Plan Overview

Title: Evaluation of the effects of natural products niga-ichigosideo F1 and 2β, 3β, 19α-trihydroxyursolic acid on human cells: cytotoxicity, genotoxicity, cell cycle monitoring, induction of apoptosis and gene expression analysis.

Creator: Edson Maistro

Affiliation: São Paulo State University (unesp.br)

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Project abstract:
The Rubus imperialis (Rosaceae) plant occurs in abundance in the southern region of Brazil, where it is known by the popular names of amora-branca, amora-do-mato or amora-brava. This plant is often used in folk medicine for the treatment of diabetes, and for its gastroprotective, anti-inflammatory and healing effects. Previous studies on the aerial parts extract of this plant have shown clastogenic/aneugenic effects in mouse cells after administration of higher doses. The niga-ichigoside F1 and 2β, 3β, 19α-trihydroxyursolic acid are two of the major compounds found in the R. imperialis extract. Niga-ichigoside F1 has been shown to have a potent analgesic and gastroprotective effect, and 2β, 3β, 19α-trihydroxyursolic acid has a potent antiulcerogenic effect. Considering the therapeutic potential and the observation of genetic toxicity of R. imperialis extract, it is essential to investigate whether the two major compounds found in this extract are related to the observed toxicity. In view of the above, this research project aims to identify the potential cytotoxic and genotoxic effects of niga-ichigoside F1 and 2β, 3β, 19α-trihydroxyursolic acid in HepG2/C3A cells (with active liver metabolizing enzymes) and human leukocytes, and the effects of these compounds on the progression of the cell cycle, as well on the induction of apoptosis in HepG2/C3A cells (human hepatoma). It also aims to investigate the possible mechanism of action of the exposure of these liver cells to the respective compounds, by analyzing the expression of some of the main genes of biochemical pathways for xenobiotic metabolism, DNA damage repair, cell cycle and apoptosis. For this, the first stage of the work will seek to observe the cytotoxicity/cell viability effects of both compounds, by the MTT test and trypan blue staining, genotoxicity by the comet and micronucleus tests, and effects on the cell cycle progression and induction of apoptosis, by flow cytometry. Once these results have been obtained, the second stage of the research will cover, through real-time RTq-PCR analysis, the investigation of the relative expression of some of the main genes involved in the above mentioned processes, which have the potential to allow understanding of mechanisms of action of test substances on human cells in culture. The development of this project will allow the training of qualified human resources at master's and scientific initiation level, and will allow the continuity of the development of several other projects for graduate and undergraduate students at the Laboratory of Toxicological Genetics at UNESP in Marília.

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Evaluation of the effects of natural products niga-ichigosideo F1 and 2β, 3β, 19α-trihydroxyursolic acid on human cells: cytotoxicity, genotoxicity, cell cycle monitoring, induction of apoptosis and gene expression analysis.

Data Collection

What data will you collect or create?
Data will be obtained from the cultured lymphocytes and HepG2/C3A human cells treated with different concentrations of both test compounds: niga-ichigoside F1 and 2β, 3β, 19α-trihydroxyursolic acid. The data will be shown the effects of these compounds in the context of cytotoxicity, genotoxicity, (with active liver metabolizing enzymes) and human leukocytes, and the effects of these compounds on the progression of the cell cycle, induction of apoptosis, and gene expression in HepG2/C3A cells (human hepatoma).
All data collected will be in tables and laboratory chains.

How will the data be collected or created?
The data will be collected from cultured human cells (lymphocytes and HepG2/C3A) exposed to both test compounds.

Documentation and Metadata

What documentation and metadata will accompany the data?
Graphics, figures, and tables.

Ethics and Legal Compliance

How will you manage any ethical issues?
All procedures were approved by the Human Ethics Committee of Animal of the Faculty of Philosophy and Sciences, UNESP (protocol number CAAE: 18800419.8.0000.5406).

How will you manage copyright and Intellectual Property Rights (IP/IPR) issues?
As a result of this project, articles will be published. Copyright will be determined jointly with the members of our research group.

Storage and Backup

How will the data be stored and backed up during the research?
Data will be backed up with copies in hard drives in laboratory computers, as well as the university offers google Drive, which assists in data storage.

How will you manage access and security?
All researchers involved in the project will have access to the data, but only the researcher responsible and coordinator will edit and update the backup.

Selection and Preservation

Which data are of long-term value and should be retained, shared, and/or preserved?
The data will be shared with the scientific community through publications. In addition, they will serve to originate future projects.

What is the long-term preservation plan for the dataset?
We store these data in drives of laboratory and laboratory chains.
Data Sharing

How will you share the data?
The data will be used for participation in a scientific congress. And, after a great understanding of the results, the data will be published in the form of an article.

Are any restrictions on data sharing required?
After the publications, no they aren't.

Responsibilities and Resources

Who will be responsible for data management?
The data acquired during the project will be managed by the responsible researcher and the project coordinator.

What resources will you require to deliver your plan?
The project will be necessary for collaboration with other researchers to collect and analyze the data obtained from human cultured cells.