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Evaluation of the genotoxicity of *Euterpe oleraceae* Mart. (Arecaceae) fruit oil (açaí), in mammalian cells *in vivo*

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ABSTRACT

E. oleracea is a tropical plant from the Amazon region, with its fruit used for food, and traditionally, as an antioxidant, anti-inflammatory, hypocholesterolemic, for atherosclerotic disease, and has anticancer properties. The oil of the fruit has antidiarrheic, anti-inflammatory and antinociceptive activities, but without genotoxicity evaluation. Therefore, the aim of this study was to evaluate the genotoxic potential of *E. oleracea* fruit oil (EOO), in rat cells. Male Wistar rats were treated with EOO by gavage at doses of 30, 100 and 300 mg/kg, for 14 days, within a 24 h interval. The DNA damage in the leukocytes, liver, bone marrow and testicular cells, was assessed by the comet assay, and the clastogenic/aneugenic effects in the bone marrow cells, by the micronucleus test. Our phytochemicals characterization of the EOO showed the presence of vanillic, palmitic, γ-linolenic, linoleic, oleic, cinnamic, caffeic, protocatechuic, ferulic, syringic acids, and flavonoids quercetin and kaempferol rutinoside as the main constituents. Both cytogenetic tests performed showed that EOO presented no significant genotoxic effects in the analyzed cells, at the three tested doses. These results indicate that, under our experimental conditions, *E. oleracea* fruit oil did not reveal genetic toxicity in rat cells.

1. Introduction

Plants have for a very long time, formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years, and continue to provide mankind with new remedies. They have gained a significant increase in popularity, as complementary and alternative medicines for the prevention and treatment of different infectious and non-infectious diseases in human therapy (Kim et al., 2015; Gurib-Fakim, 2006). According to the literature review data, natural products and their derivatives, represent 50%–80% of all the drugs in clinical use in the world (Sen and Samanta, 2015; Petrovska, 2012).

Euterpe oleracea Mart. (Arecaceae family) is a plant whose fruit is commonly known as "*açaí*". This fruit is used traditionally in the Brazilian folk medicine to treat anemia, diarrhea, malaria, pain, inflammation, hepatitis, and kidney diseases (Caetano et al., 2014; de Bem et al., 2014; Vásquez et al., 2014; Souza et al., 2011; Leão et al., 2007). Moreover, açaí is considered a dietary food supplement, in appreciation to its high content of natural antioxidant and can be considered as one of the new superfruits (Yamaguchi et al., 2015). Açaí fruits have been used as a functional food due to its nutritional benefits and therapeutic promise and, for this reason, it is being studied currently, by researchers worldwide (Bonomo Lde et al., 2014). Most of the Amazonian population consume açaí juice daily, and this makes it a fruit of great economic importance (Murrieta et al., 1999). A study by Khayat (2005) showed that, the daily intake of açaí fruit acted as a coadjuvant, to reduce the risk of coronary atherosclerotic disease in a population of the State of Pará in Brazil. Extracts from açaí fruit, induced a vasodilator effect in the rat mesenteric vascular bed, and this suggests its possible use in the treatment of cardiovascular diseases (Rocha et al., 2007). With specific regard to the açaí fruit oil, antidiarrheic action was proven (Plotkin and Balick, 1984) and Favacho et al. (2011) reported anti-

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inflammatory and antinociceptive activities.

Considering the fact that genetic toxicity is a crucial endpoint in the safe testing of plants as it addresses potential mutagenicity, which has implications for risks of both genetic disease and cancer; and that to the best of our knowledge, there are no data in the literature about the genetic toxicity of *E. oleracea* fruit oil, the aim of this present study was to investigate the genotoxic and clastogenic/aneugenic potential of this oil in different rat cells, using the comet and micronucleus assays, respectively. Concomitantly, the main chemicals present in this oil were determined.

2. Material and methods

2.1. Chemicals

Doxorubicin (DXR, Oncodox®, Meizler) was used as the DNA damage agent in the comet and micronucleus assays, and was prepared by dissolving it in sterile water. The other main chemicals were obtained from the following suppliers: Normal Melting Point (NMP) agarose (Cat. No. 15510–019: Invitrogen) Low Melting Point (LMP) agarose (Cat. No. 15517–014: Invitrogen), sodium salt *N*-lauroyl sarcosine (L-5125: Sigma) and ethylenediaminetetraacetic acid (EDTA) (Merck). EOO was dissolved in Tween 80.

2.2. Plant material

The *E. oleracea* Mart. (Arecaceae family) fruit oil (EOO) was kindly provided by the company Açaí do Amapá Agro-Industrial Ltda Sambazon, located in the city of Macapá, in Amapá State, Brazil. The extraction method consisted of a standardized method used by the company, which cannot be published, because of patent protection.

2.3. Phytochemical analysis

The Açaí fruit oil was dissolved in methanol:water (1:1) with 0.1% formic acid for reading in a positive mode and in methanol:-water solution (1:1) with 0.1% ammonium hydroxide to read in the negative mode. The solutions were directly infused into ESI in the Mass Spectrometer (MS). The ESI-MS spectra and ESI-MS/MS were acquired using positive ion mode for the acidic solution, and negative ion mode for the alkaline solution.

The chromatographic separation was performed using an Acquity UPLC BEH hybridizes column (Ethylene bridged hybrid) C18 with dimensions of 50 mm × 2.1 mm and particle size of 1.7 μm at room temperature. The mobile phase consisted of methanol:formic acid 0.1% 65/35 v/v with flow of 0.7 mL.min^{-1} and injection volume of 5 μL.

The phytochemical analyses were performed, using an Ultra Performance Liquid Chromatography (UPLC) Waters, Acquity model, coupled to a mass spectrometer TQD model, Waters, with ionization electrospray source and mass analyzer type triple quadrupole -TDQ. To perform the sequential mass analysis, argonium was used as collision gas. The Masslynx software was used for data acquisition and processing. Analyses were initially monitored in full-scan mode of mass detector and then the desired signals were selected for collision induced dissociation.

2.4. Animals and dosing

Experiments were carried out in 4–5 weeks old male Wistar rats, weighing about 100 g–120 g. The animals were acquired from the animal's house of the Universidade Estadual Paulista (UNESP), Botucatu, São Paulo state, Brazil, and kept in polyethylene boxes, in a climate controlled environment (22 ± 4 °C, 55 ± 5% of relative humidity) with a 12 h light–dark cycle (7:00 a.m. to 7:00 p.m.). Food (NUVILAB CR1-NUVITAL) and water were available *ad libitum*. The animals were divided into five experimental groups, with six animals in each group. The EOO (30 mg/kg, 100 mg/kg or 300 mg/kg b.w.) was diluted in vehicle (1% Tween 80) and administered by gavage daily for 14 consecutive days, at 24 h interval. In this procedure, each animal was weighed individually and then the calculated dose, was solubilized in 0.4 mL of the vehicle being administered. These doses were selected based on its traditional use in Brazil (25 ml–30 ml daily) (http://beneficiosnaturais.com.br/oleo-de-acai-beneficios-e-propriedades/), and also, on our preliminary acute toxicity studies in rats. At the dose of 300 mg/kg, some animals began to display signs of toxicity such as diarrhea and bristling of the hair. For this reason, higher doses were not tested. The negative control group received only vehicle by gavage, and the positive control group received an intraperitoneal injection of doxorubicin (DX R) at 16 mg/kg body weight. On the 15th day, 24 h after the administration of the last treatment, the rats were anesthetized with xylazine and ketamine (4 mg/kg b.w., i.p.), and the peripheral blood from the tail was collected to the comet assay. Immediately after this, the animals were euthanized by cervical dislocation, and the liver, bone marrow and testicle cells were collected for comet assay, and the bone marrow from another femur for micronucleus test. The Animal Bioethics Committee of the Faculdade de Medicina de Marília (CEUA/FAMEMA, Marília, São Paulo state, Brazil) approved this present study on the 31st of January, 2013 (protocol number 1659/12), in accordance with the federal government legislations on animal care.

2.5. Comet assay

The comet assay (SCGE) was carried out through the method described by Speit and Hartmann (1999), which was based on the original work of Singh et al. (1988), and includes modifications introduced by Klaude et al. (1996), as well as some additional modifications. Briefly, peripheral blood samples from the vein in the tail, and liver, bone marrow, and testicular cell samples were washed with saline solution, in an ice bath. A small portion (diameter of about 4 mm), was transferred to a Petri dish containing 1 ml of Hank's solution (pH of 7.5) and then homogenized gently with small pinches and a syringe to avoid clumps of cells. An aliquot of 20 μl was removed from the supernatant of each cell type to determine cell viability. Cell counting was performed using a hemocytometer. Cell viability was determined by trypan blue dye exclusion. The number of trypan blue-negative cells was considered as well as the number of viable cells, and it was greater than 85%. Another equal aliquot of cells from each animal was mixed with 120 μl of 0.5% low melting point agarose at 37 °C, and rapidly spread on two microscope slides per animal, precoated with 1.5% normal melting point agarose. The slides were coverslipped and allowed to gel at 4 °C for 20 min. The coverslips were gently removed, and the slides were then immersed in cold, freshly prepared lysis solution consisting of 89 ml of a stock solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH set to 10.0 with 8 g of solid NaOH, 890 ml of distilled water and 1% sodium lauryl sarcosine), plus 1 ml of Triton X-100 (Merck) and 10 ml of DMSO (Merck). The slides, which were protected from light, were allowed to stand at 4 °C for 1 h, and then placed in the gel box, positioned at the anode end, and left in a high pH (>13) electrophoresis buffer (300 mM NaOH–1 mM EDTA, prepared from a stock solution of 10 M NaOH and 200 mM, pH 10.0, EDTA) at 4 °C for 20 min, prior to electrophoresis, to allow DNA unwinding. The electrophoresis run was carried out in an ice bath (4 °C) for 20 min at 300 mA and 25 V (0.722 V cm^{-1}). The slides were then submerged in a neutralization buffer (0.4 M Tris–HCl, pH 7.5) for 15 min, dried at room