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First cytotoxic, genotoxic, and antigenotoxic assessment of *Euterpe oleracea* fruit oil (açaí) in cultured human cells

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ABSTRACT. *Euterpe oleracea* Mart., popularly known as "açaí", is a tropical fruit from the Amazon region where it has considerable economic importance. Açaí has been used as food and for several medicinal purposes. Despite the widespread use of this fruit, there is

a lack of data regarding the safety of using this fruit oil exclusively. Therefore, we evaluated the *in vitro* cytotoxic, genotoxic, and antigenotoxic effects of *E. oleracea* fruit oil (EOO) in cultured human lymphocytes (non-metabolizing cells) and HepG2 cell line (human hepatoma) (metabolizing cells) by using MTT, comet, and micronucleus assays. A wide range of EOO concentrations was tested with a preliminary MTT assay, which allowed selecting five concentrations for comet and micronucleus assays: 2.5, 10, 100, 500, and 1000 µg/mL. The results showed that none of the EOO tested concentrations presented cytotoxic effects. The genotoxic assessment revealed an absence of significant DNA and chromosome damage in human lymphocytes and HepG2 cells but did not show chemoprotection against the DNA damage induced by methyl methanesulfonate and benzo[a]pyrene, used as DNA-damaging agents.

Key words: Açaí oil; Arecaceae; Comet assay; HepG2 cells; Micronucleus test

INTRODUCTION

Cancer is still one of the most worrying human health problems, is responsible for roughly one-quarter of global deaths, as well as has the most devastating economic impact of any cause death in the world. The interventions for cancer prevention and control are done through policies of the primary prevention, early detection, diagnosis, treatment, and palliative care (WHO, 2002; UICC, 2008; Rocco et al., 2015).

Considering that mutational events are involved in the first steps of carcinogenesis, if the damage is removed by repair enzymes before it can interfere with the process of DNA replication and introduce mutations, it will decrease cancer incidence (Bauer et al., 2015). The scientific community has been focusing on the study of compounds in the diet that may protect DNA, not forgetting the obligatory assessment of the genotoxic potential of these compounds.

Euterpe oleracea Mart. is a plant from Arecaceae family and its fruit is commonly known as "açaí" or "açaí berry". The açaí fruit has a vital economic importance in the South America, being commercialized as frozen pulp, juice, or wine (Murrieta et al., 1999). This fruit is also used in folk Brazilian medicine to treat anemia, diarrhea, malaria, pain, inflammation, hepatitis, and kidney diseases (Leão et al., 2007; Souza et al., 2011; Caetano et al., 2014; de Bem et al., 2014; Vásquez et al., 2014). Specific açaí fruit oil therapeutic potentials regarding anti-inflammatory, antinociceptive, and antidiarrheic activities were reported (Plotkin and Balick, 1984; Favacho et al., 2011).

Our previous chemical characterization of the açaí fruit oil, used in the present study, has shown the presence of vanillic, palmitic, γ-linolenic, linoleic, oleic, cinnamic, caffeic, protocatechuic, ferulic, and syringic acids, and the flavonoids quercetin and kaempferol rutinoside as main constituents (Marques et al., 2016). Favacho et al. (2011) also reported the presence of the fatty acids, oleic, palmitic, and palmitoleic compounds as the major constituents of the açaí fruit oil. According to Del Pozo-Insfran et al. (2004), the presence of bioactive substances, such as phenolic acids, flavonoids, and anthocyanins, attributes antioxidant properties and antitumorigenic potential to this fruit.

Considering the significant economic importance of *E. oleracea* fruit as a functional food, its phytotherapeutic potential, and lack of studies involving the genetic toxicity exclusively of the fruit oil in human genetic material, as well as its potential for chemoprotection against DNA-damaging agents, the aim of this study was to investigate the cytotoxic, genotoxic, and antigenotoxic effects of *E. oleracea* fruit oil (EOO) in human lymphocytes and HepG2 cells through the thiazolyl blue tetrazolium bromide (MTT), comet assay, and cytokinesis-block micronucleus (CBMN) assays.

MATERIAL AND METHODS

Plant material

The EOO (Arecaceae family) was kindly provided by the Açaí do Amapá Agro-Industrial Ltda. (Sambazon), Macapá city, Amapá State, Brazil. The extraction method consisted of a standardized method used by the company, which cannot be published, because of patent protection. The phytochemical characterization of the oil was performed by our research group and published in Marques et al. (2016).

Chemical agents

Dulbecco's modified Eagle's medium (DMEM) (Gibco) was used with HepG2 cells, and RPMI-1640 medium (Gibco) was used with human lymphocytes. Methyl methanesulfonate (MMS) (Aldrich, CAS number 66-27-3) and benzo[a]pyrene (BaP) (Sigma-Aldrich) were used as the positive control, due to its potential for DNA damage, recognizable in the comet and micronucleus assays. The other main chemicals were obtained from the following suppliers: trypsin (Sigma-Aldrich), phytohemagglutinin (PHA) (Sigma-Aldrich), cytochalasin-B (Sigma-Aldrich), normal melting point (NMP) agarose (Invitrogen), low melting point (LMP) agarose (Invitrogen), fetal calf serum (Gibco), ethylenediaminetetraacetic acid (EDTA) (Merck), Triton X-100 (J.T. Baker), trypan blue (Sigma-Aldrich), MTT/ethidium bromide (Sigma), dimethyl sulfoxide (DMSO) (Sigma-Aldrich), Giemsa (Synth), and Histopaque-1077 (Sigma-Aldrich). EOO was dissolved in 1% DMSO.

Culture of human cells

In this study, human peripheral blood lymphocytes (PBL) (non-metabolizing cells) collected from 3 healthy (2 males and 1 female) non-smoking volunteers aged 18 to 27 years old, and a human hepatoma cell line (HepG2) having phase 1 and 2 enzymes (metabolizing cells) (Hewitt and Hewitt, 2004) were used. HepG2 cells were obtained from the Cell Bank of Rio de Janeiro, Brazil. Donors of PBL provided written informed consent at the time of donation for the use of their blood sample in this study. The Ethics Committee of the Universidade Estadual Paulista (UNESP), in Marília town, Brazil, approved the present study in December 2012 (protocol No. 0603/2012).

The PBL were cultivated in RPMI (Roswell Park Memorial Institute) culture medium, at 37°C, 5% CO_2, and 95% relative humidity and the HepG2 cells were cultivated in DMEM supplemented with 10% fetal bovine serum (Gibco) and antibiotics (Gibco), at 37°C, 5% CO_2, and 95% air in a humidified incubator.

MTT assay

The MTT test (CAS No. 298-93-1; Sigma) was performed with leukocytes and HepG2 cells, according to the protocol of Mosmann (1983), with some modifications. In each well of a 96-well plate, 2 x 10^4 cells were seeded. After a period of 24 h for cell stabilization, the medium was removed and 200 μL culture medium plus 1% DMSO (without serum) was added to the control and culture medium without serum plus the test mixture and 1% DMSO (diluent). The concentrations of EOO tested were 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, 800, and 1000 μg/mL. Incubation was for 4 and 24 h, then the test substance was removed, and 150 μL MTT solution (5 mg/mL) was added. The plate was incubated for 4 h, in an incubator at 37°C. After this period, the MTT solution was discarded and, in each well, 100 μL DMSO was added. The plates were then read in the spectrophotometer with a microplate reader (Epoch-Biotech) with a 540-nm filter.

Comet assay

The comet assay was performed according to Tice et al. (2000) in PBL and HepG2 cell line. First, the preparation of slides was carried out precoating with a thin uniform layer of NMP agarose. Approximately 2 x 10^5 cells per well were put on a 24-well plate and incubated for 24 h for cell attachment (in the case of HepG2). The açaí oil treatment was performed using 2.5, 10, 100, 500, and 1000 μg/mL, 150 μM positive control MMS, and the negative control was the own culture medium used in the respective cells, all incubated for 4 h. To evaluate the antigenotoxic potential of EOO, the cells were pre-incubated for 1 h at 37°C with 150 μM MMS. After this incubation period, the cells were exposed, for 4 h, to the EOO concentrations. Concentrations were selected based on the absence of toxicity in trypan blue exclusion test (data not shown) as well as MTT assay. After a 4-h treatment, cells are removed and placed with 120 μL LMP agarose on the precoating microscope slide. Slides were immersed in a vat with 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 NaOH, 890 mL distilled water, and 1% sodium lauryl sarcosine), plus 1 mL Triton X-100 (Merck) and 10 mL DMSO (Merck). Slides were left for 1 h for lysis. The slides were transferred to electrophoresis box, positioned at the anode end, and covered with alkaline buffer (pH > 13) (300 mM NaOH and 1 mM EDTA solution), where the slides remained for 20 min, for DNA denaturation followed electrophoresis in an ice bath (4°C) for 20 min at 25 V and 300 mA (0.722 V/cm). Slides were then submerged in a neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 15 min, dried at room temperature, and fixed in 100% ethyl alcohol for 10 min and stored at least for an overnight before staining. For staining, slides were rinsed in distilled water, covered with 30 μL 1X ethidium bromide staining solution, and protected with a coverslip. The material was evaluated immediately at 400X magnification using a fluorescence microscope (Olympus) with a 515-560-nm excitation filter and a 590-nm barrier filter. All experiments were performed three times and in duplicate.

The extent and distribution of DNA damage indicated by the single-cell gel electrophoresis assay were conducted by examining at least 100 randomly selected and non-overlapping cells (50 cells per coded slide) per culture well in a blind analysis. These cells were scored visually, according to tail size, into the following 4 classes: class 0 - no tail; class 1 - tail shorter than the diameter of the head (nucleus); class 2 - tail length 1 to 2 times greater than the diameter of the head; and class 3 - tail length more than twice the diameter of the

head. Comets with no heads with nearly the entire DNA in the tail or with a wide tail were excluded from the evaluation because these probably represented dead cells (Hartmann and Speit, 1997). The total score for 100 comets, which ranged from 0 (no damage) to 3 (severe damage), was obtained by the sum of multiplying the number of cells in each class by the damage class on a scale of 0-300 arbitrary units.

CBMN assay

The CBMN assay was performed according to the protocol described by Fenech (2000). Two cell types, human PBL and HepG2 cells, were used in this protocol. Experiments were carried out in human PBL from two donors, made in duplicate, and in HepG2 cells made in triplicate. Cell culture was done in a 25-cm^2 flask and added different concentrations of the tested oil (2.5, 10, 100, 500, and 1000 µg/mL). Two different drugs were used as positive controls, 150 µM MMS for the human PBL and 2 µM benzo[a]pyrene for the HepG2 cells.

Whole blood samples (0.4 mL) were added to 5 mL culture medium supplemented with 10% fetal calf serum. PHA was added to each culture flask at 10 µL/mL, and PBL were incubated at 37°C, 95% air and 5% CO_2 in a humidified incubator for 72 h. Forty-four hours after starting PBL culture, the human PBL were exposed to different concentrations (2.5, 10, 100, 500, and 1000 µg/mL) of EOO. Four hours after test compound addition, cytochalasin-B (6 µg/mL) was added to each culture. The cells were harvest by centrifugation (5 min at 850 *g*), and pellets were resuspended in a chilled hypotonic solution of 0.075 M KCl for 5 min. Subsequently, cells were washed once with 5 mL cold methanol:acetic acid solution (3:1) (v/v). The fixation procedure was applied three times. Formaldehyde (1%) was added to the last fixative to preserve the cytoplasm. The cell suspension was placed onto slides and stained with a solution of 5% Giemsa dye in phosphate buffer (pH 6.8) for 5 min.

For micronucleus test using HepG2 cells, cells had been grown for one complete cell cycle (24 h) and treated for 24 h with the test compound. Then, the cells were washed, harvested and incubated again with cytochalasin B for 28 h. After this period, the same protocol described above to PBL was performed.

Following standard criteria (Fenech, 2000), micronucleus analysis was performed on coded slides by scoring 1000 binucleated cells for each culture flask. For the analysis, an optical microscope (Zeiss, Primo Star) at 100X magnification was used. As a measure of cytotoxicity, the nuclear division index (NDI) was calculated following the formula: NDI = [M1 + 2(M2) + 3(M3) + 4(M4)] / N, where M1-M4 indicate the number of cells with 1-4 nuclei as assessed in 500 cells (N) (for each culture).

Statistical analysis

The data obtained in the comet assay and CBMN (with HepG2 cells) were submitted to analysis of variance (ANOVA) and Tukey-Kramer multiple comparison tests, and the data obtained from the CBMN assay (with lymphocytes) were analyzed by the Student *t*-test, both using the GraphPad Prism® software (version 5.02). A value of P < 0.05 was considered statistically significant for all the parameters evaluated.

RESULTS AND DISCUSSION

E. oleracea Mart. is a botanical species that is widely used as food and its fruit,

known as açaí berries, presents considerable potential for the development of new medicines, being therapeutically used by rural inhabitants especially in Amazon region (Calvazara, 1972; Rodrigues et al., 2006). Our literature review showed that there are no previous studies involving the *in vitro* assessment of the genotoxic or antigenotoxic effects of EOO.

In the present study, we used an MTT assay to evaluate the cytotoxicity of EOO. The assay is based on the fact of the tetrazolium salt (MTT) is cleaved only in living cells with active mitochondria that can be read on a scanning multiwell spectrophotometer (Mosmann, 1983). Figures 1 and 2 show the results obtained by this test using lymphocytes and HepG2 cells, respectively. The concentrations of EOO tested were 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, 800, and 1000 µg/mL, and the results after 4- and 24-h exposure to EOO showed that none of the tested concentrations induced cytotoxic effects in both cell types analyzed (cell viability was above 80%).
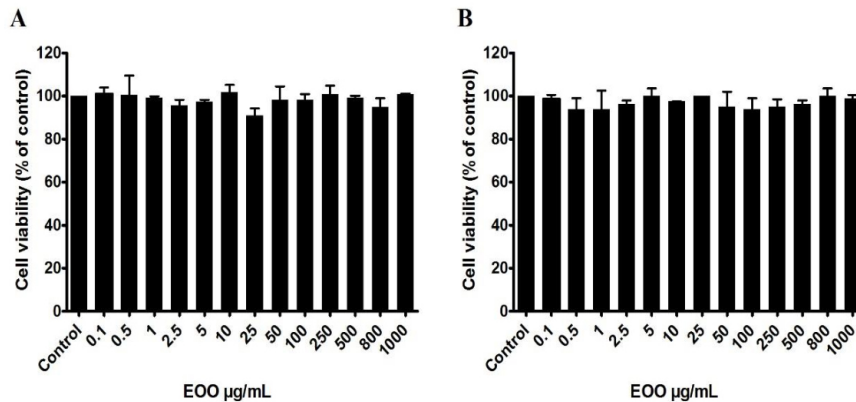

Figure 1. Cell viability based on the colorimetric MTT assay in human lymphocytes exposed to different concentrations of *Euterpe oleracea* oil (EOO) for 4 and 24 h.
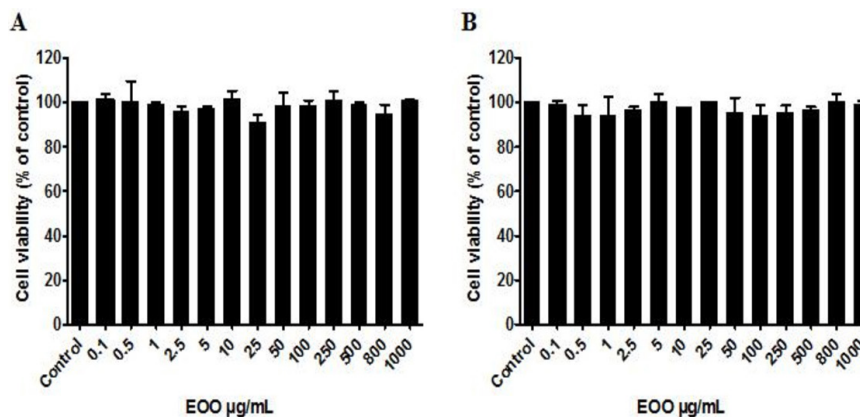

Figure 2. Cell viability based on the colorimetric MTT assay in HepG2 cells exposed to different concentrations of *Euterpe oleracea* oil (EOO) for 4 and 24 h.

Dias et al. (2014) evaluated the cell growth inhibition of açaí polyphenolic extract against colon cancer HT-29 and SW-480 cells and observed that 50-200 μg/mL of the extract inhibited the growth of SW-480 cells preferentially, without toxicity in the non-malignant CCD-18Co colon fibroblast cells. The mechanisms involved in this cell growth inhibition included downregulation of pro-oncogenic-specific proteins.

On the other hand, Silva et al. (2014) observed that bark, seed, and total açaí fruit hydroalcoholic extracts showed cytotoxicity (and antitumorigenic potential) in the human breast cancer cell line (MCF-7), but not in colorectal adenocarcinoma cells, also by using the MTT assay. The differences in the extract composition, protocols, and cell types analyzed could explain the differences in the cytotoxic effects observed in these two studies between themselves and our present study.

Choosing five concentrations of the results mentioned above with MTT assay, we assess the genotoxic and antigenotoxic property of EOO by the comet assay. The alkaline version of the comet assay detects low levels of DNA damage, including breakage of single-stranded and double-stranded DNA, alkali-labile sites, and DNA-DNA and DNA-protein crosslinks (Tice et al., 2000). The results obtained for genotoxic evaluation of EOO in human PBL and HepG2 cells are shown in Figures 3A and 4A, respectively. The cell viability of both cells at all concentrations was greater than 85% using trypan blue staining, which confirms the absence of cytotoxicity observed by the NDI in the CBMN. For the five concentrations tested, none of the two cell types tested showed a significant increase in the scores of the DNA damage compared to the negative control. A significant increase in DNA damage (scores) on both cell types was observed only for a positive control MMS, indicating the validity of this test in the detection of genotoxic effects. The antigenotoxic assessment showed a non-significant reduction in the extent of DNA damage for both cell types exposed to the five concentrations of EOO plus MMS, compared with the MMS-treated culture alone (Figures 3B and 4B), indicating the absence of chemopreventive effects of EOO against MMS.
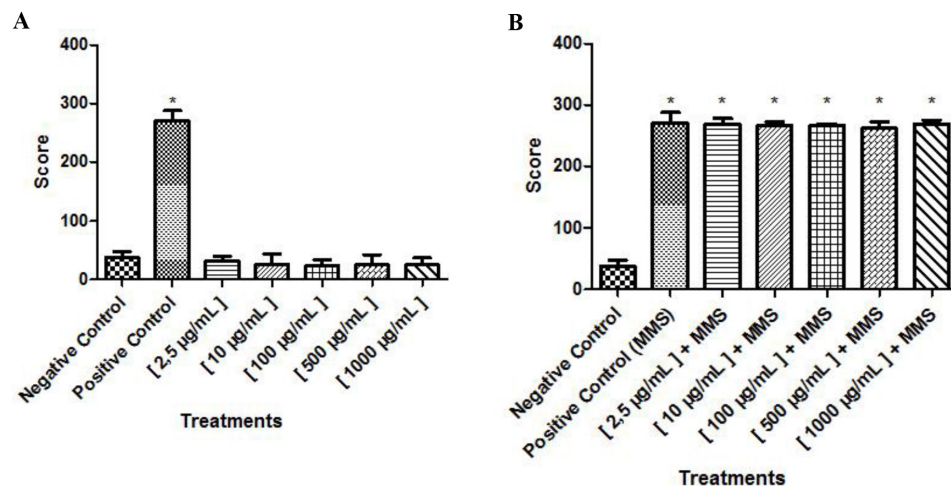

Figure 3. Genotoxicity (**A**) and antigenotoxicity (**B**) of *Euterpe oleracea* oil (EOO) in culture of human lymphocytes by comet assay. *P < 0.001 (ANOVA/Tukey post-test) when compared to negative control. Data are reported as mean values obtained from three independent experiments. Score = DNA damage index.
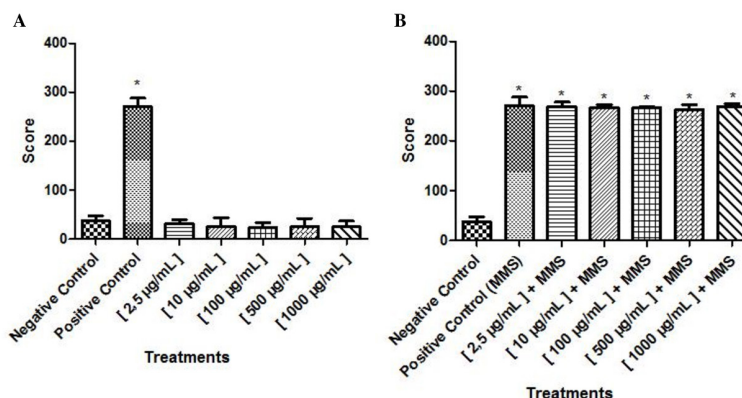
Figure 4. Genotoxicity (**A**) and antigenotoxicity (**B**) of *Euterpe oleracea* oil (EOO) in culture of HepG2 cells by comet assay. *P < 0.001 (ANOVA/Tukey post-test) when compared to negative control. Data are reported as mean values obtained from three independent experiments. Score = DNA damage index.

The other endpoint evaluated in the present study was the capacity of the EOO to produce clastogenic/aneugenic effects in human cells *in vitro* by CBMN. This test is part of a series of test on genetic toxicology, and detect micronuclei in the cytoplasm of interphase cells, due to the formation of acentric chromosome fragments (clastogenic effect) or whole chromosomes that are unable to migrate to the poles during cell division (aneugenic effect) (OECD TG 487, 2014). Our results of clastogenic/aneugenic evaluation of EOO in PBL and HepG2 cells are provided in Tables 1 and 2. There were no statistically significant differences (P > 0.05) in the mean number of binucleated cells with micronuclei between the negative control and the cultures treated with the five concentrations of EOO alone, indicating an absence of clastogenic/aneugenic effects of the tested oil. As expected, cell cultures treated with MMS or BaP (positive control) showed a high mean number of binucleated cells with micronuclei when compared to the negative control (P < 0.001). Besides, cells cultured with different concentrations of EOO simultaneously with the positive control drugs did not lead to a significant reduction in the mean number of binucleated cells with micronuclei when compared with the flask cells cultured with MMS or BaP alone.

Table 1. Cytokinesis-block micronucleus (CBMN) assay in cultures of human lymphocytes treated with *Euterpe oleracea* fruit oil (EOO) and the respective controls.

Treatment (µg/mL)	Total No. in 1000 BN cells			NDI
	MNi	NPBs	NBUDs	
Vehicle control	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.02 ± 0.01
Positive control	10.0 ± 2.0*	3.0 ± 1.0*	2.5 ± 0.5*	2.22 ± 0.05
EOO (2.5 µg/mL)	1.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	2.16 ± 0.00
EOO (10 µg/mL)	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.17 ± 0.01
EOO (100 µg/mL)	1.0 ± 1.0	0.5 ± 0.5	0.0 ± 0.0	2.09 ± 0.20
EOO (500 µg/mL)	1.5 ± 0.5	0.5 ± 0.5	0.5 ± 0.5	2.20 ± 0.09
EOO (1000 µg/mL)	1.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	2.20 ± 0.00
MMS + 2.5 µg/mL EOO	10.5 ± 0.5*	3.5 ± 1.5*	2.0 ± 1.0*	2.00 ± 0.07
MMS + 10 µg/mL EOO	9.5 ± 0.5*	3.0 ± 1.0*	1.0 ± 0.0*	1.99 ± 0.07
MMS + 100 µg/mL EOO	8.5 ± 1.5*	3.5 ± 1.5*	1.5 ± 0.5*	1.92 ± 0.18
MMS + 500 µg/mL EOO	12.0 ± 0.0*	3.5 ± 0.5*	2.5 ± 0.5*	1.80 ± 0.20
MMS + 1000 µg/mL EOO	11.0 ± 2.0*	4.0 ± 0.0*	1.5 ± 0.5*	1.85 ± 0.12

Values are reported as means ± SD. BN, binucleated cell; MNi, micronuclei; NPBs, nucleoplasmic bridges; NBUDs, nuclear buds; NDI, nuclear division index; N = 2. Vehicle control, 0.5% dimethylsulfoxide; Positive control, 150 µM methylmethanesulfonate (MMS). Data are based on three independent experiments. *Significant different from the negative control (P < 0.05).

Table 2. Cytokinesis-block micronucleus (CBMN) assay in cultures of HepG2 treated with *Euterpe oleracea* oil (EOO) and the respective controls.

Treatment (µg/mL)	Total No. in 1000 BN cells			NDI
	MNi	NPBs	NBUDs	
Vehicle control	8.3 ± 0.4	2.3 ± 0.4	10.3 ± 0.4	1.71 ± 0.03
Positive control	28.6 ± 1.2*	9.3 ± 1.6*	16.6 ± 1.6*	1.68 ± 0.00
EOO (2.5 µg/mL)	7.6 ± 0.9	5.6 ± 2.6	12.0 ± 2.9	1.69 ± 0.02
EOO (10 µg/mL)	10.0 ± 0.8	1.3 ± 0.4	9.0 ± 2.1	1.66 ± 0.08
EOO (100 µg/mL)	9.3 ± 1.2	5.3 ± 3.0	9.0 ± 2.4	1.77 ± 0.09
EOO (500 µg/mL)	11.6 ± 3.0	5.6 ± 2.8	10.6 ± 0.4	1.73 ± 0.02
EOO (1000 µg/mL)	8.6 ± 2.0	5.0 ± 2.1	10.3 ± 3.2	1.62 ± 0.08
BaP + 2.5 µg/mL EOO	30.3 ± 1.2*	9.0 ± 1.4*	18.3 ± 0.9*	1.64 ± 0.08
BaP + 10 µg/mL EOO	28.3 ± 0.4*	8.6 ± 1.2*	17.6 ± 1.2*	1.81 ± 0.08
BaP + 100 µg/mL EOO	28.3 ± 1.6*	9.0 ± 0.8*	18.0 ± 1.4*	1.65 ± 0.04
BaP + 500 µg/mL EOO	28.6 ± 1.2*	9.3 ± 1.2*	19.6 ± 1.8*	1.71 ± 0.03
BaP + 1000 µg/mL EOO	30.3 ± 2.4*	9.3 ± 0.9*	19.3 ± 0.9*	1.72 ± 0.08

Values are reported as means ± SD. BN, binucleated cell; MNi, micronuclei; NPBs, nucleoplasmic bridges; NBUDs, nuclear buds; NDI, nuclear division index; N = 2. Vehicle control, 0.5% dimethylsulfoxide; Positive control, 2 µM benzo[a]pyrene (BaP). Data are based on three independent experiments. *Significant different from the negative control (P < 0.001).

The *in vitro* results obtained in the present study by using the comet and CBMN assays confirm the results of *in vivo* studies reported by Marques et al. (2016). These authors investigated the genotoxicity of this same EOO in leukocytes, liver, bone marrow, and testicular cells of Wistar rats, after 14 consecutive days of treatment, and also observed that EOO presented no significant DNA damage in the cells analyzed. Similar results were also observed for the genotoxic evaluation of *E. oleracea* fruit pulp extract, when Ribeiro et al. (2010), also after treating mice during 14 consecutive days, reported the absence of genotoxic effects of açaí pulp in mouse bone marrow, liver, and kidney cells, by using micronucleus and comet assays.

Schauss et al. (2010) evaluated the safety of an açaí-fortified fruit and berry functional juice beverage (MonaVie Active®) done *in vivo* and *in vitro* using some tests to analyze of genotoxicity and mutagenicity. MonaVie Active® was negative for mutagenic effects of the bacterial reverse mutation assay, the chromosomal aberration assay, the mammalian cell mutation assay (L5178Y), and the *in vivo* micronucleus study. However, the toxicological evaluation of *Euterpe edulis*, which can be considered a superfruit, showed positive mutagenicity results detected by *Salmonella typhimurium* TA97 strain (AMES test) at low doses, as well as positive results that were also obtained for the mammalian erythrocyte micronucleus assay (Felzenszwalb et al., 2013). The authors concluded that the pulp of *E. edulis* contains compounds with the capacity to induce mutagenicity and clastogenic/aneugenic effects.

The antigenotoxic assessment of açaí oil and açaí pulp showed different results. In the present study, we observed that açaí oil did not present chemoprevention against the DNA damage induced by MMS and BaP. However, Ribeiro et al. (2010) observed protective effects of açaí pulp in cells of mice of an *in vivo* experiment, in both acute and subacute treatments, when administered before doxorubicin. On another study, da Silva Santos et al. (2014) observed that methanolic extract of açaí fruit protects astrocytes against manganese neurotoxicity, but at high concentrations, the "pro-oxidant" effects of its constituents likely prevail. The differences of the antigenotoxic potential observed could be due to the difference in the *in vitro* and *in vivo* designed experiments, due to different DNA damage agents used, due to differences in the chemical constituents of açaí pulp and açaí oil, and due to a combination of these factors.

The chemical constitution of the *E. oleracea* fruit pulp and the *E. oleracea* fruit oil is not identical. Açaí pulp presents carotenoids, anthocyanins, phenolic acids, and flavonoids (Ribeiro et al., 2010). Açaí oil presents polyphenols, fatty acids (vanillic acid, palmitic acid, γ-linolenic acid, linoleic acid, and oleic acid), phenolic acids (cinnamic and caffeic acids), flavonoids (quercetin and kaempferol rutinoside), and other acids identified as protocatechuic acid, ferulic acid, and syringic acid (Marques et al., 2016). The data obtained in the present study and the data available in the literature indicate that açaí oil and açaí pulp, despite some differences in the chemical constitution, did not present genotoxic effects. However, its antigenotoxic assessment showed different potentialities and needed to be better investigated in further studies.

In conclusion, under the experimental conditions employed in the present study, we observed by the comet assay and by the cytokinesis-block micronucleus test that the fruit oil of *E. oleracea* is not genotoxic or clastogenic/aneugenic in cultured human lymphocytes and HepG2 cells. Besides, this tested oil did not present chemopreventive effect against the genotoxicity of MMS and BaP in the simultaneous treatment protocol. However, we think that further studies are needed to better assess the antigenotoxic potential of the açaí fruit oil, due to its phytochemical composition with antioxidant constituents and positive chemoprevention observed in açaí pulp.

Conflicts of interest

The authors declare no conflict of interest.

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