needed to develop a standardized exercise pattern, with appropriate intensity and duration, for reducing tumor progression and improving the quality of life of patients.
Keywords: physical exercise; glioblastoma; genomic stability; DNA damage; micronucleus

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OCCUPATIONAL EXPOSURE TO INHALATIONAL ANESTHETICS IS ASSOCIATED WITH DNA DAMAGE AND EXPRESSION OF ANTIOXIDANT GENES

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Abstract:

Inhalational anesthetics are commonly used in surgeries and inevitably contaminate the air/workplace with waste anesthetic gases (WAGs), and therefore operating room personnel are occupationally exposed to. International recommendation of WAG exposure is up to 2 parts per million (ppm) for halogenated anesthetics. Literature shows controversial findings related to DNA damage in relation to occupational exposure to WAGs. Reactive oxygen species (ROS) can cause damage to proteins, lipids and DNA, so oxidative stress can cause DNA damage. Genes such as *NRF2, HO1 and SOD2* have important roles against oxidative stress. However, expression of those three genes was not evaluated facing WAG exposure. Thus, this cross-sectional study aimed to evaluate the environmental exposure of WAGs (operating rooms without proper scavenging system) and the impact on DNA damage level and the expression of antioxidant genes. After approval by the Research Ethics Committee, 70 professionals who worked an average of 6 years in surgical center and who were mainly exposed to WAGs sevoflurane and isoflurane comprised the exposed group and 67 volunteers matched by sex, age and body mass index with the exposed group were recruited as a control group. DNA damage was evaluated in mononuclear cells by the comet assay while gene expression was detected by quantitative Real Time Polymerase Chain Reaction (qPCR) in leukocytes. WAG concentrations were assessed by a portable infrared spectrophotometer in professional's breathing zone and results showed a mean of 11 ppm (5.5 times higher than international threshold) of inhalational anesthetics. There were no significant differences between groups in relation to demographic and anthropometric data and for *HO1* expression ($p > 0.05$). The exposed group presented higher levels of DNA damage ($p = 0.03$) and overexpression of *NRF2* ($p = 0.008$) and *SOD2* ($p = 0.004$) compared to control group. In conclusion, the findings suggest that a high exposure to inhalational anesthetics leads to DNA damage and modulates the antioxidant genes expression to overcome ROS damage.

Keywords: anesthetic gases; monitoring; DNA damage; comet assay; gene expression.

Support / Acknowledgment

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IMPACT OF GENETICS, EPIGENETICS AND ANTIOXIDANT RESPONSE IN OCCUPATIONAL EXPOSURE TO ANESTHETIC GASES

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Abstract:
Professionals who work in operation rooms may be daily exposed to trace concentrations of inhalational anesthetics when patients undergo general inhalational anesthesia. It is known that genetics and epigenetics play important roles in identifying mechanisms by which occupational exposure can cause adverse health effects. In fact, there is no consensus regarding the genotoxicity of inhalational anesthetics. Although it is known that methylation can modulate gene expression, there are no studies in the literature on epigenetics and occupational exposure to inhalational anesthetics. Considering that *OGG1* plays an important role in repair of oxidative DNA damage; that *SIRT1* regulates a variety of cellular processes including silencing transcription by deacetylating histones, and that *NRF2* is a key factor in regulating the antioxidant response, understanding genetic and epigenetic pathways may give us new insights into the molecular effects. In addition, antioxidant capacity allows assessing the body's response, providing evidence of oxidative damage related to this occupational exposure. Thus, for the first time, this study jointly evaluated whether occupational exposure to trace concentrations of inhalational anesthetics is associated with DNA damage, methylation, changes in *OGG1*, *NRF2* and *SIRT1* expressions and antioxidant capacity. After the institutional review board approval, 43 professionals occupationally exposed to the most widely used inhalational anesthetics, which concentrations were above international recommended thresholds (halogenated anesthetics ≥ 7 ppm and 165 ppm of nitrous oxide) and 43 volunteers (control group) were recruited. All participants signed the informed consent form and answered a detailed questionnaire. Peripheral blood samples were collected; PBMCs were used for the alkaline comet assay (genotoxicity) while DNA was extracted from whole blood for assessment of global methylation by relative quantification of 5 mdC by HPLC. After RNA extraction from blood, *OGG1*, *NRF2* and *SIRT1* expressions were detected by qPCR. In addition, plasma antioxidant capacity was measured by ferric reducing capacity (FRAP) using spectrophotometry. All data were analyzed using the Student's t test, except for FRAP and *OGG1* that were analyzed by Mann-Whitney test. Demographic data did not differ between groups ($p>0.05$). No difference between groups was observed in relation to global DNA methylation ($p>0.05$). Conversely, the exposed group showed increase in DNA damage ($p=0.02$), *OGG1* ($p=0.009$), *NRF2* ($p=0.02$) and *SIRT1* ($p=0.01$) expressions and in FRAP levels ($p=0.04$) when compared to the control group. Thus, our findings suggest that professionals highly exposed to trace concentrations of inhalational anesthetics showed oxidative, molecular and antioxidant responses, resulting in DNA damage and modulation in *OGG1* and *NRF2* expressions, but no effect in global DNA methylation. Additionally, as *SIRT1* expression was overexpressed, we highlight the need for future studies that assess other epigenetic mechanisms with DNA damage and anesthetic exposure.
Keywords: anesthetic gases; DNA damage; DNA methylation; gene expression; oxidative stress

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Financial support: São Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq)

INVESTIGATION OF THE MUTAGENIC POTENTIAL OF *VITIS LABRUSCA* JUICES OBTAINED FROM IRRADIATED GRAPES IN WISTAR RATS

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Abstract:

The grape, *Vitis labrusca*, contains a variety of phenolic compounds, including resveratrol. Studies have shown that the resveratrol present in grapes has antioxidant and anti-inflammatory properties and may play an important role in disease prevention and health promotion. Some works have shown that it is possible to raise resveratrol levels by irradiating fruits with UV-C (510W). However, research investigating the mutagenic potential of juices obtained from irradiated fruits is scarce. In this sense, the present study evaluated *in vivo*, in *Rattus norvegicus*, Wistar strain, the mutagenic potential through acute treatment, of grape juices (*Vitis labrusca* L.) cultivar Concord, conventional (CONV) and organic (ORG), with and without treatment with UV-C in bone marrow cells through the Micronucleus Test. For this purpose, grape juices were prepared and administered to the animals via gavage in three concentrations (0.5; 0.75 and 1mL/100g body weight (b.w.); the control group and cyclophosphamide received, respectively, 1mL of water via gavage and CP (1.5mg/100g b.w.) via intraperitoneal. Five animals per group were used, and 2,000 polychromatic erythrocytes (PCE) were analyzed to investigate the presence of micronuclei and 1,000 cells between PCE and normochromatic erythrocytes (NCE) for cytotoxicity. The results were subjected to analysis of variance (oneway ANOVA) followed by Tukey's test ($\alpha=0.05$, $p<0.05$, $n=5$) with the aid of the GrafPad Instat program. The results obtained showed that the groups treated with the different types of grape juices did not induce an increase in the frequency of micronucleated cells, with the exception of the group treated with 0.5mL of organic grape juice that exhibited a statistical difference when compared to the control. In addition, the results of juices irradiated with UV-C did not differ statistically from juices that were not irradiated. For the PCE/NCE ratio, none of the juice treatments showed statistically significant differences when compared to the control. Therefore, in the present study, no differences were found between juices obtained from the conventional and organic cultivation system, as well as those obtained from UV-C treated fruits in relation to mutagenic and cytotoxic potential.
Keywords: UV-C; Resveratrol; organic grape; conventional grape.

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INVESTIGATION OF THE CYTOTOXIC AND MUTAGENIC POTENTIAL OF EXTRACTS FROM THE GENUS *VANILLA SPP.* OF COMMERCIAL INTEREST

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Abstract:
Vanilla is one of the most important spices in the world, with great economic value. The genus *Vanilla* has approximately 120 species, with *Vanilla planifolia* G. Jackson being the main species used in the global production of this item present in the food, perfumery and pharmaceutical industries. However, with the great exploitation, several species of Vanilla are under threat of extinction, creating a scenario of crisis in the production of Vanilla. The genus *Vanilla* have a description of medicinal use by traditional people and some studies have already confirmed its antiproliferative activity. It is of great interest to investigate species with potential to occupy this market and that are of natural occurrence in Brazilian territory, such as *Vanilla chamissonis* Klotzsch and *Vanilla bahiana* Hoehne. With this purpose, methanolic extraction of the two mentioned species was carried out, together with the species *V. planifolia*. Analyzes of the mutagenic potential were performed through the Ames Test and cell viability assays were conducted by verifying the effect of the extracts on mitochondrial activity WST assay in human hepatocarcinoma cells, HepG2, and mouse liver fibroblast cells, FC3H. In the Ames test, the assays were performed with concentrations in the range of 0.5 and 5000 ug/pl and on strains TA97a, TA98, TA100, TA102 and TA1535. None of the studied species showed mutagenicity, with the exception of *Vanilla planifolia* at the highest concentration in TA98 strain A possible suggestion for this result is that the phenolic content of the extract together with other organic compounds with antioxidant potential could generate a saturation that is inherent in biological systems, in which free radical scavenging compounds act as antioxidant agents or as pro-oxidant agents. The viability tests were carried out in a range of doses from 0.05 to 5000 ug/ml and with 24, 48 and 72 hours exposures. None of the species induced cytotoxicity, being possible to observe a reduction in cell viability only in the highest concentration, 5000 ug/ml, of the three species and in the two tested cells. Observing these results, it is noted that the species, did not generate mutagenicity or cytotoxicity, which is a promising data in the evaluation of new compounds for human use, whether for food or pharmaceutical purposes.
Keywords: Mutagenicity; Citotoxicity; Vanilla.

OCCUPATIONAL PROFILE OF RURAL WORKERS EXPOSED TO PESTICIDES IN THE IRRIGATED FRUIT FARMING IN THE SUB-MID REGION OF SÃO FRANCISCO

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Abstract:
The practice of using pesticides in family farming is a common phenomenon both in Brazil and in several countries around the world. Among these elements are the lack of instruction in using Personal Protective Equipment (PPE), low education, lack of knowledge of the risks involved and non-adherence to safety guidelines. The sub-medium of the São Francisco Valley (VSF) is known for being one of the main poles of irrigated fruit growing. In this context, this study aimed to carry out the analysis of the occupational profile of rural workers in family agriculture regarding the handling and use of pesticides. This is a cross-sectional study, for data collection a semi-structured questionnaire was used with 20 family farmers in the city of Petrolina-PE, all male, where absolute frequency was used as a tool for building the profile. Of the 20 rural workers, 40% were aged 30-45 years and 30% had incomplete primary schooling. As for the use of pesticides, 23.4% use methonyl, classified as an insecticide, with moderate toxicology (level 3) and banned in 47 countries, with 10.64% the insecticide Cyhalothrin and the herbicide Paraquat Dichloride, with toxicology level 3 and level 1 (extremely toxic) where they are banned in 58 and 29 countries respectively. They are the second most used pesticide by family farmers. These pesticides were obtained from authorized points of sale in the city of Petrolina-PE. According to information from 95% of rural workers, they receive guidance on the use of these products, and 75% of these instructions are provided by agricultural technicians. In addition, 50% of the spraying of pesticides is carried out using animal traction, with the agronomist's prescription and respecting the recommended withdrawal periods. These farmers report that they find it dangerous to work with pesticides, but 10% smoke, 5% drink water, 5% eat food while applying the product. When we relate the frequency (average time) of exposure to pesticides and the use of PPE, it was observed that 50% are exposed 1 to 2 times a week and that 76.6% use complete PPE and 29.4% use it incorrectly or do not use it PPE, therefore, 75% report discomfort when using it, even though 100% of workers describe that they find it important to use PPE during application. As for handling, farmers report that they wash the PPE separately from other parts of the house. Constant exposure to pesticides puts smallholder farmers at risk for both acute and chronic health problems. The results of this research show that low education, weekly exposure and non-use or inappropriate use of PPE are risk factors considered associated with intoxication. It is crucial to strengthen public health initiatives and promote educational programs that serve small farms, offering appropriate training, with the aim of reducing risks to the health of workers.
Keywords: Agriculture; Occupational exposure; Agrochemicals; Public health.

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VARIATION OF THE MITOCHONDRIAL DNA COPY NUMBER (MTDNACN) IN BRAZILIAN FARMERS EXPOSED TO PESTICIDES

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Abstract:
Pesticides are used broadly worldwide to control the pest. This use has the potential to cause adverse effects on human, animal, and environmental health. And also, has been associated with increased production of free radicals and reactive oxygen species, inducing oxidative stress, which can lead to genomic instability. A marker for instability is the mitochondrial genome copy number (mtDNAcn), that have been altered in several types of human diseases, as types of tumors. The aim of this study was to evaluate mtDNAcn variation among individuals occupationally exposed to pesticides. Were carried out real-time PCR assays using a total of 154 individuals (78 exposed and 76 non-exposed). No statistically significant differences in mean age were observed between the exposed (49.12 ± 9.99 years, range 25.20 - 65.00 years) and non-exposed (47.46 ± 10.85 years, range 23.8 - 64.80 years) groups. Of the individuals who had been exposed, with a mean exposure time to pesticides of 30.1 ± 13.4 years, and 42.5% reported the use of personal protective equipment. Individuals exposed to pesticides showed a significant reduction in mtDNAcn (1.11±0.37mtDNAcn/genome) when compared to non-exposed individuals (1.30±0.33mtDNAcn/genome; $p=0.001$). In the multivariate analysis, subjects who reported the use of haloxyfop and copper sulfate showed an increase, ($\beta=0.200$, $p=0.053$) and decrease ($\beta=-0.2$, $p=0.021$), respectively, in mtDNAcn compared to those who reported non-exposure to these pesticides. Therefore, our findings suggest that chronic exposure to pesticides led to changes in mtDNAcn.
Keywords: genome mitochondrial; mtDNAcn; pesticides expositor; rural workers.

ARAUCARIA BROWN PROPOLIS: *TOXICOLOGICAL AND CHEMOPREVENTION ANALYSIS*

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Abstract:
Brazilian brown propolis (BBP) refers mostly to propolis from the south region, in areas dominated by Araucaria forests. Some biological activities have been attributed to this type of propolis, such as leishmanicidal, anti-inflammatory, nociceptive, and antimicrobial. However, studies on Brazilian brown propolis are scarce, especially when compared to green propolis. In this sense, the present study aimed to carry out toxicological evaluations that contribute to the safe use of BBP for human health. Furthermore, its chemopreventive potential was investigated. The cytotoxicity of extract was evaluated by two assays, clonogenic cell survival and XTT, using V79 cells. BBP significantly reduced cell colonies at concentrations greater than or equal to 10 µg/mL, while IC_{50} equivalent to 83 and 21.4 µg/mL were observed after 24 h and 48 h of treatments, respectively, by the XTT assay. Cell cultures treated with the highest tested concentration of BBP, 7.5 µg/mL, show a significantly lower nuclear division index than the negative control, indicating cytotoxicity through micronucleus test. For the Salmonella/microsome assay, BBP did not show mutagenicity when evaluated in strains TA98 and TA100. However, the highest concentration of BBP tested, 4000 µg/plate, led to a significant increase in revertant colonies in TA102 strain, in the absence of metabolization. The evaluation of acute toxicity of BBP in a zebrafish model revealed LC_{50} equivalent to 8.83 mg/L. Considering the evaluation of the chemopreventive potential, the concentration of 2.5 µg/mL of BBP significantly reduced the chromosomal damage induced by the mutagen doxorubicin in V79 cell cultures. The antioxidant activity observed by the DPPH assay may be responsible, at least in part, for the antigenotoxic effect of BBP. Therefore, the evaluated brown propolis exhibited cytotoxic and mutagenic activity at higher concentrations, while at lower concentrations it displays a chemopreventive effect on doxorubicin-induced mutagenicity. The data from the present study contribute to a better understanding of the biological activities of BBP.
Keywords: Genotoxicity; antioxidant activity; micronucleus; Ames test; zebrafish

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Carcinogenesis / Oncogenetics

ALLELIC VARIANTS AND IMMUNOSTAINING PROFILE IN CXCL12/CXCR4 AXIS: AN INVESTIGATION OF ASSOCIATION WITH PROGNOSIS IN PROSTATE CANCER

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Abstract:

Prostate cancer (PCa) is the malignant neoplasm that most commonly affects men and is an important cause of death. It can be detected by changes in serum levels of Prostate Specific Antigen (PSA) and digital rectal examination, but often symptoms do not appear until advanced stages and metastases. The *C-X-C Motif Chemokine Ligand 12/C-X-C Motif Chemokine Receptor 4 (CXCL12/CXCR4)* axis acts in cell migration and may be involved in the metastatic process. In this context, the aim of this study was to evaluate the allelic variants rs1801157 *(CXCL12)* and rs2228014 *(CXCR4)* and the immunostaining of CXCR4 protein as candidates for prognostic markers in PCa. Samples (n=60) were divided according to prognostic parameters (with and without metastasis at diagnosis) in tree groups: better prognosis, worse prognosis with metastasis at diagnosis and worse prognosis without metastasis at diagnosis, and immunostaining was evaluated by indirect immunohistochemistry, considering tumoral and adjacent tissues from the same patient (n=120). A significant association was found between the C allele of rs2228014 *(CXCR4)* and the extraprostatic extension. For CXCR4 immunostaining a weak labeling and a cytoplasmic localization predominated, as well as a significant difference between malignant versus adjacent tissue, with higher protein expression in the malignant tissue. A significant association was found between CXCR4 tumor immunostaining with TNM staging (T2b-T2c) and PSA level (> 20ng/mL). None of the allelic variants affected CXCR4 immunostaining. Prognostic groups did not differ in allelic variant frequency or immunostaining profile. Findings suggest that CXCR4 receptor may be one of the ways to worsen the prognosis of prostatic cancer.

Keywords: Genetic polymorphism; Immunohistochemistry; rs2228014 *(CXCR4)* ; rs1801157 *(CXCL12)*.

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BLOCKAGE OF MACROAUTOPHAGY MEDIATES CROSSTALK AMONG CHAPERONE-MEDIATED AUTOPHAGY, NRF2 AND FERROPTOSIS

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Abstract:

Glioblastoma is the adult-type diffused glioma with the worst prognosis. Stated the low therapeutics advances since the Stupp protocol in 2005, which introduces temozolomide as standard chemotherapy, the average survival time remains only 15 months. Moreover, the frequent drug resistance developed in glioblastoma treatment is one of the biggest obstacles to extending patient survival. Beyond the well-established temozolomide resistance mechanisms, autophagy is a mechanism highly conserved that cancer cells use to adapt to the stress induced by chemotherapy agents, including the different types: macroautophagy (MA) and chaperone-mediated autophagy (CMA). Although studies indicate that autophagy inhibition can be promising for improving the treatment response, it is necessary to carry out extensive investigations into the compensation that may exist between MA and CMA. Furthermore, it is essential to consider the connection between autophagy and the nuclear factor-erythroid 2 related factor 2 (NRF2), which on one hand promotes chemoresistance but, on the other hand, it is an important predictor of sensitivity to ferroptosis inducers, a newly discovered non-apoptotic type of cell death. Thus, we aimed to analyze the different sensitivity to temozolomide between the established glioblastoma (U251) knockout lineages for key proteins of macroautophagy (ATG7) and chaperone-mediated autophagy (LAMP2A); verify the functional crosstalk between MA and CMA; and clarify the association of autophagy and NRF2, which may reveal innovative alternatives. Thereby, viability and clonogenic assays were conducted through XTT reagent and violet crystal pigment respectively, and our results demonstrated that U251 ATG7 KO cells were more resistant to TMZ treatment and the elimination of LAMP2A (U251 ATG7/LAMP2A KO) was able to reverse this phenotype. The western blot analyses indicated a compensatory increase of LAMP2A protein in U251 ATG7 KO cells, while no increase of ATG7 protein in U251 LAMP2A KO cells was observed. In addition, it was noticed an increase in NRF2 protein in U251 ATG7 KO and LAMP2A KO cells when compared with the U251 WT and ATG7/LAMP2A KO, which displayed similar levels of this protein. Finally, it was observed that the cells with higher levels of NRF2 were more sensitive to the ferroptosis inducer RSL3. The statistical analyses of the data were performed by one-way ANOVA, with proper checks of their assumptions. Thereby, it is possible to infer that the blockage of macroautophagy triggers temozolomide resistance in U251 cells through the compensatory increase of CMA suggested by the elevated level of LAMP2A protein in U251 ATG7 KO cells and the reversion of the resistance phenotype in U251 ATG7/LAMP2A KO. Furthermore, both single knockouts promoted accumulation of NRF2 protein, and an increased sensitivity to RSL3, which may indicate a critical role of NRF2 and ferroptosis in the autophagy pathway. These results encourage further investigation into the functional universe of the interplay among MA, CMA, NRF2, and the responses to traditional and novel therapeutic approaches.

Keywords: Glioblastoma; Autophagy; NRF2; Chemoresistance; Temozolomide

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IN VITRO EVALUATION OF THE INHIBITORY EFFECT OF THE FLAVONE CIRSIMARIN ON THE MIGRATION OF PROSTATE TUMOR CELLS (DU-145)

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Abstract:
Prostate cancer is the fourth most common cancer in both sexes worldwide, the second most common in men, and the fifth leading cause of death. Due to the increasing incidence and limited treatment options, new therapeutic strategies are needed. Traditionally, plant species have been used in various therapeutic preparations, and among all phytochemicals, flavonoids stand out for their low toxicity and minimal adverse effects. In this context, it has already been observed that the flavone cirsimarin, extracted from the plant Scoparia Dulcis Linn, is cytotoxic to breast tumor cells (MCF -7) and can inhibit their migratory activity. Therefore, the aim of this study was to investigate the in vitro effect of the flavone cirsimarin on prostate tumor cell migration. Wound healing migration assay was performed on cell cultures in monolayer (2D) of brain metastatic human prostate adenocarcinoma cells (DU -145) in different concentrations of cirsimarin (1, 5, 10, 20, 40, 80, and 160 µM) at times of 0, 24, 48, and 72 h. The results of the assay were obtained by using the QC software. Images were documented with QCapture Pro 7 software, migration area was measured with ImageJ software, and statistical analysis was performed with GraphPad Prism 8 software using the ANOVA test followed by Tukey's post-test for samples with normal distribution. The compound was shown to inhibit cell migration starting at a concentration of 20 µM at all time points tested (24, 48 and 72 hours). It is concluded that the flavone cirsimarin appears to have an anti-migratory effect on prostate tumor cells, suggesting that it is a potential therapeutic alternative for metastasis. However, further studies need to be conducted to verify the actual mechanism of action of this compound.
Keywords: Flavonoids; Cell culture; Prostate cancer; Cell migration.

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We would like to say thank for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Finance Code 001), Federal University of Maranhão and the Graduate Program in Genetics and Molecular Biology of the State University of Londrina.

EFFECTS OF THE FLAVONE CIRSIMARIN ON THE VIABILITY AND PROLIFERATION OF HUMAN GINGIVAL FIBROBLASTS IN A TWO-DIMENSIONAL (2D) AND THREE-DIMENSIONAL (3D) MODEL

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Abstract:
Cancer is currently the main public health problem in the world, and the treatments used have many adverse effects on patients. Natural compounds are alternatives to synthetic chemotherapeutics used in the clinic. The flavone cirsimarin (CIR) extracted from the aerial parts of the medicinal herb Scoparia dulcis has already demonstrated antitumor activity in cultured breast cells (MCF-7); however, its effects on non-tumor cells are not yet approved. Thus, the objective of the present study was to analyse the effects of CIR on non-tumor human gingival fibroblasts (HGF) through two-dimensional (2D) and three-dimensional (3D) cultures, the latter capable of more accurately mimicking in vivo tissues. The influence of CIR (1, 5, 10, 20, 40, 80, and 160 μM) on viability in a 2D model was evaluated using resazurin and acid phosphatase assay after 24, 48, and 72 h of treatment and lactate dehydrogenase (LDH) release assay after 48 and 72 h of treatment. In the 3D model, the integrity and area of the treated spheroids were analysed and photographed every 72 h until 216 h. At the end of this assay, the resazurin test was performed. The results were analysed using the ANOVA test and the Dunnett (viability) or Sidak (integrity) post-test. The results obtained in a 2D model showed that in the resazurin assay, the CIR (80 and 160 μM) reduced viability at all treatment times. In the acid phosphatase assay, after 24 h of treatment, CIR reduced cell viability only at concentrations of 80 and 160 μM, while at 72 h there was a reduction in viability at all concentrations analysed. The results of the LDH assay did not show statistical significance, indicating any induction of membrane disruption and enzyme release in any of the treatments. Regarding resazurin in a 3D model, there was a reduction in cell viability at concentrations of 40, 80, and 160 μM, demonstrating similarity to the results obtained in a 2D model. At these concentrations ≥40μM, the spheroids were smaller than the negative control after 72 h of treatment, suggesting induction of cell death by cirsimarin and consequent disintegration of these dead cells from the 3D structure, corroborating the results of the resazurin assay. Thus, in contrast to results previously observed in breast tumor cells, the flavone CIR reduced cell viability only at the highest concentrations in normal FGH cells, demonstrating very interesting selective effects. These results could be promising in the search for new anticancer drugs with reduce adverse effects.
Keywords: 2D culture; 3D culture; Cirsitakaoside; phytochemicals.

IRF-5 RS3757385 POLYMORPHISM PREDICTS SUSCEPTIBILITY AND RISK OF RECURRENCE IN BLADDER CANCER

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Abstract:
Bladder cancer (BC) is the most recurrent neoplasm that affects the urinary system and occupies the 10th position among the most common cancers in the world. National data inform that, on average, there are 10,640 cases/year among men (prevalent) and women. Under this condition, investigating factors that may affect susceptibility to BC, determining the prognosis, and predicting the chances of recurrence is extremely relevant. The IRF-5 protein (Interferon Regulatory Factor 5) regulates type I Interferons, which are associated with the induction of inflammatory cytokines and apoptosis. The best-elucidated way of acting IRF-5 is by stimulating the transcription of pro-apoptotic genes, such as BAK1, BAX, CASP8, and DAPK2. IRF-5 was also described as a required Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) ligand in the apoptosis of cancer cells, acting in a step before the activation of CASP8, which TRAIL induces. So far, there are no elucidations about the alterations in the expression of IRF-5 caused by rs3757385 (T>G) in the bladder; however, this same SNP demonstrated that the cardiac tissue of individuals with the homozygous mutated genotype (GG) showed an increase in IRF-5 expression, followed by the heterozygous genotype (TG) and the homozygous reference (TT). The present study verified the frequency of the rs3757385 polymorphism in the IRF-5 gene located on chromosome 7 in BC patients and control individuals to assess its role in BC susceptibility and prognosis. The two groups were evaluated for occupational exposure, alcoholism, smoking, and patients for prognostic factors such as tumor histology (invasive and non-invasive) and relapse risk. 294 patients and 294 controls were genotyped by real-time PCR using a TaqMan® probe. Among the patients, 50.5% had the GG homozygous genotype, 49.3% are heterozygotes (TG) and 50.9% TT homozygotes. In the control group, the GG, TG, and TT are 49.5%, 50.7%, and 49.1% individuals, respectively. The multinomial regression analysis using the SPSS software showed significant results for susceptibility in the heterozygous genotype (TG) and patients "not exposed to pesticides" (OR=0.389, CI=0.193-0.786). Regarding prognosis, the Dominant model (GG+TG) and the variable "Recurrence within one year" were significant (OR=0.364, IC=0.171-0.773). Our analyzes showed that in the codominant model, heterozygous individuals (TG) not exposed to pesticides had protection for BC. In the dominant model, the polymorphism confered protection against BC relapse within 1 year (OR=0.364, CI=0.171-0.773). Our results showed a relevant role for the rs3757385 polymorphism of the IRF-5 gene in the susceptibility and follow-up of BC.
Keywords: Inflammation; Urothelial cancer; Single nucleotide polymorphism.

Support / Acknowledgment

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HUMULENE INCREASES THE EXPRESSION OF *CXCL8* BUT NOT IL-8 SECRETION IN THE TRIPLE-NEGATIVE BREAST CANCER CELL LINE BT-20

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Abstract:
Triple-negative breast cancer (TNBC) is a molecular subtype of breast cancer defined by the absence of hormone receptors, estrogen (ER), and progesterone (PR), as well as negative for epidermal growth factor receptor 2 (HER-2). The disease is characterized by its aggressive behavior, high recurrence, metastasis, and mortality rates. Target therapies for breast cancer are not effective for TNBC subtype because they lack the expression of these receptors. Therefore, there is a need for a better understanding of the disease to develop new therapeutic strategies. Plant-derived and plant-isolated compounds, such as camptothecin-derived semi-synthetic drugs, have been used in cancer treatment for years. Sesquiterpenes have been studied for their various biological and pharmacological activities, including antitumor and immunomodulatory effects. The sesquiterpenes humulene (CAS: 6753-98-6) and caryophyllene (CAS: 87-44-5) are commonly found together in plant essential oils. The objective of this study was to evaluate the *in vitro* effects of humulene and caryophyllene, isolated or in combination, in TNBC-derived cell lines (BT-20, BT-549, MDA-MB-231, MDA-MB-436) at different concentrations (6.25, 12.5, 25.0, 50.0 and 100.0µM). The cell viability was analyzed by MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide). BT-20 cells were selected for the wound healing assays and further analysis of the expression levels of the *CXCL8* gene and its encoded product (cytokine Interleukin-8, IL-8), determined by quantitative real-time RT-PCR and ELISA, respectively. The experiments were done in triplicate and two independent biological replicates. The statistical significance of the data compared with untreated controls was determined by the ANOVA/Dunnett's multiple comparison tests or Kruskal-Wallis' test. The significance level was 5% and the statistical test was performed using Prism 10 software (GraphPad Software, Inc., USA). The cytotoxic effect was observed in BT-20 cells at the highest concentration of humulene and caryophyllene combined. The cell migration rates of BT-20 cells were not influenced ($p>0.05$) after exposure to 50µM of α-humulene and/or β-caryophyllene. However, our results showed that treatment with 50µM of α-humulene for 96h significantly increased ($p=0.01$) the expression levels of the gene *CXCL8*, whereas it did not reflect ($p>0.05$) alterations in IL-8 secretion under the same experimental conditions. It is known that IL-8 has tumor-promoting effects in various types of cancer. In breast cancer, literature shows that this cytokine influences the epithelial-to-mesenchymal transition of adjacent epithelial cells, which favors the development of a metastatic phenotype, and the *in vitro* invasive potential is reduced when IL-8 receptors are inhibited. Interleukin-8 also can modulate the tumor microenvironment by recruiting immune suppressive cells to infiltrate tumor bulk. Our discrepant results between *CXCL8* mRNA expression and IL-8 secretion in BT-20 cells highlight the need to better understand the mechanism of IL-8 secretion, the elements involved in its intracellular retention, and the disruption of intracellular protein trafficking in TNBC. Further studies are needed to understand the effect of humulene on the expression of *CXCL8* in BT-20 and to elucidate the intracellular retention of IL-8 in TNBC.
Keywords: sesquiterpene; cytokine; interleukin-8.

Support / Acknowledgment

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CYTOTOXIC AND CYTOSTATIC PROFILE OF THE FLAVON CIRSIMARIN IN TUMOR PROSTATE DU-145 AND NON-TUMOR PNT-2 CELLS GROWN IN MONOLAYER (2D)

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Abstract:
Cases of cancer have gradually increased, one example being prostate cancer, which is the most common type of cancer in men in Brazil. Due to the limitations of existing treatments and the increase in their incidence, new therapeutic strategies are needed, reinforcing the importance of studying possible products that can combat the progressive development of tumors. Traditionally, plant species are used in various therapeutic preparations, and among all phytochemicals, flavonoids stand out. The flavone cirsimarin, extracted from the plant species *Scoparia dulcis* Linn, has demonstrated its effectiveness in the treatment of diabetes, hypertension, hemorrhoids, and wounds. Recently, the anticancer action of the extract of the aerial parts of *Scoparia dulcis* L. was demonstrated, showing a cytotoxic effect in cells of the breast tumor lineage (MCF-7) cultivated in 2D and 3D cell culture models, in addition to inhibition of the migratory capacity of these cells. Therefore, the present evaluated *in vitro* (2D) the effects of the flavone cirsimarin in different concentrations (1, 5, 10, 20, 40, 80, and 160 µM) in tumor (DU-145) and non-tumor (PNT-2) prostate cells, considering the cell viability (resazurin and acid phosphatase) at different times of treatment (24, 48 and 72 h), liberation of Lactate Dehydrogenase (LDH) by membrane disruption (after 72 h of treatment) and cell death assay by triple staining (after 24 h of treatment). Cirsimarin decreased DU-145 tumor cell viability in the two assays, resazurin and acid phosphatase, with the following CC50 values obtained after 24 h of treatment 6.5 µM and 22.9 µM; after 48 h of treatment 3.4 µM and 12.9 µM; and after 72 h of treatment 6.6 µM and 71.3 µM, respectively. As for the PNT-2 cells, the respective CC50 values after 24 h of treatment were 44.6 µM and 79.9 µM. The cytotoxicity was higher for the tumor lineage with a selectivity index equal to 7.17 for resazurin and 3.49 for phosphatase assays compared with PNT-2 cells. In the LDH assay, the results showed that cirsimarin did not induce death with membrane damage, with a CC50 >160 µM. This fact was proved through the triple staining death assay, in which the flavone induces death by apoptosis (without membrane disruption and PI labeling) from the concentration of 10 µM. Furthermore, as cirsimarin concentrations increase, cell viability decreases and the number of apoptotic cells increases. With this study, it was possible to verify that flavone cirsimarin has cytotoxic effects in tumor and non-tumor prostate cell lines, being more cytotoxic in tumor cells. In addition, the compound demonstrates that it does not induce cell death by necrosis, causing damage to the cell membrane, but by a type of programmed death, such as apoptosis. Therefore, the use of this compound appears to be promising, indicating that further studies should be carried out to evaluate the anticancer potential of the flavone cirsimarin.
Keywords: Flavonoids; Phytochemicals; Cell Viability; Cell Culture.

Support / Acknowledgment

We would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Finance Code 001), Federal University of Maranhão, Graduate Program in Genetics and Molecular Biology and the State University of Londrina.

EVALUATING THE ROLE OF AUTOPHAGY IN ESOPHAGEAL SQUAMOUS CELL CARCNOMA PROGRESSION AND THE POTENTIAL INVOLVMENT OF *TP53*

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Abstract:

Esophageal squamous cell carcinoma (ESCC) is a poor prognosis tumor and characterized by its molecularly heterogeneity, with mutation in *TP53* gene being the only alteration found at high frequency. *TP53* is responsible for orchestrating responses to various cellular stressful stimuli, maintaining genomic integrity. Thus, it regulates important cellular processes such as autophagy, an essential catabolic process for maintaining cellular homeostasis, and its imbalance is associated with carcinogenesis. In cancer, stresses stimuli are associated with tumor progression and capable of triggering autophagy, acting as a survival pathway for tumor cells. The knowledge about autophagy mechanisms involved in ESCC carcinogenesis is scarce. Therefore, our goal is to understand the molecular basis of autophagy in ESCC and the involvement of p53 in regulating this process. First, to assure experiments quality and efficacy of the different treatments performed, we assessed cell viability and proliferation (CCK-8 assay), *CDKN1A* expression (RT-qPCR) to determine the efficacy of p53 function reactivators, and LC3II labelling (immunofluorescence) to determine the use conditions of autophagy modulators in ESCC TE-1 cell line. TE-1 presents a thermosensitive *TP53* mutation resulting wild-type (wt) or mutant (mut) protein expression, depending on the culture temperature. Next, we evaluated *in vitro* whether *TP53* mutation impacts on autophagy and apoptosis rates, as these are closely linked process, both regulated by p53. To this end, ESCC cell lines were treated with autophagy modulating compounds - Torin-1 (autophagy activator), 3-MA (early-stage autophagy inhibitor) and Hydroxychloroquine (HCQ - inhibit late-phase autophagy) - and of p53 function - APR-246 (reactivates mut p53 protein function) and Nutlin-3a (wt p53 function enhancer), either alone or in combination. We have measured caspases 3/7 activity by luminescence (Caspase-Glo® 3/7 Assay System) to evaluate apoptosis in TE-1 wt or mut p53 cells upon combined treatments. Apoptosis rates were increased by treatment with both APR-246 and Nutlin-3a at 48h. Autophagy modulators do not change apoptotic levels. However, they do increase caspases rates when combined with p53 function reactivators, and there is no significant difference between wt or mut p53 cells. To assess autophagy flux, we used an Autophagy LC3-antibody based kit (Cytek) to track LC3 (a key marker of autophagy) levels within the cell. Under basal conditions, autophagic flux is increased in mut p53 cells when compared to the wt ones. In mut p53 cells, torin-1 treatment significantly increased autophagic flux, whereas combined treatment with APR-246 reverted this phenomenon. HCQ treatment led to decreased autophagic flux, whereas combined treatment with APR-246 did not significantly change it. In wt p53 cells, Nutlin3-a increased autophagic flux, while Torin-1 reverted these levels, both alone or in combination with Nutlin-3a. Conversely, HCQ showed a milder autophagic flux inhibition effect than in mut p53 cells, and combined treatment with Nutlin-3a was not capable of reverting it. 3-MA did not show relevant results in both cells. These differences in autophagic flux results between the two cells should be based on p53 action. Next, we will evaluate parameters of tumor malignancy and mechanisms by which p53 may be involved in autophagy imbalance and ESCC progression.

Keywords: Autophagy; *TP53*; Esophageal Squamous Cell Carcinoma.

CHARACTERIZATION OF THE ANTITUMOR ACTIVITY OF A CURCUMIN-RESVERATROL MOLECULAR HYBRID IN MURINE MELANOMA

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Abstract:

Melanoma is a potentially fatal type of cancer due to its metastatic and chemoresistance profile to conventional treatments, such as chemotherapy. Although immunotherapy and target-directed therapies have increased the chances of cure for patients, new approaches are still needed that minimize the problems related to selectivity, resistance, and low response rate. In this sense, substances synthesized based on the chemical structure of natural compounds have emerged as effective chemotherapeutic agents. The present study aimed to investigate the antitumor effects of PQM-162 [(E)-3-(4-hydroxy-3-methoxyphenyl)-N'-((E)-4-methoxybenzylidene) acrylohydrazide], a resveratrol and curcumin hybrid derivative. For the antimelanoma evaluation, the reduction in tumor weight and volume was quantified after melanoma induction in C57BL/6 rodents using the B16F10 cell line. The animals were treated with PQM-162, at a dose of 2 mg/kg/body weight (b.w) administered subcutaneously (s.c) to male C57BL/6 mice for 5 consecutive days. In addition, systemic toxicity (body weight, water consumption, blood creatinine levels) and genotoxicity in blood and bone marrow cells through micronucleus assays were evaluated. The obtained data were statistically analyzed by variance (ANOVA) analysis for completely randomized experiments, with the calculation of the F statistic and its respective P value. PQM-162 showed no systemic toxicity signals, an increase in the ratio of polychromatic and normochromatic erythrocytes (PCE/NCE) were observed in groups treated with PQM-162 when compared to treatment with cisplatin alone (7 mg/kg b.w. s.c for 5 days). Bone marrow genotoxicity was significantly lower in the group of animals that received PQM-162 when compared to the group that only received cisplatin. More studies are being carried out in order to investigate the molecular mechanisms involved in the antitumor activity. Therefore, under the experimental conditions used in this evaluation, the data suggest that PQM-162 can be considered a promising candidate molecule for anticancer therapy due to the antitumor effect displayed without systemic toxic effects.

Keywords: melanoma; antitumor; curcumin; resveratrol; molecular hybrid

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POLYMORPHISM OF THE UGT2B7 RS7438135 INCREASES THE RISK OF BLADDER CANCER WHEN ASSOCIATED WITH HYPERTENSION AND DAILY MEDICATION CONSUMPTION

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Abstract:
Urothelial cancer is a malignant neoplasm that affects the organs of the urinary system, including the bladder (BC), reaching men 4 times more frequently than women. In this context, studies involving factors that can influence BC, such as susceptibility, parameters of good and bad prognosis, and predicting chances of recurrence, are important. UGT2B7 codes for a protein belonging to phase II drug metabolism, participating in detoxifying endogenous and exogenous molecules and metabolites of oxidative stress. Genetic polymorphisms in genes such as UGT2B7 may influence the process of metabolizing xenobiotics and antioxidant defense. Thus, the present study analyzes the frequency of the polymorphic variant rs7438135 (G>A) in the UGT2B7 gene in patients with BC. It compares it with the frequency in control individuals free of this neoplasm. Besides, we associated the results with the variables habit of smoking, alcoholism, occupational exposure, diabetes, hypertension, continuous medication consumption, history of cancer in the 1st-degree family, and history of cancer in the family 2nd and 3rd-degree between patients and controls. The genotype frequencies were associated with the prognostic variables (tumor invasion, tumor grade, and recurrence) for the patients. For this purpose, 295 patients and 295 controls were genotyped using the real-time PCR technique using the TaqMan® probe. All individuals participated voluntarily, signed an informed consent form, and completed a personal questionnaire. The statistical analyzes employed were multinomial logistic regressions to assess the impact of the rs7438135 polymorphism of the UGT2B7 gene on the incidence or not of BC and for the mentioned variables. The results were analyzed using the IBM SPSS Statistics 21 program. The frequencies of the homozygous (GG) reference genotype were 16.9% in the patient group and 17.3% in the control group. For the heterozygous genotype (GA) the frequencies were 48.5% and 45.8 % in the patients and controls, respectively. The mutated homozygote (AA) were 34.6% and 36.9 % in patients and controls. The results of the regression did not show any significant value (p>0.050) for BC susceptibility. However, signed values were found in the interaction of the recessive model and some variables. A protective effect was observed for patients not exposed to pesticides with the AA genotype (OR=0.480 - CI= 0.242-0.954). Carriers of the AA genotype have 50% more glucuronidation activity, which may explain the observed protective effects. Patients who have hypertension and present the genotype (GG+GA) (OR=1.922, CI=1.078-3.426), patients with daily medication consumption (OR=1.736, CI=1.031-2.923), and patients who do not consume daily medications with the same genotype (GG +GA) (OR=1.800, CI= 1.014-3.196) are in risk. No significant association was observed with the prognostic parameters in patients. This risk could be explained by the low activity of the enzyme in carriers of the G allele, as it results in poor metabolism, thus leading to the accumulation of non-excreted compounds. Our results indicate that, in the recessive model, the rs7438135 polymorphism confers susceptibility to BC by associating it with hypertension variables and daily medication consumption.
Keywords: SNP; 842G>A; Metabolism; Urinary System.

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NRF2 ACTIVATION CONTRIBUTES TO CHEMOTHERAPY RESISTANCE IN TUMOR CELL LINES CULTIVATED IN A THREE-DIMENSIONAL MODEL

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Abstract:
Cancer stands out as one of the leading causes of death nowadays, and its treatment is a worldwide concern. Therapeutic interventions focus on radiotherapy and chemotherapy, which lead to the activation of several cell death pathways. Nevertheless, efficient regulatory mechanisms associated with molecular elements such as the nuclear factor erythroid 2-related factor 2 (NRF2) and some of its protein targets have favored cellular stress control and consequent resistance to treatment. Studies in this field have brought to light important findings, however, most of the carried out experiments are based on monolayer cultures, which, despite constituting an incredibly important model, do not fully mimic important characteristics of the tumoral environment. The present study aims to comparatively analyze the chemotherapy resistance phenotype presented by cancer cell lines in bi-dimentional (2D) and three-dimensional (3D) culture models, addressing the elucidation of possible protein targets involved in cellular protection. To this end, we first standardized spheroid cultivation for human glioblastoma (T98G) and alveolar adenocarcinoma (A549) cell lines using the liquid overlay technique. Agarose gel was used as a way to establish a coat that prevents the adhesion of cells to the plate, favoring their aggregation into a three-dimensional mass. The obtained spheroids and the monolayered cells were then treated with the preferred chemotherapeutic agents, temozolomide (TMZ) (2 replicates) and cisplatin (3 replicates), respectively. The captured viability was subsequently analyzed. We observed that both lineages proved to be more resistant to treatment in the three-dimensional model. Thus, we chose to analyze the expression of NRF2 and its molecular targets, in the different culture models. That was made through the Western Blot protein quantification technique. Our results showed that both cell lines present higher NRF2 expression in the three-dimensional arrangement. Regarding its molecular targets, it was noted that T98G cells have higher expression of the heme oxygenase-1 gene (HMOX1) and of the multidrug resistance protein 1 (MRP1) in the 3D model, while A549 cells present a similar picture for MRP1 and little variation between the culture patterns when regarding HMOX1. It is worth noting, however, that A549 is endowed with a considerably high concentration of HMOX1 in the 2D model when compared to T98G. In order to observe the role of these targets in the modulation of resistance, we treated the T98G cell line, in the 2D model, with TMZ and an HMOX1 inhibitor (ZnPP). We observed a considerable decrease in cell viability when HMOX1 is inhibited, which will be further investigated. In light of the effect of NRF2 on oxidative stress, the role of MRP1 in the detoxification of endogenous substances and toxins, such as in multi-drug resistance (MDR) and the cytoprotective action of HMOX1, our results suggest that the increased expression of these targets may assist in explaining the resistance phenotype observed in the three-dimensional arrangement, as demonstrated by the findings here presented. Thus, the modulation of these factors may constitute an interesting approach to circumvent the challenge concerning chemotherapy resistance.
Keywords: Cancer; Spheroids; Multidrug Resistance; Heme-oxigenase-1.

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PRO-FERROPTOTIC GENES PLAY A CRITICAL ROLE IN OVERCOMING DRUG RESISTANCE IN NRF2-ADDICTED TUMOR CELLS.

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Abstract:
Cancer is one of the primary causes of death on a global scale. Despite significant advancements in cancer treatment through the development of chemotherapy drugs, the main cause of treatment failure in metastatic cancer is attributed to drug resistance. Tumor cells can upregulate oxidative stress defense programs to overcome the toxicity of chemotherapy drugs, leading to resistance. NRF2, an important transcription factor that regulates antioxidant response genes, has been closely associated with drug resistance and poor clinical outcomes. Another key mechanism of drug resistance involves acquiring genetic mutations to evade apoptotic cell death. Consequently, alternative caspase-independent cell death pathways, such as ferroptosis, have emerged as potential treatment approaches to eliminate drug-resistant cancer cells. However, the molecular mechanisms of ferroptosis and its interplay with NRF2 in cancer remain to be fully elucidated. Therefore, this study aims to analyze the relation between NRF2 and ferroptosis in specific cancer entities. To achieve this, we examined several cellular responses in two human glioma cell lines U251MG and T98G, one human lung carcinoma cell line A549, and one human breast cancer cell line MCF7. In our analysis, the T98G and A549 cell lines demonstrated higher levels of NRF2 expression and activity, as well as increased glutathione levels, compared to U251MG and MCF7 cells. Interestingly, we observed by cell viability analyses that NRF2-addicted cells exhibited greater sensitivity to the ferroptosis inducer RSL3 in a dose-dependent manner, along with elevated levels of lipid peroxidation measured by BODIPY 581/591 C11 probe, indicating that high NRF2 levels do not necessarily imply ferroptosis resistance. To investigate the differential mechanisms of ferroptosis sensitivity in these cells, we analyzed the expression of ferroptosis-related proteins through western blot analyses. First, we observed that MCF7 cells were found to lack endogenous ACSL4 expression, contributing to its ferroptosis resistance, since this enzyme is crucial for ferroptosis induction. Conversely, the ferroptosis-sensitive cells (T98G and A549) displayed low levels of GPX4, which acts as the main ferroptosis inhibitor mechanism. Also, both cell lines displayed higher expression of NRF2 targets MRP1 and HMOX1, which are commonly associated with tumor survival and drug resistance. On the other hand, recent studies have described the pro-ferroptotic role of these NRF2 target genes. In fact, high levels of MRP1 promote glutathione efflux, disrupting the mechanism of defense from ferroptosis, whereas elevated HMOX1 levels lead to excessive cellular iron availability strongly contributing to ferroptosis. Thus, the vulnerability of cancer cells to ferroptosis through NRF2 pro-ferroptotic targets represents an Achilles' heel that could be exploited to reverse drug resistance in cancer by using ferroptosis inducers. In this regard, we evaluated the potential additive effect of ferroptosis induction in combination with chemotherapy treatment in T98G and A549 cells, and we observed a significant decrease in cell viability after the combinatorial treatment. Altogether, our data suggests that elevated NRF2 expression does not predict resistance to ferroptosis, and more importantly, NRF2 can upregulate genes that influence ferroptosis susceptibility, such as MRP1 and HMOX1, which play a critical role in counteracting drug resistance in NRF2-addicted cells through ferroptosis therapy. This mechanism of sensitivity to ferroptosis in NRF2-addicted cells holds great potential as a strategy to combat cancer progression.
Keywords: Drug resistance; NRF2; Ferroptosis; Cancer.

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BCL-2 AND H2AX IMMUNOSTAINING PROLIFE IN BLADDER CANCER: AN INVESTIGATION WITH TUMOR AGGRESSIVENESS

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Abstract:
Bladder cancer is a highly malignant neoplasm of the urinary tract worldwide and has an elevated rate of tumor recurrence, mainly affecting males. However, this disease lacks markers capable of predicting tumor recurrence and aggressiveness. It is known that the BCL2 apoptosis regulator (BCL-2) gene encodes a protein that acts in regulation of programmed cell death, a fundamental process in development of malignant tumors. Although H2A variant histone (H2AX) encodes a histone responsible for signaling the double-strand DNA damage that contributes to the recruitment of repair factors and can also influence the formation of the malignant neoplasm. Within this context, the objective of the present work was to analyze the immunostaining prolife of both proteins in 30 tissue samples from patients with malignant bladder tumors (12 invasives and 18 non muscle invasives) and correlated with prognostic parameters: recurrence, staging, tumor grade and muscular invasion. For this purpose, monoclonal antibodies against BCL-2 and H2AX proteins were subjected to the indirect immunohistochemistry (IHC) technique, followed by semiquantitative analysis by a pathologist, using a scoring system. It was considered: (+) as weak marking, (++) as intermediate marking and (+++) as strong marking. Positive controls were performed in all batches (positive tonsil tissue) and analysis of lymphocytic infiltrate per patient was also conducted as a reference for both antibodies. Adjacent non-tumor tissue (n=9) from the same patients were evaluated as a comparison standard. Comparative analyses between the immunostaining profiles and prognostic parameters were performed using the Kendall's Tau test and logistic regression, with a significance level of 5%. The adjacent non-tumors tissues mostly lacked immunostaining for both proteins (66.77% BCL-2 and 83.3% H2AX). It was observed that the BCL-2 immunostaining pattern was cytoplasmic, although H2AX was nuclear and that the lymphocytic infiltrate served as an internal staining control for both proteins. BCL-2 immunostaining was seen predominantly in non-invasive tumor samples with a weak pattern. Although, H2AX staining was more abundant in invasive tumor samples, mainly demonstrating an intermediate pattern. It is also noteworthy that H2AX showed a significant positive correlation ($p=0.031$, Tau= 0.378) with muscle invasion parameter. The remaining prognostic parameters (staging, recurrence and tumor grade) didn't show any significant correlation. The increase in the sample number may elucidate if these proteins play a relevant role in the tumor invasion, as well as in other relevant parameters associated with the prognosis of malignant bladder tumors.
Keywords: Bladder cancer; Immunohistochemistry; Apoptosis; DNA repair; Prognosis

EFFECT OF TYROSINASE ENZYME INHIBITION AND OF $^1O_2$ GENERATION IN VIABILITY OF MELANOMA CELLS

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Abstract:
Melanoma is the most aggressive type of skin cancer that originates in melanocytes, the cells that produce melanin. Melanin biosynthesis, called melanogenesis, begins with the oxidation of L-tyrosine by the enzyme tyrosinase and is followed by other steps that produce this pigment. Melanin may play a photoprotective or photosensitizing role under UV radiation and visible light, contributing to the removal and generation of reactive oxygen species (ROS), respectively. Among ROS, singlet molecular oxygen ($^1O_2$) is a potent oxidant, reacts with all types of biomolecules and can lead to cell death. $^1O_2$ formed through the type II photosensitization process has been applied for the treatment of several types of skin cancer, named photodynamic therapy (PDT); however, melanin may also be involved in the resistance of melanoma to this therapy. In this ways, we evaluated if the absence of melanin production interferes with the subsequent treatment by photosensitization in the viability of murine melanoma cells (B16-F10). In order to get melanoma cells that do not produce, we performed the knockout of the TYR gene (TYR-KO), which codes for the tyrosinase enzyme by CRISPR/Cas9. Then, wild-type B16-F10 (WT) and TYR-KO cells were submitted to melanogenesis stimulation with L-tyrosine and NH_4Cl and/or generation of $^1O_2$ by photosensitization process with rose bengal acetate (RBAc) and visible green light. Two distinct and complementary methods (MTT and crystal violet staining) were performed to investigate the cell viability at 0 and 18 h after the treatments. Using the MTT method it was observed that cell viability decreased by 35% in B16-F10 WT treated cells with RBAc immediately after (0 h) the irradiation process and after 18 h the decrease was higher (93%). Stimulation of melanin production by L-tyrosine + NH_4Cl, followed by RBAc treatment and irradiation did not affect cell viability at 0 h and caused approximately 70% reduction in viability after 18 h of irradiation. In this case, it was possible to observe that pigmentation modified cellular response to photosensitization process. In B16-F10 TYR-KO cells, there was a reduction around 30-40% in cell viability in cells treated with RBAc, with or without L-tyrosine + NH_4Cl already at 0 h. In addition, 18 h after the irradiation process, loss of viability was even greater, 90 and 94%, in relation to control under irradiation. Using crystal violet staining, the percentage of adhered cells reduced approximately 70% after the treatment with RBAc in B16-F10 WT after 18h of irradiation, while in cells also treated with the melanogenesis stimulation the decrease was only 15% in relation to control under irradiation. In B16-F10 TYR-KO, the reduction of adhered cells observed was closer for both conditions, 65% in cells treated with RBAc and around 50% in cells with the combination of treatments. Therefore, the results indicated that in cells without melanin production, especially after 18 h, the photosensitization process was more effective in decreasing cell viability compared to B16-F10 WT cells, which is mainly explained by the absorption of incident light by melanin and a possible melanosome trapping of the photosensitizer.
Keywords: Melanin; Melanoma; Singlet Molecular Oxygen; Photosensitization.

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COMBINED EXPRESSION OF *JHDM1D/KDM7A* GENE AND LONG NON-CODING RNA *RP11-363E7.4* AS BIOMARKER FOR UROTHELIAL CANCER PROGNOSIS

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Abstract:
Bladder cancer is the ninth most common neoplasia worldwide. It is a heterogeneous disease, comprising several tumor subtypes with differences in histology, gene mutations, prognosis, and sensitivity to therapies. Classification of high-or low-grade tumors is based on cytological atypia and cellular architecture. Long non-coding RNAs (lncRNAs) have attracted attention because of their significant roles in tumorigenesis and cancer progression. Thus, we conducted a marker lesion study to investigate whether gene/lncRNA expression in urothelial carcinoma tissues may be used to differentiate between low-grade and high-grade tumors. RT-qPCR was used to evaluate the expression of *JHDM1D* and the lncRNAs *JHDM1D-AS1, CTD-2132N18.2, SBF2-AS1, RP11-977B10.2*, *CTD-2510F5.4*, and *RP11-363E7.4* in 20 histologically diagnosed high-grade and 10 low-grade tumors. The results showed significant overexpression of *JHDM1D* in high-grade tumors, and a moderate (positive) correlation between *JHDM1D* and *JHDM1D-AS1,* and *CTD-2510F5.4* and *CTD2132N18.2*. The strongest relationship was observed for *JHDM1D* and *RP11-363E7.4,* with an AUC of 0.826. In conclusion, the results indicated that the combined expression of *JHDM1D* and *RP11-363E7.4* may be an attractive prognostic biomarker and a promising target for urothelial tumor therapy. Funding: CNPq (310905/2020-6) and CAPES
Keywords: JHDM1D; bladder tumor; RP11-363E7.4; gene expression..

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IMMUNOSTAINING INVESTIGATION OF METALLOPROTEINASES 2 AND 9 IN PROSTATE TISSUES AND THEIR RELATIONSHIP TO CANCER PROGNOSIS

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Abstract:
Metastasis is the main problem in the treatment of prostate cancer (PCa), and for it to occur, proteolytic enzymes must remodel the extracellular matrix (ECM) surrounding the tumor. The most important group of enzymes with this action include the matrix metalloproteinases (MMPs), which act on various substrates cleaving ECM components. Among the 23 types of MMPs, MMP-2 and MMP-9 stand out, the so-called gelatinases, which are considered the most important enzymes for ECM degradation and are involved in the process of tumor invasion and metastasis. The aim of the present study was to evaluate the immunostaining profiles of MMP-2 and MMP-9 in samples from 60 PCa Brazilian patients using the indirect immunohistochemical technique. The study was approved by the Ethics Committee for Research on Human Subjects at the State University of Londrina - Brazil, under number 176/2013 (CAAE: 19769913.0.0000.5231). One hundred seventy eight tissue samples from malignant tumor (n=60), adjacent non-tumor (n=58) and ECM (n=60) were evaluated by a pathologist according to the immunostaining intensity: (-) absence of staining, (+) weak staining, and (++ and +++) strong staining. To compare the immunostaining between malignant tumor and adjacent non-tumor tissues, the McNemar test for related samples was used. For analysis of correlations between protein staining and clinical-pathological parameters and between staining of different proteins, the Kendall Tau-b test was used. All statistical analyzes were performed using IBM® SPSS®. MMP-2 and MMP-9 showed positive immunostaining in both cytoplasmic regions of the malignant tumor and in the adjacent tissues. In addition, positive staining for both proteins was observed in the ECM. The MMP-2 immunostaining in tumor cytoplasm was statistically more intense than in ECM ($p=0.001$), but it did not correlate with any clinical-pathological parameter. The MMP-9 staining was similar in all cell compartments (malignant tumor cytoplasm, adjacent non-tumor cytoplasm and ECM) but showed significant positive correlations with prognostic groups ($p=0.038$; Tau=0.253), ISUP grade ($p=0.044$; Tau=0.249), extraprostatic extension ($p=0.025$; Tau=0.309) and biochemical recurrence ($p=0.048$; Tau=0.306). It was also observed MMP-2 versus MMP-9 protein correlation in adjacent non-tumor ($p=0.001$; Tau=0.412), malignant tumor ($p\leq0.001$; Tau=0.461) and ECM ($p\leq0.001$; Tau=0.443) tissues. Our results suggest that MMP-2 does not seem to be able to predict tumor prognosis in the PCa samples from present study. MMP-9 protein may play a role in prognostic aspects and seems to constitute a candidate marker for tissue invasion, essential in the PCa metastatic process.
Keywords: MMP-2; MMP-9; Immunohistochemistry; Metastasis.

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INVESTIGATION OF THE ANTI MELANOMA POTENTIAL OF A RUTHENIUM(II) COMPLEX CONJUGATED TO THE CINNAMIC ACID

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Abstract:
Although advances in therapy have been promising, cancer treatment still present many challenges since chemotherapy is nonspecific and generates critical adverse effects for patients. Among the most aggressive cancers, with high mortality, although low incidence, is melanoma, a heterogeneous cutaneous tumor originating from melanocytes which is difficult to treat. Thus, the identifying new drug candidates that can improve treatment proposals is relevant. The metal complexes with a structural center based on Ruthenium II (Ru II) have shown promising antitumor activity due to selective inhibition of tumor cell proliferation. Its octahedral geometry characterized by a greater number of coordination sites, allowed us to conjugation the Ru(II) with trans-cinnamic acid, a phenolic compound found in many vegetables, wine and coffee, producing a derivative of Ru(II) containing trans-4-trifluoromethyl-cinamic acid, the Transcinam [Ru(L2)(dppb)(bipy)]PF6. Previous in vitro studies demonstrated positive results in inhibiting cell proliferation, invasion and migration. Therefore, this study aimed to evaluated the antitumor activity of Transcinam [Ru(L2)(dppb)(bipy)]PF6 in murine syngeneic model of metastatic melanoma. B16F10 cells were inoculated into the back of male C57BL/6 mice and the animals were treated with five consecutive days with different doses of Transcinam [2.5; 5 and 10 mg/kg body weight (b.w.), subcutaneously (s.c)] and a Cisplatin (CDDP 7 mg/kg b.w. s.c.) group was included. To evaluated the toxicity effect, the animals body weight and water consumption were evaluated daily while the parameters of tumor weight and volume were quantified to evaluate the anti-melanoma effect. The bone marrow and peripheral blood cells were collected in order to evaluated the genotoxicity. Transcinan showed no sistemic toxicity signals. An increase in the ratio of polychromatic and normochromatic erythrocytes (PCE/NCE) were observed in groups treated with the Transcinam when compared to the CDDP group, which indicates selectivity. Transcinan showed antimelanoma effect from the reduction in tumor weight of the treated groups compared to the implant control group (IC). The results obtained in this work provide preliminary information for the development of prototype drugs for the treatment of melanoma.
Keywords: chemotherapy; melanoma; metal ions.

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EVALUATION OF THE ANTINEOPLASTIC POTENTIAL OF SOLAMARGINE AGAINST HUMAN GLIOBLASTOMA

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Abstract:
Glioblastoma (GB) is one of the most treatment-resistant cancers, presenting a survival rate of approximately 5.8% in 5 years after diagnosis. Solamargine (SM) is an isolated compound from *Solanum lycocarpum*, also known as "fruta-do-lobo", which is a Brazilian plant species from the biome called Cerrado. This compound has been studied for its antitumor, antiproliferative, and anti-inflammatory potential. Due to the need for new drugs to treat GB, natural products such as SM could be promising candidates. Therefore, the present study evaluated the antineoplastic potential of the steroidal glycoalkaloid SM in human glioblastoma cell lines (U-87MG, U-251MG, T98G, and KNS-42). Cell viability was evaluated using the XTT colorimetric assay after 24 hours in normoxia and hypoxia conditions. Cytotoxicity was also evaluated using the clonogenic efficiency assay. The results showed that SM presents IC_{50} ranging from 7.26 to 9.48 µM in human glioblastoma cells under normoxic conditions. There was a decrease in cell viability when cell cultures were treated with SM under hypoxic conditions, with IC_{50} ranging from 6.75 to 8.92 µM. Differences were significant for the T98G cell line. Through the clonogenic efficiency assay, SM exhibited IC_{50} ranging from 5.27 to 7.52 µM. Then, apoptosis and cell cycle analyzes were conducted in the U-87MG cell line, in which greater SM cytotoxicity was observed. Treatment with SM IC_{50} (7.26 µM) in the U-87MG cell line resulted in 56% of the cell population undergoing apoptosis. Moreover, SM was able to arrest the U-87MG cell cycle at G2/M phase, with 46.28% of the cell population in this phase. Therefore, SM showed cytotoxic effect on different cell lines of human glioblastoma, which involves the induction of apoptosis and cell cycle arrest induced by the glycoalkaloid. This natural product has shown promising antineoplastic activity on glioblastoma cells.
Keywords: Glycoalkaloid; Antitumor; Natural products; Cytotoxicity.

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EVALUATION OF THE ROLE OF HMGA1 IN THE PROGRESSION OF ESOPHAGEAL ADENOCARCINOMA

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Abstract:
Esophageal cancer is highly incident and lethal, being the 7th most incident and 6th most deadly tumor in men worldwide. It is subdivided in two mains histological types, squamous cell carcinoma (SCC) and esophageal adenocarcinoma (EAC) which, despite affecting the same organ, exhibit different etiopathogenesis, affected populations, geographic distribution and molecular alterations associated with tumor progression. Even with considerable differences, both tumor subtypes present the same treatment approach, poor prognosis and a 5-year survival between 15-20%. This highlights the need for new therapeutic approaches. A deep understanding of the tumor associated molecular mechanisms provides powerful tools for the identification of potential therapeutic targets. In this sense, the *HMGA* family of genes emerge, since their overexpression occurs in several tumors, correlates with a worse patient's prognosis and the modulation of their expression impacts tumor progression *in vivo*. *HMGA* genes encode the HMGA1 and HMGA2 proteins, which act as modulators of chromatin structure, participating in the transcriptional regulation of target genes. In this way, they directly and indirectly regulate a range of biological processes, such as proliferation, apoptosis, cell cycle, migration, etc. Our group has been studying the role of HMGA proteins in esophageal tumors and has identified, in primary analysis, the overexpression of HMGA1 in samples from patients with EAC. Therefore, the aim of the present study was to evaluate the role of HMGA1 in the progression of EAC. Using an *in vitro* model of transient *HMGA1* silencing in two EAC cell lines, OE19 and OE33, the data obtained demonstrated an impact of modulating *HMGA1* expression on processes associated with tumor malignancy, such as proliferation, cell death, migration, and invasion. To investigate the potential mechanism associated with such effects, molecular data from patients with EAC deposited in The Cancer Genome Atlas (TCGA) were reanalyzed to determine which differentially expressed genes (DEG) in EAC would have their expression levels correlated with those of *HMGA1*. Among the DEG identified as positively or negatively correlated with *HMGA1* expression, pathway enrichment analysis highlighted the cell cycle as the main biological process deregulated in EAC and associated with the *HMGA1* expression. In this context, *CCNA1*, *CCNB1*, *CCNB2* and *CCNE1* genes, which encode, respectively, the cyclins A2, B1, B2 and E1 were selected for subsequent analysis. Further, gene prediction analysis identified a greater number of potential consensus HMGA1 binding sites in the promoter region of the *CCNE1* gene compared to the other evaluated and, in line with these results, *HMGA1* inhibition *in vitro* resulted in modulation of the mRNA expression of *CCNE1* in the EAC cell lines. The cell cycle profile was not significantly impacted by *HMGA1* silencing. However, there is a trend towards an increase in the percentage of cells in the G0/G1 phases after 24 and 48 hours of silencing in the OE33 cell line, which present higher *HMGA1* and *CCNE1* levels than the OE19 cell line, suggesting the participation of HMGA1 in the regulation of this phenomenon. Together, all these results suggest that HMGA1 could contribute to the progression of EAC.

Keywords: Esophageal Cancer; Esophageal Adenocarcinoma; HMGA; Tumor Porgression.

RUTHENIUM EXHIBITS ANTI-MELANOMA ACTIVITY IN *IN VITRO* AND *IN VIVO* EXPERIMENTAL MODELS

Matheus Reis Santos de Melo [1]; Arthur Barcelos Ribeiro [1]; Gabriela Fernandes [1]; Iara Silva Squarisi [1]; Gabrielle Pupo Vieira [1]; Monize Martins da Silva [2]; Alzir Azevedo Batista [2]; Denise Crispim Tavares [1]

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Abstract:

Melanoma is the most aggressive and lethal type of skin cancer due to its characteristics, such as high metastatic potential and a low response rate to existing treatment modalities. Therefore, new drug prototypes are being studied to address the treatment problem for patients with melanoma. Among these prototypes, metallopharmaceuticals like ruthenium are promising alternatives due to their antitumor properties and low systemic toxicity. In this context, the present study evaluated the antineoplastic effect of the ruthenium(II) hexafluorophosphate complex of 2-mercaptothiazoline-di-1,2-bis(diphenylphosphine)ethane (RuMTZ) on human melanoma (A-375) and murine (B16-F10) cell lines, as well as in an orthotopic allogeneic tumor model. The potential antiproliferative activity was evaluated using the XTT tetrazolium assay and cell migration method. Western blot analysis performed on cell cultures evaluated apoptosis and DNA damage through cleaved caspase 3 and γH2AX proteins, respectively. In *in vivo* experiments, when tumor volume reached 100 cm^3, C57BL/6 mice were subcutaneously treated with RuMTZ (5 mg/kg) for five consecutive days. In addition to the tumor volume being evaluated during treatment, the weight and frequency of mitoses in the tumor tissue were analyzed after euthanasia of the animals. Apoptotic cell death and DNA damage were also evaluated in tumor tissue by immunohistochemistry. The data obtained showed that RuMTZ exhibits selective cytotoxic activity, with the lowest IC_{50} (2.4 μM) observed for B16-F10 cells. RuMTZ also inhibited cell migration in 35.17% and 43.87% for A-375 and B16-F10 cells, respectively, by wound healing assay. *In vivo* studies revealed that RuMTZ led to a significant decrease in tumor tissue weight (94.90%) and mitotic frequency (57.14%). Molecular analyzes conducted on cell cultures and tumor tissues from animals treated with RuMTZ showed a significant increase in cleaved caspase 3 and γH2AX proteins. These findings indicated that the anti-melanoma activity of RuMTZ is related, at least in part, to the induction of apoptosis and DNA damage. Therefore, RuMTZ exhibited promising antineoplastic activity against melanoma.

Keywords: Cleaved caspase 3; γH2AX; Cytotoxicity; Thiazoline.

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ADVANCES IN ANTITUMOR EFFECTS USING LIPOSOMAL CITRININ IN INDUCED BREAST CANCER MODEL

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Abstract:

Tumor-targeted drug delivery systems such as liposomes have emerged as an essential advance in cancer treatment, ensuring sufficient drug levels accumulating at the tumor site, in addition to the possibility of enhancing cytotoxicity and antitumor effects of natural products such as citrinin. Citrinin (CIT) is a mycotoxin with promising biological activities as a candidate for antitumor drug, however, with some limitations due to its physicochemical properties. Therefore, the study aimed to evaluate the antitumor and toxicogenetic effects of liposomal nanoformulations containing citrinin in animal breast carcinoma induced by 7,12-dimethylbenzanthracene (DMBA). *Mus musculus* virgin females were divided into 6 groups treated with: (1) olive oil (10 mL/kg); (2) 7,12-DMBA (6 mg/kg); (3) citrinin, CIT (2 mg/kg), (4) cyclophosphamide, CPA (25 mg/kg), (5) liposomal citrinin, LP-CIT (2 µg/kg), and (6) LP-CIT (6 µg/kg). Metabolic, behavioral, hematological, biochemical, histopathological and toxicogenetic bioassays (micronucleus test and comet assay) were performed. DMBA and cyclophosphamide induced behavioral changes, not observed for free and liposomal citrinin. No hematological and biochemical changes were observed for LP-CIT. However, free citrinin reduced monocytes and caused hepatotoxicity, these effects justify the synthesis of nanoformulations for this natural compound. During treatment, significant differences were observed regarding weight of the right and left breasts treated with DMBA compared to negative control. Treatment with CPA, CIT and LP-CIT reduced the weight of both breasts with better results for liposomal citrinin. Furthermore, CPA, CIT and LP-CIT presented genotoxic effects for tumor, blood, bone marrow and liver cells, although less DNA damage was observed for LP-CIT compared to CIT and CPA, when the damage index and frequency values were analyzed. Blood cells damage induced by LP-CIT was repaired during treatment, unlike CPA that caused unrepaired genotoxic effects during such treatment period. With the application of the micronucleus test, in breast, bone marrow and liver cells, it was possible to observe that CIT, differently from that observed for CPA, did not induce clastogenic effects with micronucleus formations for bone marrow and liver cells. However, in tumor cell analyses, it was observed that the highest concentration of the nanoformulation induced a significant increase in mutagenic effects, differently from what was observed in free citrinin (CIT). In addition, the erythrocyte ratio showed that only the antineoplastic CPA showed cytotoxic effects on the blood cells evaluated when compared to the vehicle Thus, CIT showed antitumor effects, but with toxic effects on normal cells. However, the LP-CIT showed advantages for its use as a model of nanosystems for antitumor studies.

Keywords: Fungal metabolites; cytotoxicity; nanotechnology; breast cancer.

Support / Acknowledgment

Universidade Federal do Piauí

CXCL12/CXCR4 ALLELIC VARIANTS DO NOT INFLUENCE CXCR4 TISSUE IMMUNOSTAINING OR CLINICOPATHOLOGICAL PARAMETERS OF CERVICAL CANCER

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Abstract:
Cervical cancer is the third most common cancer in women worldwide and inflammation is a crucial component for tumor progression, although other cofactors must be present for the development of a malignancy, such as individual genetic factors. In this context, CXCL12 and CXCR4 genes may have a single nucleotide variant (SNV) rs1801157 and rs2228014, respectively, which are involved in survival, angiogenesis and invasion of malignant cells. The goal of the present work was to verify a possible association between the single nucleotide variants with clinicopathological features (type of tumor, histology grade and stage) and CXCR4 expression in tumor tissue. SNVs were assessed by PCR followed by restriction fragment length polymorphism for 90 patients and immunohistochemistry was performed in 35 formalin-fixed-paraffin-embedded cervical tumor tissues. No significant difference in the genotype distribution was found for the assessed variables. Neither the main effect of SNVs nor the interaction term (GA + AA by CT + TT) were associated with the evaluated clinicopathological characteristics. Regarding CXCR4 staining, no significant association was observed with the evaluated clinicopathological features. Evaluation of CXCL12 rs1801157 and CXCR4 rs2228014 and immunostaining showed no significant relationship between the degrees of CXCR4 immunostaining and each model of CXCL12 and CXCR4 SNVs, although a strong CXCR4 immunostaining was observed in patients presenting the CXCL12 AA genotype (p=0.05). This is the first time that CXCL12 and CXCR4 SNVs were analyzed in order to verify possible association with CXCR4 immunostaining and clinicopathological features in cervical cancer.
Keywords: rs1801157; rs2228014; cervical cancer; immunohistochemistry.

Support / Acknowledgment

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SELECTIVE CYTOTOXIC ACTIVITY OF A PHOSPHINIC RUTHENIUM COMPLEX IN MELANOMA CELLS

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Abstract:
Cancer is one of the leading cause of death in the world with the prevalence of >10 million mortalities annually. Among the various types of cancer, the melanoma is the most aggressive and lethal due to its high metastatic potential and low response rate to existing treatments. In this context, metallopharmaceuticals have demonstrated promising antitumor properties. Therefore, the present study evaluated the cytotoxicity of a phosphinic ruthenium complex - $[Ru(2mq)(dppen)_2]PF_6$, namely RuPP, on human melanoma cells (A-375), as well as its influence on cellular morphology and inhibition of cell migration. Cytotoxicity was assessed using the XTT assay, with concentrations ranging from 0.78 to 100 μM. In order to evaluate the effect of RuPP on cell morphology, cell cultures were treated for 24 hours with 0.625, 1.25, 2.5, 5.0, 10 and 20 μM. Finally, the ability of RuPP to inhibit cell migration was analyzed at the concentrations of 0.1, 0.2 and 0.4 μM by wound healing assay. The results showed IC_{50} equivalent to 3.54 μM in A-375 cells. Considering that the IC_{50} observed in non-tumor cells (HaCat) was 16.32 μM, RuPP revealed a selectivity index equal to 4.61. Cell cultures treated with RuPP at concentrations of 5, 10 and 20 μM led to the loss of adherence, decreased cell density, and presence of dead cells. Significant migration inhibition rates were obtained for treatments with 0.2 and 0.4 μM RuPP for 24 hours (40% and 60%, respectively). Therefore, RuPP demonstrated selective cytotoxicity against A-375 tumor cells. Additionally, RuPP was able to induce morphological alterations and inhibit cell migration in the human melanoma cell line, under the experimental conditions employed.
Keywords: cellular morphology; cellular migration; metallopharmaceuticals.

Support / Acknowledgment

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ANALYSIS OF INTERECTION BETWEEN GENES *TP53* AND *HMGA2* IN AUTOPHAGY REGULATION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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Abstract:
Esophageal squamous cell carcinoma (ESCC) is a highly prevalent and lethal tumor, presenting remarkable molecular heterogeneity, being *TP53* gene mutation the most frequently observed alteration in this tumor. Our group has identified another important molecular alteration associated with ESCC development: overexpression of *HMGA2*, a member of the High Mobility Group A Family, being its depletion capable of reversing the malignant phenotype *in vitro*. *HMGA* members alter chromatin conformation and interact with transcription factors, thereby regulating gene expression. *TP53* and *HMGA2* play fundamental roles in the regulation of various and overlapping cellular mechanisms. Considering the importance of *TP53* loss and *HMGA2* overexpression in ESCC, one could hypothesize that there is an interaction between them in regulating cellular mechanisms involved in ESCC development. In this sense, autophagy stands out as an interesting process, since it is associated with therapeutic response in various tumors and regulated by both *TP53* and *HMGA2*. Autophagy is an important physiological cellular process, however, its imbalance is associated with cancer, where it acts as a survival pathway for tumor cells. The knowledge about autophagy in ESCC is still limited. Considering the role of *TP53* and *HMGA2* in autophagy control and the high frequency of alterations in these two genes in ESCC, the aim of this study is to investigate their interaction in autophagy regulation in this tumor. To this end, a list of 113 genes involved in different stages of the autophagic process was generated based on literature curation. To gain insights on whether *TP53* and/or *HMGA2* would regulate the "autophagy genes", and consequently autophagic process in ESCC, we performed *in silico* analyses by using the molecular data from ESCC patients' available in The Cancer Genome Atlas (TCGA). Firstly, the expression profile of the 113 "autophagy genes" was evaluated, according to *TP53* mutational status, by using TCGA RNA-Seq data from 81 patients (seven = *TP53* wild type and 74 = *TP53* mutant). Within the ESCC group harboring *TP53* mutation, eight differentially expressed genes (DEGs) were identified (Benjamini-Hochberg test, adjusted p-value<0.05). In the same dataset, correlation analyses between the expression levels of the 113 "autophagy genes" with those of *HMGA2* were conducted, revealing 10 statistically significant correlations (Spearman's correlation, p<0.05). Considering the DEGs identified according to *TP53* mutational status and those showing significant correlation with *HMGA2* expression, an intersection of six autophagy-related genes was identified. To confirm their potential regulation by *TP53* and/or *HMGA2*, reanalyzes of ChIP-seq data from the Gene Expression Omnibus (GEO) database (GSE174442; GSE86164) were performed, showing p53 binding onto the regulatory regions of two out of the six investigated genes: *ULK3* and *RETREG1*. To validate the expression of these genes from the *in silico* findings through RT-qPCR, an ESCC cell line harboring a thermosensitive *TP53* mutation (expression of wild-type or mutant protein based on the culture temperature) with stable *HMGA2* knockdown will be used. As the next step, p53 binding onto the promoter region of *ULK3* and *RETREG1* genes will be assessed in the established cell model by chromatin immunoprecipitation (ChIP) assay.
Keywords: *TP53*; *HMGA2*; Autophagy; ESCC.

INVESTIGATION OF THE ROLE OF THE DNA REPAIR ENZYME THYMINE DNA GLYCOSYLASE AND THE TUMOR SUPPRESSOR GENE *TP53* IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA DIFFERENTIATION PROCESS

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Abstract:
Esophageal Squamous Cell Carcinoma (ESCC) is a highly prevalent and lethal tumor characterized by molecular heterogeneity. *TP53* gene mutations are the only frequent alteration, occurring early in ESCC and found in over 80% of patients. The p53 tumor suppressor protein is responsible for maintaining cellular homeostasis and loss of *TP53* function is associated with carcinogenesis. Our group has previously demonstrated the direct transcriptional regulation of Thymine DNA Glycosylase (TDG), a DNA repair enzyme, by p53. *TP53* mutations negatively impact the tumor suppressor functions of TDG, which is involved in base excision repair (BER), addressing DNA mismatches, specifically G:A transitions at CpG sites, the most prevalent mutations in cancer. Additionally, TDG acts as a transcriptional co-regulator, contributing to cellular differentiation. Dysregulation of differentiation processes is associated with malignant transformation. Given the high frequency of *TP53* mutations in ESCC and their impact on TDG activity, understanding the role of TDG in ESCC differentiation and the involvement of p53 in this process becomes crucial. To investigate ESCC differentiation, we developed 3D-organoid culture models, providing a reliable in vitro system, from TE-1, an ESCC cell line carrying a temperature-sensitive *TP53* mutation, which allows the expression of wild-type-like p53 protein at 32ºC and mutant protein at 37ºC. Due to TDG transcriptional regulation by p53, mutant p53-expressing TE-1 cells exhibited reduced TDG expression and transcriptional activity. This enable us to compare ESCC differentiation parameters in the organoids based on *TP53* mutational status and TDG levels. To confirm the influence of TDG, we generated TDG overexpressing and silenced TE-1 cells and organoids. In future experiments, we will treat TE-1-derived-organoids expressing mutant p53 with APR246, a p53 reactivating drug. Our next objective was to explore the mechanism through which TDG influences ESCC differentiation. As TDG acts as a transcriptional co-regulator, particularly in association with the retinoic acid receptor (RAR) pathway, that regulates a set of genes associated with development and differentiation, we conducted a literature search and identified 25 genes directly regulated by retinoic acid (RA). To narrow down the genes for further analysis, we performed in silico analyses using ESCC patient's data from The Cancer Genome Atlas database. We investigated the correlation between the expression levels of the 25 genes and TDG (Pearson/Spearman Correlation Analyses) as well as TP53 mutational status (T Test Analysis). Three genes (*HOXA4*, *ILR2RA*, and *TGM2*) exhibited significant correlation ($p < 0,05$) with TDG, while four genes (*HOXA4*, *CRABP2*, *HSD17B1*, and *RARB*) were associated with *TP53* mutational status. Notably, *HOXA4* showed a negative correlation with TDG and was overexpressed in *TP53*-mutated samples. Our subsequent steps involve evaluating the expression of the identified genes in TE-1-derived-organoids treated with RA pathway inducers by RT-qPCR analysis. Overall, our research will help elucidating the role of TDG and its interaction with p53 in ESCC differentiation, shedding light on the ESCC differentiation process and its progression. In summary, understanding the interplay between TDG and *TP53* in ESCC is of great importance, given that this exploration serves as a key to unlock the molecular intricacies of this often-overlooked disease.
Keywords: Cancer; DNA repair; *TP53*; TDG; Esophageal Squamous Cell Carcinoma

PREVALENCE OF BREAST CANCER PATIENTS WITH INDICATION OF GENETIC TESTING FOR HEREDITARY BREAST AND OVARIAN CANCER SYNDROME IN A CANCER HOSPITAL

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Abstract:
Breast cancer is the most prevalent cancer among women worldwide; about 5 to 10% are related to hereditary syndromes, where Hereditary Breast and Ovarian Cancer (HBOC) is the most prevalent. Pathogenic genetic variants in BRCA genes are associated with an increased risk for HBOC. The patient's family history and some cancer characteristics can strongly predict the development of breast and ovarian cancers. Identification of patients at risk for HBOC, following international criteria, have been used to identify candidates for genetic testing. These patients may benefit from clinical cancer prevention and/or early diagnosis. This study aimed to define the prevalence of breast cancer patients that fulfilled HBOC criteria for genetic testing. Through a cross-sectional study, we evaluated 100 breast cancer patients undergoing treatment at an oncological hospital in Paraná. An interview was conducted using a structured questionnaire to collect data about cancer's personal and clinical history. Clinical data were collected through the patient's medical records. NCCN (National Comprehensive Cancer Network) and ASCO (American Society of Clinical Oncology) guidelines 2023 were used to identify patients at risk for HBOC. This project was approved by the ethics committee (CAAE 64029222.0.0000.0102). The data was summarized through descriptive statistics analysis. The medium age of breast cancer diagnosis was 53,3 years, ranging from 20 to 86 years old. A total of 70 (70%) breast cancer patients met the criteria for HBOC syndrome. About 58% of them met the criteria for both ASCO and NCCN. Patients with HBOC had an early age of diagnosis (less than 50 years). Of those diagnosed at any age, 10 (14,3%) patients were eligible for HBOC syndrome for having triple-negative tumors, 5 (7,14%) for bilateral breast cancer, and 5 (7,14%) for positive breast and/or ovarian cancer family history. According to our results, a high proportion of breast cancer patients met criteria for HBOC, and may benefit from genetic testing. These results underline the importance of identifying breast cancer cases that fulfill criteria for genetic testing to better promote their correct clinical management.
Keywords: oncogenetics; hereditary breast and ovarian cancer; genetic testing.

Support / Acknowledgment

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Germ Cells and Hereditary Effects

OPTIMIZING PROTOCOLS TO EVALUATE CELL VIABILITY AND DNA DAMAGE IN SPERM CELLS OF A TROPICAL AQUATIC AMPHIPOD

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Abstract:
Genotoxic evaluation in germ cells is fundamental to access chemical hazards, so there is a need to develop new tests and alternative models, because the established ones are usually performed in rodents. The amphipod *Parhyale hawaiensis* has been considered an important model for embryonic development, regeneration, sensory biology, and more recently ecotoxicology. It has a circumtropical distribution, is easily cultivated in the laboratory and can be exposed via water or feeding. We had already optimized the comet assay and micronucleus test in somatic cells (hemocytes) of *P. hawaiensis*. Our next step is to develop protocols to evaluate viability and genotoxicity in germ cells. The aim of this work was to develop an *in vivo* viability test and an *in vitro* comet assay for sperm cells. To collect the sperm cells, adult organisms were anesthetized with clove oil (0,06% in artificial seawater) and dissected under a stereomicroscope to remove the testes. Testes were dilacerated in artificial seawater and weighted. For the viability test, male organisms were exposed for 24h to Disperse Red 1 and emodin, both dissolved in dimethyl sulfoxide (DMSO) and tested at a maximum of 0.01% DMSO in the media, resulting in 3.15mg/L of Disperse Red 1 and 0.15 and 0.3mg/L of emodin as exposure concentrations. After collection, sperm cells suspension of each organism was stained with SYBR-14 (stains live cells in green) and propidium iodide (stains dead cells in red) and counted under a fluorescence microscope. For both compounds sperm viabilities were >80% and not significantly different from the control (DMSO 0.01%). To increase the concentration of the solvent in the media, allowing increased concentrations of the toxicants, we exposed the organisms to different concentrations of DMSO (0.01, 0.1 and 1%) in comparison to artificial seawater. We observed that the sperm viabilities were >90% for all DMSO concentrations. More experiments with the same toxicants, 10 and 100 times more concentrated will be done to verify their ability to affect cell viability. For the *in vitro* comet assay, first we evaluated two different lysis (1 and 18h) and unwinding (15 and 40min) times, and the best result were obtained using 18h lysis and 15min unwinding. Sperm cells were collected from males and exposed them to H_2O_2 (0, 15, 30, 60 e 90µM) for 30 min; ethyl methane sulfonate (EMS) (10 e 100µM) for 30min; and UV light (short wave, 15W) for 10 and 30sec. We observed an increase in the % DNA in tail of the cells treated with H_2O_2 (60 e 90µM), EMS (100µM) and both UV exposure times. *P. hawaiensis* seems to be a good alternative test organism to evaluate sperm cells' viability and genotoxicity. Efforts are now being done to develop an *in vivo* protocol to verify DNA damage in sperm cells.
Keywords: crustacean; *Parhyale hawaiensis*; comet assay; sperm viability; genotoxicity

Support / Acknowledgment

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Epigenomic

MITOCHONDRIAL DNA AND THE OXIDATIVE STRESS RESPONSE MECHANISM IN HELA AND 143B

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Abstract:
Mitochondria is an essential organelle for cellular functioning through the production of energy via oxidative phosphorylation, helping to maintain cellular homeostasis. Mitochondrial DNA (mtDNA) contains 37 genes, coding for 22 tRNAs, 2 rRNAs and 13 mRNA, but the absolute majority of mitochondrial component proteins are encoded by nuclear DNA, as well as all the proteins necessary for mitochondrial replication, transcription, translation and repair. Given the importance of the organelle, it is imperative that its maintenance is carried out correctly and that it remains intact for proper cell functioning and, therefore, it is necessary to understand mechanisms that alter its functioning, such as the regulation of mitochondrial DNA integrity and mitochondrial gene expression. This project aims to understand how epigenetic modifications occur in mtDNA that are not very well elucidated, focusing on cytosine methylation, a frequent epigenetic alteration catalyzed by DNA methyltransferase enzymes (DNMTs) that were recently found in mitochondria of mammalian cells. Furthermore, the modulation of these enzymes affect the mtDNA methylation pattern, indicating the possibility that they are related to the establishment and/or maintenance of mtDNA methylation, suggesting a potential functional link between methylation and gene expression. To characterize cell signaling and its relationship with mtDNA, human cervical tumor (HeLa) cells in their wild-type and altered form, with a decreased amount of mtDNA (HeLa Rho-) and human osteosarcoma (143B) cells were used in its wild form and in its form without mtDNA (Rho0) in experiments of cell survival and proliferation in an environment of oxidative stress caused by hydrogen peroxide (H2O2), a reactive species of natural oxygen of cellular metabolism in different concentrations. Preliminary results indicated that wild-type 143B and Hela cells are more resistant to H2O2 damage at all concentrations used compared to their mtDNA-depleted forms for presenting a higher survival rate in the experiment in direct comparison. Considering that p53, also known as "guardian of the genome" receives the signaling of H2O2 levels and activates mitochondrial biogenesis factors such as PCG1α and NRF1 which in turn would stimulate the replication of mitochondria and increase the expression of DNMT1 and its recruitment to mitochondria, we repeated the experiment using a p53 blocker, pifithrin α, to block the cascade activated by p53 and thus, consequently, it was expected that mitochondrial DNMT1 levels would be decreased. In this case, the results shows that 143B and HeLa, both in its wild-type form and in its form without mitochondria, are more resistant to damage caused by peroxide when treated with pifithrin α , indicating that, with the inhibition of p53, there was a decrease in the apoptotic pathway and consequent higher survival rate. However, when analyzing both results, we observed that even rho cells treated with pifithrin α survived more when compared to untreated rho cells, both survived less than the pair with normalized mitochondrial DNA levels, indicating a direct relationship between response to oxidative stress and mtDNA. To confirm whether there is indeed a change between H2O2 levels, DNMTs and mitochondrial biogenesis, cDNA samples were collected from all cell cultures used in the experiments and the next steps are based on performing an RT-PCR to obtain an expression library, focusing on establishing the presence and concentration of mitochondrial DNMTs, confirmed by immunofluorescence assays and the comparison of the results obtained between cell models and different treatments to which they were submitted. Therefore, as it is an extremely important organelle but with characteristics and relationships that have not been fully elucidated, greater attention is needed and all new discoveries in science are well received. The project in question, so far, comes confidently with expected results and is opening new discussions to investigate the characterization of integration and communication of the mitochondria and mitochondrial DNA in the cellular models used.
Keywords: mitochondria; mtDNA; peroxide; signaling; stress

MITOCHONDRIAL DNA LEVELS AND P53 SIGNALING MODULATE CELLULAR OUTCOME TO H2O2 EXPOSURE

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Abstract:

The mitochondrial DNA (mtDNA) localizes in the mitochondrial matrix in a nucleoprotein complex known as nucleoids. The human mtDNA encodes 13 proteins, 2 rRNAs and 22 tRNAs. Since all proteins encoded by mtDNA are subunits of the oxidative phosphorylation complexes, regulation of mtDNA expression is essential for mitochondrial respiration and cellular homeostasis. Emerging data indicate that mtDNA expression can be regulated by mtDNA methylation, and recent studies found evidence of mitochondrial isoforms of DNA methyltransferases (DNMTs) and ten-eleven translocation methylcytosine oxigenases (TETs). Moreover, data indicating that p53, PCG1α and NRF1 signaling can modulate DNMT1 translocation to the mitochondria, together with our previous data showing that the p53/PGC1α axis can modulate mitochondrial redox homeostasis led us to investigate whether mtDNA methylation is involved in oxidative signaling and mitochondrial homeostasis. Thus, we asked whether H2O2 and the p53/PCG1α/NRF1 axis can modulate DNMTs translocation to the mitochondria, promote mtDNA methylation and impact mitochondrial function. Initially, in order to characterize the role of mtDNA in the cellular responses to the signaling pathways of interest we measured clonogenic survival of osteosarcoma (143B) and HeLa cells lacking (Rho0) or depleted of mtDNA (Rho-). Our results show that cells depleted of mtDNA are more sensitive to H2O2 treatment when compared with wild type cells, indicating that mtDNA has an important role in H2O2 signaling and apoptosis resistance. Proliferation assays confirmed that mtDNA levels modulate the cellular responses to redox imbalance. In addition, p53 inhibition by pifithrin α protected 143B Rho0 cells from H2O2 citotoxicity, showing that the mtDNA modulation of the cellular outcomes depend on p53 signaling. Additionally, we confirmed the presence of putative DNMT1, DNMT3a and DNMT3b isoforms in mitochondrial extracts from Hela and 143B cells by western blot and immunofluorescence. In conclusion, our results show that cellular responses to H2O2 and p53 inhibition depend on mtDNA levels. The mitochondrial localization of DNMTs suggests the possible involvement of mtDNA methylation in the responses to oxidative stress e p53 signaling.
Keywords: mtDNA; Epigenetics; Cytosine methylation; Oxidative stress; Methyltransferases

Support / Acknowledgment

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EXPRESSION AND FUNCTIONAL ANALYSIS OF PLASMA MIRNAS IN WORKERS WITH OCCUPATIONAL EXPOSURE TO PESTICIDES

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Abstract:
Pesticides are compounds with extensive applications in agriculture, therefore, are often present in various human environments. Exposure to pesticides has been associated with negative effects on human health, including the development of pulmonary, cardiovascular, and neurodegenerative diseases, as well as several types of cancer. Given this problem, pesticide exposure becomes a public health concern, especially when it comes to occupational exposure of workers exposed to pesticides. Currently, studies have demonstrated the role of miRNAs as biomarkers of a variety of diseases and chemical compounds. However, little is known about the expression profile of miRNAs in individuals occupationally exposed to pesticides. Therefore, this study aimed to evaluate the expression profile of miRNAs in individuals exposed to pesticides and identify the potential biological role of differentially expressed miRNAs through predicted target genes. The study included 46 men (23 exposed and 23 unexposed). During recruitment, a questionnaire on sociodemographic and exposure characteristics was applied, together with the collection of biological samples. The mean exposure to pesticides of the exposed group was 33.1 ± 13.7 years. The expression profile of plasma miRNAs was assessed by means of the nCounter® miRNA Expression Assays (NanoString Technologies). As a result, we found 31 differentially expressed miRNAs between the exposed and unexposed groups ($p<0.005$). Our results demonstrated that the 31 miRNAs were negatively regulated in the exposed group. After, using logistic regression analysis, we identified a molecular signature composed of 4 miRNAs with a predictive value of AUC of 0.89. A total of 244 target genes for differentially expressed miRNAs were predicted using mirDIP, a microRNA Data Integration Portal. Gene Ontology (GO), pathway (KEGG), and diseases (DisGeNET) analyses were conducted through predicted target genes. A total of 298 biological processes, 38 cellular components, and 19 molecular function terms were found. The KEGG enrichment analysis revealed 16 pathways, including MAPK, Rap1, and PI3K-Akt signaling pathways, microRNAs in cancer, and pathways in cancer. The gene-disease interaction networks resulted in a network consisting of 30 diseases. The main diseases noted were neurological diseases and various types of cancer. Based on these studies we can conclude that the population exposed to pesticides shows a downregulation of many miRNAs that may be involved in the development of several diseases. In addition, we can see that identification of epigenetic biomarkers such as miRNAs must have a considerable impact on preventing the development of multiple diseases. However, further studies are required to identify the role of alterations along the pathways, determining the effects of pesticides on human health.
Keywords: Pesticides; miRNAs ; Occupational exposure; Biomarkers; Molecular signature

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PLASMA EXTRACELLULAR VESICLE MIRNAS ARE MORE SUITABLE BIOMARKERS FOR LOCALIZED AND METASTATIC PROSTATE CANCER THAN CELL-FREE MIRNAS OBTAINED FROM WHOLE PLASMA.

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Abstract:
Prostate cancer (PCa) is the most common cancer in older men, and its high incidence is related to the number of first-degree relatives affected, age at diagnosis, accumulation of DNA damage, and decline in DNA repairome during ageing. However, its etiology, the molecular mechanisms involved in carcinogenesis, and its progression are not well-known. For the clinical diagnosis, the standard approach is represented by solid biopsy, an invasive procedure. To overcome this challenge, liquid biopsy (LB) assays are on developing because it is rich in molecules, especially in transcriptomic information provided by genetic markers. In this sense, circulating biomarkers, have been included. Non-invasive biomarkers should distinguish between indolent and aggressive pathology, contributing to reducing the risk of overdiagnosis/overtreatment of PCa. Several miRNAs implicated in PCa have been identified in the literature and show great potential for early PCa detection. MiRNAs can exist in the extracellular space as cell-free miRNAs (cf-miRNAs) or incorporated into extracellular vesicles (EV-miRNAs). In this sense, this study evaluated cell-free miRNAs (cf-miRNAs) and extracellular vesicles (EV-miRNAs) to understand which source could provide a better biomarker for PCa. A total RNA sample was isolated from whole plasma and plasma EVs obtained from 30 patients (20 with localized PCa, 10 with metastatic PCa (mPCa)), and 15 controls. The EVs analysis by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA), and Western Blotting (WB), confirmed that they presented all the essential requirements for being classified as such. Through liquid biopsy (LB) and RT-qPCR using Taq-Man probe we evaluated the relative expression of four miRNAs known to have a diagnostic potential for PCa: miR-21-3p, miR-200c-5p, miR-375-5p, and miR-1290-3p. The miRNAs relative expressions in the three groups were compared, and their association with clinicopathological parameters were analyzed. To evaluate the diagnostic potential of miRNAs, ROC curve analysis was performed. Regarding the miRNA expression, EV-miRNAs showed higher expression than cf-miRNAs; all EV-miRNAs analyzed had diagnostic potential, with areas under a curve bigger than 0.7. Among the analyzed miRNAs, EV-miR-21-3p, EV-miR-375-5p and EV-miRNA-1290-3p were upregulated in both PCa and mPCa groups, distinguishing them from the control group, whereas EV-miR-200c-5p was upregulated only in mPCa compared to control group. When we analyzed the expression of miRNAs in relation to clinicopathological parameters, we found that in the patients group, PCa showed upregulation of EV-miR-200c-5p, EV-miR-375-5p and EV-miR-1290-3p and ISUP ≥ 3 compared to ISUP ≤ 2. The same analysis in the mPCa group indicated only the cf-miR-200c as upregulated. The EV-miR-1290-3p was upregulated in patients of PCa group that presented PSA $\geq$ 10ng/mL compared to PSA< 10ng/mL. This miRNA was also upregulated in the mPCa patients' group that presented bone marrow metastasis. In conclusion, we demonstrated that EV-miRNAs provide a more reliable source of miRNAs than the whole plasma and that the identification and use of EV-miRNAs as biomarkers for PCa diagnosis and prognosis is a promising avenue of research.
Keywords: Liquid biopsy; exosomes; cell-free; microRNA; biomarkers

IMMUNE CELL INFILTRATION AND DYSREGULATED IMMUNE-ASSOCIATED LONG NONCODING RNAS CONTRIBUTE TO TUMOR HETEROGENEITY IN ORAL CAVITY SQUAMOUS CELL CARCINOMAS

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Abstract:
Oral cavity squamous cell carcinoma (OSCC) is a complex and dynamic disease characterized by clinicopathological and molecular heterogeneity. Approximately 2% of oral cavity cancers are human papillomavirus (HPV) positive, while HPV-negative tumors are often attributable to alcohol and tobacco consumption. HPV-positive patients exhibit better prognosis and response to chemotherapy alone or combined with radiotherapy. Spatial and temporal heterogeneity of cell subpopulations has been associated with cancer progression and implicated in the prognosis and therapy response. Emerging evidence indicates that aberrant epigenetic profiles in OSCC may foster an immunosuppressive tumor microenvironment by modulating the expression of immune-related long noncoding RNAs (lncRNAs). Herein, we characterized the OSCC immune microenvironment by exploring methylome data. 46 matched OSCC and normal adjacent tissue samples were retrospectively obtained from patients who underwent surgery at A.C. Camargo Cancer Center, São Paulo, Brazil. The study was approved by the institutional Ethics Committee (Protocol # 1876/14) and all patients provided written informed consent before the sample collection. DNA methylation analysis was performed using a genome-wide platform (Infinium HumanMethylation450 BeadChip). Using an online calculator, DNA methylation (DNAm) age was determined for these samples, and epigenetic age acceleration was calculated by subtracting chronological age from DNAm age. According to this measure, patients were separated into age-accelerated or age-decelerated. DNA methylation data of 344 OSCC and 34 normal samples was retrieved from The Cancer Genome Atlas (TCGA) database, and differential methylation analyses were carried out in the internal and external datasets. Reference-based computational deconvolution (MethylCIBERSORT) was applied to infer the immune cell composition of the bulk samples from both cohorts. No significant association was identified between DNAm age acceleration and HPV status, alcohol or tobacco consumption. The expression levels of genes encoding immune markers and differentially methylated lncRNAs were investigated using TCGA OSCC and normal samples. We identified 21,085 differentially methylated probes (DMPs) in our dataset (13,342 hypo- and 7,743 hypermethylated probes) and 39,245 DMPs in the TCGA-OSCC cohort (19,932 hypo- and 19,313 hypermethylated probes). OSCC specimens presented distinct immune cell composition, including the enrichment of monocyte lineage cells, natural killer cells, cytotoxic T-lymphocytes, regulatory T-lymphocytes, and neutrophils. B-lymphocytes, effector T-lymphocytes, eosinophils, and fibroblasts were diminished in tumor samples. The infiltration patterns of these nine cell populations showed no statistical difference when comparing HPV-positive (N=14) and -negative (N=32) OSCC cases. The hypomethylation of three immune-associated lncRNAs (*MEG3*, *MIR155HG*, and *WFDC21P*) at individual CpG sites was confirmed by bisulfite-pyrosequencing. Also, the upregulation of a set of immune markers (*FOXP3*, *GZMB*, *IL10*, *IL2RA*, *TGFB*, *IFNG*, *TDO2*, *IDO1*, and *HIF1A*) was detected. The immune cell infiltration, immune markers alteration, and dysregulation of immune-associated lncRNAs reinforce the impact of the immune microenvironment in OSCC. These concurrent factors contribute to tumor heterogeneity, suggesting that epi-immunotherapy could be an efficient alternative to treat OSCC.
Keywords: oral cancer; tumor immune microenvironment; lncRNAs; DNA methylation; deconvolution

Support / Acknowledgment

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Applied Toxicological Genetics

DEVELOPMENT OF LIPOSOMAL FORMULATIONS CONTAINING CITRININ AND ITS NANOTOXICOGENETIC EVALUATION IN BREAST TUMOR CELL LINES

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Abstract:
Research of new medicines based on natural products and the application of nanosystems has been explored for treatment of cancer. The citrinin mycotoxin (CIT), obtained from the fungus *Penicillium citrinum*, has already demonstrated potential cytotoxic effects in in vitro and in vivo studies, so the aim of present study was to synthesize liposome-type nanoformulations containing CIT, evaluating its cytotoxic and genotoxic capacity in cell lines tumor (MDA-MB-231 - human breast cancer) and non-tumor (MCF-10AA - human mammary gland line), in order to improve the availability and anticancer efficacy of compound in question. The synthesized liposomal formulations (LP-CIT) were characterized using the following parameters: macroscopic appearance, pH, particle size (PS), polydispersion index (PDI) and zeta potential (ZP). Furthermore, tests of CIT content, encapsulation efficiency and liposome stability were carried out. The synthesis of nanosystem presented citrinin-containing liposomes with small unilamellar vesicles (SUV), with diameter and PDI of 143.3 and 0.3 nm, respectively, and surface charge of +28.5 mV compatible with applications in in vitro tests and in vivo. Liposomal formulations together with isolated citrinin showed cytotoxic effects for all cell lines evaluated in MTT and trypan blue test, with lower IC_{50} values for nanocarriers. The isolated cyt presented IC50 of 19.51; 10.56 and 9.18 µg/mL and LP-CIT of 2.75; 0.77 and 0.20 µg/mL for L929, MDA-MB231 and MCF-10A lines, respectively. Flow cytometry demonstrated the non-cytotoxicity of empty liposomes (20 µg/mL in 72h exposure time) and the ability of CIT and LP-CIT (0,1; 1; 5 µg/mL in 72h exposure time for both) to induce cell death in tumor and non-tumor mammary cells, with better results for nanoformulations. In addition, CIT and LP-CIT (1 µg/mL in 72h exposure time for both) showed cytotoxicity due to the loss of mitochondrial membrane potential in MDA-MB-231 cells and increased protein expression of apoptotic genes caspase 9 and BAX. Finally, CIT and LP-CIT (0,5; 1; 2 µg/mL in 72h exposure time for both) showed genotoxicity by comet assay on MDA-MB-231 and MCF-10A tumor cells. Therefore, the synthesized liposomal formulations maintained the cytotoxic and genotoxic effects of CIT, improving its biological effects in the breast tumor cell lines studied.
Keywords: Nanotechnology; pharmaceutical formulations; neoplasms; marine metabolites; toxicogenetic effects

HIGH GLUCOSE LEVELS DECREASE CELL VIABILITY AND SENSITIZE PC12 CELLS TO OXIDATIVE DAMAGE

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Abstract:
Diabetes is a chronic metabolic disease defined by the increase in blood glucose levels that affects 422 million people worldwide. Long term exposure to high glucose can induce modifications on β-pancreatic cells, triggering the insulin resistance process and establishing a feedback loop between high glucose levels and insulin resistance, which increases the systemic levels of oxidative stress and inflammation. Since the brain has a high amount of easily peroxidizable unsaturated fatty acids, high oxygen consumption and a lower amount of antioxidant enzymes is highly vulnerable to oxidative damage. Thus, hyperglycemia-induced oxidative stress may cause neuronal dysfunction and apoptotic cell death, factors that are associated with the development and progression of diabetes and diabetic neuropathy. Therefore, this project aims to analyze the effects of the in vitro exposure of PC12 cells to high glucose levels. The hypothesis is that exposure to high glucose levels will cause cytotoxic effects on PC12 cells, possibly sensitizing them to H2O2-induced oxidative damage, leading to DNA fragmentation and cell death. PC12 cells were cultured under hyperglycemic conditions, with glucose concentrations of 25, 50, 75 and 100 mM for 24, 48, 96 and 120 h, to mimic the effects observed in diabetic patients. The oxidative damage was induced by H2O2 at concentrations of 25, 50, 75, 100 and 150 µM during 30 minutes. The percentages of cell viability were achieved through the XTT assay. The results from three independent experiments showed that the isolated glucose 100 mM (for 120h) treatment induced a significant decrease in cell viability. For the H2O2 treatment, following 24 h of recovery, cell viability was evaluated and the results showed a significant decrease for the higher concentrations (75, 100 and 150 µM). For the combined treatments, 100 mM glucose and H2O2 25 or 50 µM, we also obtained a significant decrease in cell viability compared to respective controls, treated only with H2O2, suggesting that the treatment with high glucose concentrations increased the PC12 cells susceptibility to oxidative damage. The cell cycle analysis was also performed (three experiments, evaluation by flow cytometry). However, the treatments with glucose and/or H2O2 did not induce significant differences relative to controls. Additional experiments are currently in development to analyze the effects of high glucose concentrations regarding the induction of DNA damage and clonogenicity. It is expected that the information obtained in this project may provide a better understanding regarding the association between hyperglycemia and oxidative stress, as it has been observed in patients with diabetes.

Keywords: hyperglycemia; diabetes; oxidative stress; hydrogen peroxide.

ANNONA CRASSIFLORA INHIBITS COLONIC PRENEOPLASTIC LESIONS INDUCED IN RATS

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Abstract:
Popularly known as marolo or araticum, Annona crassiflora is a native and endemic species to Brazilian Cerrado whose fruits have high sensorial, nutritional, bioactive, and economic potential. Recent scientific findings about this species have attracted interest from different industrial sectors. The compiled data showed that araticum fruit parts contain a wide range of bioactive compounds, particularly phenolic, alkaloids, annonaceous acetogenins, carotenoids and phytosterols, moreover that these phytochemicals contribute to different biological activities verified in araticum fruit extracts/fractions, including antioxidant and anti-inflammatory effects. Despite the promising discoveries, toxicological studies that confirm the safety of consumption and demonstrate its effect on lesions to genetic material are few. In this sense, this study aimed to investigate the influence of Annona crassiflora treatment against carcinogen 1,2-dimethylhydrazine (DMH) induced pre-neoplastic lesions in Wistar rat colon. For that, a comparative study was developed using the fruit pulp (ACP) as well as a pulp extract (ACPE in methanol:acetone:water 7:7:6 v.v.). Total phenolic compounds and flavonoids were determined. In addition, its antioxidant activity was measured by DPPH and ABTS assays. Wistar rats received ACP at 0.7, 1.4 or 2.8 g/kg body weight (b.w)/day added in standard ration and 0.05, 0.11 and 0.23 g/kg b.w./day of ACPE for 30 days. Four subcutaneous injections of DMH (40 mg kg p.c) were used to induce pre-neoplastic lesions that were assessed by the Aberrant Crypt Foci (ACF) assay. During the experiment, water consumption and body weight were measured, while serum glucose levels were also monitored at the beginning and the end of the treatment. After the euthanasia, the colons were excised and the ACF and Aberrant Crypt (AC) were quantified. The results indicated that the treatments with ACP and ACPE do not demonstrate signals of toxicity, since they did not modulate water consumption and weight gain. Serum glucose levels were also unchanged. The ACP and ACPE effectively inhibited pre-neoplastic lesions induced by DMH administration at all concentrations tested. The ACP showed high content of total phenolic compounds [9.16 ± 0.36 mg in gallic acid equivalent (GAE/g)] and total flavonoids [7.26 ± 0.37 mg catechin equivalent (CE/g)], representing about 85% of the phenolic compounds content while the ACPE showed 29.52 ± 0.13 mg GAE/g of total phenolic compounds and 25.52 ± 1.51 mg CE/g of total flavonoids. The ACP also presented considerably bioactive amines content, mainly tyramine (31.97 mg/kg), putrescine (20.65 mg/kg), and spermidine (6.32 mg/kg). A significant antioxidant activity through the DPPH radical scavenging activity were showed, whose results was 65.94 ± 5.96 µmol TE/g (Trolox equivalent) for ACP and 91.48 ± 6.51 µmol TE/g for ACPE, respectively. Therefore, this study shows that the promising effect of Annona crassiflora in the prevention of colonic carcinogenesis is due, at least in part, to the high levels of bioactive compounds, especially amines and phenolic compounds that, because of their antioxidant activity, can protect the genetic material against oxidation caused by carcinogens.
Keywords: araticum; chemoprevention; colon carcinogenesis; bioactive compounds.

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EFFECTS OF THE PRE-TREATMENT OF DIALLYL DISULFIDE AGAINST ETHYL CARBAMATE CYTOTOXICITY AND GENOTOXICITY IN HUMAN CACO-2 AND HEPG2 CELLS

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Abstract:
Ethyl carbamate (EC) is formed as a by-product of fermentation in foods and beverages, including, cheese, yoghurt, wine and whiskies. EC has been classified as a Group 2A carcinogen by the IARC. However, only few studies have assessed the toxicity of EC in humans cells, and it is necessary to evaluate its effects using human liver and colon cells. Several experimental studies have demonstrated that diallyl disufide (DADS), an organosulfur compound derived from garlic (*Allium sativum*), at low concentrations, has significant activities against cytotoxicity, genotoxicity and carcinogenicity. This study assessed the possible protective effect of DADS on EC-induced cytotoxicity and genotoxicity using human colorectal adenocarcinoma cell line (Caco-2) and hepatocellular carcinoma cell line (HepG2). In the pre-treatment, Caco-2 or HepG2 cells were treated with various DADS concentrations (10 - 40 µM) and incubated for 24 h. Then, EC was added to a final concentration of 80 mM, and the cells were incubated for 24 h. Cell viability was tested by resazurin reduction assay and DNA damage was measured by comet assay. Resazurin assay result showed that pre-treatment with DADS for 24 h effectively increase Caco-2 cells viability when compared to CE treatment alone ($p<0.05$). However, pre-treatment with DADS did not prevent significant increases in cytotoxicity induced by CE in HepG2 cells ($p>0.05$). After treatment with CE for 4 or 24 h, surprisingly only a minimum non-significant increase in DNA damage was observed in Caco-2 and HepG2 cells, when compared to negative control ($p>0.05$). DADS treatments did not induced DNA damage assessed by comet assay in both cell lines. Our data indicate that DADS showed protective effects of preventing CE-induced cytotoxicity in Caco-2 cells. Therefore, DADS merits investigation as a potente nutraceutical against CE-mediated toxicity.
Keywords: Protective effect; Comet Assay; DNA damage.

Support / Acknowledgment

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ANNONA TOMENTOSA REDUCES THE VIABILITY, PROLIFERATION, AND MIGRATION OF BREAST CELLS CULTIVATED IN MULTICELLULAR TUMOR SPHEROIDS

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Abstract:
Breast cancer is a multifactorial disease consisting of uncontrolled proliferation of abnormal breast cells to form tumors that may migrate and invade other body parts during metastasis. Considering that current treatments such as surgeries, radiotherapies, and chemotherapies may result in severe side effects and tumor resistance, there is an increasing need for new antitumoral agents, such as naturally occurring compounds. These agents may be less toxic to patients and can act synergistically with conventional therapies to decrease the therapeutic doses needed and adverse effects. Therefore, the present study evaluated the effects of the alkaloid fraction obtained from *Annona tomentosa* R.E.Fr, a plant widely used in folk medicine and previously described to have anti-nociceptive and anti-inflammatory effects. Cell viability, proliferation, and migration of breast cancer cells (MCF-7) were evaluated in multicellular tumor spheroids (MCTS), a three-dimensional model that better mimics the tumor microenvironment. The fraction was diluted in dimethylsulfoxide 0,25% (Solvent Control) to the final concentrations of 10, 50, 100, 150, 200, and 250 µg/mL, and for the positive control, it was used docetaxel 100µM. After 72 h of MCTS's treatments, it was performed the clonogenic and the resazurin assays followed by the cell death assay (96 h), through the addition of the PI (red) and Hoechst 33342 (blue) fluorescent dyes to identify necrotic and viable cells, respectively. The anti-migratory properties of the fraction were evaluated by the cell migration in an extracellular matrix (bovine skin gelatin) after 96 h of treatments, through photomicrographs taken every 24 h, followed by the addition of the fluorescent dyes. For the evaluation of the effects of the *Annona tomentosa* in the MCTS area, integrity, and morphology, the spheroids were photographed and treated every 72 h until 216 h, and at the end of the integrity assay, it was performed the resazurin assay. It was observed that after 72 h of treatments, the alkaloid fraction (≥10 µg/mL) significantly reduced the colony formation but did not decrease cell viability by the resazurin assay. The labeling with fluorochromes showed a decrease in Hoechst intensity in relation to solvent control. Additionally, it was observed that *Annona tomentosa* (≥ 100µg/mL) inhibited cell migration and increased the number of PI-stained cells throughout the migration area. Finally, the spheroid integrity assay showed that the alkaloid fraction reduced the spheroid area and disaggregated them after 216 h of treatment, which goes following the resazurin assay (IC_{50}= 189.1 µg/mL). Our results demonstrate that the alkaloid fraction of *Annona tomentosa* is a potential anticancer agent, and its effects should be further investigated.
Keywords: anticancer; 3D culture; Phytochemicals; Annonaceae; Cerrado

Support / Acknowledgment

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MUTAGENIC AND ANTIMUTAGENIC EVALUATION OF THE RESIDUAL AQUEOUS FRACTION FROM ANTARCTIC MOSS *SANIONIA UNCINATA*

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Abstract:

The Antarctic moss *Sanionia uncinata* is a source of antioxidants, and the photoprotective activity of its organic extracts has been investigated. We reported previously that residual aqueous fraction (AF) showed significant *in vitro* sunlight protection. It absorbs the UV-vis spectrum, increasing sunlight protection factor values of UV-filter benzophenone-3 and octyl-methoxycinnamate, and does not induce embryotoxicity in zebrafish's early life stage. The cell death observed by WST-1 e LDH assays allowed the establishment of non-toxic doses of AF, and phototoxicity was not detected. In 3D cell culture compared to monolayers, we showed that human HaCaT keratinocytes were more resistant to cell death induced by AF via cell membrane disruption and mitochondrial enzymes. The present study aimed to evaluate the mutagenic and antimutagenic potential of the AF from Antarctic moss, using TA98 and TA100 strains, which can detect frameshifts and base-pair substitution, respectively. The *Salmonella*/microsome mutagenicity assay was carried out using the pre-incubation with and without an exogenous metabolism (-/+S9). To characterize desmutagenic and bioantimutagenic mechanisms, pre-, co-, and post-treatments were carried out to evaluate the antimutagenic potential of AF (-S9). *Salmonella*/microsome mutagenicity assay results showed that AF did not induce mutagenicity in either strain, with and without S9. In antimutagenicity assays, AF significantly decreased the number of revertants colonies compared to the positive control (4NQO) in post-all treatments. The 4NQO is a potent mutagen and carcinogen that induces DNA lesions by producing reactive oxygen species, generating superoxide radicals or hydrogen peroxide, which can cause DNA mutation of guanine residues inducing mainly C:G at A:T transitions. The AF induces a critical protective action to DNA, reached a 67% reduction in induced colonies of TA98 strain in the co-treatment. Therefore, the results suggest that the FA of Antarctic moss was not able to induce point mutation in TA98 and TA100 strains. In addition, it showed protective activity against 4NQO, suggesting a chemopreventive agent action, strongly inhibiting oxidative damages by direct effects.

Keywords: Mutagenicity; Antimutagenicity; Photoprotection; Oxidative damages.

MUTAGENICITY EVALUATION OF A NEW PHARMACEUTICAL FORMULATION OF ECHINODORUS MACROPHYLLUS BASED ON ETHNOPHARMACOLOGICAL EVIDENCE

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Abstract:
The use of medicinal plants on popular and modern medicine is a common topic in Brazilian culture, especially when utilizing Brazilian medicinal plants, which the knowledge of the supposed effects are passed down on generations. Hence, those plants need a deeper and thorough chemical-pharmacological investigation, based on three reasons: the need for scientific confirmation of the effects on health; to study its toxicologic safety of the plants extracts due to the presence of environmental contaminants; and to explore which molecules of the extract bring such benefits to the health. *Echinodorus macrophyllus*, a Brazilian medicinal aquatic plant species in the Alismataceae family, is widely used in popular medicine, but rarely studied, therefore, it was made necessary to study the extract of the aforementioned plant and to check the possible toxic effects of its preparations. Thus, the aim of the present study was to ensure the toxicological safety, as well as to identify the presence of biomarkers of the Alismataceae family with bioactive activity. A 100 mg/mL ethanolic extract (55%, ethanol:water) extract was prepared. The identification of the presence of biomarkers in the extract was done by a high performance liquid chromatography coupled with a diode array detector (HPLC-DAD). Isoorientin, vitexin, isovitexin, rutin, gallic acid and chlorogenic acid were used as standard biomarkers. Henceforth, the co-injection of those standards revealed the presence of isoorientin, vitexin, isovitexin and rutin, and, unfortunately, the absence of gallic acid and chlorogenic acid in the sample. For further investigation of the toxicological safety, the stock extract was diluted with distilled water to the concentration of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. These dilutions were evaluated by its toxical safety through the Ames test. The Ames test was performed using five strains of Salmonella typhimurium: TA97a, TA98, TA100, TA102 and TA1535. Each strain used was tested with and without exogenous metabolic activation (S9 mix). A two-fold increase in the spontaneous revertants associated with a clear dose-dependent behavior and statistical difference ($p < 0.05$) were used to assume the extract mutagenicity. As a conclusion of the experiments, and based on standardized criterias of mutagenicity, the *E. macrophyllus* was found to be non-mutagenic and not toxic in the Ames test. Moreover, the substances we found in the HPLC-DAD have health benefits, such as the isoorientin having similar properties to its isomer, the orientin, with both reported as anticoagulant, antithrombotic and anti-microbiotic effects; and, the isomers vitexin and isovitexin both have antioxidant, anti-inflammatory, anticancer and neuroprotective effects; lastly, the rutin has many pharmacologic properties, acting on cardiac diseases, diabetes, bacterial infections and inflammations. Thus, the results suggest potential health benefits associated with the consumption of *Echinodorus macrophyllus* extract, once it contains substances that benefit the health, without potential associated genotoxic risks.
Keywords: Ames test; Chromatography; *Echinodorus macrophyllus*; mutagenicity.

CHEMOPREVENTIVE AND MUTAGENIC EFFECTS INVESTIGATION OF NEW FOOD PRODUCTS BASED ON HONEY AND BACCHARIS DRACUNCULIFOLIA EXTRACT

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Abstract:
The consumption of healthy foods and natural products plays a crucial role in maintaining good health and promoting therapeutic effects, making it an essential method for disease prevention. In this context, cancer chemoprevention emerges to delay the development of new cases using natural or synthetic chemical agents of low toxicity. Honey has drawn attention due to its flavonoid content and its potential anticancer effects, being able to interrupt the cell cycle, increase apoptosis and modulate the immune response. The Baccharis dracunculifolia, a medicinal plant native to the Brazilian cerrado, is also known for its antioxidant and anti-inflammatory properties derived from its phenolic compounds and flavonoids, exhibiting an antimutagenic characteristic. Both honey and B. dracunculifolia extract have clear chemopreventive effects demonstrated by the literature. However, our research group developed a honey-based food product enriched with field B. dracunculifolia ethanolic extract (HBDE) whose effects needed to be evaluated. The association of both creates an expectation of increased influence of one over the other, enhancing their chemopreventive effects. So, the present study aimed to evaluate the chemopreventive and mutagenic effects of HBDE. Two different products with honey as their main ingredient were enriched, respectively, one with extracts from the leaves (HBDLE) and other inflorescences (HBDIE) of B. dracunculifolia. Male Swiss mice (Mus musculus) were treated with HBDLE and HBDIE at 10%, 5% and 2.5%, with isolated honey and isolated extracts (BDLE and BDIE) at 10% for 15 days. Micronucleus (MN) assay on peripheral blood were performed on the 2nd day and on the 14th day, to assess the possible mutagenicity and at the end (16th day), after doxorubicin [DXR, 20 mg/kg body weight (b.w.)] injection, to evaluate its chemopreventive effect. The animal weight, water consumption, and blood glucose levels were monitored to assess toxicological safety and the impact on health. Total phenolic compounds and flavonoids were determined. In addition, its antioxidant activity was measured by DPPH and ABTS assays. Our findings demonstrated that the parameters of weight and water consumption did not show significant changes throughout the treatment. Besides, HBDIE 5% caused a reduction in glycemic variation compared to the negative control group (NC). No alterations in the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Furthermore, there was no difference in the MN's frequency between the treated groups and the NC until the 14th day, but after DXR administration, the MN's frequency in the groups HBDIE 10% and 5% was significantly different from both NC and DXR groups. The HBDFE and HBDIE showed, respectively total phenolics compounds [0.72 ± 0.79 and 1,01 mg $\pm$ 0.11 mg in gallic acid equivalent (GAE/g)] and total flavonoids [0.28 ± 0.02 and 0,61 $\pm$ 0.09 mg catechin equivalent (CE/g)]. A significant antioxidant activity through the ABTS radical scavenging activity were showed, whose results was 8.46 ± 0.34 µmol TE/g (Trolox equivalent). Our studies suggest chemopreventive potential for HBDIE due the levels of bioactive compounds and antioxidant activity.
Keywords: Chemoprevention; Cancer; *Baccharis dracunculifolia*; Honey.

STUDY OF THE ROLE OF *TP53* IN THE REGULATION OF THE ANTIPROLIFERATIVE EFFECTS OF *CIS*-TRIMETHOXYSTILBENE IN THE BREAST TUMORAL CELL LINES MCF-7 AND MDA-MB-231

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Abstract:

Natural products are the primary sources for obtaining molecules with biological potential for the development of new drugs. Among these molecules, a group called stilbenes stands out. Resveratrol, a widely studied stilbene with significant pharmacological potential for cancer therapy, has been limited in its advancement in more advanced clinical trials due to its low bioavailability. To overcome this problem, methoxylated derivatives of resveratrol, such as *cis*-trimethoxystilbene (*cis*-TMS), have been synthesized, which have shown higher bioavailability and much more efficient antiproliferative activity in cellular assays compared to their precursor, resveratrol. Despite the remarkable antiproliferative action of *cis*-TMS at very low concentrations (below 2.5 μM) in tumor cells, it is unclear whether the regulation of this biological activity is related to the action of the p53 protein, encoded by the *TP53* gene. Thus, the aim of this study is to answer the following question: Does the antiproliferative activity of *cis*-TMS depend on p53?. The methodology initially involved evaluating the interaction between cis-TMS and p53C using spectrofluorimetry and dynamic light scattering (DLS), followed by the genotoxic analysis of the molecule in the tested cell lines and assessing their repair potential through histone γH2A.X labeling and comet assay. In spectrofluorimetry, the protein was exposed to concentrations ranging from 2.5 to 10 μM of *cis*-TMS, showing a dose-dependent effect where an increase in concentration led to a decrease in protein luminescence. However, only the lowest tested concentration did not interact with p53C, resulting in no change in the emitted luminescence intensity compared to the isolated protein. Regarding DLS, it was observed that the interaction of the bioactive compound *cis*-TMS at a concentration of 2.5 μM with p53C did not cause significant changes in its dimensions and surface charge when compared to the values of p53C and the control solvent, DMSO. As for the investigation of the genotoxic effect of *cis*-TMS, it was observed that the concentration of 2.5 μM statistically induced double-strand breaks only in the MCF-7 cell line ($p<0.0001$), with approximately 30.1% presence of γH2A.X, which differed from MCF-10A and MDA-MB-231, which exhibited 8.1% and 8.3% presence, respectively, compared to the negative control (NC). Therefore, this same concentration is capable of causing irreparable damage in all cell lines since even after 24 hours of treatment withdrawal, the MCF-7 ($p<0.01$ *vs.* NC), MCF-10A, and MDA-MB-231 ($p<0.001$ *vs.* NC) cell lines were unable to repair the induced damage. Based on the preliminary results of this study, we can conclude that *cis*-TMS causes irreversible damage to genetic material and induces double-strand breaks, as observed in MCF-7. Furthermore, even in the presence of p53C protein, the compound does not cause conformational changes at a concentration of 2.5 μM, suggesting that it does not induce protein aggregation or directly modulate the central domain of the protein. However, further studies will be conducted to obtain more information and understanding about the mechanism of action of this compound.

Keywords: natural products; cancer; p53; stilbene.

Support / Acknowledgment

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INVESTIGATION OF CYTOTOXIC AND GENOTOXIC ACTIVITIES OF GLYPHOSATE-BASED HERBICIDES ON GLIOBLASTOMA CELL LINES.

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Abstract:

Glyphosate is the world's most widely used herbicide, which has a low toxicity rating despite substantial evidence of adverse health effects. Furthermore, glyphosate-based herbicides (GBHs) contain several other chemicals, some of which are known to be harmful, while many are regarded as trade secrets. It is unknown that chemicals might be contributing to the health negative effects, as glyphosate is present in the form of potassium salt. The controversy remains regarding whether GBHs are more potent than glyphosate alone in activating cellular mechanisms that drive genetic stability. One central protein involved in maintaining genetic stability is P53, a tumor suppressor protein. P53 acts as a key regulator of the cellular response to stress and DNA damage. Mutations in the *TP53* gene, which encodes P53, are common genetic alterations found in various types of cancer. Therefore, this study aimed to evaluate the cytotoxicity and genotoxicity of GBH in two glioblastoma cell lines: U87MG (proficient for TP53) and U251MG (mutant for TP53). Additionally, the study aimed to identify the main proteins involved in the response to GBH exposure using Systems Biology in a network containing P53 and another network without P53. The MTT assay was used to study the toxicity of GBH in the cell lines, the clonogenic assay was used to investigate cell survival, and the Comet Assay was used for genotoxicity evaluation. For data analysis, bioinformatics tools such as STRING 11.0 and STITCH 5.0 were applied, serving as a basis for designing binary networks in the Cytoscape 3.6.0 program. Comparisons were made between control and treatment groups using one-way analysis of variance (ANOVA) followed by Dunnett's test. Values of $p \leq 0.05$ were regarded as statistically significant. From the *in vitro* test analyses, we observed a decrease in cell viability at doses starting from 10 ppm, a dose considered safe by ANVISA and CODEX. DNA damage was observed by the Comet Assay at concentrations of 10 ppm and 30 ppm for the U251MG and U87MG cell lines, respectively. The network generated with systems biology showed that the presence of P53 is important for the regulation of biological processes involved in genetic stability and neurotoxicity, processes that did not appear in the network without P53.

Keywords: glyphosate; Comet assay; TP53; in vitro; System Biology

Support / Acknowledgment

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EVALUATION OF THE *IN VITRO* GENOTOXICITY AND MUTAGENICITY OF SYNTHETIC Β-CARBOLINE ALKALOIDS WITH SELECTIVE CYTOTOXIC ACTIVITY AGAINST OVARIAN AND BREAST CANCER CELL LINES

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Abstract:

The family of β-carboline alkaloids comprises both natural and synthetic products, exhibiting remarkable structural diversity and pharmacological properties. The aim of this work was to evaluate the *in vitro* cytotoxic, genotoxic, and mutagenic potentials of two synthetic β-carboline alkaloids developed as prototypes of antitumor agents (NQBio-06 and NQBio-21). The studies were carried out employing the human cell lines TOV-21G (ovarian adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), and WI-26-VA4 (nontumor pulmonary fibroblast), used as reference of normal cells. To the evaluation of genotoxicity (comet assay) and mutagenicity (cytokinesis-block micronucleus assay) of the synthetic β-carboline alkaloids, the studies were performed with and without metabolic activation with S9 fraction (2%). All experiments were repeated independently at least three times, and an analysis of variance (ANOVA) was conducted followed by Tukey's multiple comparison post-test to analyze the results obtained. The level of $p < 0.05$ was considered statistically significant. To assess the effects of the synthetic compounds on the cell viability, the MTT assay was performed, and the results obtained showed that NQBio-06 presented higher cytotoxicity in the ovarian cancer cell line TOV-21G (IC_{50} = 2.5 µM, selectivity index = 23.7). NQBio-21 presented an IC_{50} of 6.9 µM and a selectivity index of 14.4 against MDA-MB-231 breast cancer cells. Comet assay results showed that NQBio-06 did not induce chromosomal breaks *in vitro,* but the compound NQBio-21 was genotoxic in studies with and without metabolic activation (S9 fraction). The cytokinesis-block micronucleus assay showed that both compounds were mutagenic. In addition, metabolic activation enhanced this effect *in vitro*. These results suggest that the mechanisms underlying the cytotoxicity of NQBio-06 and NQBio-21 are related to DNA damage induction and that the use of S9 enhanced these effects. Additionally, the compound NQBio-06 exhibited negative results in the comet assay but induced micronuclei, suggesting its potential as an aneugenic agent. These findings underscore the promise of β-carboline compounds as a source for developing new chemotherapeutic agents for anticancer treatments. However, further studies are imperative to gain a better understanding of the mechanisms of action and safety profiles of these compounds, both *in vitro* and *in vivo.*

Keywords: Comet Assay; Micronucleus Assay; *in vitro*.

Support / Acknowledgment

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DIFFERENTIATION CAPACITY OF THE SH-SY5Y HUMAN NEUROBLASTOMA CELL LINE BY Y-27632 ROCK INHIBITOR

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Abstract:
A great interest has arisen regarding the role of ROCK2 protein, a positive PTEN regulator, in cellular functions, particularly in neurons, once the inhibition of this protein leads to the activation of PI3K/AKT pathway. This pathway is implicated in cell survival and also in neurogenesis and neuritogenesis of the brain. Many studies have shown that the use of Y-27632, a ROCK inhibitor, is capable of inducing neuritogenesis and neurodifferentiation in several cell models. However, to date, there are no studies demonstrating that this inhibitor is capable of inducing neuronal differentiation in SH-SY5Y human neuroblastoma cells. SH-SY5Y cells are an important model in the field of neuroscience, and are classically differentiated into mature neurons by using the retinoic acid (RA). However, alternative methods that are able to differentiate them in mature neurons are of great relevance. Therefore, in this study we tested the hypothesis that the inhibition of the ROCK2 protein could lead to the inactivation of PTEN and, consequently, the activation of PI3K/AKT signaling, promoting neurodifferentiation and neuritogenesis in SH-SY5Y cells. Our aim was to characterize the potential of the compound Y-27632 (ROCK inhibitor) to induce neurodifferentiation of SH-SY5Y cells, in order to establish a new method for the differentiation of this model. Thus, cells were treated with Y-27632 (5 µM) for 7 days, with the treatment medium changed every 2 days. Our results showed that after 7 days, ROCK inhibition promoted morphological changes typical of neurons in SH-SY5Y cells, such as a significant increase ($p<0.001$) in neurite size (Y-27632: 43.9 µm; negative control: 23.9 µm); a significant reduction ($p<0.001$) in the diameter of the cell cytoplasm (Y-27632: 22,9 µm; negative control: 26,9 µm); and a significant increase ($p<0.001$) in the percentages of differentiated neurons (Y-27632: 63%; negative control: 24%). The confirmation that SH-SY5Y cells were differentiated into terminally differentiated neurons can be achieved through the detection of the β-III-tubulin protein, which is considered a universal marker of neurons. Thus, after treatment with Y-27632, positive cells for anti-β-III-tubulin staining were detected by immunofluorescence; in addition, a propensity for an increase (although not significant, $p > 0.05$) of the expression of this marker was also observed. Our results also showed that the compound Y-27632 did not alter mitochondrial mass, intracellular and mitochondrial ROS production, cell cycle progression or cell proliferation index of SH-SY5Y cells. These findings indicate that the inhibition of ROCK may be a potential target for promoting neurodifferentiation, leading to the induction of neurite outgrowth, and neuronal differentiation in the SH-SY5Y human neuroblastoma cell model. Therefore, the use of Y-27632 can be an interesting alternative for the differentiation of these cells.
Keywords: ROCK2 inhibition; PI3K/AKT pathway; Neurodifferentiation; Neurogenesis.

Support / Acknowledgment

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KALANCHOE DAIGREMONTIANA: ETHNOPHARMACOLOGICAL INSIGHTS FROM METABOLOMICS, CHEMOPREVENTIVE AND ANTICANCER ACTIVITY.

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Abstract:

Kalanchoe daigremontiana Raym.-Hamet & H. Perrier, commonly known as Mother of Thousands, is a succulent plant with a long history of traditional medicinal use. This study investigated the metabolomic profile, pharmacokinetic properties, antimutagenic activity and anticancer potential of aqueous extracts obtained through decoction and cold processes, according to the most popular ways of preparing this phytomedicine. Using UHPLC-MS approach we compared the metabolomic profiles of aqueous extracts obtained through cold extraction (Kd1) and decoction (Kd2) processes. Both extraction methods extracted the same pool of compounds from *K. daigremontiana*, albeit with remarkable differences in their relative intensities. Using metabolomics and *in silico* pharmacokinetic analysis, we identified several promising compounds with favorable ADMET properties in the aqueous extract compositions. Notable compounds included corchorifatty acid F, 9,12,13-TriHOME, 19-oxodesacetylcinobufagin, hellebrigenin, and L-malic acid. These findings suggest the potential therapeutic relevance of *K. daigremontiana* in developing novel drug candidates. To further access *K. daigremontiana* aqueous extracts safety and biological activities, we have conducted the *Salmonella* microsome assay to assess mutagenicity and found that Kd extracts were not mutagenic. Moreover, the extracts exhibited a protective effect against genotoxicity linked to C-G/A-T substitution through intra and extracellular interactions, particularly against 4NQO. The antimutagenic results in bacterial model showed that Kd1 was able to activate DNA repair system in the TA100 strain, suggesting its potential role in DNA damage repair mechanisms and that the previous subtle differences presented in metabolomics profiles may be able to play a role in the extracts potential.Furthermore, the cytotoxic effects of *K. daigremontiana* extracts on gastric and hepatic cancer cell lines were evaluated trough cell viability assays and both extracts demonstrated dose-dependent cytotoxic effects on the HCG-27 cell line (derived from stomach cancer) with better performance of Kd1 with EC_{50} 1149 ± 1.51 ng/mL at 24 h, 750 ± 1.25 ng/mL at 48 h and 668 ± 1.44 ng/mL at 72 h. None of the extracts were cytotoxic to FC3H cells (up to 5×10^6 ng/mL). These results shed a light at the efficacy of *K. daigremontiana* in cancer context, demonstrating the antimutagenic and antitumoral activities of its extracts, mainly the cold ones, and the observed protective effects against genotoxicity and the ability to induce cancer cell death suggest a synergistic composition of active phytometabolites in the cold extracts. These findings contribute to the validation of the ethnopharmacological use of *K. daigremontiana* and provide a scientific basis for its further exploration as a source of novel therapeutic agents in the treatment of gastric and liver cancer.

Keywords: *Kalanchoe daigremontiana*; natural product; anticancer; chemopreventive; metabolomics

Support / Acknowledgment

FAPERJ, CNPQ, CAPES, UERJ e UNIRIO.

EMBRYOTOXICITY AND VISUAL-MOTOR RESPONSE IN ZEBRAFISH (DANIO RERIO) INDUCED BY RETENE

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Abstract:
Retene or RET (1-methyl-7-isopropylphenanthrene), a non-priority Polycyclic Aromatic Hydrocarbon (PAH), is the most prevalent from cellulose burning, although its toxic effects are not clearly elucidated. In this work, using zebrafish embryos as a experimental model, we investigated the embryotoxicity and neurotoxicity of RET. Five RET concentrations were used: 100 µg /L, 250 µg/L, 500 µg/L, 750 µg/L, and 1000 µg/L, and also two negative controls: sistem water control (WC) and DMSO 0.1% (NC). 4mg/L of 3,4- Dichloroaniline were used as a positive control. The eggs and larvaes were evaluated for external morphology every 24, 48, 72 and 96 hours post fertilization (hpf). Larvaes with 7dpf previously treated with RET were exposed to a string moving stimuli to measure optomotor response and a black ball moving stimuli to measure Avoidance behavior. RET did not alter significantly the survival rate for any group analyzed. However, our results demonstrate that the treatment with all five concentrations of RET induced morphological defects and caused changes in optomotor development and behavior, suggesting a neurotoxic effect. Taken together, these findings reinforce the need to better track environmental pollutants in aquatic ecosystems, especially those whose toxic potentials remain underestimated.
Keywords: Retene; Zebrafish; Embryotoxicity; Optomotor behavior; Neurotoxicity

Support / Acknowledgment

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INVESTIGATION OF CURCUMIN MECHANISMS ON CELL PROLIFERATION IN HELA SPHEROIDS

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Abstract:
Since resistance to clinically available chemotherapeutic agents is the main problem in cancer treatment, searching for new drugs is an important way to overcome this issue. Curcumin is a phytochemical extracted from *Curcuma longa* L., which is a well-known molecule and presents antioxidant and anti-inflammatory properties. This molecule has been studied to investigate its antiproliferative effects. 3D drug screening is an innovative tool for drug prediction since this model mimics cancer microenvironmental conditions not present in cell monolayer models. In spheroids, hypoxia acts on cells, stimulating the production of factors involved in cell survival leading to cellular heterogeneity, and creating pH and nutrient gradients, the cells on spheroids interact with each other and produce extracellular matrix, which is important to their signaling communication. This characteristic mimics tumor microregions and micrometastasis. Therefore, this work aimed to investigate curcumin mechanisms on cell proliferation of HeLa cell spheroids. To assess curcumin's cytotoxicity, a resazurin assay was performed on a monolayer model to determine the appropriate concentration for testing on a 3D model. Spheroids were created by cultivating 5×10^3cells/well in non-adherent 96 well plates for six days. A comet assay was conducted to evaluate genotoxicity. Spheroid growth was monitored for 72 hours using ANaSP and ReViSP software. Afterward, spheroids were assessed for proliferation recovery. A clonogenic assay was performed to determine the potential for colony formation. Hoechst 33342 was used to observe chromatin compaction, while Rhodamine 123 assessed mitochondrial metabolism activity. RT-qPCR was performed to verify mRNA expression levels. The results were evaluated using REST software and expressed as fold-change. Data normality was confirmed through the Shapiro-Wilk test. One-way ANOVA with Dunnett's post-hoc and two-way ANOVA with Tukey's post-hoc was used when applicable, with $p \leq 0.05$. In the cytotoxicity assay, within 24 hours of treatment, curcumin exhibited cytotoxicity in a dose-dependent manner, reducing viability by 62.04% at the highest concentration tested (50 μM). Under the condition of this study, the IC50 value (20.61 μM - selected concentration to treat spheroids) was calculated through non-linear regression analysis. Curcumin reduced spheroid growth by 9.41% at 24h, 19.5% at 48h, and 25.35% at 72h. It also decreased proliferation recovery and colony formation (by 61.95%). Hoechst 33342 showed more condensed chromatin in curcumin-treated spheroids, and Rhodamine 123 indicated lower mitochondrial metabolism activity. There was no evidence of genotoxicity. Curcumin reduced the mRNA expression of *PARP1*(-2.14x), *BECN1* (-2.86x) and *CASP9* (-2.11x) genes. *PARP1* is essential for cell proliferation maintenance. The down-regulation of *CASP9* and the regular expression of *CASP3/8* evidence that curcumin acts through caspase-independent pathways. These findings suggest that curcumin has the potential to be a therapeutic phytochemical with cytotoxic effects.
Keywords: 3D; Gene Expression; Genotoxicity; Toxicology; Antiproliferative Effects

Support / Acknowledgment

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INFLUENCE OF ANGIOTENSIN-CONVERTING ENZYME (ACE) GENE SINGLE NUCLEOTIDE POLYMORPHISM (SNP) RS4291 ON LOSARTAN TREATMENT IN HYPERTENSIVE PATIENTS

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Abstract:

Arterial hypertension is a chronic multifactorial disease characterized by persistent systolic ≥ 140 mmHg and/or diastolic ≥ 90 mmHg pressure. The angiotensin converting enzyme (ACE), through the renin-angiotensin-aldosterone system (RAAS), participates in the regulation of blood pressure, sodium availability and water balance. The presence of polymorphisms in the ACE gene can trigger a greater predisposition and establishment of hypertension. The T allele of the rs4291 ACE polymorphism has already been reported as being the central risk allele for the onset of the disease. The treatment of hypertension consists mainly of the use of antihypertensive drugs belonging to the classes of thiazide diuretics and angiotensin II receptor antagonists, such as hydrochlorothiazide and losartan, respectively, main drugs prescribed in the Brazilian Health Systems (SUS). Individualized therapy, characteristic of pharmacogenetics, may prevent occurrence of important adverse events and, simultaneously, favor an favorable prognosis for hypertensive patients. This study aimed to evaluate the association of genetic polymorphisms of the angiotensin-converting enzyme (ACE) rs4363, rs4291 and rs4335 and the response to antihypertensive drugs in hypertensive individuals from Ouro Preto/MG. A case-control study was carried out with 143 hypertensive, 87 individuals under treatment with losartan and 75 with hydrochlorothiazide recruited from the Clinical Analysis Laboratory of the Faculty of Pharmacy of the Federal University of Ouro Preto (UFOP) who responded to a questionnaire including sociodemographic, clinical and behavioral data. Good responders to pharmacological therapy were defined as those who used losartan and/or hydrochlorothiazide for at least 3 months and who had mean arterial pressure <140/90 mmHg in medical records. Poor responders presented mean arterial pressure ≥140/90mmHg even after treatment. Blood samples were collected for evaluation of the biochemical and molecular profiling. Biochemical analyzes were performed on the serum using UV/Vis spectrophotometry. Identification of the rs4363, rs4291 and rs4335 ACE variants was performed by real-time PCR using the TaqMan® system. Univariate logistic regression test was performed to compare categorical data and the association between polymorphisms and drug response between good and poor responders. P<0.05 was considered significant. All analyzes were performed using the STATA 13.0 software. Results showed there was no influence of the rs4363, rs4291 and rs4335 ACE polymorphisms on the response to hydrochlorothiazide therapy. However, regarding the losartan, the AT or TT genotypes of rs4291 were more frequent in the group of good responders (54,9%), indicating that individuals with at least one T allele are 2.8 times more likely to be good responders (95% CI 1.12-6.80, p=0.026) compared to those with the AA genotype. In conclusion, patients who carry the risk alelle (rs4291) for arterial hypertension control blood pressure more effectively when using losartan as antihypertensive therapy. These results show the importance of pharmacogenetic studies in order to detect genetic characteristics, classifying individuals as good and poor responders to the pharmacological treatment, which would reduce costs for the public health system. Financing: Fapemig (APQ-03555-22), CNPq (310905/2020-6) and CAPES (001).

Keywords: Hypertension; pharmacogenetics; losartan; genetic polymorphism; angiotensin converting enzyme gene

Support / Acknowledgment

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PEGYLATED LIPOSOMAL FLUOPSIN C INDUCES GENE EXPRESSION CHANGES IN RESPONSE TO CYTOTOXICITY AND GENOTOXICITY IN HEPG2/C3A SPHEROIDS

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Abstract:
Fluopsin C (FlpC), a copper-containing antibiotic from *Pseudomonas aeruginosa*, exhibits potent cytotoxic and antiproliferative effects on cancer cells. However, its clinical application is limited by high toxicity. To overcome this limitation, FlpC can be incorporated into PEGylated liposomes (PL). This nanopharmacological strategy enables targeted delivery to the tumor microenvironment, reducing systemic toxicity and preserving drug efficacy. In this regard, Multicellular Tumor Spheroids (MCTs) derived from human hepatocytes, which mimic the architecture, physiological responses, and gene expression of solid tumors, can be employed as a toxicological model to evaluate the effects of PL-FlpC. Therefore, this study aimed to evaluate the effects of PL-FlpC on cytotoxicity, antiproliferation, and metabolism in monolayer and MCTs cultures of HepG2/C3A cells. For all experiments, empty liposomes were used as vehicle control (VC). The data from three experimental replicates were analyzed using ANOVA, followed by Dunnett's test ($p \leq 0.05$). For RT-qPCR analysis, a Student's t-test was performed ($p \leq 0.05$), considering fold-change ≥2 or ≤-2. In monolayer, cytotoxic concentrations were determined using a resazurin reduction-based assay (PL-FlpC= 0.05-0.8 μM; T=24h). PL-FlpC exhibited dose-dependent cytotoxicity (IC50= 0.5 μM). A real-time cell analysis system, based on changes in electrical impedance, generated a genotoxic curve pattern with PL-FlpC (IC50; T=24h). Time-lapse microscopy observations revealed cellular changes induced by PL-FlpC (IC50; T=24h), including cell cycle arrest, loss of adhesion morphology, the emergence of endoplasmic reticulum-like structures, cell swelling, and cell death. Additionally, fluorescence microscopy revealed altered mitochondrial arrangement and accumulation of lipid droplets. The following assays involved spheroids, thus MCTs were generated using the Liquid Overlay Technique (2×10^4 cells/well) for six days with MEM complete medium, and treated with PL-FlpC (½IC50, IC50, and 2×IC50). Genotoxicity was confirmed by the alkaline comet assay (n=5; T=3h). Cell cycle analysis (propidium iodide staining) in flow cytometry revealed cell cycle arrest at the S phase with IC50 and 2×IC50. Apoptosis analysis (Annexin-V/7-AAD) in flow cytometry demonstrated that PL-FlpC induced necrotic cell death at IC50 and 2×IC50, with a respective increase of 12.6% and 13.2% compared to the VC. Spheroid growth rate (mm3) was assessed at 24, 48, and 72h relative to time 0. PL-FlpC (IC50 and 2×IC50) attenuated ($p \leq 0.05$) spheroid growth compared to the VC at all time points. Interestingly, after 72h, IC50 and 2×IC50 exhibited 31.2% and 44.6% lower growth rates than VC, respectively. The spheroids collected from the growth assay were subsequently subjected to a clonogenic assay (n=5; T=14d). IC50 and 2×IC50 led to a reduction of colony numbers by 24.4% and 46.7%, respectively. The molecular mechanisms involved in the effects of PL-FlpC were investigated by analyzing the cDNA of mRNA-polyA from spheroids (n=20; T=12h; PL-FlpC=IC50) using RT-qPCR. Genes of xenobiotic metabolism (*CYP1A1*, *CYP2B6*, *CYP2C9*, *HNF4-α*, and *GST*), DNA damage response (*GADD45A*), cell cycle regulation (*CDKN1A*), and cell death (*SLC711A*, *RIPK1*, *BBC3*, *M-TOR*, and *SQSTM1*) were upregulated, while the *PCNA* gene (cell proliferation) was downregulated. In conclusion, these findings highlight the potential of PL-FlpC for future research and optimization of PL-FlpC as a targeted therapeutic agent to enhance efficacy in the treatment of solid tumors.
Keywords: Cancer; Secondary Metabolites; Targeted Therapy; Necrosis; Xenobiotic

Support / Acknowledgment

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IN VITRO TOXICITY AND MUTAGENICITY OF ZINC OXIDE: COMPARATIVE ANALYSIS OF NANOPARTICLE AND BULK FORM

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Abstract:
The understanding of nanotechnology has been comprehensive to improve the use of nanoparticles in order to ensure quality and safety. However, it is already known that they cause relevant consequences for both the environment and human health. Zinc Oxide (ZnO) nanoparticles are frequently used metal nano-oxides , as well as their massive form. Thanks to their antimicrobial capacity and other beneficial applications, they are found in commercial products such as sunscreens acting as a UV-A and UV-B radiation filter. Nanoscale particles are more likely to cause damage to cells and, after being absorbed, a high production of reactive oxygen species (ROS) occurs due to the release of free Zn2+, resulting in subsequent cell damage. Taking into account that these damages may vary according to the structural form of the particle, the objective of this study is to demonstrate the toxicological and mutagenic differences between the nanometric and massive forms. ZnO NP and its bulk form were diluted in dimethylsulfoxide (DMSO) to carry out the tests and used concentrations from 0.005 to 50 µg/pl. In the Ames Test, after 72 hours of incubation of the samples with *Salmonella enterica sorovar Typhimurium* from TA98, with frameshift mutation and TA102, which detects A:T /G:C base pair substitution mutation , it was observed the induction of a significant increase of revertant colonies for both samples. The results concerning TA102 strain, at the highest concentration tested, in the absence of metabolic activation with S9 Mix 4%, the nanoparticles presented a range of 879 ± 3 revertants and, in the presence of activation, 1223 ± 4. Respectively, the massive form presented 636 ± 14 and 1,119 ± 13. In the TA98 strain, without metabolic activation, the nanoparticles induced 72 ± 9 and with activation 75 ± 4. The massive form presented 59 ± 8 and 75 ± 3. However, in the other strains used - TA97a, TA100, TA104 and TA1535 - no results suggestive of mutagenicity were observed. In the colorimetric assay of cell viability of Water Soluble Tetrazolium (WST-1) the Human Hepatocarcinoma cell line (HepG2) was used. After 24h, 48h and 72h of incubation of the cells with the samples, gradual cell death was observed, with emphasis on the cytotoxicity of the massive form of Zinc Oxide with the respective LC50 data for the nanoparticle in 24h, 48h and 72h respectively: 15.03; 13.69 and 23.44µM. For the massive form, 3,196; 2.002 and 10.19µM. Finally, the respective study demonstrates, by the Ames Test, the induction of mutagenicity caused by Zinc Oxide nanoparticles as well as its massive form. It is also observed, in terms of cell viability, that both samples showed considerable levels of cytotoxicity. However, the massive form showed a lower LC50 level compared to the nanoparticle. This fact indicates the greater cytotoxicity of this molecular form of Zinc Oxide. With this, it is concluded that additional information is important in order to guarantee greater authenticity and toxicological safety in the use of Zinc Oxide both in its nanometric form and in its massive form.
Keywords: Nanotechnology; Zinc Oxide Nanoparticle; Nanoparticle.

ORGANOTERULAN RF07 CYTOTOXICITY AND CELL DEATH MECHANISM AGAINST BREAST CANCER CELLS

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Abstract:
Despite advances in cancer therapy, the search for novel anticancer drugs more specific and less toxic is still one of the biggest interests in the medical field. In this regard, understanding the drug´s mechanisms of action are as important as its effect against cancer cells. Previous pre clinical studies presented promising effects of the organoterulan RF07 so that understanding its mode of action and pathways could better filter its use. For this, *in vitro* experiments to evaluate RF07 cytotoxicity and cell death were carried out using the human breast cancer cell line MDA. The MTT assay was used to identify the cytotoxic concentration (CC_{50}); flow cytometry for classifying cell death mechanism and western blot for apoptotic pathway determination. The RF07 CC_{50} was 4,78 µM, showing that low concentrations have strong impact against MDA cell line. Cell death mechanism are influenced by the drug within the organism, and RF07 at 1, 2.5 and 5 µM, increased early apoptosis in a concentration-dependent manner, with 4.27, 78.33 and 86.33 %, respectively. These results were significantly better than cisplatin, used as positive control. Conversely, necrosis was almost 20 times higher for cisplatin than RF07. The determination of apoptotic pathway was made using western blot assay, and a significant increase of caspases 3 and caspase 9 and decrease of caspase 8 indicated the intrisic pathway as the RF07 pathway for cell death in MDA cell line. Preliminary results show that RF07 not only induce apoptosis as a cell death mechanism, but also do that through the intrinsic pathway.
Keywords: organometals; breast cancer; apoptosis; protein expression; antitumoral

ANTITUMOR ACTIVITY OF DIALLYL DISULFIDE AGAINST *IN VITRO* 3D HT-29 SPHEROIDS

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Abstract:
Colorectal cancer (CRC) is the third most common cancer and the second most deadly cancer globally. Early detection of CRC allows for complete cure through surgery and subsequent medication. However, the recurrence rate is high, and drug resistance in cancer cells contributes to treatment failure. The chemotherapeutic drug 5-fluorouracil (5-FU) is a primary systemic treatment option for CRC. It works by inhibiting the S phase of the cell cycle, blocking DNA synthesis, and triggering cell death. However, the main drawback of 5-FU is the development of drug resistance. Therefore, innovative therapeutic interventions are necessary to overcome drug resistance and improve treatment response. Several nutraceuticals, including curcumin, epigallocatechin gallate, and resveratrol, are undergoing different phases of clinical trials for CRC treatment. Epidemiological studies have provided clear evidence linking garlic consumption to a decreased risk of CRC. Diallyl disulfide (DADS), a major organosulfur compound found in garlic, has shown promising antitumor activity against various types of tumor cells. Multicellular spheroids have become a widely accepted approach for in vitro cancer models and testing substances with antitumor activities. In this study, we investigate the efficacy of DADS in inhibiting the growth of the human colorectal adenocarcinoma cell line HT-29 using three-dimensional (3D) spheroidal models. HT-29 cells were grown as spheroids, and the effects of DADS or 5-FU on growth, area, morphological integrity, and cell viability were evaluated. Spheroids were generated using ultra-low-attachment plates, and their morphology and diameters, both untreated and treated with DADS (200 - 800 µM) or 5-FU (100 - 1,000 µM), were observed and measured using microscopy. The first photomicrograph of each spheroid was obtained 72 hours after treatment with DADS or 5-FU, and the area was measured using AxioVision 3.1 software. Cell viability was assessed using a resazurin assay. Results demonstrated that all concentrations of 5-FU significantly inhibited the growth of HT-29 spheroids ($p<0.05$). Treatment with DADS (800 µM) decreased cell viability compared to the negative control ($p<0.05$). Our findings indicate that the antitumor activity of DADS is comparable to that of 5-FU. These results suggest that DADS merits further investigation in preclinical studies for CRC treatment.
Keywords: Garlic; colorectal cancer; 5-fluorouracil.

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DIOSGENIN INDUCES MULTINUCLEATION IN NCI-H460 LUNG CARCINOMA CELLS BY INHIBITION OF CYTOKINESIS

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Abstract:
Diosgenin, a steroidal sapogenin found in plants of the genus *Dioscorea* spp., has shown promising antiproliferative activity in various tumor cell lines. In lung cancer cells, diosgenin can induce DNA damage, apoptosis, cell cycle arrest and increased cell sensitivity to the chemotherapy drug cisplatin. However, this phytochemical can induce the formation of multinucleated tumor cells, known for their chemoresistance and higher susceptibility to genomic instability. Given the unknown specific effects of diosgenin on the formation of multinucleated lung carcinoma cells, this study aims to assess the activity of diosgenin in inducing multinucleation in NCI-H460 lung carcinoma cells. The assay results were presented as means of three biological replicates and compared to the vehicle control (VC, 0.22% ethanol). Statistical significance was determined using ANOVA followed by Dunnett's test ($p<0.05$). For RT-qPCR data, significance was determined using Student's t-test ($p<0.05$) and log2(fold change) ≥ 1 or ≤ -1. Resazurin was used as an indicator of cell viability through its enzymatic conversion to resorufin. After 24h, cells showed a dose-dependent reduction ($p<0.05$) in viability at the concentration tested (30-70 µM). Inhibitory concentration (IC) values 12.5 (31.28 µM), 25 (36.42 µM) and 50 (44.36 µM) were determined for subsequent assays. The dynamics of cell proliferation were monitored through the cell index (CI), which reflects changes in impedance, using Real-Time Cell Analysis (RTCA). After 6h and 12h, there was a reduction ($p<0.05$) of CI with $IC_{12.5}$ (-10.52% at 6h and -11.96% at 12h), IC_{25} (-1.19% at 6h and -11.04% at 12h) and IC_{50} (-20.32% at 6h and -25.57% at 12h). While after 24h, there was a reduction ($p<0.05$) of CI only with IC_{50} (-25.40%). The dynamics of response to treatment were monitored by time-lapse microscopy for 24h with IC_{50}. Out of a total of 200 cells counted, 9.5% underwent cell fusion after the formation of the cytokinesis cleavage furrow. Nuclear DNA content was assessed by flow cytometry using propidium iodide. The frequency of cells with twice the DNA content, compared to cells in the G1 phase of the cell cycle, were higher ($p<0.05$) with $IC_{12.5}$ (27.4%), IC_{25} (30.07%) and IC_{50} (29.3%), compared with the VC (18%), after 24h. The percentage of multinucleated cells was determined after 24h via Giemsa staining. The percentage of mononuclear (%MN) cells decreased ($p<0.05$) in $IC_{12.5}$ (91.43%), IC_{25} (86.80%) and IC_{50} (78.93%), while the percentage of binucleated (%BN) cells increased ($p<0.05$) in $IC_{12.5}$ (7.20%), IC_{25} (10.93%) and IC_{50} (18.50%), in comparison with the respective VC (MN = 98.47%; BN = 1.43%). The percentage of cells with more than 2 nuclei increased ($p<0.05$) in $IC_{12.5}$ (1.37%), IC_{25} (2.27%) and IC_{50} (2.57%) compared to VC (0.10%). Genes involved in regulating the mitotic phase of the cell cycle were evaluated using RT-qPCR after 12h with IC_{50}. Cytokinesis regulatory genes *RAB35*, *OCRL*, *AURKB* and *BIRC5* were down-regulated ($p<0.05$ and log2(fold change) ≤ -1). Our study suggests that diosgenin induces multinucleated lung tumor cells by inhibiting cytokinesis, which highlights potential adverse effects in its application for lung cancer treatment.
Keywords: Cell Culture; Cytokinesis Failure; Phytochemical; Sapogenin.

Support / Acknowledgment

The authors want to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Brasil), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/Brasil), FINEP (Financiadora de Estudos e Projetos/Brasil), and Fundação Araucária-PR (Fundação de Amparo à Pesquisa do Estado do Paraná/Brasil) for support infrastructure.

ASSESSMENT OF THE ANTIPROLIFERATIVE POTENTIAL OF THE PEPTAIBOLS TRICOCONIN VI AND VIII IN MCF-7 AND MDA-MB-231 BREAST CANCER CELLS

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Abstract:

Natural products and their metabolites play a crucial role in the development of new drugs. In this context, the peptaibols Tricoconin VI and VIII (TK-VI and TK-VIII, respectively), substances isolated from endolichenic fungi of the *Hypocrea sp*., have aroused significant interest. Although TK-VI has been reported for its antibacterial, antifungal, antiviral, antimalarial, and antiproliferative activities in tumor cells, little is known about the biological activities of TK-VIII. Considering the current demand for antitumor agents due to the increasing number of cancer cases and the urge to combat chemoresistance, this study aimed to evaluate the antiproliferative activity of the peptaibols TK-VI and TK-VIII in the tumor cell lines MCF-7 and MDA-MB-231. Additionally, we sought to compare their cytogenotoxic effects with the normal human fibroblast cell line GM07492-A. To assess short and long-term cytotoxicity, XTT and clonogenic survival assays were performed using concentrations ranging from 2.5 µM to 80 µM and 5 µM to 20 µM, respectively. Based on the results, the IC_{50} (minimum inhibitory concentration) of TK-VI and TK-VIII was determined to be 7.96 µM and 10.02 µM in the MCF-7 cell line, 7.93 µM and 5.46 µM in the MDA-MB-231 cell line, and 7.68 µM and 6.17 µM in the GM07492-A cell line. We observed a reduction in cell survival at all tested concentrations in the tumor cell lines, as well as at concentrations of 10 µM in GM07492-A (TK-VI), along with 5 µM and 7.5 µM in MCF-7 and MDA-MB-231, respectively, together with a reduction by the concentration of 5 µM in GM07492-A (TK-VIII). To characterize the damage at the genetic level, we analyzed the score and frequency of DNA lesions by the comet assay. TK-VI caused statistically significant damage to the DNA of tumor cell lines at all tested concentrations, while damage was only observed in the normal cell line at concentrations of 7.5 µM and 10 µM. TK-VIII, on the other hand, induced damage at all tested concentrations in the GM07492-A and MDA-MB-231 cell lines, but only at concentrations of 5 µM and 10 µM in MCF-7. To investigate the cell death pathway induced by these peptaibols, we conducted assays to detect cytomorphological cell death and cell cycle arrest. Both substances were capable of induce cell death through apoptosis in all cell lines, but only TK-VIII significantly affected the G2/M phase of the cell cycle in the normal cell line GM07492-A. Based on the results obtained in this study, we can conclude that the peptaibols TK-VI and TK-VIII reduced cell viability and survival fraction, induced apoptosis and DNA damage. These findings confirm the cytotoxicity and genotoxicity of these substances, highlighting their potential as candidates for antitumor drugs.

Keywords: Cytotoxicity; genotoxicity; breast cancer; natural products.

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ANTIGENOTOXIC AND ANTIMUTAGENIC EVALUATION OF CRAFT IPA BEER IN MICE

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Abstract:
Beer is one of the most consumed fermented alcoholic beverages worldwide, being a source of carbohydrates, amino acids, minerals, vitamins, and polyphenols. Moderate beer consumption can have beneficial effects on human health and provide protection against chronic diseases such as cardiovascular diseases, neurodegenerative diseases, and cancers. Craft beers, such as IPA, have a greater variety of phenolic compounds compared to other types, due to their high hop proportion, which contains antioxidant, anti-inflammatory, and antimicrobial substances. Therefore, the aim of this study was to evaluate the genotoxic/antigenotoxic and/or mutagenic/antimutagenic effects of consuming craft IPA beer. Sixty-four adult male Swiss mice were used, divided into 8 groups: Group 1 and 2 - Water; Group 3 and 4 - IPA; Group 5 and 6 - 7% Alcohol; Group 7 and 8 - Hop. The animals received water, IPA beer, 7% alcohol, and hop ad libitum in the hydration bottle for 30 days. After this period, the animals underwent genetic evaluations using the ex vivo and in vivo Comet Assay. For the ex vivo evaluation, blood samples from the animals in groups 2, 4, 6, and 8 were collected on the 30th day of treatment and exposed to hydrogen peroxide (H2O2). For the in vivo evaluation, the alkylating agent cyclophosphamide (CP) was administered at a concentration of 50 mg/kg to groups 2, 4, 6, and 8 after blood collection for the ex vivo assay, while a 1 mL/kg saline solution was administered intraperitoneally to control groups 1, 3, 5, and 7. After 4 hours, a new blood sample was collected from all groups for the Comet Assay. On the 31st day, 24 hours after CP and saline administration, the animals were euthanized for dissection of the brain, liver, and heart to perform the Comet Assay, and the femurs were dissected to obtain bone marrow for the Micronucleus Test. In the genotoxicity assay and the mutagenicity test, the results showed that the groups treated with IPA beer, 7% alcohol, and hop did not exhibit genotoxic and mutagenic activity in the blood, brain, heart, and liver. The antigenotoxic activity of IPA beer and hop was observed in both the in vivo and ex vivo models, showing a reduction in DNA damage caused by CP in a similar manner. Regarding antimutagenic activity, there was no significant difference between the groups and the formation of micronuclei induced by CP. In conclusion, our results demonstrated that moderate chronic consumption of IPA beer and hop infusion exhibited antioxidant and antigenotoxic activity in mice.
Keywords: antigenotoxic; antimutagenic; craft ipa beer; beer.

JAMBU EXTRACT SIGNIFICANTLY REDUCES CELL VIABILITY OF HUMAN HEPATOBLASTOMA CELLS WHEN COMPARED TO NORMAL FIBROBLAST CELLS

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Abstract:
Liver neoplasms are the third leading cause of death from cancer in the world. The use of immortalized liver tumor cell lines has become a widespread practice not only for cancer research but also for the study of applied toxicology to the safety of new substances. Currently, the most used liver tumor cell for testing antitumor substances is Hep-G2 (human hepatoblastoma) which has gained popularity due to its ability to simulate the metabolization and expression of proteins in the transport of phase I, II and III substances. In the Amazon, a South American biome that has one of the richest biodiversity on the planet, several plants are the target of research for prospecting new bioproducts. One of these plants is Acmella oleracea (Jambu), rich in important secondary metabolites, such as phenolic compounds, tannins and Epicanthal. The extraction and purification of these substances is a crucial step for its use. The supercritical extraction (ES) method shows relevant efficiency, since during the extraction process it is possible to determine the extractor standards, so that the solvent used can carry a specific target. Furthermore, it is also necessary to guarantee the usability of new compounds obtained by any means, including ES. Thus, the purpose of this study was to conduct cytotoxicity tests using the MTT assay to verify the levels of cell viability in culture of neoplastic cells and normal cells after exposure to the Jambu extract obtained by ES. For this assay, Hep-G2 cells and murine fibroblast (L-929) were used, a metabolizing and a sensitive cell lineage respectively, widely used for initial screenings of new substances. The cells were grown in DMEM culture medium, supplemented with 10% SBF, 100µg/ml of Pen/Strep and kept in an incubator at 37°C and 5% CO_2. After stabilization, the cells were seeded in sterile 96-well plates and exposed to concentrations of Jambu supercritical extract (6.25-1000µg/ml) at 24 and 48 hours, with doxorubicin (50µg/ml) used as positive control (CP), DMEM medium as the negative control (CN) and 1% DMSO as dilution vehicle control. Statistical analysis of variance as well as IC_{50} determination was performed using GraphPad Prism software. With the two-step analyses, the IC_{50} obtained was 386.8µg/ml (±2.9µg/ml) for L-929 and 113.5µg/ml (±1.9µg/ml) for Hep-G2, thus indicating that the extract needs a much lower concentration to reduce the viability of a tumor cell in relation to a normal cell. In addition, the behavior of the extract is similar in the two strains, inducing cell proliferation at low concentrations and, at higher amounts, they have an effect of acute cytotoxicity, being possible to see significant differences in all concentrations in the two times evaluated. Thus, it is observed that the Jambu extract has a relevant potential for studies related to antitumor activities.
Keywords: MTT; Amazon; Bioproducts.

Support / Acknowledgment

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A NOVEL HYBRID ACETYLCHOLINESTERASE INHIBITOR INDUCES NEURODIFFERENTIATION AND NEUROPROTECTION IN PC12 CELLS THROUGH PI3K/AKT PATHWAY

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Abstract:
Alzheimer's disease (AD) is the most common form of dementia, affecting more than 50 million people worldwide, the main class of compounds used to treat AD is the acetylcholinesterase inhibitors (AChEIs), but these compounds provide limited efficacy. In this context, the aim of this study is to analyze the neuroprotective effects of TA8Amino and TAHB3, novel hybrid compounds synthetized from donepezil and tacrine, already characterized as AChEIs. We studied PC12 cells that were differentiated in mature neurons and treated with an oxidative agent, aiming to analyze cellular responses. Intending to study the neuroprotective potential of the two hybrids (AChEIs), cells were differentiated into mature neurons for seven days using NGF (50 ng/ml), following a pre-treatment (48 h) with the compounds using IC50 concentrations, 5 µM for TAHB3 and TA8Amino and 10 µM for donepezil and tacrine, and then submitted to H2O2 (150 µM) treatment to induce oxidative damage. Analyses of cell viability were carried out after 24h of recovery, using the XTT assay, we also performed the evaluation of cell cycle kinetics and cell death by using flow cytometry. The results showed that the hybrid compounds were not cytotoxic to PC12 cells, and did not alter the cell cycle progression, or cell death rates. It was also demonstrated that only TAHB3 caused a neuroprotective potential against H2O2 induced-oxidative damage; while cells treated with H2O2 showed 58% of viability, cells preteated with TAHB3 presented 82%; the pre-treatment with TA8Amino, donepezil and tacrine did not show a significant increase in cell viability compared to H2O2. We also studied the effects of the compounds on PC12 neurodifferentiation, by performing cell treatments with the AChEIs for seven days, and morphological and quantitative analyses of cell differentiation and neurite outgrowth. The results indicated that TAHB3 induced a significant increase in the differentiation of PC12 cells (from 12.3% to 54.3%) and caused an increase in neurite length after treatment with TAHB3. β-III-tubulin expression (Western blot analysis), which is a marker of mature neurons, was found significantly increased, thus confirming the neurodifferentiation induced by TAHB3. However, TA8Amino did not exhibit significant alterations in cell morphology, or cell differentiation. Western blot analyses also demonstrated that the levels of P-AKT and P-PTEN increased after the treatment with the AChEIs, especially after treatment with TAHB3, suggesting that the compounds are acting through the PI3K/AKT pathway, which is known for its role on cell survival and neurodifferentiation. Therefore, this data is compatible with previous results from our research group and suggest the therapeutical potential of the novel donepezil-tacrine hybrid TAHB3. Additional experiments are currently in development to confirm those effects and to identify the molecular mechanisms underlying the activity of TAHB3.
Keywords: Alzheimer's disease; Donepezil-tacrine hybrids; Acetylcholinesterase inhibitors; Neurodifferentiation; Neuroprotection

Support / Acknowledgment

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IMPACT OF VETERINARIAN OCCUPATIONAL EXPOSURE TO HIGH ISOFLURANE POLLUTION ON GENETIC INSTABILITY AND INFLAMMATORY STATUS

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Abstract:
Professionals who work in veterinary operating rooms (VOR) are occupationally exposed to waste anesthetic gases (WAG) due to the use of inhalational anesthesia and lack of scavenging systems in VOR, especially in less developed countries. Among WAG, isoflurane is widely used in veterinary anesthesiology practice and has been poorly investigated for its possible association with DNA damage in exposed professionals. In addition, only a few and controversial studies have evaluated the effects of occupational WAG exposure on the inflammatory profile in professionals who work in human OR and are exposed to a mixture of WAG. Considering the lack of studies in veterinary professionals, this study aimed to evaluate genetic instability and inflammatory status by detecting IL-6, IL-8 and IL-10 markers (both in serum and as gene expression) in veterinarians exposed to the WAG isoflurane. After the Research Ethics Committee approval, 36 professionals who work in VOR of the university hospital of UNESP/Botucatu-SP and were occupationally exposed to WAG isoflurane for at least one year and 34 volunteers (non-exposed group) were recruited. All participants signed the Informed Consent Form and answered a detailed questionnaire. A real-time infrared spectrophotometer determined the concentrations of WAG isoflurane in VOR and values were far above the international recommended threshold. Blood samples were collected from both groups and used for analysis. The cytokinesis-block micronucleus (MN) assay was performed to detect the genetic instability marker (MN) in peripheral blood lymphocytes while inflammatory markers were assessed by flow cytometry (serum levels) and by qPCR (gene expression). Demographic data did not statistically differ between groups. MN frequency of the exposed group was higher compared to the unexposed group ($p=0.04$). For serum inflammatory markers, only the pro-inflammatory cytokine IL-8 was significantly higher in the exposed group when compared to the unexposed group ($p=0.002$). There were no statistically significant differences between groups for *IL-6*, *IL-8* and *IL-10* expressions. These findings suggest that high exposure to WGA isoflurane in VOR contribute to genetic instability and modulate a pro-inflammatory response (serum IL-8), demonstrating the potential risk to veterinarians.
Keywords: health professionals; anesthetic gas; micronucleus assay; cytokines; gene expression

Support / Acknowledgment

Financial support: São Paulo Research Foundation (FAPESP) and São Paulo State University (UNESP), Medical School, Botucatu.

ISOLATION, SPECTRAL CHARACTERIZATION AND TOXICOGENETIC AND ANTIOXIDANT EVALUATION OF THE ALKALOID FRACTION OBTAINED FROM GUATTERIA FRIESIANA LEAVES

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Abstract:
Some of antineoplastic agents used for cancer treatment are derived from natural products. Among them, Alkaloids, a diverse group of low-molecular-weight nitrogen-containing compounds, have been the first line treatment for different tumors, including lung, breast, hematological and prostate cancer. Phytochemical investigations have shown the plant species Guatteria friesiana (W.A. Rodrigues) Erkens & Maas (Annonaceae family) is rich in alkaloids. In this regard, the study aimed to obtain and characterize the fraction rich in alkaloids from G. friesiana leaves (FRAGf) and to evaluate the toxicogenetic and antioxidant activity of this fraction in preclinical studies. For this, FRAGf was isolated by preparative thin layer chromatography and characterized by spectroscopic (IR, UV-Vis, 1H and 13C 1D/2D NMR) and spectrometric (DI-ESI-ITMS) techniques. Toxicogenetic tests were carried out through the MTT, Allium cepa and cytokinesis blocking micronucleus test (CBMN), and the antioxidant tests through Saccharomyces cerevisiae strains, ABTS and DPPH. In relation to the alkaloids found in the fraction we identified atherospermidine, 4-dehydroxyguatteriopsiscine, 3-hydroxymelosmidine and 9-methoxy-4-dehydroxyguatteriopsiscine. The alkaloid fraction showed cytotoxic potential with IC50 values = 4.32 µg/mL for L929 cells (non-tumor fibroblast cell line) and IC50 = 10.39 and 7.41 µg/mL for MDA-MB-232 and MCF 7 cells (human breast tumor cell lines), respectively. Furthermore, the alkaloid fraction showed mutagenic effects and cell death in MDA-MB-232 tumor cells by the CBMN test. In plant cells, inhibition of root growth of A. cepa, reduction of the mitotic index and increased chromosomal alterations were observed in all evaluated concentrations. The antioxidant effect to eliminate ABTS and DPPH radicals presented EC50 values of 20.70 and 17.88 µg/mL, respectively. For S. cerevisiae concentrations above 5 µg/mL presented antioxidant effects when compared to isolated H2O2. Thus, the fraction of alkaloids from G. friesiana leaves showed cytotoxic, mutagenic and antioxidant potential in preclinical studies, with potential for in vivo antitumor studies
Keywords: Antineoplastic; phytochemicals; alkaloids; Cytotoxicity; Mutagenic damage

Genomic Instability

OPI1-MEDIATED TRANSCRIPTIONAL MODULATION ORCHESTRATES GENOTOXIC STRESS RESPONSE IN BUDDING YEAST

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Abstract:
In budding yeast, the transcriptional repressor Opi1 regulates phospholipid biosynthesis by repressing expression of genes containing inositol-sensitive upstream activation sequences (UAS_{INO}). Upon genotoxic stress, cells activate the DNA Damage Response (DDR) to coordinate a complex network of signaling pathways aimed at preserving genomic integrity. Here, we reveal that Opi1 is important to modulate transcription in response to genotoxic stress. We find that cells lacking Opi1 exhibit hypersensitivity to genotoxins, along with a delayed G1 to S-phase transition and decreased gamma-H2A levels. Transcriptome analysis using RNA-seq reveals that Opi1 plays a central role in modulating essential biological processes during MMS-associated stress, including repression of phospholipid biosynthesis and transduction of mating signaling. Furthermore, we observe increased mitochondrial DNA instability in *opi1Δ* cells upon MMS treatment. Notably, we show that constitutive activation of the transcription factors Ino2-Ino4 is responsible for genotoxin sensitivity in Opi1-deficient cells, and the production of inositol pyrophosphates by Kcs1 counteracts Opi1 function specifically during MMS-induced stress. Overall, our findings highlight Opi1 as a critical sensor of genotoxic stress in budding yeast, orchestrating gene expression to facilitate appropriate stress responses.
Keywords: genotoxic stress; RNA-seq; inositol pyrophosphates; mitochondria; cell cycle

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WORKERS OCCUPATIONALLY EXPOSED TO PESTICIDES AND THE MICRONUCLEI FREQUENCY EVALUATION

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Abstract:
Brazil is one of the largest agricultural producers in the world and consequently, one of the largest consumers of pesticides. It is known that the occupational contact with pesticides leads to a greater genomic instability when compared to non-exposed individuals. This instability induces alterations, which are related to several diseases' development, such as cancer. A range of studies use the micronucleus test (MN) that can be purposed as genotoxicity and mutagenicity biomarkers in the exposed population. The aim of this study is to evaluate genomic instability by the micronuclei frequency workers exposed to pesticides compared to unexposed individuals. For this, the micronuclei frequency was performed in oral mucosal cells. We used a cytobrush to collect the sample that were putted it in Surepath solution. After storage, the cells centrifugation and resuspension in mucosal buffer, they were transferred to slides and stained with Feulgen and Fast Green. Then 1000 cells per slide were counted and two slides per individual were analyzed by microscopy. In our preliminary results, forty-eight occupationally exposed individuals were matched with 40 unexposed ones according to gender, age, smoking status, and alcoholism. The exposed group has a mean age of 48 ± 17 and non-exposed group 40 ± 9. Both groups are composed mostly of males, self-declared white, non-smokers, and use alcoholic beverages. In the first analysis they were divided into two groups according to the exposure time: 5 to 20 years and more than 30 years. It is possible to observe that the micronuclei frequency significantly increased in the exposed group when compared to the unexposed one. Nevertheless, there is no difference between micronuclei frequency when the groups were divided for exposure time. Conclusion: The genotoxicity caused by these substances can be evaluated by oral mucosal micronuclei frequency a cheap, fast, reproducible, and minimally invasive assay. These findings can help possible identify btiomarkers, that will benefit the comprehension of chemically induced diseases development, such as cancer.
Keywords: pesticides; micronuclei; occupational exposure; genomic instability; rural workers

MYELOTOXICITY EVALUATION IN MICE EXPOSED TO A MIX OF PESTICIDES WITH GLYPHOSATE, METHAMIDOPHOS, AND MANCOZEB

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Abstract:
Large-scale food production involves the use of agricultural pesticides to combat weeds, insects, or even fungi that accelerate degradation during transport and storage. At least 51% of vegetables consumed by the Brazilian population present pesticide residues. Because the human diet uses several types of foods, we hypothesized that exposure to a mixture of pesticides at a dose equivalent to the acceptable daily intake (AID) recommended by a regulatory agency may interact with the genome favoring chromosomal instability. We selected an herbicide (glyphosate), an insecticide (methamidophos), and a fungicide (mancozeb) that were detected as residues in Brazilian vegetable foods. Thus, the present study tested whether a short-term exposition to a pesticide mixture may induce myelotoxicity detected by micronuclei induction. Pesticides were mixed according to AID. The mice doses were adjusted to the human equivalent dose (0.35 mg/Kg p.c. glyphosate; 0.03 mg/Kg p.c. methamidophos; 0.037 mg/Kg p.c. mancozeb). Twenty male Swiss mice were treated with the pesticide mixture (N = 10) or water (N = 10) by gavage for 7 days. Cytotoxicity was evaluated in 1,000 total erythrocytes (immature and mature erythrocytes) from bone marrow per animal. Micronuclei frequency was assessed in 4,000 immature erythrocytes for each animal. The groups were compared by Student t-test with $\alpha = 5\%$. The animal ethics committee approved the procedures (CEUA/UEG 005/2019 e 006/2020). The pesticide mixture did not induce cytotoxicity estimated by the ratio of immature erythrocytes to mature erythrocytes when compared to water treated group ($P > 0.05$). For mutagenesis, it was not observed an increase in the frequency of micronucleated immature erythrocytes in the pesticide-exposed group ($P > 0.05$). Unfortunately, our data did not indicate chromosomal instability. We need to be cautious with the negative results observed in the mutagen test. It is possible that a long-term exposition may induce chromosomal alterations. In conclusion, our data showed the absence of myelotoxicity during a short-term exposition to a mix of pesticides with glyphosate, methamidophos, and mancozeb in animals.
Keywords: Diet; Micronuclei; Food residue.

Support / Acknowledgment

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THE ROLE OF CYCLIN-DEPENDENT KINASE BUR1/CDK9 DURING REPLICATION STRESS

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Abstract:
In eukaryotic cells, the cyclin-dependent kinase 9 (CDK9) is a positive regulator of transcription elongation. Although previous studies have implicated CDK9 in the control of DNA replication stress (RS) response when the fork replication is paused or stalled compromising the DNA replication, but underlying mechanism remains poorly understood. Our study aims to shed light on the functions of Bur1, an essential kinase that encodes CDK9 in yeast, during RS. Contrary to previous reports, we found that the *bur1-107* hypomorphic mutant not only exhibits deficiency in RS response but also in transcriptional elongation. Through genetic interaction assays and biochemical approaches, we demonstrate that the function regulated by Bur1 in RS appears to be dependent on its cyclin Bur2. Interestingly, our study uncovered a surprising and novel role of Bur1 in checkpoint-deficient mutants. Specifically, we found that in cells deficient for *MEC1* treated with low doses of HU, Bur1 elicits a highly toxic phenotype characterized by increased cell death, acceleration of S-phase progression, and accumulation of double-strand DNA breaks. However, in cells deficient for *DUN1*, *BUR1* plays a positive role in RS resistance, suggesting its involvement in the control of transcription of ribonucleotide reductase (RNR) and consequently affecting the dNTPs synthesis. In conclusion, our findings unveil Bur1's role in RS response and its pleiotropic influence on diverse cellular processes, leading to distinct phenotypes depending on the genetic background of the cell. This study provides valuable insights into the complex mechanisms by which cyclin-dependent kinases Bur1/CDK9 contribute to cellular responses to replication stress.
Keywords: *Saccharomyces cerevisiae*; replication stress; DNA damage response; CDK9.

ASSESSING GENOME INSTABILITY IN OBESE AND HYPERTENSIVE ZSF1 RATS

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Abstract:

Obesity increases the risk for complex diseases, such as cardiovascular diseases, osteoarthritis, and many types of cancer. It has been hypothesized that the underlying mechanism involves chronic low-grade inflammation, which is characterized by the production of reactive oxidative species (ROS) that can damage DNA. The accumulation of DNA oxidation will trigger several base-excision repair (BER) DNA glycosylases, and therefore, we hypothesize that BER may play a role in obesity and can become a new biomarker for obesity related health risks. To comprehend the levels of BER activity in multiple organs that can be affected by obesity and hypertension, BER activity was assessed in liver, adipose tissue, skeletal muscle, and lungs of Zucker fatty and spontaneously hypertensive (ZSF1) 35-36 weeks old rats; including obese (n=9) and lean hypertensive (n=11) rats. BER activity was compared to healthy control Wistar rats (n=8); 22-23 weeks old. BER activity was assessed by means of the comet-based *in vitro* DNA repair assay and gene expression was analysed for the main BER related genes; *Ogg1*, *Apex1* and *Parp*. Preliminary results showed higher BER activity in liver tissue from obese and lean ZSF1 rats compared to non-hypertensive controls, but this did not reach statistical significance. This trend was confirmed by increased expression of BER-related enzymes in lean/obese vs. healthy rats. The quantification of BER activity by the modified comet assay in the other tissues is being optimized to take into account tissue-specific cellular densities, morphology, and protein content. Thus, we will account for cell number by assessing the number of nuclei per tissue lysate. The lysate concentration will be adjusted to the same number of nuclei, rather than same protein concentration, to ensure proper comparison between tissues. Overall, our pilot data showed differences in BER activity between lean and obese animals.

Keywords: BER; Obesity; Animal Study; Hypertension; Genome Instability

Environmental Mutagenesis

EVALUATING GENOTOXICITY OF THE DYE EMODIN AND THE SOLVENT EFFECT IN THE COMET ASSAY OF AN AQUATIC AMPHIPOD

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Abstract:
Emodin ($C_{15}H_{10}O_5$) is an anthraquinone isolated from plants and fungi, ranging in color from yellow to red, and is used as a dye in the textile industry. Despite its use, emodin presented mutagenicity *in vitro* in the *Salmonella*/microsome assay. Recently our research group observed that emodin increased the micronucleus frequency, but no DNA damage was observed in the comet assay. These tests were performed in the hemocytes of the aquatic crustacean Parhyale hawaiensis in an *in vivo* test. In both studies emodin was dissolved at its limit of solubility in DMSO and tested using a maximum of 0.01% of solvent concentration in the media, providing emodin concentrations of 0.15 and 0.30 mg/L. The objective of this work was to evaluate emodin 99% purity in the comet assay at higher concentrations but for that, higher concentrations of DMSO in the exposure media needed to be used. To determine which would be the maximum concentration of DMSO for the comet assay, adults (8 months) were exposed for 96 h, at concentrations of 0.1 and 1% and compared to the negative control (artificial seawater). Ethyl methane sulfonate (EMS) 2 mM was used as positive control. Comet assay was performed using the alkaline comet assay protocol, slides were stained with ethidium bromide, photographed under a fluorescence microscope and 100 comets were analyzed per gel. %DNA in the tail was calculated using Comet Score version 2.0 and Kruskal-Wallis test (software Past 4.03) was used to compare the tested conditions. Cell viability was also evaluated using the trypan blue exclusion method and was >80% for all conditions. DMSO at 0.1% and 1% did not have increase the %DNA tail in relation to the control. Since no significant differences were observed at both tested concentrations, adults of *P. hawaiensis* were exposed to emodin at 3 and 30 mg/L to verify DNA damage using the comet assay at the same conditions. However, after 24 h of exposure all organisms died. Therefore, due to the observed toxicity it was not possible to evaluate the genotoxic effect of emodin at higher concentrations. Nevertheless, our work demonstrated that it is possible to increase the concentration of DMSO up to 1% in future assays when solvents are needed for dissolution of the test samples.
Keywords: DNA damage; anthraquinone; *Parhyale hawaiensis*; crustacean.

Support / Acknowledgment

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INTERGENERATIONAL EFFECTS OF C.I. DISPERSE RED 1 AZO DYE IN MALE MICE

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Abstract:
In light of the rising prevalence of infertility in the general population, reproductive health is presently receiving considerable focus. Although there is evidence that environmental xenobiotic contaminants have a significant impact on gametogenesis, the molecular mechanisms underlying decreased fertility are still unknown. C.I. Disperse Red 1 (DR1) is a textile azo dye and an environmental pollutant that is occasionally discharged into human-consumable water sources. This study aimed to investigate the effect of DR1 on sperm (concentration, vitality, acrosome integrity, motility, and mitochondrial activity) of exposed mice (F0) and their progeny (F1). F0 mice were placed into six groups of ten animals each. 1 - Negative control: oral administration of filtered water; 2 - Positive control: intraperitoneal administration of three doses of n-etil-n-nitrosourea (100 mg/kg body weight); 3 Vehicle control: orally administered with 0.5% dimethilsuphoxide (DMSO); 4, 5 and 6 - orally administered DR1 at concentrations of 5, 50 or 500 µg/kg/b.w. (concentrations equivalent to those identified in a Brazilian river because to the influence of textile industries). Animal treatments lasted fourteen days, and mating occurred thirty-five days later. Sperm from F0 was collected two days after mating, while sperm from F1 was collected eight weeks after birth. In both generations, motility was altered at all concentrations; in F1 mice, a decrease in sperm concentration was observed at a dose of 50 g/kg/b.w., acrossomal damage was observed at doses of 50 and 500 g/kg/b.w., and abnormal mitochondrial activity was observed at all concentrations of DR1. In conclusion, DR1 was capable of causing intergenerational toxicological damage, indicating that contamination of water with these substances may be hazardous to reproductive health.
Keywords: Textile azo dye; germ cell; sperm abnormalities.

Support / Acknowledgment

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GENOTOXIC EFFECTS IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) EXPOSED TO CONCENTRATIONS OF AL AND MN DETECTED IN GROUNDWATER INTENDED FOR HUMAN CONSUMPTION

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Abstract:

Environmental exposure to metals can induce genotoxic effects in cells and can affect the health of the exposed population. Previous studies realized by our group reported higher concentrations than those given by the CONAMA 396/2008 regulation for Al and Mn (0.4-0.8 and 0.1-1.7 mg/L, respectively) in groundwater in cities in the interior parts of Mato Grosso do Sul. In this context, the aim of this study, was to investigate the genotoxicity effects of Aluminum (Al) and Manganese (Mn) of peripheral blood mononuclear (PBMCs) cells exposed to these metals previously quantified concentrations in groundwater intended for human consumption by means of the micronucleus and comet assay tests. Therefore, nuclear alterations, micronucleus (MCN), and DNA damage were analyzed in PBMCs cells exposed to Al (0.2, 0.6, and 0.8 mg/L) and Mn (0.1, 0.3, 1.0, and 1.5 mg/L) for 48h. This study was approved by the Committee of Ethics and Research with Humans of the Federal University of Grande Dourados (2.556.885/2018). The Kruskal-Wallis test, followed by Dunn's test, were performed using the R Studio platform for micronucleus and comet assays. With respect to genotoxic damage, the number of nuclear buds and MCN increased significantly ($p<0.05$) in PBMCs cells exposed to Al for 48h, across all tested concentrations. Additionally, in the comet assay test, the percentage of DNA increased in the tail by approximately two-fold in treatment with 0.6 and 0.8 mg/L of Al when compared to the percentage of DNA in the tail observed in the negative control (NC). With regards to tail length, treatment with 0.2, 0.6, and 0.8 mg/L of Al promoted a more than four-fold increase in tail length compared to the NC. Additionally, treatment with 0.6 and 0.8 mg/L of Al increased the percentage of DNA in the tail by approximately two-fold when compared to that observed in the NC. Mn exposure causes significant increase ($p<0.05$) in the number of nuclear buds was observed starting from 0.3 mg/L of Mn. With respect to MCN, a significant increase ($p<0.05$) was observed at all concentrations. However, Mn treatment did not cause a significant increase in the number of nucleoplasmic bridges in PBMCs cells. As for DNA damage evaluated by the comet assay, a significant increase in the tail DNA (%) and tail length was observed with all concentrations of Mn when compared to the NC. Our results also showed that even concentrations allowed by the Brazilian legislation for Al (0.2 mg/L) and Mn (0.1 mg/L) in groundwater intended for human consumption can cause genotoxicity in PBMCs cells. Therefore, our results reinforce the need for revision of the established regulatory standards, as well as the parameters and tests used for groundwater quality assessment for human consumption, to ensure that the health of a population is not compromised due to chronic exposure to Al and Mn through the ingestion of water.

Keywords: Environmental exposure; comet assay; micronucleus; regulatory standards; cell culture

Support / Acknowledgment

CAPES, UFGD, FUNDECT, CNPq

CYTOTOXIC AND GENOTOXIC EFFECTS ON *ASTYANAX LACUSTRIS* INVOLVING THE TEMPLO® HERBICIDE AT DIFFERENT CONCENTRATIONS

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Abstract:
A part of the Brazilian GDP is driven by agribusiness, however, to be produced on a large scale, agriculture needs inputs and technologies to increase production. Of these inputs, the most used are agrochemicals, to reduce weeds and pests in the plantation. In 2021 alone, 562 new pesticides, components, and the like were released, many of these, it is not known for sure what risks they can cause to human health and the environment. Glyphosate is the most used herbicide, representing about 60% of the world market for non-selective herbicides and 62% of the agrochemicals consumed in Brazil in 2016, which is higher than the sum of the other 7 best-selling agrochemicals, being applied pure or with various combinations of active principles. Fish are considered good indicators of water quality and can be used to assess, the mutagenic and genotoxic effects of the xenobiotic environment. Faced with the need to discover the toxic potential of the Templo® herbicide in fish, the present study evaluated the cytotoxic and genotoxic effects of this diluted herbicide at concentrations of 3 µl-1, 6 µl-1, 9 µl-1 and 12 µl-1 in *Astyanax lacustris*, using the micronucleus test (MN), comet test and nuclear morphological changes (AMC) from peripheral blood of individuals, as well as possible chromosomal changes from kidney cells. In all tests, the fish were exposed to water contaminated with the Templo® herbicide for 4 days. Of all the analyzed groups, for the MN and AMC tests, the specimens exposed to the highest concentrations showed the highest number of alterations. In total, 202 MN and 10553 AMC were found. When compared with the control group, all the other four groups had statistically significant changes, as well as, when making comparisons between the concentration groups, all of them have statistically significant changes. DNA damage evidenced by the comet assay was also dose-dependent, that is, as exposure concentrations increased, DNA damage also increased, causing all concentrations to have a significant difference when compared to the control group. The chromosomal alterations did not go through statistical data, however, it can be observed that, as the herbicide concentration increased, the damage to the chromosomes also increased. Furthermore, from the concentration of 3 µL to the concentration of 12 µL, chromosomal GAPs were found. After exposure to the herbicide, significant damage to Astyanax lacustris cells was revealed. The damage caused to erythrocytes (MN and AMN), DNA (comet assay), and chromosomes, found in the present research, demonstrate the mutagenic potential of the contaminant, since, even at the lowest concentration tested, the damage was significant. Even with the present data, it is still extremely important to assess the toxicity of agrochemicals in the environment.
Keywords: Chromosomal alterations; Comet essay; Ecotoxicity; Micronucleus; Pisces

Support / Acknowledgment

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EXPOSURE TO CRACK COCAINE INDUCES GENOTOXICITY AND DEGENERATIVE CHANGES IN MULTIPLE ORGANS OF WISTAR RATS

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Abstract:
Crack cocaine is one of the most widely used illicit drugs worldwide, with consumption and widespread dissemination seen as a major public health problem. The aim of the present study was to investigate the effects of subacute crack cocaine exposure in multiple organs of rats. The present study was approved by the ethics committee of the 'Federal University of Sao Paulo' (n° 2372290921). All procedures were carried out under the ethical principles to animal research, according to the 'National Board of Control for Animal Experimentation, following national and international guidelines. A total of 24 male Wistar rats were distributed into four groups (n=6), as follows: Control Group (CTRL - animals without any intervention); Experimental Group 1 (G1): crack cocaine at 25 mg: Experimental 2 (G2): crack cocaine at 50 mg and Experimental 3 (G3): crack cocaine at 100 mg. All experimental groups were exposed to crack cocaine smoke once a day for 5 consecutive days. The following parameters were evaluated: genotoxicity, and histopathological changes in liver, kidney and bone marrow. The results showed that crack cocaine induced histopathological changes in G2 and G3 groups from kidney and liver. In immunohistochemistry, 8-OHdG was seen in all groups; nevertheless, only G1 and G2 groups had an increase immunoexpression in relation to CTRL group, with a statistically significant difference ($p<0.05$) in kidney. In addition, the frequency of micronucleated polychromatic erythrocytes (MNPcE) was higher in all experimental groups exposed to crack cocaine (G1, G2, and G3), with a statistically significant difference ($p<0.05$) when compared to control group. Taken together, the results show that exposure to crack cocaine induces degenerative changes and genotoxicity in liver, kidney and bone marrow demonstrating a clear dose-response relationship.
Keywords: crack cocaine; liver; kidney; bone marrow; micronucleus

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INFLUENCE OF LNCRNA *SBF2-AS1* IN THE EFFECTS OF CHRYSIN TREATMENT IN BLADDER CANCER CELLS

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Abstract:

Bladder cancer is the tenth most common type of cancer in the world and has high rates of recurrence and progression. Platinum-based chemotherapy is a widely used protocol for the treatment of bladder cancer, but it is associated with acute toxicities for patients. Studies have shown the antitumoral effect of chrysin (CRIS) on bladder cancer and biomolecular investigations have increased the understanding and functionality of long non-coding RNAs (lncRNAs) to carcinogenesis. Among the lncRNAs of importance, *SBF2-AS1* has already been associated with tumor malignancy in cancer. In this context, this study aimed to generate information that may contribute to the elucidation of the role and importance of lncRNA *SBF2-AS1* concomitantly with CRIS treatment in bladder cancer. Bladder cancer cells J82 and UM-UC3 were treated with CRIS alone (10 uM, 20 uM, and 40 uM) or in combination with siRNA specific to silence *SBF2-AS1*. The cytotoxicity test (XTT) was used to measure cell viability after treatment and the long-term effects were evaluated by the clonogenic survival assay. Cell morphology and migratory capacity were observed by light microscopy after treatments. The results showed in the cytotoxicity test, for both cell lines, it was possible to observe a similar and significant reduction in cell viability after treatment with CRIS alone or combined with SBF2-AS1 silencing. All treatments reduced clonogenic survival in both cell lines compared to untreated cells. In addition, in J82 cells, there was a significant reduction in siRNA + 10 uM CRIS compared with siRNA alone. The morphological analysis showed a reduction in cell density and the presence of irregularities in the cells in the treatments with CRIS, siRNA, and combined treatments. Regarding cell migration, all treatments inhibited this process in both cell lines compared to untreated cells. In J82 cells, there was a higher reduction in cell migration in combined treatment compared with the respective concentration of CRIS alone. According to the results, our study showed that lncRNA *SBF2-AS1* is intrinsically related to bladder cancer progression and also showed some effects on CRIS sensitization, supporting further studies as novel therapeutic strategies.

Keywords: bladder cancer; *SBF2-AS1*; long non-coding RNAs.

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COMPARATIVE TOXICITY AND GENOTOXICITY EVALUATIONS OF COAL AND COAL ASH USING DIFFERENT BIOLOGICAL ASSAYS

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Abstract:
Coal combustion used for electricity strongly impacts the environment, including air quality, water, soil, causing impairs in the fauna and flora besides increasing the greenhouse gases. This study aimed to assess toxic and genotoxic effects of coal and coal ash collected from a coal-fired power plant near the one of the largest coal reserves of Southern America. The toxicity was evaluated in *Caenorhabditis elegans* and *Daphnia magna* Straus and the genotoxicity was evaluated *in vitro* using alkaline Comet assay and *Salmonella*/microsome assay. LC_{50} to the coal was 52 mg/mL while to coal ash was 34 mg/mL for *C. elegans*, and the body length of worms exposed was significantly reduced in a dose-dependent way. Coal ash decreased the survival of *D. magna* reaching 90% in a chronic exposure for 21 days. Both coal and coal ash showed genotoxic effects in HepG2 cells using Comet assay, and in the presence of FPG enzyme detecting oxidized guanine, a significant increase of DNA damages was observed. However, coal and coal ash were not able to induce gene mutations in TA97a, TA100, and TA102 *Salmonella typhimurium* strains. The toxic effects were related to inorganic elements (Mg, Al, Si, K, Ca, Ti, Cr, Fe, Zn and Ga) which were found in greater quantities in the coal ash than coal. In conclusion, *C. elegans*, *D. magna*, and HepG2 cell line were proved efficient to detect toxicity and genotoxicity caused by these complex mixtures. The FPG-enzyme-modified Comet assay results suggest oxidative DNA damages induced by coal and coal ash. The findings reinforce the need for protective measures in handling coal and to mitigate the exposure to coal ash generated by its combustion.
Keywords: Coal mining; DNA damage; Genotoxicity; HepG2 cell; Mineral coal

Support / Acknowledgment

This study was supported by the Universidade Luterana do Brasil (ULBRA), Universidade La Salle (UNILASALLE), Universidade FEEVALE, Conselho Nacional de Desenvolvimento Científico e Tecnologia (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior do Brasil (CAPES; Finance Code 001), Brazil.

DID MUTAGENIC ACTIVITY IN SURFACE WATERS CHANGE DURING THE COVID-19 PANDEMIC PERIOD?

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Abstract:
Water pollution is a matter of concern worldwide, mostly as a consequence of industrial and anthropogenic activities. CETESB has a water quality monitoring program, established in 1974, that includes chemical, microbiological, hydrobiological, and toxicological analyses of surface-water samples from all over the State of São Paulo. The results have been used by the Agency to assess the quality trends of freshwaters to prioritize pollution control actions, identify the most degraded sites, diagnose the conditions of source waters used by the drinking water treatment plants, and verify the conditions for aquatic life protection and other uses of water such as irrigation, livestock, and recreation. During the COVID-19 pandemic, starting in the first quarter of 2020, a decrease in the industrial activity was expected, as well as an improve in water quality. We chose 3 sampling sites from the monitoring program to verify whether lockdown during the pandemic influenced the quality of the waters. The Water Quality Index (WQI) classifies waters in very good, good, fair, poor and very poor, taking into account pH, temperature, dissolved oxygen, biochemical oxygen demand, nitrogen, phosphorus, turbidity, solids, and coliforms. We analyzed the WQI calculated for each site, and the Salmonella/microsome results. The WQI did not show differences between the pre-pandemic, pandemic and pos-pandemic periods, regardless of the site. Mutagenicity results, on the other hand, reflected the fluctuation of industrial activity. In site 1, impacted by industrial loads, the mutagenicity observed in the beginning of the pandemic indicated lower mutagenic potencies, compared to 2019 and years before, suggesting a decrease in industrial production. In the subsequently months, mutagenic activity increased, implying that industrial activity was being reestablished, reaching pre-pandemic values by the end of 2021. Sites 2 and 3 had occasional positive mutagenic responses before 2020, but none during the pandemic. Unexpected results were observed beginning in 2021, with sites showing mutagenic activity in most of the samplings. This could suggest a sudden increase of industrial and other potential polluters activities, maybe attempting to compensate the restriction period. Qualitatively, our observations indicate that the mutagenic activity found in the 3 sites can be related with discharges of industrial sources, which in turn had no impact on WQI, that fluctuated between fair and good during the period studied, and is more related to sanitary parameters. These findings demonstrate the relevance of incorporating diverse parameters in water quality monitoring, as the integrated analysis of various aspects contributes to a more comprehensive environmental characterization.
Keywords: Mutagenicity; Surface Waters; COVID-19; Environment; Ames test

IN VITRO INVESTIGATION OF THE CYTOTOXIC ACTIVITY OF THE NATURAL COMPOUND NARINGIN ON TUMOR CELL LINES (786-O, MCF-7 AND HEPG2/C3A)

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Abstract:
Flavonoids are plant-derived compounds used as dyes, flavor enhancers, and fragrances. Among them is naringin, a subclass of flavanones, found in many vegetables, especially in citrus fruits, and responsible for the bitter taste. Numerous studies have already demonstrated various biological activities of flavonoids, such as antioxidant, anti-inflammatory, and antitumor effects, among others. Therefore, it is important to better understand the effects caused by this compound to gain a deeper understanding of its mechanisms of action and its antitumor activity. Hence, the objective of this study was to assess the cytotoxic potential of the flavonoid naringin on human renal carcinoma (786-O), breast carcinoma (MCF-7), and liver carcinoma (HepG2/C3A) cell lines using the MTT Cytotoxicity Assay after 24 hours of exposure. For the execution of this assay, cells were cultured in culture flasks with DMEM medium supplemented with 10% fetal bovine serum (FBS). After cultivation, cells were seeded at a concentration of 1×10^4 cells/well in 96-well plates. Following a 24-hour stabilization period, the plates received the following treatments: cytotoxic agent Doxorubicin (18 µM) for the 786-O cell line, and Methylmethanesulfonate (100 µM) for the MCF-7 and HepG2/C3A cell lines; control (DMEM with 10% FBS); and concentrations of naringin at 50, 100, 150, 200, and 250 µM. After 24 hours of exposure, these treatments were discarded, and MTT salt (0.167 mg/mL) was added to the wells. The cells were incubated for an additional 4 hours, and after this time, the MTT was discarded, and dimethyl sulfoxide (DMSO) was added to solubilize the formed formazan crystals. Absorbance readings were taken at 550 nm using a microplate reader, and absorbance values were subjected to analysis of variance (ANOVA), followed by Dunnet's test (α=0.05, $p<0.05$), conducted in the statistical program GraphPad Instat version 3.02. The results of cell viability showed that in the 786-O cell line, there was a statistically significant difference from the control starting at a concentration of 100 µM. A difference was also observed in HepG2/C3A, however, only at the concentration of 250 µM. However, no concentration resulted in viability lower than 80% in both cultures. For the MCF-7 cell line, no naringin treatment showed a statistically significant difference compared to the control, but it's worth noting that this cell line is known for its resistance to various treatments. Thus, despite the distinct results, naringin was not capable of reducing cell viability below 80% in any cell line. Based on the obtained data, it is important to investigate whether at higher concentrations and longer exposure periods, naringin would yield different results.
Keywords: Flavonoid; Flavanone; MTT Assay.

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MUTAGENIC CHARACTERIZATION *IN VITRO* AND *IN VIVO* OF HIGH-PURITY DYES: PROVIDING RELIABLE DATA FOR BETTER *IN SILICO* PREDICTIONS

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Abstract:
Dyes are everywhere. That's why studies on their mutagenicity have been carried out over the years. However, the uncertainty about their purities, solubility issues, and lack of molecular confirmation may have compromised some of the published results. The Wax Weaver Library (MWDL) houses around 98.000 samples of dyes, including azo dyes, characterized by the functional group (-N=N-), one of the most widely used in several applications. Five dyes (purity >97%) were selected, three azo, one diazo, and one diazoamine were selected from the MWDL and named 32, 36, 41, 58, and 73. The purification and chemical structures confirmation had been performed using a high-performance liquid chromatography and quadrupole-time-of-flight mass spectrometry (HPLC-Q-TOF MS). Tests were performed to verify their mutagenicity *in vitro* (Salmonella/microsome assay and micronucleus test in HepG2) and *in vivo* (comet assay in the aquatic crustacean *Parhyale hawaiensis*). According to the recent literature, the use of TA98 and TA100 is considered enough to detect the mutagenicity of the majority of chemicals. The strain YG1041 is not included in any recommended testing battery but it has shown high sensitivity to azo dyes. Therefore, it was included in this work, besides TA98 and TA100. Dyes were dissolved in dimethyl sulfoxide (DMSO) at the limit of their solubility and tested in concentration-response experiments in the miniaturized version of the Salmonella/microsome assay, microplate agar (MPA) with and without metabolic activation (rat liver S9 5%, Aroclor induced). All dyes were mutagenic for YG1041, only one positive for TA98 and none for TA100. The micronucleus assay results are underway, but so far, the diazoamine dye (58) did not induce an increase in micronuclei frequency but reduced nuclear division index and was cytotoxic at the highest tested concentration. For the *in vivo* studies, adults of *Parhyale hawaiensis* were exposed to all dyes in a preliminary experiment (single concentration, provided by the dilution of the stock solution at a maximum of DMSO 0.01% in the media). After 96 h exposure, the hemolymph was collected, slides prepared, and DNA damage was analyzed by the alkaline comet assay. Slides were stained with ethidium bromide (4%) and photographed under a fluorescence microscope. A total of 100 comets per gel were scored using the software CometScore v. 2.0. Results were negative for all dyes. We concluded that the inclusion of the YG1041 was responsible for the detection of the mutagenicity of all 5 dyes. The negative results obtained for the comet assay maybe are due to the low concentrations tested. Comet assay experiments in concentration-response using higher dye concentrations will be performed, as well as the micronucleus test with *P. hawaiensis in vivo*. We hope to provide data for the literature to enhance the quality of *in silico* prediction tools for dyes.
Keywords: Salmonella/microsome assay; micronucleus; comet assay; HepG2; azo dyes

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A NEW STATISTICAL MODEL APPROACH FOR REPEATED MEASURES IN COMET ASSAY

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Abstract:
Repeated measures overtime provide an extra variability from the same individuals and the consequence is correlated data. This type of data require a specific statistical analysis due to the correlation among the response variables. In this case, the most articles with comet assay do not use properly statistical analysis, such as non parametric tests (Kruskal Wallis), t test or even though ANOVA. In this work, we propose a new statistical model to comet assay with repeated measures. Data from an antigenotoxicity test by comet assay was used to propose the new statistical model. Damage frequency (DF) and damage index (DI) dataset were obtained after 7-days of pretratment by an antigenotoxicity agent. The dataset were collected in three different times: 0h, 1h and 24h after etoposide administration as mutagenic agent. Negative and positive control groups were performed such as comet assay protocols. For statistical analysis, we considered a model that includes a random effect to explain the correlation among the response variable. In this sense, the generalized linear mixed model for proportion and continuos data was the most appropriate to statistical analysis. The results showed difference among treatment groups and this model was properly to the dataset. To confirm it, residuals analysis was performed to check the model adequability. The mixed model explain two kinds of effects. First, the fixed effect of the controlled variables, such as the treatments. Second, the random effect from the not observed extra variablity, such as genetic effect. To perform the properly statistical analysis is so important to decision making and implicate in a better accurate conclusion.
Keywords: proportion and continuos data; generalized linear mixed model; single cell gel electrophoresis.

MUTAGENICITY OF FUNGAL EXTRACTS FROM *CORTINARIUS SEMISANGUINEUS* AND *CORTINARIUS SANGUINEUS* USED AS DYES EMPLOYING THE SALMONELLA/MICROSOME ASSAY

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Abstract:
The use of natural dyes is an ancient practice of mankind. One of the BioColour project's (biocolour.fi) aim is to develop natural dyes extracted from plants and fungi to be used in more sustainable dyeing practices. Some of these extracts are from the webcap fungi *Cortinarius semisanguineus* and *Cortinarius sanguineus*, widely used as dye sources by crafters in Northern Europe and America as well as Australia. Both extracts contain several color-producing anthraquinones, one of the largest groups of natural dyes. Some anthraquinones are mutagenic e.g., emodin, which is present in *C. sanguineus* but not in *C. semisanguineus*. Both extracts also contain, among other compounds, dermorubin and dermocybin which were not mutagenic when tested in the Salmonella/microsome assay. This study aimed to test the mutagenicity of these two fungal extracts using the Salmonella/microsome assay and compare with the mutagenicity of emodin. Anthraquinones of each fungus were obtained as the main fraction, and in powder form, from the dried fungal bodies using an enzymatic hydrolysis process. Emodin, 99% purity, was also tested for comparison. The strain TA1537 was selected because it is the most sensitive to anthraquinones. The powder form extracts were dried and then dissolved in dimethyl sulfoxide (DMSO) and the highest concentrations tested was based on the limit of solubility (*C. sanguineus* 63.0 ng/L - *C. semisanguineus* 169.6 ng/L). Emodin was tested in parallel (60.0 ng/L). The three samples were tested in concentration-response experiments in a miniaturized protocol of the Salmonella/microsome assay, the microplate agar (MPA) with and without metabolic activation (rat liver S9 10%, PB induced). Data were statistically analyzed using an ANOVA, followed by a linear regression analysis using the Bernstein model. *C. sanguineus* extract was as mutagenic as emodin, in the presence of metabolic activation, even though it only contained around 50% of emodin. This result suggests probably there are other mutagenic compounds present in this extract, which need to be chemically identified and tested. *C. semisanguineus* did not show mutagenicity, both with and without metabolic activation for TA1537. The next step is to test *C. semisanguineus* extract with other strains recommended by OECD e.g., TA98, TA100, TA97a, and TA102 to confirm the negative results. *C. sanguineus* extract was mutagenic and emodin did not fully explain its mutagenicity. *C. semisanguineus* extract needs to be further tested to confirm its negative response.
Keywords: Salmonella/microsome assay; Microplate Agar Protocol; Anthraquinones; Emodin.

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CYTOTOXIC EFFECTS OF THE FLAVONOID ACACETIN ON BLADDER TUMOR CELLS

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Abstract:
Bladder cancer is the 10th most commonly diagnosed cancer worldwide, accounting for approximately 213,00 cancer-related deaths in 2020. Classification of high- or low-grade tumors is based on cytological differentiation and is an important prognostic factor: high-grade neoplasias are usually muscle-invasive tumors (MIBC), associated with worse prognosis. Platin-based therapy is the standard chemotherapy protocol for MIBC, but its high toxicity and drug resistance are hindrances to successful therapy. Natural compounds have been widely studied as an alternative for antitumor strategies with low systemic cytotoxicity. Acacetin, a natural flavonoid compound found in several plants, seeds, and flowers, presents anti-inflammatory, anti-infectious, cardio-protective, and anti-tumor activity. Studies have demonstrated that acacetin inhibits cell proliferation, cell invasion, and tumor progression and growth in several cancers such as gastric, prostate, breast, and lung carcinomas, but its effects on bladder cancer are poorly understood. Therefore, our objective was to assess acacetin's selectivity and cytotoxic effects on high-grade bladder tumor cells. For that purpose, T24, UM-UC-3, and J82 high-grade bladder tumor cells, with mutated *TP53* gene, were treated with 6 different acacetin concentrations (12.5, 25, 50, 100, 200, and 250 µM). MRC5 human fibroblast cell line was treated on the same way to evaluate the selectivity index of acacetin. XTT assay was used to evaluate cell viability after 24h and 48h of acacetin treatment. The selectivity index (SI) was calculated by the formula: SI = IC50 on MRC5 cells/IC50 on tumor cells, where IC50 is the concentration required to kill 50% of the cell population. A SI value above 2 indicates selectivity. Effects of acacetin treatment on cell morphology were evaluated by observation in an inverted optical microscope, at 40 or 100x magnification. The results showed that for T24 and UM-UC-3 cell lines, all of the acacetin concentrations used significantly reduced cell viability after 24 and 48h of treatment; for J82 cell line, acacetin concentrations over 50 µM significantly reduced cell viability after 24 and 48h of treatment. MRC5 cell viability after 24 and 48h of treatment was only significantly reduced with acacetin concentrations over 100 µM. Moreover, acacetin concentrations of 12.5 and 25 µM significantly increased cell viability on MRC5 fibroblast cell line. Regarding acacetin's selectivity, IC50 to MRC5 cells was higher than IC50 in all bladder carcinoma cell lines after 24 and 48h of treatment, suggesting selectivity of acacetin for tumor cells. The SI value for UM-UC-3 cells was 3.5 and 8.91 (after 24 and 48h, respectively), confirming the selectivity of acacetin treatment for this cell line. Morphology alterations such as rounded cells, dead cells, and a reduction of cell density were observed in all tumor cell lines after treatment, corroborating with the cytotoxicity data. In conclusion, the flavonoid acacetin presents cytotoxicity activity and moderate selectivity for high-grade bladder cancer cells. Thus, it can be explored as a new therapeutical approach for bladder cancer treatment. Funding: UFOP, FAPEMIG, CAPES, CNPQ.
Keywords: Acacetin; Bladder cancer; Anticancer activity.

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Universidade Federal de Ouro Preto, FAPEMIG, CAPES, CNPQ.

REVISITING THE MUTAGENICITY OF ALIZARIN IN THE MICROPLATE AGAR PROTOCOL, A MINIATURIZED VERSION OF THE *SALMONELLA*/MICROSOME ASSAY, USING DIFFERENT S9 CONCENTRATIONS

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Abstract:
The natural dye alizarin (1,2-dihydroxyanthraquinone; $C_{14}H_8O_4$) is a reddish substance, generally extracted from the plant *Rubia tinctorum*. Alizarin is considered an efficient and economical biomaterial, being used in food packaging and textiles dyeing. There are already records in the literature about its mutagenicity using the *Salmonella*/microsome assay, with strains TA100, TA1537 and TA2637 mainly in the presence of metabolic activation. In the literature it has also been found that the anthraquinone group dyes require a higher concentration of S9 in the metabolization mix than that routinely employed (5%). In addition, TA1537 was the most sensitive strain. The objective of this work was to confirm the mutagenicity of alizarin 97.1% purity, using a miniaturized version of the *Salmonella*/microsome assay, the microplate agar (MPA), with the strain TA1537 using two different concentrations of S9 in the mix (10% and 30%). The S9 fraction used was induced by Phenobarbital/5,6-Benzoflavone, PB/BNF, Moltox®. The dye was dissolved in dimethylsulfoxide (DMSO) at its maximum solubility and tested in concentration-response experiments (0.15 to 38.61 ng/µL). Negative control was DMSO, and positive controls were 9-aminoacridine (9AA) without S9 and 2-aminoanthracene (2AA) with S9. Data was analyzed by ANOVA followed by a linear regression using the Bernstein model. Alizarin was mutagenic only in the presence of S9 concentrations (10% and 30% S9), with an increased response when 30% S9 was used. Alizarin is probably causing DNA intercalation, a common mechanism for anthraquinones, due to the planarity of the molecule. We confirmed the mutagenicity of alizarin in the miniaturized *Salmonella*/microsome assay and, we also demonstrated the importance of using different concentrations of S9 to evaluate anthraquinones. Although alizarin is of natural origin, its use does not seem to be a sustainable alternative. Next steps will be the evaluation of its mutagenicity in vivo using the micronucleus test with the marine amphipod *Parhyale hawaiensis* and its acute toxicity with *Daphnia similis*, *P. hawaiensis* and with *Danio rerio* in the fish embryo test.
Keywords: Ames test; natural dye; TA1537.

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MUTAGENICITY EVALUATION OF A NEW BIOACTIVE PRODUCT BASED ON TECHNICAL CASHEW NUT SHELL LIQUID (TCNSL) INTENDED FOR THE CONTROL OF *AEDES AEGYPTI*

Kryslla Barbosa Rocha de Carvalho [1]; Sabrina Rodrigues da Luz [1]; Bruno do Amaral Crispim [2]; Fabiana Gomes da Silva Dantas [3]; Fábio Kummrow [4]; Kelly Mari Pires de Oliveira [2]; Alexeia Barufatti [2]

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Abstract:
The resurgent arboviruses such as Dengue, Zika, Yellow Fever, and Chikungunya are caused by viruses whose main vector is the *Aedes aegypti* mosquito. The technical cashew nut shell liquid (tCNSL) is an agro-industrial by-product with recognized insecticidal activity. However, the hydrophobic properties of this by-product limit its use as a larvicide. To enable its use in mosquito breeding sites, we partially neutralized tCNSL with sodium hydroxide (NaOH) and developed a water-soluble bioactive product (tCNSLNa50). tCNSLNa50 showed larvicidal activity against *Ae. aegypti* with an LC_{50} = 5.94 mg/L. However, to enable the use of this bioactive, it is also necessary to evaluate its safety. The Salmonella/microsome assay (Ames test) is the most widely used standard *in vitro* test to detect genetic mutations and is one of the necessary tests to evaluate the safety of new larvicides. Thus, our objective was to evaluate the mutagenicity of the bioactive tCNSLNa50 using the *Salmonella*/microsome assay. Assays were performed using strains of *Salmonella enterica serovar* Typhimurium TA98 and TA100, in the presence and absence of metabolic activation (S9 mix). The sample was considered mutagenic when the mutagenicity ratio (MR) was equal to or greater than 2 in at least one of the concentrations tested and when there was a dose-response relationship. Concentrations with an MR ≤ 0.7 were considered cytotoxic. The results indicated that there was no significant increase ($p>0.05$) in the number of revertant colonies of *S. Typhimurium* strains TA98 and TA100 in the presence and absence of S9 mix for any of the tested doses (50-5000 µg/plate) when compared to the negative controls. The concentrations tested did not show cytotoxicity and the RM<2 indicates that the bioactive tCNSLNa50 does not have the potential to cause gene mutations by base pair substitution and frameshift. Strains TA98 and TA100 are the most used strains for mutagenicity assessment as they detect more than 90% of the mutagens, thus these results are safe in demonstrating that the bioactive tCNSLNa50 at the concentrations tested are not able to induce gene mutations. Genotoxicity assessments are essential components of safety assessments for all substances intended for use as larvicides. Thus, the absence of mutagenicity and cytotoxicity are factors of paramount importance due to the intention to implement the bioactive tCNSLNa50 as a biolarvicide against *Ae. aegypti*.
Keywords: Larvicide; agro-industrial by product; safety; genotoxicity; Ames test

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CYTOGENETIC CHANGES IN ORAL MUCOSAL CELLS OF HIV-INFECTED CHILDREN AND ADOLESCENTS UNDERGOING ANTIRETROVIRAL TREATMENT

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Abstract:
Currently, 79 million people have been infected worldwide with the human immunodeficiency virus (HIV), whose predilection for immune system cells, induces acquired immunodeficiency syndrome (AIDS), has been responsible for the death of 39 million people since the beginning of the epidemic. Still, it is responsible for 88% of contaminations of children up to 13 years. From this age, the main route of contamination is sexual. Antiretroviral therapy (ART) started in the late 80s, has been constantly evolving, acting especially on the replication of the virus in several stages and combining different classes of antiretrovirals. Antiretroviral drugs are classified according to their mechanism of action; there are more than 25 drugs divided into six types: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, integrase inhibitors, and entry inhibitors. The objective of this study was to evaluate possible cytogenetic changes in children and adolescents with HIV on ART, through the micronucleus test in oral mucosa. The prospective study consisted of 40 individuals, 21 from the HIV group and 19 from the control group. Children and adolescents with HIV were recruited. The inclusion criteria were: < 18 years and to consent in participating of the study. The exclusion criteria were systemic comorbidities, presence of oral lesions, smoking, alcohol consumption and X-ray exposure 15 days prior to sample collection. A gentle scraping was performed on the inner portion of the jugal mucosa on both sides. A total of 2,000 cells per slide were analyzed, for determination of mutagenicity parameters, as follows: micronuclei (MN), binucleation (BN), nuclear buds (NB). For measuring cytotoxicity, the following metanuclear changes were evaluated: pyknosis (PK) karyolysis (KL) and karyorrhexis (KR), in a double-blind manner. The repair Index (RI) was also evaluated in this setting. The HIV group showed high frequencies of MN ($p=0.05$), binucleated cells ($p=0.001$) and nuclear buds ($p=0.03$). In the cytotoxicity parameters, represented by the cell death phases, there was an increase with statistical difference ($p \leq 0.05$) in the KR frequency ($p= 0.05$). Additionally, RI was decreased in the HIV group. These results indicate that HIV-infected individuals undergoing ART have cytogenetic changes in oral mucosal cells.
Keywords: HIV; Micronucleus; Antiretroviral therapy; Oral mucosa; Children and Adolescents

EVALUATION OF THE POTENTIAL CYTOTOXIC AND GENOTOXIC EFFECT OF AGENTS PRESENT IN DRY TOBACCO LEAF USING HUMAN HEPATOCARCINOMA CELL LINE (HEPG2)

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Abstract:
The cultivation of tobacco in the southern region of Brazil stands as a highly significant agro-industrial activity with considerable socioeconomic impact. As the second-largest tobacco producer globally, Brazil holds a prominent position in this sector. Additionally, the state of Rio Grande do Sul serves as the nation's leading tobacco exporter. The tobacco production process involves several steps, from planting to sorting the dry leaf. Throughout the process of tobacco production, farmers are exposed to a variety of substances, such as pesticides, nicotine, and tobacco-specific nitrosamines (TSNAs). Notably, two specific TSNAs, namely N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) due to their proven association with cancer in humans. The aim of this study was to assess the cytotoxic and genotoxic effects of the aqueous extract derived from dried tobacco leaves, as well as the individual compounds nicotine, NNN, and NNK, utilizing the human hepatocarcinoma cell line (HepG2). In this study, cytotoxicity was evaluated using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay, while genotoxicity was assessed using the alkaline comet assay. The concentrations of the samples tested were as follows: the aqueous extract (ranging from 0.312 to 5 mg/mL), nicotine (0.0078 mg/mL), NNN and NNK (0.001 mg/mL). For the MTT assay, the positive control consisted of DMSO (20%), while the comet assay utilized 4NQO (0.060 μM) as the positive control. The negative control included the culture medium. All tests were conducted in triplicate. Regarding the results, the MTT assay revealed that mitochondrial viability exceeded 80% in most samples, except for the highest concentration of the aqueous extract, indicating a certain level of cytotoxicity. In terms of genotoxicity, as assessed by the alkaline comet assay, significant effects were observed in the highest concentration of the aqueous extract, as well as in the agents nicotine, NNN, and NNK. These results emphasize the importance of understanding the potential health risks associated with tobacco production and exposure to its byproducts. Further in vitro studies are warranted to comprehensively understand the mechanisms underlying DNA damage caused by tobacco-related substances.
Keywords: cytotoxicity; genotoxicity; tobacco; nitrosamines; comet assay

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GENOTOXICOLOGICAL EVALUATION OF MAROLO SEED FLOURS

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Abstract:
Marolo (Annona crassiflora M.) belonging to Annonaceae family. It is a species rich in phytochemicals, thiamine, riboflavin, vitamin A, C and E, as well as minerals and carotenoids. The presence of these chemical compounds contributes to the various biological activities of the plant, including the antioxidant capacity, antimutagenic, antimalarial and antibacterial activity. Parts that are not conventionally consumed, such as seeds and peels, have received special attention in the development of new food products, with nutritional, economic and functional advantages. In view of the growing therapeutic interest in substances present in different food formulations, the present study aimed to evaluate the mutagenicity and the possible chemopreventive effect of marolo seeds flours (MSF). Two types of flour were investigated, a fruit seed flour (MSF) and a fruit seed film flour (MSFF). Total phenolic and flavonoids were prior determined spectrophotometrically using the Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively. In addition, the antioxidant activity of these flours was measured by DPPH and ABTS assays. For the genotoxicological evaluation, the 30 male mice with 6 weeks years old received standard ration supplemented with 3.5, 7, 14 and 28 mg body weigh (b.w) of MSF and MSFF for 16 consecutive days and the mutagenicity was evaluated in peripheral blood samples at times of 48h, 7 and 14 days by micronucleus test. To evaluated the chemopreventive effect, on the 14th day, the doxorubicin [DXR 20 mg/kg p.c, intraperitoneal (i.p)] was administered and peripheral blood samples were collected. Rodent weight, water and food consumption was monitored during the experiment. In addition, blood glucose levels were quantified before the start of the experiment and at the end. The data was statistically analyzed by analysis of variance (ANOVA). Our results demonstrated that the antioxidant potential according to the ABTS assay, in trolox equivalents, was 323.13±35.77.02 µmol/g for MSFF and 4.34±1.37 µmol/g for MSF, whereas, at DPPH method was 165.98±8.06 µmol/g for MSFF and 7.21±0.60 µmol/g for MSF. For total phenolics, in gallic acid equivalents, MSFF showed 54.28±1.17 mg/g and MSF 1.52±0.10 mg/g. Regarding total flavonoids, in catechin equivalents, MSFF demonstrated 50.86±2.95 mg/g and MSF 0.76±0.19 mg/g. It was possible to verify a significant difference in the water and food average consumption in the different groups that received flours (MSFF and MSF) when compared to the negative control group. Blood glucose levels did not show significant differences between treatments, not even between the initial and finalmeasurements. From the statistical analysis we found that MSFF and MSF were not cytotoxic and mutagenic. The doses of 3.5 and 7 mg/kg p.c of MSFF exhibited chemopreventive potential, reducing, respectively, 41,2% and 33,9%, the frequency of micronucleated polychromatic erythrocytes (MNPCEs). For MSF, it was possible to verify a significant reduction of MNPCEs in all treatments, being, respectively, 23.97%, 16.47%, 28.12% and 53.36%, for 3.5, 7, 14 and 28mg/kg p.c. These findings suggest that the ability to inhibit DNA damage demonstrated by MSFF and MSF are due to their total phenolic and flavonoid content and are related to their antioxidant activity.
Keywords: *Annona crassiflora M.*; phytochemicals; chemoprevention; genotoxicity.

Support / Acknowledgment

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EVALUATION OF THE GENOTOXIC POTENTIAL OF SPRING WATER SAMPLES USING *ASTYANAX LACUSTRIS*

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Abstract:
Springs are places of extreme importance for the conservation of water resources, as the water table rises in these places, originating fresh surface water bodies. The areas of springs belonging to the Dourados River Basin (DRB) are subject to several threats from anthropic activities, which promote the degradation of riparian vegetation in its surroundings, making the springs susceptible to the entry of toxic chemicals. Agriculture is one of the most important sources of water contamination in this watershed, contributing to the presence of pesticides, metals, and other organic and inorganic toxicants in aquatic environments. Our objective was to evaluate the genotoxic potential of water samples from three spring areas located in the DRB (SI, SII, and SIII) using the test with the fish species *Astyanax lacustris*. In each spring a 50 L water sample was collected and then the samples were transported to the laboratory and placed in aquariums (1 aquarium per spring) with 10 fish each. Dechlorinated water was used as a negative control (NC). After 96h of exposure, five fish were anesthetized and the caudal fin was cut for blood sample collection. The occurrence of nuclear changes and the frequency of micronucleus were analyzed in duplicate blood smears with 1000 cells on each slide, totaling 2000 cells per fish. DNA damage was assessed by the comet assay. For nuclear alterations, the genotoxicity index (GI) was calculated, which represents the sum of all alterations found. In the comet assay, the Arbitrary Unit (AtU) and the total cells with alteration (TCA) were evaluated. The normality of the data was verified using the Shapiro-Wilk test, and were classified as non-parametric data, thus using the Kruskall-Wallis,with Dunn posteriore test ($p \leq 0.05$). We found three types of nuclear alterations: nuclear invagination (NI), nuclear budding (NB) and binucleated cell (BC). Micronuclei were not found. None of the samples showed an increase in the frequency of BC ($p > 0.05$) in relation to the NC. All samples showed significant differences in NB frequencies ($p \leq 0.05$). For samples collected in SI and SII, the frequencies of NI differed significantly ($p \leq 0.05$) from CN. The GI obtained for all samples differed significantly from the NC ($p \leq 0.05$). In the comet test, the water samples from the three springs showed a statistically significant difference ($p \leq 0.05$) for AtU and TCA in relation to the NC, demonstrating that these springs are contaminated with genotoxic chemicals. Considering the intense agricultural activity around the three springs studied, we can suggest that the genotoxic contaminants present in the water are mainly related to the use of agrochemicals (mainly pesticides and fertilizers) that are transported to the aquatic environments. In addition, it was possible to verify that these contaminants negatively interfere with cell division in exposed fish, leading to different types of DNA damage. Therefore, these areas of springs are undergoing environmental disturbances that could trigger irreversible damage to the aquatic biota.
Keywords: nuclear alterations; comet test; agricultural expansion.

Support / Acknowledgment

CAPES, UFGD, FUNDECT, CNPq

CYTOGENETIC CHANGES IN ORAL MUCOSA CELLS FROM INDIVIDUALS SUBMITTED TO ORAL HIV PRE-EXPOSURE PROPHYLAXIS (PREP) USE

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Abstract:

The different existing methods to avoid contamination by the human immunodeficiency virus (HIV) have not yet been enough to eradicate the disease. Since its form of transmission was discovered, incessant recommendations have been used in order to prevent infection and spread of AIDS, such as mechanical barrier (condoms), the non-sharing of needles, the decrease of high-risk behaviors, regular testing for HIV, prompt treatment of other sexually transmitted infections and prevention of transmission by HIV-positive individuals with regular use of antiretroviral therapy (ART). Since 2016, the World Health Organization (WHO) has published a guide of recommendations closely related to AIDS and has pointed oral pre-exposure prophylaxis (PrEP) as part of the combined prevention strategy (biomedical and behavioral) to HIV infection for people at high risk, being this based on the continuous intake of antiretroviral drugs in HIV-negative people as to reduce the risk of acquiring HIV infection. The objective of this study was to evaluate possible cytogenetic changes in individual's submitted to oral HIV pre-exposure prophylaxis (PrEP) use. This study consisted of 37 individuals, 17 from the PrEP group and 20 from the control group. A total of 2,000 cells per slide were analyzed, for determination of micronuclei (MN), binucleation (BN), nuclear buds (NB), and cytotoxicity parameters: pyknosis (PK) karyolysis (KL) and karyorrhexis (KR) through the micronucleus test in oral mucosa, in a double-blinded manner. The repair Index (RI), was also evaluated in this setting. The results showed that PrEP group increased ($p \leq 0.05$) the frequency of MN ($p=0.0001$), BN ($p=0.001$) and NB ($p=0.07$). In the cytotoxicity parameters, represented by the cell death phases, there was an increase with statistical difference ($p \leq 0.05$) in the KR frequency ($p= 0.001$). Additionally, the repair system efficiency decreased in the PrEP group. As a conclusion, these results suggest that individuals undergoing PrEP use may have mutagenic and cytotoxic effects in oral mucosal cells.

Keywords: Cytogenetic; Oral mucosa; HIV pre-exposure prophylaxis; HIV.

OXIDATIVE GENOMIC DAMAGE IN HUMANS EXPOSED TO HIGH INDOOR RADON LEVELS IN NORTHEAST BRAZIL

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Abstract:
Radon gas inhalation is the main source of exposure to ionizing radiation by humans. There is still a lack of knowledge concerning the chronic and indirect effects of exposure to this carcinogenic factor. A small city named Lajes Pintadas in Rio Grande do Norte state, Northeast Brazil, was built in a region with rocks such as granite, migmatites and pegmatites which have traces of uranium and thorium in their formation, consequently releasing Rn into the environment. The mean indoor Rn levels are approximately 300 Bq/m^3, reaching maximum values of 4000 Bq/m^3. This city can be considered a medium-high background radiation area (HBRA) since an annual dose of 5.0^3 mSv/year could be estimated only for Rn exposure to this population.Therefore, the aim of this work was to analyze the levels of oxidative genomic damage in inhabitants of a HBRA (N = 82) in Northeastern Brazil and compare them with people living in a low background radiation area (LBRA) (N = 46). 8-hydroxy-2-deoxyguanosine (8-OHdG) was quantified in urine, Ser326Cys polymorphism was determined in the *hOGG1* gene, and indoor radon was measured. HBRA houses had 6.5 times higher indoor radon levels than those from LBRA (p-value < 0.001). The 8-OHdG mean (95% confidence interval) were significantly different, 8.42 (5.98-11.9) ng/mg creatinine and 29.91 (23.37-38.30) ng/mg creatinine for LBRA and HBRA, respectively. The variables representing lifestyle and environmental and occupational exposures did not have a significant association with oxidized guanosine concentrations. On the other hand, lower 8-OHdG values were observed in subjects that had one mutant allele (326Cys) in the *hOGG1* gene than those who had both wild alleles (Ser/Ser (p-value < 0.05). It can be concluded that high radon levels have significantly influenced the genome oxidative metabolism and *hOGG1* gene polymorphism would mediate the observed biological response.
Keywords: natural radiation; oxidative stress; 8-hydroxy-2-deoxyguanosine; radon.

Support / Acknowledgment

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Nutrigenomic

TOXICOLOGICAL SAFETY OF A MANGO PEEL FLOUR, ITS ANTIOXIDANT AND ANTICANCER POTENTIAL OVER HUMAN BREAST CANCER CELLS

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Abstract:

Mango (*Mangifera indica* L.) is a fruit of the Anacardiaceae family and is cultivated worldwide, with production estimated in 42 million tons in 2021 and 63 million tons projected for 2030. On the other hand, processing of mangoes produces different yields of mango waste, with the peel accounting for 11% of the waste of this crop, which translates into near 6.3 million tons of waste. However, the literature demonstrates that plant residues may be a source of bioactive compounds with anticancer activities. Also, it is necessary to improve the shelf life of these materials, with the drying process relying as a viable technique to this objective, making the flour elaboration from plant residues desirable. Mango peel is a promising raw material for the preparation of flour due to its nutritional composition in terms of macro and micronutrients. However, as a fruit residue, it is necessary to verify the safety regard its consumption. Thus, the aim of the present study was to develop a mango peel flour, access its toxicological safety, antioxidant and antimutagenic activities and citotoxicity. Mango peel was sanitized, washed, selected by appropriate parts, milled, dried, and passed for a second millage giving birth to the mango peel flour. Mango peel flour was submitted to two extractors (water and 70% ethanol) which were tested by their total phenolic content by Folin-Ciocalteu method, as well as different *in vitro* antioxidant assays (DPPH+, ABTS+, FRAP, and ORAC). Additionally the aqueous extract was screened through the Ames test (TA98 and TA100 strains), with and without exogenous metabolic activations (s9mix) for its toxicological safety, as well as its antimutagenic activity and cytotoxicity was evaluated against MCF-7 and MDA-MB-231 human breast cancer cell lines for 24h, 48h and 72h of treatment. The mango peel flour was considered safe as it did not present a dose-dependent behavior and twice the number of spontaneous revertants in Ames test. For both breast cancer cell lines, the 48h of treatment had the best result, with the lethal concentration of 50% of the cells (LC50) being 23.0 ± 6.7 mg/mL for MCF-7 and 15.7 ± 3.0 mg/mL for MDA-MB-231. These results suggest that the mango peel flour is a promising product elaborated from a mango by-product, which collaborates with the reduction of environmental impact of this crop as well as may be a great source of antioxidant and anticancer compounds.

Keywords: *Mangifera indica* L.; Toxicological safety; Anticancer activity; Breast cancer.

METABOLOMIC ANALYSIS AND *IN VITRO* ASSESSMENT OF ANTIMUTAGENIC POTENTIAL OF *EQUISETUM HYEMALE* AQUEOUS EXTRACT

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Abstract:
Equisetum hyemale L., commonly known as horsetail, is recognized in traditional folk medicine worldwide for its use in the treatment of various diseases such as hypertension, cystite, cancer, and other illnesses, primarily administered in the form of infusions. Despite the widespread benefits associated with its use, there is a lack of information regarding the biological mechanism, effectiveness, and potential health risks. Such evidenced effects are generally associated with secondary metabolites, which are chemical compounds that vary according to the species, genus, and family of the plant. In this regard, there is the presence of phenolic compounds, such as flavonoids, in some species of the *Equisetum* genus. These phenolic compounds possess biological activities, including antibacterial, antifungal, and antiviral properties, as well as exhibiting antioxidant and anti-inflammatory responses. However, a comprehensive understanding of its medicinal properties, phytochemical composition of the extract, and potential biological effects is still needed. The aim of study was to investigate the antimutagenic potential of the aqueous extract of *E.hyemale* and the phytochemical composition, through metabolomic analyses. For the antimutagenicity assay, strains of *Salmonella enterica* serovar Typhimurium were used in three different treatment methods: pre, co and post-treatment. To evaluate the antimutagenic potential of the extract against an oxidative mutagen, the 4-nitroquinoline-1-oxide (4NQO) was used with the strains TA98, TA100 and TA1535 without metabolic activation. Against alkylating agents, Methyl Methanesulfonate (MMS) was used in absence of metabolic activation with the strain TA100, and Cyclophosphamide (CPA) were used in the presence of exogenous metabolic activation (S9 mix 4%) with the strains TA98 and TA100. For the metabolomic analysis, the sample was submitted to UHPLC-MS/MS acquisition thought a Thermo Scientific instrument equipped with a heated electrospray ionization probe. The UHPLC-MS/MS data were pre-treated using the software MzMine3 and exported to Global Natural Products Social Molecular Networking (GNPS), which the tool "Data Analysis" and "Library Search" were used to identify the compounds. In the antimutagenicity assay it was possible to observe that the extract demonstrated antimutagenic effects against DNA damage caused by 4NQO in all strains analyzed. In the TA98 strain, there was a reduction of mutagenicity up to 32% in the pre-treatment, 55% in the co-treatment and 37% in post-treatment. In the TA100 there was a reduction up to 35% in the co-treatment, and in the TA1535 a reduction up to 63% in pre and co-treatment. Against the alkylating agent MMS, in the TA100 strain there was a reduction up to 35% in the post-treatment. Against CPA, in the TA98 strain, there was a reduction up to 30% in post-treatment, and in TA100 strain, a reduction up to 78% in pre-treatment and 80% in post-treatment. In metabolomic analysis, it was possible to identify 9 present compounds. The most abundant ones were Meglutol, Harmol Hydrochloride, and 5,6-dihydrouracil, which are related to cholesterol reduction, cytotoxicity in tumor cells, increased autophagy, among others. The remaining compounds mainly exhibit antioxidant, anti-inflammatory, antibacterial, and antiviral effects. Thus, it can be observed that the *E.hyemale* extract possesses desirable biological effects, but further investigation is still required.

Keywords: horsetail; antimutagenicity; metabolome.

CELL VIABILITY ASSESSMENT OF COLORECTAL CANCER CELLS FOLLOWING EXPOSURE TO DIALLYL DISULFIDE AND 5-FLUOROURACIL

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Abstract:
Colorectal cancer (CRC) is the second deadliest cancer, and ranks third in incidence. In 2020, there was an estimated 1.9 million new cases and 900 thousand deaths worldwide. Changes in our modern society, such as adopting a sedentary lifestyle, low consumption of grains and fiber along with alcohol, cigarettes and red or processed meat intake contribute to the main risk factors for CRC. Surgical intervention and chemotherapy are the primary methods to improve patient survival. 5-fluorouracil (5-FU), an antimetabolite drug, is widely used for a various cancer, and is considered the first-line treatment for CRC, alone or in combination with other drugs. However, one of the biggest challenges that remains, is the development of acquired resistance to 5-FU in CRC tumor cells. The mechanisms responsible for 5-FU resistance include regulation of drug transporters, induction of epithelial-mesenchymal transition, dysregulation of microRNA and epigenetic changes. Therefore, finding alternative approaches to cancer treatment is urgently needed. The nutraceutical diallyl disulfide (DADS), extracted from garlic, has shown anti-tumor activities against many cancer cells. It has been demonstrated that DADS induce apoptosis, suppresses DNA adducts, regulates cell cycle arrest and inhibits metastasis. This study aims to evaluate the cellular viability of two CRC cell lines, Caco-2 and HT-29, and a non-tumoral cell line, HUVEC, treated separately with DADS (25-600µM) and 5-FU (5-100µM) for 24 hours. Viability was assessed using resazurin and neutral red assays. Our results indicate that the tumoral lines responded similarly to each treatment. HT-29 cells appear to be more sensitive to DADS in both assays, while the viability of the non-tumoral cells was only reduced only at the two highest concentrations of DADS. Using public databases, we also identified, genes whose expression was altered by treatment with DADS or 5-FU. We selected genes whose expression altered by 5-FU conferred resistance to the drug and could be reversed by combined treatment with DADS. The main genes identified were FOSL1, MDM2, BIRC3, HMOX1, KLF2, and TP53, along with other genes involved in critical cellular processes such as, apoptosis, proliferation, and the cell cycle. These findings offer valuable insights into the interactions between the nutraceutical DADS and the chemotherapeutic drug 5-FU, and can potentially become a useful tool in improving cancer treatment.
Keywords: Colorectal cancer; Diallyl disulfide; Cytotoxicity.

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EPIGENETIC MODULATION OF HUMAN HISTONE DEACETYLASES BY ROYAL JELLY DERIVED FATTY ACIDS MIGHT UNDERLIE THEIR NUTRACEUTICAL PROPERTIES

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Abstract:
Honeybee-derived products are commonly used as traditional complementary medicine, especially in phytotherapy and diet because of their powerful healing properties. Royal jelly (RJ) is a complex mixture secreted by nurse bees that is responsible for feeding young worker larvae during a short time and for the entire life of queen bees. Importantly, the differential diet during larval development with RJ determines the morphological, reproductive, and longevity features between queens and workers, although no single chemical compound seems to be responsible for these differences. In this context, honeybees are unique models to study how diet influences gene expression and shapes the phenotype. Previous studies have indicated that RJ derived compounds, such as phenyl butyrate and trans-10-hydroxy-2-decenoic acid (10-HDA) are weak histone deacetylase inhibitors (HDACi). In this context, this study aims to understand the diversity of royal jelly derived fatty acids and predict their interactions with human histone deacetylases using computational-based approaches. A list of 32 fatty acids derived from royal jell was retrieved from the scientific literature (PubMed database, pubmed.ncbi.nlm.nih.gov) and classified into two groups: mid-chain 8-12 carbons long and those with short-chain and/or aromatic compounds. The molecular structures were analyzed using the ChemMine Tools Web Server (chemminetools.ucr.edu), the physicochemical properties were calculated using OpenBabel Descriptors, and the chemical space was illustrated using the Constelation Plot strategy. The molecular docking was performed on AutoDock Vina, and ligand-protein interactions were visualized on the Protein-Ligand Interaction Profiler (https://plip-tool.biotec.tu-dresden.de). The target protein used was the crystallographic model of human HDAC2 complexed with a well-known HDAC, suberoylanilide hydroxamic acid (SAHA). The complementary analysis of drug-like compounds was performed using the Platform for Unified Molecular Analysis - PUMA (http://132.248.103.152:3838/PUMA) and SwissAdme (swissadme.ch). The 32 fatty acids were clustered into a few cores despite their similarities. The 10-HDA and its precursor, the 10-HDAA (10-hydroxydecanoic acid), together represent 60-80% of the total organic acids in RJ. Interestingly, the molecular docking predictions indicated that phenyl butyrate has lower affinity (-5.3 kcal/mol) than 10-HDA and 10-HDAAA (-5.8 and -6.1 kcal/mol, respectively) for the catalytic domain of HDAC2. Moreover, other MCFAs such as dihydroferulic acid and 9-oxo-2-decenoic acid (9-OXO) presented promising orientations inside the enzyme catalytic domain and their binding energy values were -7.6 and -6.5 kcal/mol, respectively. These ligands share common interactions with the amino acid's residues PHE155, PHE210, LEU276, TYR308, ASP104, ASP181, ASP269, GLY154, GLY306, HIS145, HIS146, HIS183, and the zinc ion (401). Based on the similarities between the predicted interactions within the enzyme active domain and in the energy values, our data indicate that the royal jelly-derived fatty acids might inhibit HDACs acting collectively to compete with the enzyme substrate. Notably, these compounds have drug-like properties including permeabilization through the gastrointestinal tract and blood-brain barrier. The comprehension of the biological properties of specific classes of chemical compounds derived from honeybee products may contribute to safe consumption, and to be used in drug discovery and medicinal chemistry, improving their positive effects on human health.
Keywords: HDACi; natural products; food supplements.

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THE EFFECTS OF A NON-PROTEIN DIET ON THE HIPPO GENE PATHWAY IN THE BRAIN OF HONEY BEE (*APIS MELLIFERA* WORKERS)

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Abstract:
There is evidence that restrictive diet has a pathophysiological pattern in early cellular aging and in the development of pathological processes, since it can provide cellular responses and defense against free radicals that can lead to DNA damage. This early cellular aging is associated with the activity of several signaling pathways, such as the hippo pathway, which is a highly conserved cascade kinase, mainly studied in insects and mammals. This pathway regulates cell fate decisions in response to DNA damage, and may induce cell death depending on the phosphorylation status of the Yorkie protein. The core of the insect pathway is a kinase cascade consisting of four proteins including Expanded, Hippo, Salvador and Mats. These are the main proteins that are known to control Yorkie phosphorylation. The honey bees represent an adequate model for studies of morpho-physiological changes dependent on food content and age. Young adult bees (~1-15 days old) consume a protein-rich diet and older ones (> 16-days old) consume a carbohydrate-rich diet. In this work, we analyzed the effects of a non-protein diet (NPD) on the expression levels of Hippo pathway genes in brains of adult honey bee workers (*Apis mellifera*). Newly-emerged workers were kept in an incubator and fed with a NPD (100% sucrose, experiment) or protein diet (PD = 30% pollen+70% sucrose, control) for 7 days. For comparisons, young (7 days-) and older (28 days-old) bees were also collected directly from the colonies. The brains were dissected, following the extraction of the total RNA and the synthesis of cDNAs. Transcript expression analyses (n=9 samples of individual brains per group) were performed by semi-quantitative RT-PCR and optical densitometry. The actin encoding gene was used as a normalizer, and statistical significance was assessed by the T- test or Mann-Whitney ($p<0.05$). We found that the expression of Expanded, Hippo, Mats, Salvador and Yorkie genes were similar ($p>0.05$) between PD and 7-days groups, as well as between NPD and 28-days groups. However, these patterns were statistically different from each other ($p<0.05$). Thus, our comparative findings indicate some feature of aging process in the brains of bee workers that have consumed a protein-free diet, suggesting DNA damage and an apoptotic-degenerative state, but these effects still need additional experiments and analyses of other parameters.
Keywords: Hippo pathway; food content; aging; DNA damage; honey bee

ANTIGENOTOXIC EVALUATION OF VOLUNTARY PHYSICAL EXERCISE IN THE HIPPOCAMPUS OF OFFSPRING OF FEMALE MICE THAT CONSUMED HIGH FRUCTOSE DURING PRE-PREGNANCY AND PREGNANCY

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Abstract:
The brain may be a target tissue in fetal programming, as the fetal brain is a particularly plastic tissue due to changes in structure and function produced by in-utero conditions, which may have long-term implications for the offspring. For example, the placenta can transport fructose, so the fetal programming process is thought to be driven by fructose and its metabolites. In this context, new alternatives for protection against the damage caused to fetal programming are extremely relevant. It is known that physical exercise has antioxidant activity and protects DNA. Thus, the objective of the study was to evaluate whether the practice of voluntary physical exercise associated with chronic consumption of fructose, from the pre-gestational period and/or until the pregnancy period, is capable of protecting the hippocampus from genotoxic alterations in female mothers and offspring (females and males). We used 70 Swiss female mice at 21 days of age, which received fructose (20%) in the hydration bottle and/or practice voluntary physical exercise (VPE) for 8 weeks (pre-pregnancy). Females were first divided into 4 groups: G1 - Water; G2 - Water + VPE; G3 - Fructose; G4 - Fructose + VPE. After 8 weeks of treatment, they were randomly subdivided into 7 experimental groups and mated to the male for 7 days for the interventions. The groups were: G1 - Water; G2 - Water + VPE; G3 - Water + VPE/Water; G4 - Fructose; G5 - Fructose + VPE; G6 - Fructose + VPE/Water + VPE; G7 - Fructose + VPE/Water. After the lactation period, the offspring were separated by sex in the 7 experimental groups according to sex and treatments performed on the females. The mothers were euthanized after lactation, and the offspring at 60 days of age for genetic evaluation in the hippocampus. This study was approved by the Animal Ethics Committee (CEUA) of the University of Southern Santa Catarina (approval No. 023/2019-1). The quantification of fructose in sera was determined by High-performance liquid chromatography (HPLC) and was performed on samples from female matrices in pre-pregnancy and pregnancy, as well as their offspring at 7 and 60 days of age. Through the Comet Assay, we observed that VPE was able to modulate DNA damage caused by maternal fructose in the fetal brain and hippocampus of female mice and their offspring. Furthermore, we observed in female offspring, a decrease in APE1 levels in the fructose group about the water group, and that VPE increased the levels of APE1. In male offspring, we observed an increase in OGG1 levels in the fructose + VPE group compared to the fructose group. Thus, we observed that fructose was genotoxic in the hippocampus and that VPE was able to reverse DNA damage. In conclusion, this work demonstrates that dietary and physical habits can influence maternal and offspring health. There are genetic mechanisms involved in metabolic programming from intrauterine life that can impact the health of offspring in adulthood, specifically in the hippocampus.
Keywords: Fetal programming; Fructose; Physical exercise; Genotoxicity; Hippocampus

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HYPERCALORIC DIET SUPPLEMENTED WITH SPERMIDINE: STUDY OF THE EFFECTS ON DNA

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Abstract:

For healthy aging, good nutrition is a determining factor as it can reduce molecular damage accumulated throughout life. Diets based on ultra-processed foods, rich in sugar and calories, are linked to DNA damage, as they are risk factors for cancer. Components naturally present in food, added to the diet in adequate amounts, can prevent the early occurrence of DNA damage. Spermidine is a polyamine with several biological functions already demonstrated in the literature without research results at the DNA level. The present study aimed to evaluate the effects of spermidine supplementation in rodents fed a hypercaloric diet, rich in sucrose, before and after the induction of DNA damage. The study was carried out using male Swiss mice (n=3), divided into the following experimental groups: 1) Negative Control [NC, standard diet (SD)], 2) Positive Control [PC, Methylmetasulfonate (MMS - 40 mg/kg body weight (bw)], 3) Hypercaloric diet [HD - SD plus 20% sucrose in the form of refined sugar diluted in water], 4) ESP 10 mg/kg bw, 5) ESP 30 mg/kg bw, 6) DH + ESP 10 mg/kg bw, 7) DH + ESP 30 mg/kg bw. The animals were treated for a total of 45 days and, to assess mutagenicity, blood samples were collected after 48 hours and 42 days of treatment. To evaluate the chemopreventive potential, on the 44th day the MMS was administered intraperitoneally (ip) to induce mutagenicity in all groups except the NC and bone marrow cell samples were collected 24 hours later. During the experimental period, weight, water, feed and sucrose consumption and naso-anal length were evaluated. Blood glucose was monitored on the 1st and 43rd day. In general, the results obtained showed that animals in the groups that received sucrose reduced food consumption and water intake when compared to the negative control group (NC). There was no significant difference in glycemic variation and gain in abdominal circumference between treatment groups. The polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) ratio in peripheral blood and bone marrow did not showed significant differences in any of the treatments. The frequency of micronucleated polychromatic erythrocytes (PCEMNs) in peripheral blood revealed absence of mutagenicity of treatments. The bone marrow micronucleus assay demonstrated a statistically significant reduction in the frequency of PCEMNs in the animal's group that received treatment with SPD 30 mg/kg bw when compared to MMS. On the other hand, in the treatment groups that received SPD 10 and 30 mg/kg bw associated with DH, a significant increase in the frequency of PCEMNS was identified. Our results indicate that the SPD can act as a chemopreventive, but when the treatment was associated with a HD, the mutagenicity was potentiated. These evaluations contribute to clarifying the effects of spermidine on induced DNA damage and provide information for future studies.

Keywords: Longevity; Spermidine; Hypercaloric diet; chemopreventive.

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KETOGENIC DIET INHIBITS PRENEOPLASTIC COLON LESIONS BY MODULATION OF ENDOGENOUS ANTIOXIDANTS AND ANTI-INFLAMMATORY AND CELL PROLIFERATION PATHWAYS

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Abstract:
Lifestyle is related to quality and time of life, with diet being one of the factors that can exert a positive influence on the development of diseases, including cancer. Colorectal cancer (CRC), third most diagnosed cancer worldwide, is associated with low fiber diets, excessive consumption of red meat, and saturated fats. The ketogenic diet (KD) is characterized by high fat, sufficient amounts of protein and low carbohydrate content. It has been shown that KD can act on the carcinogenesis process by modulating insulin levels due to anaerobic glucose (Warburg effect). However, data about the influence of KD quality on this process are scarce. In this sense, this study aimed to evaluate the effects of KD in the colorectal tumorigenesis promotion induced in rats. The study was performed using two diet models based a lipid profile: a) rich in saturated fats (KD) and b) rich in medium-chain triglycerides and unsaturated fats (MCTKD). In vitro analyses were performed to determine antioxidant potential and total flavonoid and phenolic content. In vivo, preneoplastic lesions were induced in the Wistar rat colon with the carcinogenic agent 1,2 dimethylhydrazine [DMH 40mg/kg body weight (b.w.).] for 6 weeks. After, the animals were subjected to the two diet models (KD and MCTKD) for 6 weeks. Weight, abdominal circumference, naso-anal length, water and food consumption were monitored daily. Before the beginning and at the end of the experimental period, the of glucose and ketosis levels were measurement. To evaluate the chemopreventive potential, the Aberrant Crypt Foci (ACF) and Aberrant Crypts (AC) in the rat colonic mucosa and bone marrow genotoxicity were quantified. To investigated the possible mechanisms involved in the protective effect we performed the relative quantification of the transcripts by RT-PCR (real time polymerase chain reaction) and the proteins by immunohistochemistry. Our results showed that the KDs did not show antioxidant potential in vitro. None of the treatments showed influence on body composition and water and feed intake did not indicate toxicity. As expected, the KD and MCTKD induced the state of ketosis but without modulating glucose levels and were able to reduce chromosomal damage in the bone marrow caused by the carcinogen DMH. Colonic mucosa analyses demonstrated that KD and MCTKD were able to reduce the frequency of ACF and AC. The KD treatment increased iNOS, IGF-1, NRF-2, GST and SOD mRNA levels while MCTKD treatment increased NRF-2, GST and SOD and decreased iNOS and IGF-1 mRNA levels. The KD also increased the expression of COX-2 and PCNA, and decreased β-catenin while the MCTKD decreased the expression of COX-2, PCNA and β-catenin protein markers. Our findings suggest a chemopreventive potential of KDs in colorectal carcinogenesis, which acts on anti-inflammatory, endogenous antioxidant defense, and cell proliferation pathways. The most evident effect was observed in the MCTKD group, indicating that the quality of the lipid profile is relevant for the protective effect.
Keywords: ketogenic diet; chemoprevention; precancerous lesions.

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INFLUENCE OF A BEETROOT PEEL FLOUR EXTRACT OVER THE CLONES FORMATION OF BREAST CANCER CELLS, ITS TOXICOLOGICAL SAFETY AND BETALAINS PROFILE DESCRIPTION

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Abstract:
Cancer is a global health problem worldwide. From the non-communicable chronic diseases, cancer still remain as one of the major causes of death, specially breast cancer, which is the major cause of cancer death in women. As a multifactorial disease, cancer can be affected by food habits as well as social habits as smoking. Alongside the growing necessity of the development of products that may help with cancer treatment, relies the necessity of the reuse of vegetable residues, making part of the 11th and 12th Sustainable Development Goals proposed by the United Nations in 2015. In addition, vegetable residues are widely reported as a great source of bioactives, including anticancer agents. It was previously reported that a beetroot peel flour has a strong antioxidant and anticancer activity against breast cancer cells, alongside a diverse phytochemical profile, which includes the betalains as their main pigment as well as other bioactive compounds as flavonoids. Thus, the aim of the present work were: a) to verify the influence of a beetroot peel flour over the clone formation ability of breast cancer cells by the clonogenic assay; b) to verify the toxicological safety thru the Ames test (TA97a, TA98, TA100, TA102, and TA1535 strains, with and without exogenous metabolic activation - S9mix) and; c) to explore the betalains profile of the flour thru liquid chromatography coupled with high-resolution mass spectrometry. A 50mg/mL aqueous stock solution of a beetroot peel flour (BPF) was made and four different concentration were diluted for use in the clonogenic assay and Ames test (2.5, 5.0, 10.0 and 20.0 mg/mL). The BPF present a strong anti-clonogenic behavior, with a strong dose-dependence of the clones formation ($R^2 = -0.9$). In addition, the BPF extracts did not presented any genotoxic behavior thru the Ames test with and without metabolic activation by rat liver homogenate (s9mix), being considered safe for consumption. The betalains profile presented different forms of betalains, grouped as betacyanins (betanin and neobetanin) and betaxanthins (indicaxanthin, miraxanthin II, valine-iso-betaxanthin, vulgaxanthin I, vulgaxanthin II, and vulgaxanthin IV), in accordance with the literature that demonstrates that betaxanthins are the betalains forms most present in the beetroot peel. The betalains profile observed may explain the reduction in clones formation. These betalains are reported in the literature as influencers of different metabolic pathways in cancer cells leading to cell death and/or reduction of the metastatic ability, including cell cycle arrest thru downregulation of cyclins and upregulation of caspases expression (betanin), influence over DNA metilation and gene expression (indicaxanthin), and modulation of energy metabolism thru inhibition of lactate dehydrogenase enzyme by molecular docking (miraxanthin II and vulgaxanthin I). Due the results, the BPF is considered a great source of compounds with antiproliferative activities and a safe product for the consumption.
Keywords: *Beta vulgaris* L.; Betacyanins; Betaxanthins; Ames test.

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GENOMIC INSTABILITY IN FEMALE SWISS MICE TREATED WITH SUCROSE AND MALTODEXTRIN DURING PREGNANCY, LACTATION, AND IN THEIR OFFSPRING

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Abstract:
Excessive intake of carbohydrates during fetal and neonatal life can lead to related disorders in adulthood, such as obesity, increased insulin resistance, and diabetes, which can ultimately result in genomic instability. Therefore, the objective of this study was to evaluate dietary intake, body weight, and mutagenicity in female breeding mice and their offspring. Thirty 60-day-old female Swiss mice were used, which received water, maltodextrin (10%/L), or sucrose (10%/L) in the hydration bottle during mating (7 days), gestation (21 days), and lactation (21 days). For the mating period, thirty 60-day-old male Swiss mice were used solely for reproduction (the males were kept with the females for only 7 days and received the same interventions during this period). After the lactation period, the offspring were followed until 30 days of age according to the same experimental groups, with separation between males and females. During the experiment, the dietary intake and body weight of the animals (breeding females and offspring) were measured, and subsequently, they were euthanized for the micronucleus test. Regarding the dietary intake in breeding females, an increase in liquid consumption was observed in the maltodextrin and sucrose groups compared to the control group during the mating and gestation periods. Additionally, there was an increase in calorie intake in the maltodextrin and sucrose groups compared to the water group during the mating, gestation, and lactation periods. There was no difference in weight and dietary intake among breeding females. As for the offspring of the sucrose group, weight gain was observed in male mice at 21 days of age compared to the maltodextrin group. The micronucleus test revealed that maltodextrin was mutagenic in mothers and male offspring, while sucrose was mutagenic in male and female offspring. Based on these results, it can be suggested that the consumption of maltodextrin and sucrose can alter dietary intake and body weight and exhibit mutagenic effects in breeding females and their offspring.
Keywords: pregnancy; lactation; maltodextrin; sucrose.

DNA Repair

CELL RESPONSES FROM COCKAYNE SYNDROME PATIENTS TO DNA DAMAGE CAUSED BY FORMALDEHYDE

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Abstract:
The integrity of the DNA molecule is essential for biological stability, but it is susceptible to damage caused by endogenous and exogenous threats. Cockayne syndrome (CS) is an autosomal dominant disease related to mutations in the *CSA/ERCC8* and *CSB/ERCC6* genes, which are crucial for the transcription-coupled nucleotide excision repair (TCR-NER) pathway, leading to a phenotype of progressive neurodegeneration and premature aging caused by mechanisms yet unknown. Recent studies point to endogenous formaldehyde (FA) as the agent causing genomic instability leading to neurodegenerative processes. This study aims to evaluate the response of CSB-mutated fibroblasts to DNA damage induced by FA to understand its contribution to the observed degenerative clinical characteristics in CS patients. Cells were treated with FA at different concentrations and exposure times, and performed assays to detect cell viability, clonogenic survival, DNA breakage, and cell cycle alterations. The cell viability assay (XTT) showed that sensitivity to FA increases with prolonged treatment, with the strongest effect observed for the longest period (72 h) of treatment. Clonogenic survival assay revealed that CSB cells are more sensitive to higher doses of FA. In the alkaline comet assay, we detected a significant increase in DNA breaks in CSB cells 22 h after FA treatment. Lastly, cell cycle analysis by flow cytometry revealed an increase in the G2 population in FA-treated cells, especially in CSB cells. The results indicate that CSB cells are more sensitive to FA, exhibiting greater DNA damage and accumulation in G2, suggesting that FA may be involved in the degenerative characteristics observed in CS patients. However, further studies are needed to understand the mechanisms involved in FA damage repair, which may lead to more effective therapeutic interventions. As future perspectives, we plan to investigate the effects of glutathione depletion on DNA damage and explore other DNA repair pathways and cellular protection mechanisms.
Keywords: DNA damage; Cockayne syndrome (CS); formaldehyde (FA); repair; degenerative.

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EXPLORING THE INTERPLAY BETWEEN LONG COVID AND DNA DAMAGE: UNDERSTANDING THE RELATIONSHIP AND REPAIR MECHANISMS

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Abstract:
COVID-19, caused by SARS-CoV-2 infection, has been associated with persistent symptoms in some individuals, known as long COVID. These symptoms are related to DNA damage and faulty repair mechanisms, which can contribute to the development of chronic diseases. However, there is limited understanding of the molecular alterations and underlying mechanisms that contribute to the progression and severity of this infection. Understanding these effects is crucial for comprehending the long-term consequences of the virus infection, as well as for developing effective treatment and prevention strategies. Therefore, the objective of our study is to investigate the cellular mechanisms and risk factors that contribute to genomic instability in individuals with long COVID, with a particular focus on understanding the repair mechanisms related to the persistent symptoms. To achieve this, we examined 50 individuals diagnosed with COVID-19 between January and March 2023 in the city of Campo Bom, Brazil. These individuals were divided into two groups: those with less severity and variety of symptoms, and those who experienced greater severity and variety of symptoms. To assess genomic instability, we employed the alkaline comet assay, a sensitive method for detecting DNA damage. Furthermore, we analyzed the influence of potassium bromate (100 mM), a known agent for inducing oxidative damage, on blood samples obtained from patients exhibiting different symptoms of long COVID. We conducted a cellular challenge to evaluate the repair mechanism at three-time intervals: 15, 30, and 60 minutes among individuals with lower symptom severity and higher symptom severity. There was no statistical difference in the cellular repair process at any of the times tested for patients with less severity of symptoms and patients with greater severity of symptoms. So far, these findings suggest that there is no difference in the cellular repair system of patients diagnosed with long COVID, regardless of the severity of symptoms. However, biochemical parameters point to an increase in markers of inflammatory processes in patients with greater symptom severity, such as C-reactive protein and D-dimer. Nevertheless, further studies are necessary to deepen our understanding of the complexities of long COVID and its impact on cellular repair mechanisms and inflammatory processes.
Keywords: Long Covid; Repair; genomic instability; SARS-CoV-2; inflammatory processes

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ROLE OF DNA POLYMERASE ETA IN CELLULAR SENSITIVITY TO DIFFERENT GENOTOXINS AND IN MITOCHONDRIAL FUNCTION

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Abstract:
DNA is constantly exposed to endogenous and exogenous physical and chemical genotoxic agents. DNA lesions can block replication or transcription, leading to cellular dysfunction and death, or mutagenesis. In addition to DNA repair mechanisms, cells have DNA damage tolerance mechanisms (DDT) that allow DNA replication even in the presence of lesions: template switching (TS) and translesion synthesis (TLS). In the TLS mechanism, the replicative DNA polymerase is temporarily replaced by one or two specialized DNA polymerases capable of synthesizing past the lesion, but since these polymerases lack proof-reading activity, this process is potentially mutagenic. Lesions like cyclobutane pyrimidine dimers (CPDs) can be tolerated in an error-free manner by DNA polymerase eta (Pol eta), an essential TLS polymerase. Mutations and lack of function in Pol eta causes xeroderma pigmentosum variant (XP-V), a milder form of the rare autosomal disease with clinical manifestations such as photosensitivity, several melanoma and non-melanocytic skin cancer cases since early-childhood, and more rarely ophthalmologic and neurodegenerative issues. Although important as a TLS polymerase, Pol eta has other cellular functions, acting on restart of stalled replication forks under replication stress conditions, telomere replication and prevention of under-replicated DNA at common fragile sites, among other functions. Here we investigate whether the XP-V cellular phenotypes are due to the loss of Pol eta's TLS activity or by dysregulation in cellular homeostasis related to other XPV functions, including the impact on mitochondria, as other XP complementation groups, such as XP-C cells, have altered mitochondrial function. As a model, we used SV-40 immortalized XP-V cells, isogenic cells complemented with the functional POLH gene, MRC-5 fibroblasts with the POLH gene silenced using small interference RNA (siRNA). Using XTT assay, we found that XP-V cells are significantly more sensitive to MMS and cisplatin than the complemented and MRC-5 WT. As next steps, mitochondrial function will be evaluated using Seahorse XFe24 to measure if the lack of Pol eta in basal and stress conditions affects mitochondrial function for all lineages.
Keywords: Xeroderma pigmentosum variant; Translesion synthesis; Mitochondria; DNA polymerase eta.

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DNA DAMAGE RESPONSE TO DOUBLE-STRANDED BREAKS IN MAMMALIAN MITOCHONDRIA

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Abstract:
DNA double-strand breaks (DSBs) are extremely cytotoxic and can lead to cell death or accumulation of deletions. In mammalian cells, DSBs can be repaired by homologous recombination (HR) or non-homologous end-joining (NHEJ). In HR, the MRN complex (MRE11-Nbs1-Rad50) recognizes the DSB and recruits the ATM protein kinase, through interaction with the NBS1 protein. This event leads to ATM activation and ATM-mediated phosphorylation of several proteins involved in DNA repair and cell cycle control, thus initiating the DNA repair process through HR. Mitochondrial DNA (mtDNA) accumulates large amounts of deletions with age, which has been proposed to result from incomplete repair events at DSB. However, HR is poorly characterized in mitochondria. Few studies have shown HR activity in mitochondria, mitochondrial localization of HR and NHEJ proteins and the presence of recombination intermediates (Holliday junctions) in mtDNA. Moreover, the mechanisms for damage recognition and signaling of DSBs in mtDNA remain unknown. Here we investigated the possible role of ATM and the MRN complex in the recognition, signaling and repair of DSBs in mtDNA, using the following cell lines: MRC5, AT5-BIVA, Hela, Hek293T wild-type and ATM KD, silenced by shRNA. To detect the presence of nbs1 in mitochondria, the western blotting technique was used on extracts obtained after mitochondrial isolation from the mentioned cell lines. The long-extension PCR technique was employed to detect the quantity of lesions in mtDNA after MRE11 inhibition, using the mirin inhibitor and treatment with bleomycin, an agent that induces double-strand breaks in DNA. For the bioenergetic assay, oxygen consumption was measured in Hela cells after treatment with mirin and bleomycin, using the Seahorse equipment. Mitochondrial localization of NBS1 was detected in HEK293T, MRC-5, AT5-BIVA and Hela. Interestingly, the pharmacological inhibition of MRE11 with mirin in HEK293T cells led to accumulation of mtDNA damage, but treatment with bleomycin in MRE11-inhibited cells did not cause significant increase in the number of lesions or in repair kinetics observed in cells exposed to mirin alone, indicating that MRE11 might be essential for mtDNA maintenance under physiological conditions. Initial bioenergetic results indicate that mirin inhibition does not seem to affect oxygen consumption in Hela cells, but bleomycin treatment in inhibited cells generated a significant impact on respiratory rates. Our results support the mitochondrial localization of the MRN complex and suggest that MRE11 is required for mtDNA maintenance.
Keywords: Mitochondrial DNA repair; double strand breaks; homologous recombination; MRN complex; ATM

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MRNA TRANSFECTION OPTIMIZATION IN HUMAN FIBROBLASTS USING EGFP

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Abstract:
During the COVID-19 pandemic, mRNA vaccines against SARS-Cov2 have been successfully and safely used, point out to the potential of the messenger RNA (mRNA) transfection technic. This approach represents a valuable alternative to gene therapy, since the translation of the transfected mRNA is highly efficient and only the protein of interest is generated, in contrast with other vectors. We intended to implement in the lab a strategy of mRNA molecules transfection in human fibroblasts. The protocol has been standardized using a plasmid containing the sequence of enhanced green fluorescent protein (eGFP). The plasmid was linearized, purified, *in vitro* transcribed, purified of double-stranded (ds)RNA products, capped and transfected into fibroblasts. For optimization of transfection efficiency, many conditions were tested and efficiency was evaluated by flow cytometry, as percentage of eGFP positive cells. Tested conditions were: a) commercial plasmid versus an *in house* amplified plasmid; b) *in vitro* transcription using uridine or pseudouridine; c) removal or not of dsRNA; d) presence or absence of capping; e) 2 different concentrations of mRNA; f) 3 different concentrations of lipofectamine; g) 3 different time points after transfection; h) 6 transfection kits; and i) medium replacement, or not, 2-4 h after transfection. We have notice that transfection efficiency is enhanced if dsRNA has been removed from synthetized mRNA and that capping is essential for transfection success. Similar transfection efficiencies have been observed between mRNA synthetized from a commercial plasmid or from an *in house* amplified plasmid, as long as the mRNA is fresh. However, when the mRNA is not fresh, mRNA synthetized from an *in house* amplified plasmid presents a lower transfection efficiency. Regarding nucleotide used in mRNA production, mRNA synthetized with pseudouridine has higher transfection efficiency than mRNA synthetized with uridine, and less cell death is observed. When lipofectamine concentration is constant, similar transfection efficiencies have been obtained for both mRNA concentrations tested (500 and 1000 ng). Although higher and intermediate concentration of lipofectamine have shown a similar result, being both better than the lower concentration, intermediate concentration of lipofectamine had more stable results than the higher concentration. Lastly, the frequency of eGPF positive cells peaked at 24 h, gradually decreasing at 48 h, and remaining stable until 72 h. In summary, higher eGFP mRNA expression in human fibroblasts was achieved using fresh mRNA synthetized from commercial plasmid, using pseudouridine, purified for dsRNA, and capped. Moreover, the most efficient condition concerning transfection itself was the combination of 500 ng of mRNA with intermediate concentration of lipofectamine, leading to a peak of eGFP positive cells at 24 h post-transfection (81,7 %). Testing these conditions with 6 different transfection kits, and without medium replacement after transfection, we could increase the percentage of cells expressing eGFP up to 95-96 % for 3 of the kits. We plan to use this effective mRNA transfection protocol in several studies, mainly on DNA repair, which is of particular interest to our group.

Keywords: mRNA transfection; human fibroblasts; eGFP.

UNRAVELING THE ROLE OF FORMALDEHYDE-INDUCED STRESS ON XERODERMA PIGMENTOSUM AND TRICHOTHIODYSTROPHY PATIENT-DERIVED CELLS

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Abstract:
The genome faces continuous challenges from several internal and external agents capable of inducing damage. A subset of individuals suffering from DNA repair disorders, including xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD), exhibit symptoms of neurodegeneration and premature aging features. The underlying causes of this neurological phenotype are believed to be related to transcription-coupled DNA repair stress. Since neurons are not directly exposed to ultraviolet sunlight, endogenous compounds emerge as potential candidates for triggering this condition. Recent research have shed light on the significance of the formaldehyde-detoxifying enzymes ALDH2 and ADH5 as an initial defense mechanism against the genomic threat posed by this molecule, and possibly by other aldehydes. In the present study, we aim to assess the effects of formaldehyde (FA) on fibroblasts derived from patients diagnosed with TTD and XP with CS (XPCS). Those patients carry mutations in the *XPD* gene encoding a helicase involved in both transcription and nucleotide excision repair (NER) processes. Our preliminary results using these cell lines, along with isogenic complemented fibroblasts serving as wild-type controls, indicate an increased sensitivity to FA. Increasing doses of FA (10-80 µM) exhibited a dose-dependent reduction in colony formation after 7 days of treatment in XPCS and TTD cells. Furthermore, the XTT viability assay conducted 72 h after 2 h of exposure, or 72 h of exposure, to increasing concentrations of FA (60-240 µM) revealed a notable decline in cell viability, especially on 72 h of exposure. Comet assay performed 0, 4, and 24 h after 2 h treatment with 160 µM FA demonstrated a time-dependent increase in DNA breaks in both TTD and XPCS cells. Additionally, the same dose disrupted the cell cycle, leading to an augmented fraction of G2 cells. These observations highlight the heightened sensitivity of NER-deficient cells to FA. Currently, we are investigating the impact of glutathione synthesis inhibition, a co-substrate of ADH5 in FA detoxification, and generating patient-derived fibroblasts with knockouts for *ADH5* and *ALDH2*. We hope the results of this work could contribute to a deeper understanding of the underlying biology of neurodegenerative conditions and early aging associated with NER deficiencies, shedding light on the intricate interplay between FA-induced stress and its metabolism on such diseases.
Keywords: Formaldehyde; NER; XPD; xeroderma pigmentosum; trichothiodystrophy

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FAPESP

PHOSPHORYLATION DYNAMICS OF DNA DAMAGE RESPONSE (DDR) PATHWAY IS ORCHESTRATED IN A TIME-DEPENDENT MANNER DURING *T. CRUZI* INFECTION IN NON-PHAGOCYTIC CELLS

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Abstract:
Intracellular pathogens, such as viruses, bacteria, fungi, and protozoa, often activate the DNA damage response (DDR) pathway in host cells. *Trypanosoma cruzi*, the etiological agent of Chagas disease affects ~8 million individuals worldwide, with ~70 million living at risk of infection due to the change in migratory dynamics in recent years due to the global economic crisis. During infection, *T. cruzi* alters different signaling pathways in host cells, including inhibition of apoptosis, induction of senescence, inflammatory response, alteration of the cytoskeleton, and inhibition of proliferation of infected cells. We recently demonstrated that *T. cruzi* infection modulates transcription and splicing machinery in host cells, causing alterations in the nuclear compartments. We wondered whether this interaction between *T. cruzi* and the host cell nucleus could affect the integrity of the host cell DNA. In response to DNA damage, LLC-MK2 cells infected by *T. cruzi* showed the maximum phosphorylation of H2AX occurs between 2 and 4 hours post infection (hpi), as analyzed by immunofluorescence and western blotting ($p<0.001$). Analysis by comet assay presented a high number of DNA breaks at 12 hpi ($p<0.001$). Throughout the infection, γ53BP1 protein and γATM kinase remained active ($p<0.001$), while γDNA-PKcs kinase had maximum activity at 12 hpi ($p<0.001$). In contrast, γATR and γRad50 kinases were not altered during the evaluated period suggesting that *T cruzi* infection induce double-strand breaks - DSB in the host cells. Our data showed that *T. cruzi* induces host DNA damage orchestrated by phosphorylation dynamics in a time-dependent manner. In this time, the non-homologous end junction (NHEJ) repair pathway may be activated to maintain genome integrity.
Keywords: DNA damage response pathway; Trypanosoma cruzi infection; Host cell nucleus; Host-parasite interaction.

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INTERPLAY BETWEEN BASE ALKYLATION, AAG DNA GLYCOSYLASE AND MITOCHONDRIAL DNA EXPRESSION REGULATION

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Abstract:

Living organisms face constant exposure to agents that can damage their DNA, particularly through base modifications. Mitochondrial DNA (mtDNA) may be especially vulnerable due to its location. The primary repair mechanism for mtDNA is base excision repair (BER), which deals with abasic sites, single strand breaks, and base modifications like oxidations and alkylation. BER starts with a DNA glycosylase recognizing and removing the modified base. We demonstrated earlier that the alkyladenine DNA glycosylase (AAG) is involved in repair of alkylated bases in mammalian mitochondria. Notably, compacting DNA into chromatin can hinder BER by limiting access to damage, so it could be speculated that BER would be associated with processes that require chromatin remodeling such as replication and transcription. Although there is still no clearly characterized transcription coupled BER, growing evidence indicates an interaction between DNA glycosylases, transcription factors, and proteins of the transcription machinery. This interaction results in the co-regulation of gene expression and preferential repair of lesions in transcriptionally active regions. Additionally, DNA glycosylases have expanded roles beyond repair, including the removal of epigenetic modifications that regulate transcription. While most evidence of the BER-transcription interaction pertains to nuclear DNA, the question arises as to whether similar interactions occur in mitochondria. Since the regulatory mechanisms of mtDNA expression remain poorly understood and the BER pathway is vital for this organelle, our current study aims to explore the potential reciprocal interaction between mtBER and mtDNA transcription. Specifically, we investigate whether alkylation damage to mtDNA affects mitochondrial gene expression. Initial results indicate that exposure to the alkylating agent MMS did not have significant impact on mitochondrial mRNA levels in 143B and HeLa cell lines, although it does not exclude the possibility of mtBER and mt-transcription interactions. Additionally, we will examine whether components of the mtBER pathway, especially AAG, can interact with the mitochondrial transcription machinery, including TFB2M, POLRMT, TEFM, and mTERF1. Finally, we will assess whether the functional interplay between BER and transcription can modulate mtDNA repair. The characterization of this functional interaction is essential for a better understanding of the mechanisms that maintain mtDNA stability.

Keywords: mitochondria; DNA repair; AAG; alkylation damage; transcription

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THE ROLE OF MITOCHONDRIAL TRANSCRIPTION FACTOR A (TFAM) IN PROTECTING DNA FROM OXIDATIVE DAMAGE

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Abstract:
Mitochondrial Transcription Factor A (TFAM) is a DNA binding protein that is involved in mtDNA maintenance through its role in mtDNA transcription, translation and copy number regulation. TFAM is also the primary packaging factor in mtDNA nucleoids, playing the same role as histones do in nuclear DNA. TFAM is able package the mtDNA into nucleoids through non-specific DNA binding, bending of the DNA strand, cooperative binding and cross-strand dimerization. Through these interactions with mtDNA, TFAM molecules are able to fully coat and completely package the mtDNA into nucleoids. Since the mtDNA is contained within the mitochondrial matrix it is susceptible to DNA damage by oxidants which are a byproduct of oxidative phosphorylation. In this study we investigated the role of TFAM in protecting mtDNA from oxidative damage. Incision assays in vitro showed that in the presence of TFAM, DNA suffered less oxidative damage. Preliminary experiments in HEK293T WT and TFAM KD cells also showed a decrease in survival in cells expressing less TFAM after treatment with damaging agents. This data suggests that TFAM does play a role in protecting the mtDNA from damage. Conversely, DNA binding to mtDNA could also inhibit DNA repair and maintenance, as previous results have shown that TFAM binding to DNA could block access to DNA repair proteins. This study seeks to continue to research the role of TFAM in protecting the mtDNA from damage and how it modulates binding to allow access to repair proteins, allowing for the regulation of mtDNA integrity.
Keywords: Mitochondrial DNA Repair; Mitochondrial DNA Maintenance; TFAM; Nucleoids.

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EVALUATING THE EFFECTS OF THE B-AMYLOID PEPTIDE (1-42) ON DNA DAMAGE AND REPAIR IN HUMAN NEUROBLASTOMA CELLS

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Abstract:
Alzheimer's disease (AD) is the main type of dementia that affects the elderly. With increasing life expectancy, the number of people who develop AD has increased annually, with an estimated 153 million affected people by 2050. While some causative mutations have been associated with the familial AD, both AD forms, familial and sporadic, show accumulation of DNA damage, in both nuclear (nuDNA) and mitochondrial (mtDNA) genomes, with disease progression, suggesting that DNA damage is a key event in AD pathophysiology. Deposits of amyloid beta peptide (Aβ) aggregates are one of the main hallmarks of the disease and there is evidence that Aβ has a mechanistic role in the development of the pathology through inducing oxidative stress. Considering that oxidative DNA damage in mainly repaired by the Base Excision Repair (BER) pathway, in the present study investigated the impact of Aβ on nuclear and mitochondrial DNA stability and the response to damage via the BER pathway. The treatment was performed in SH-SY5Y cells with Aβ 1-42 (24h and 48h) and H_2O_2 (24h) and DNA and RNA were extracted from recovered cells. The mRNA levels of enzymes participating in the BER pathway (NEIL1, POLB, OGG1, UDG, LIG3, APE1, FEN1, AAG) were determined by qRT-PCR and the measurement of nuDNA and mtDNA damage was performed by Long-extension PCR (XL-PCR). The results were submitted to One-way analysis of variance (ANOVA), using the GraphPad Prism software, and subsequently submitted to the Tukey test, adopting the significance of $p < 0.05$. The results showed a decrease in the expression of all BER genes evaluated in both treatments when compared to the untreated control. DNA damage analysis by XL-PCR showed that Ab treatment induced significant nuDNA but not mtDNA damage, when compared to the control, suggesting that the mtDNA might be protected from Aβ toxicity.
Keywords: Alzheimer disease; amyloid beta; mitochondria; DNA damage; DNA repair

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THE ROLE OF TCCSB IN MITOCHONDRIAL DNA REPAIR ASSOCIATED WITH TRANSCRIPTION IN *TRYPANOSOMA CRUZI*

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Abstract:

Trypanosoma cruzi is the etiological agent of Chagas disease, and it has a unique mitochondria, which makes it unique among eukaryotes. In the organisms studied so far, the presence of the nucleotide excision repair pathway in this organelle has not yet been characterized. In this study, we investigated the role of CSB, a gene involved in transcription-associated nucleotide excision repair. Cells modified for the CSB gene (single knockout or overexpressing) were exposed to Doxorubicin (directed to mitochondria - Mt-DOX, or nucleus - Dox) and cisplatin (directed to both organelles). After exposure to 16µM of Mt-Dox, TcCSB-deficient cells showed increased sensitivity 1 hour after treatment compared to the wild-type cells, but resumed their growth after 24 hours. On the other hand, TcCSB overexpressing cells exhibited increased resistance 1 hour after treatment compared to WT cells, and their growth was halted. After treatment with 16µM of Doxorubicin, which targets the nuclear DNA, it was observed that the TcCSB-overexpressing cells were more sensitive and the TcCSB-deficient mutant was more resistant 1 hour after treatment, although the former resumed their growth more quickly. When treated with 300µM of cisplatin, the same response as with Dox was observed. We then analyzed the repair kinetics with cisplatin and found that the TcCSB-overexpressing cells repaired the mitochondrial damage more rapidly, while the TcCSB-deficient cells had slower repair. We also evaluated changes in mitochondrial transcription after cisplatin treatment and observed that the TcCSB-overexpressing cells showed a decrease in transcripts immediately after treatment compared to WT and TcCSB-deficient cells. After 1 hour of treatment, the overexpressing cells had already recovered the transcript levels, while the levels continued to decline in the other strains, with a more pronounced decrease in the TcCSB-deficient strains. These results suggest the involvement of TcCSB in mitochondrial repair, and its absence somehow impairs the metabolism of kDNA in *T. cruzi*. The data show that unlike all organisms studied so far, *T. cruzi* has a DNA repair pathway associated with transcription, which may be related to the fact that it has only one mitochondrion

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Toxicogenomic and Bioinformatic

A LAWSONE SILVER SALT ACTIVE AGAINST *SPOROTHRIX* SPP. INDUCES DNA DAMAGE IN HEPG2 CELLS AND DIFERENCIAL CYTOTOXICITY IN BALB/3T3 CELLS IN MONOLAYER AND THREE-DIMENSIONS (3D)

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Abstract:
Sporotrichosis is the most frequent subcutaneous mycosis in Latin America, caused by fungus of the genus *Sporothrix*. As a neglected disease, treatment options are restricted and compromised by extensive duration, cost, side effects and emergence of resistant isolates. We previously synthesized 1,4-naphthoquinone derivatives and identified a lawsone silver salt (SL) able to inhibit *S. brasiliensis* and *S. schenckii* growth and biofilm formation. Therefore, we aimed to investigate its safety through *in silico* pharmacokinetics, genotoxicity and differential cytotoxicity studies. Pharmacokinetics and toxicity properties were predicted on pkCSM and SwissADME *in silico* platforms. Mutagenicity was assessed by the Ames Test on five *Salmonella* Typhimurium strains (TA1535, TA97a, TA98, TA100, TA102), in the absence and presence of metabolic activation. Genotoxicity was evaluated by *in vitro* micronucleus assay in HepG2 cells. Cytotoxicity was determined by WST-1 and LDH assays using BALB/3T3 fibroblasts cultured in monolayer (2D) and in three-dimensions (3D), applying cell spheroids based on alginate scaffolds. Hepatocytotoxicity was assessed on F C3H and HepG2 cells. *In silico* analyses suggested a good drug-likeness, lead-likeness and bioavailability profile. Furthermore, SL was considered permeable to the blood-brain barrier, indicating possible applications in brain fungal infections. SL was predicted to be a CYP1A2 inhibitor, related to potential drug interactions, although in terms of toxicity, SL was not predicted to be hepatotoxic, cardiotoxic, or mutagenic. Confirming the predictions, SL was not mutagenic in the Ames test, on the other hand, it induced a significant increase in micronucleus formation at the highest concentration tested (500 µM) in HepG2 cells. In both types of cell death assays, SL induced greater toxicity in BALB/3T3 cells cultured in monolayer than in 3D. At 24, 48 and 72h, 2D half-maximal cytotoxic concentration (CC_{50}) ranged from 2.9 to 24.5 µM, while 3D CC_{50} ranged from 197.5 to >5000 µM. Considering SL half-maximal inhibitory concentration (IC_{50}) of 1 µM in 48h for *Sporothrix* spp., selectivity indices of 8.9-12.9 for 2D and 2,019->5,000 for 3D were calculated for WST and LDH, respectively. In hepatocytotoxicity assays, F C3H liver fibroblasts were more sensitive to SL than HepG2 cells in both assays. F C3H CC_{50} ranged from 0.4 to 1,715 µM, while HepG2 CC_{50} ranged from 52.1 to >5,000 µM. SL reduced the viability of HepG2 cells in monolayer at higher concentrations than those observed for BALB/3T3 and F C3H, suggesting that the metabolic capacity of the cell line interferes the cytotoxicity profile of the lawsonate. In general, the three cell lines were more sensitive to cell membrane damage, evidenced by LDH release, than to mitochondrial dysfunction, suggested by WST. These two damage mechanisms were also observed in the fungistatic action of SL, previously described by our group. *In vitro* clastogenicity is often related to 1,4-naphthoquinones, however, this dataset indicates that SL genotoxic and cytotoxic concentrations were considerably higher than the inhibitory concentration for *Sporothrix*, allowing a therapeutic window applicable to *in vivo* investigations of this promising antifungal agent.
Keywords: Sporotrichosis; 1,4-naphthoquinone; Lawsone; 3D cell culture; DNA damage

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APPLICATION OF UNSUPERVISED MACHINE LEARNING IN CLUSTER ANALYSIS OF GENOMIC INSTABILITY IN WORKERS EXPOSED TO PESTICIDES

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Abstract:
In Brazil there is an increase in the consumption of pesticides, these substances cause several effects on the health of exposed workers, including cancer. The aim of this study was to perform an exploratory analysis of epidemiological and molecular data collected from exposed and non-exposed workers to identify genomic instability patterns through micronuclei, telomere length, and mitochondrial DNA copy number. The dataset includes epidemiological and molecular data from groups of individuals both exposed and non-exposed to pesticides. A total of 154 samples were collected from participants in Barretos, Colombia, and Cajobi, in the state of São Paulo, Brazil, between November 2017 and January 2019. After preprocessing and data cleaning, the dataset was reduced to 135 participants with 64 (characteristics) attributes, divided into 67 non-exposed and 68 exposed individuals. The data were organized into several groups, using the attributes such as sociodemographic, pesticide, anthropometric, behavioral, occupational, health, and genomic instability. Descriptive analysis of the data was conducted, and data projection techniques, such as Principal Component Analysis (PCA) and Interactive Document Map (IDMAP), were employed for explore patterns. Unsupervised machine learning algorithms, including K-means, Agglomerative Hierarchical Clustering, and Spectral Clustering, were used for clustering analysis. The algorithms and the number of clusters were evaluated using the Average Silhouette Width (ASW). The data projections using PCA and IDMAP did not show satisfactory results. The best clustering results were obtained from the behavioral group, specifically concerning tobacco and alcohol use, with an ASW of 0.85 using K-means with 3 clusters. Descriptive analysis showed that each cluster (zero, one, and two) is comprised by 58, 34, and 43 participants, predominantly male (91.40%, 76.5%, and 83.70%, respectively). The mean ages and standard deviations for the clusters were 47±10, 48±11, and 50±10 years. About tobacco there were 58, 34 and 0 non-smokers in each cluster (zero, one and two). Only cluster two had 43 smokers. Most of the exposed participants belong to cluster two 62%, compared to clusters zero and one (39.7% and 52.9%). The mean values and standard deviations of telomere length were 46.27±23.04 and 39.55±20.224 kb/diploid genome for clusters zero and one, and 48.84±34.93 kb/diploid genome for cluster two. Participants who smoked and were exposed to pesticides with an average age of 50 years had high telomere values, compared to other clusters. However, other instabilities, such as micronuclei and the number of mitochondrial DNA copies, showed no significant differences between the clusters. In conclusion, the results suggest that the use of machine learning with clustering algorithms shows promise in recognizing patterns of genomic instability in individuals exposed to pesticides.
Keywords: Pesticides; K-means; Machine Learning; Genomic Instability; Unsupervised

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GENOMIC INSIGHTS OF MARINE BACTERIA IN ANTARCTICA'S EXTREME ENVIRONMENTS

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Abstract:
The Antarctic continent keeps several extreme environmental conditions and specific isolation from the effects of human society. In that place, the genetic material of the organisms is under constant aggression, with various agents that damage the DNA molecule, including the ultraviolet radiation (UV) from sunlight. Other environmental conditions can also challenge the maintenance of DNA stability, such as the very low temperatures, making Antarctica an interesting place to study how organisms maintain genome integrity. Therefore, in this work, we intend to explore mechanisms that microorganisms have to survive to extreme conditions from different environments of that continent. To do that, we have sequenced the genomes of three Antarctic marine bacteria, two isolated from sediment samples from a depth of 280 m and the third from 1500 m (samples obtained at the Bransfield Strait). The greater the depth conditions in the sea, the bacteria endure higher concentrations of CO_2 under higher atmospheric pressure. The DNA from these isolates was extracted and sequenced by the Illumina platform. *De novo* genome assembly was performed with *SPAdes v3.15.4.* Assembly metrics were assessed with *QUAST v5.2.0* and genome completeness was analyzed by *BUSCO v5.3.1.* Additionally, annotation was conducted using the *Prokaryotic Genome Annotation Pipeline (PGAP)* and species identification was done based on 16S ribosomal RNA (rRNA) sequence and using the software *GTDB-Tk 2.1.0.* Functional annotation was assessed by *eggNOG.* Additionally, we have analyzed the presence of DNA repair genes of these organisms using *tblastn.* Preliminary results indicate that most of the protein functions of these bacteria are unknown. Moreover, we have found that most of the DNA repair genes are present in these bacteria, pointing out that there are many ways for them to deal with DNA damage. As a perspective, we intend to do a transcriptome of the 1500 m-bacteria in two conditions: one with atmospheric pressure and no saturation of CO_2 and other with 100 atm and 100% of CO_2, to know which genes are more expressed in the last condition.
Keywords: extreme environments; Antarctica; DNA repair.

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IN SILICO ANALYSIS OF POTENTIAL CANNABINOIDS TARGETS AS TREATMENT FOR AUTISM SPECTRUM DISORDERS THROUGH MOLECULAR DOCKING

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Abstract:
Autistic Spectrum Disorder (ASD) is a neurobiological disorder that is characterized by the presence of restricted interests, stereotyped behaviors, and changes in social and communication skills. Pharmacological treatment is routinely used for the symptomatic treatment of ASD. Currently, two medications are approved for the treatment of ASD, Risperidone and Aripiprazole, but studies have shown significant side effects. Thus, the development of new drug/receptor interactions is necessary to improve the management of these patients. The objective of this study was to evaluate the interaction between Cannabigerol (CBG) and Cannabidiol (CBD) with the main target receptors of drugs already used in the treatment of ASD. In this survey, the protein receptors that act in the central nervous system and that have higher interaction and affinity with Risperidone and Aripiprazole were selected. To obtain the ligands files, ZINC database was used. Human and native receptor structures were selected from PDB. The modeling was performed using the Swiss-Model platform and validated by the MolProbity platform. The target receptors of Risperidone and Aripiprazole were selected and molecular docking of these receptors with CBG, CBD and the synthetic dexanabinol was carried out using the AutoDock software. In silico analyzes of drug-protein complex interactions and amino acid residues involved in the interactions were performed using PyMol software. CBD and dexanabinol had their toxicity analyzed using Pass online software. The selected receptors were 5HT1A, 5HT2C, D2, D3, CB1 and CB2. Such receptors are involved in functions related to the serotonergic, dopaminergic and endocannabinoid systems. The highest binding affinity energies and the highest probability of presenting therapeutic efficacy found here are related to the receptor/ligand complexes: 5HT2C with Risperidone and CBD; CB1 with CBG and CBD; D2 with Aripiprazole and CBG. Dexanabinol showed higher favorable affinity energies for dexanabinol/CB1 complex. Toxicologic analysis revealed possible adverse and toxic effects, including irritation and euphoria for CBD, as well as sleep disturbance and drowsiness for dexabinol. Such results reinforce the potential use of CBG and CBD compounds for the treatment of ASD-related behaviors, including anxiety, hyperactivity, and repetitive movements. The affinity of CBG and CBD complexes are even better than the ones observed for Aripripazole and Risperidone, promoting less toxicity and lower side effects than the actual clinical treatment proposed for autism.
Keywords: cannabidiol; autism; molecular docking; bioinformatics.

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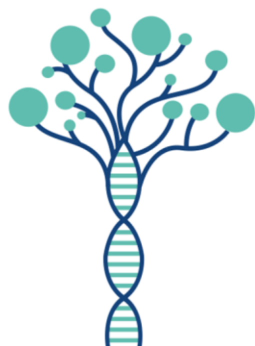

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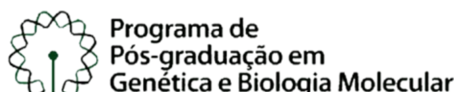

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