Revista Brasileira de Farmacognosia 29 (2019) 54–61

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Cytotoxic effects of *Euterpe oleraceae* fruit oil (açaí) in rat liver and thyroid tissues

Eduardo de Souza Marques[a], Juliano Gabriel Froder[a], Patrícia Rosa de Oliveira[b], Fábio Ferreira Perazzo[c], Paulo Cesar Pires Rosa[d], Isabel O'Neill de Mascarenhas Gaivão[e], Maria Izabel Camargo Mathias[b], Edson Luis Maistro[a,f,*]

[a] *Programa de Pós-graduação em Biologia Geral e Aplicada, Instituto de Biociências, Universidade Estadual Paulista Julio de Mesquita Filho, Botucatu, SP, Brazil*
[b] *Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista Julio de Mesquita Filho, Rio Claro, SP, Brazil*
[c] *Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, Diadema, SP, Brazil*
[d] *Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil*
[e] *Escola de Ciências Agrárias e Veterinárias e Departamento de Genética e Biotecnologia, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*
[f] *Departamento de Fonoaudiologia, Faculdade de Filosofia e Ciências, Universidade Estadual Paulista Júlio de Mesquita Filho, Marília, SP, Brazil*

ARTICLE INFO

Article history:
Received 14 September 2018
Accepted 5 December 2018
Available online 18 December 2018

Keywords:
Açaí oil
Thyroid cytotoxicity
Liver cytotoxicity
Açaí cytotoxicity

ABSTRACT

Euterpe oleracea Mart., Arecaceae, fruit (açaí) presents considerable potential for the development of new medicines due to its phytochemical composition and antioxidant activity. More recently, special attention has been given to the pharmacological potential of the fruit's oil. This study analysed the histological and histochemical effects of different dosages of açaí oil on rat's liver and thyroid cells, in order to evaluate its cytotoxic potential after administration for consecutive days. Male Wistar rats were treated with the açaí oil by gavage at doses of 30, 100 and 300 mg/kg, for 14 days, within a 24 h interval. Liver and thyroid fragments were collected for histology (hematoxylin and eosin) and histochemistry analysis (blue of Nilo (lipids), Baker (lipids), bromophenol blue (protein), PAS (polysaccharides)). The results showed that animals exposed to açaí oil presented alterations in the liver cells, where the integrity of the liver tissue was increasingly lost as the açaí oil doses increased. Nuclear pyknosis was observed in several hepatocytes, evidencing the occurrence of cell death. Alteration in the amount of lipids, polysaccharides, vacuoles in the cytoplasm, and proliferation of Kupffer cells were observed in histochemical analyzes. As for the thyroid of the treated rats, alterations were observed in the size of the follicular lumen and also in the connective tissue found between the follicles. Under the experimental conditions employed in the present study, the cytotoxicity observed in this work is worrying, specially considering the liver, when frequent or continuous damage could lead pathological disorders in this organ.

Introduction

The use of natural herbs as dietary supplements is very common in several countries (including Asian, African, European and American countries), with recent data indicating that one in three Americans incorporate them daily into their diet, specially cancer patients (Richardson et al., 2000). Even though herbal remedies are often promoted as natural and therefore harmless, they are by no means free from adverse effects (Neergheen-Bhun, 2013). Therefore, a careful assessment of their toxicological effects for human uses is mandatory.

Euterpe oleracea Mart., Arecaceae, is a plant whose fruit is commonly known as "açaí". This fruit is 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). With specific regard to the açaí fruit oil, antidiarrheic action was proven (Plotkin and Balick, 1984) and Favacho et al. (2011) reported anti-inflammatory and antinociceptive activities. Currently, the açaí fruit is well studied worldwide, with applications in the food industry and pharmaceutical and cosmetic industries (Bonomo Lde et al., 2014).

Studies regarding the chemical composition of açaí have shown the presence of polyphenolic components (such as phenolics, flavonoids and anthocyanins) with antioxidant properties (Zapata-Sudo et al., 2014). The phytochemical characterization of açaí fruit

* Corresponding author.
E-mail: edson.maistro@unesp.br (E.L. Maistro).

https://doi.org/10.1016/j.bjp.2018.12.001

oil by Ultra Performance Liquid Chromatography (UPLC) analysis revealed that vanillic acid, palmitic acid, γ-linolenic acid, linoleic acid, oleic acid, cinnamic acid, caffeic acid, protocatechuic acid, ferulic acid, syringic acid, flavonoids quercetin and kaempferol rutinoside as the main constituents (Marques et al., 2016).

Researches that evaluate the cytotoxic potential of complex mixtures (and their chemical compounds) on the cells of mammalian organs have become fundamental. Some of these organs are vital for the metabolism of the individual, such as the case of the liver that is responsible for the detoxification and inactivation of endobiotics, toxic compounds and nutrients (Ross and Pawlina, 2008; Sreelatha et al., 2009).

Thus, considering the tendency to increase the use of açaí oil as an herbal medicine for humans, the main objective of this study was to analyze the morpho-histochemical effects of different dosages of açaí oil on the liver and thyroid cells of rats in order to determine its cytotoxic potential.

Materials and methods

Plant material

The *Euterpe oleracea* Mart., Areacaceae, fruit oil was kindly provided by the company Açaí do Amapá Agro-Industrial Ltda Sambazon, located in the city of Macapá, in Amapá State, Brazil. Fruits were collected in Bailique Island, Bailique District, Amapá State, Brazil, Latitude: 1.03333, Longitude: −49.9667. 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 same sample of açaí oil used in the present work was performed by Marques et al. (2016).

Dose and experimental design

Experiments were carried out in 4–5 weeks old male Wistar rats (*Rattus norvegicus*), weighing about 100–120 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 ($22 \pm 4\,^{\circ}C$, $55 \pm 5\%$ of relative humidity) with a 12 h light–dark cycle (7 a.m. to 7 p.m.). Food (Nuvilab CR1-Nuvital) and water were available ad libitum. The animals were randomly divided into four experimental groups, with six animals in each group. The açaí oil (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 (25–30 ml daily) (http://beneficiosnaturais.com.br/oleo-de-acai-beneficios-e-propriedades/), and also, on our preliminary acute toxicity studies in rats. The dose corresponds to the amount of oil (around 10%) in the fruit extract. Considering that a regular person takes about 250–300 ml of açai daily, that means about 25–30 ml of the oil in its composition. The negative control group received only vehicle by gavage.

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.*). Excess tissue was removed with the aid of scissors, dissection tweezers, and paper towels. The animals were euthanized by cervical dislocation, and the liver and thyroid fragments were collected for histology and histochemistry analysis. The Animal Bioethics Committee of the Faculdade de Medicina de Marília (CEUA/Famema, Marília, São Paulo state, Brazil) approved this study on the 31st of January, 2013 (protocol number 1659/12), in accordance with the federal government legislations on animal care.

Histology and histochemistry

Liver (central and peripheral regions) and thyroid fragments were collected and fixed in paraformaldehyde at 4% for 24 h. They were put in phosphate buffer 10% (0.1 M, pH 7.4) for 24 h. The material was then dehydrated in ethanol solutions at crescent concentrations of 70, 80, 90, and 95% for 30 min each bath and transferred to JB4 Polaron Instruments/Bio Rad resin solution without catalyzer for 24 h. The material was then accommodated in plastic molds previously filled with resin-containing catalyzer and put in the oven for polymerization. The resin blocks containing the material were sectioned by a microtome (3 μm thickness), hydrated, and displayed on previously cleaned glass slides.

After dried, the sections were stained with Harris hematoxylin and aqueous eosin (HE) according to histological routine (Junqueira and Junqueira, 1983). To detect changes such as the presence or absence, frequency, and distribution of proteins, polysaccharides, and lipids in the livers and thyroids of animals of control and treated groups, histological sections were prepared for the histochemical analysis, as listed: liver – blue of Nilo (lipids) (Lison, 1960); liver – Baker (lipids) (Baker, 1946, modified by Sampieri, 2012); liver and thyroid – bromophenol blue (protein) (Pearse, 1985); liver and thyroid – PAS (polysaccharides) (Junqueira and Junqueira, 1983). The slides were examined and photographed in a MOTIC BA 300 photomicroscope coupled to an INTEL microcomputer.

Results

Liver histology

Control group: The results obtained by applying the histologic technique with hematoxylin and eosin (HE) (Fig. 1A and B) showed that the liver tissue of control individuals was found intact, since it was observed hepatic cords where the cells (hepatocytes) presented regular form (polyhedral, evidenced by some cell boundaries), spherical nuclei, and the presence of evident nucleoli in several cells. There are no intercellular spaces, confirming the integrity of the tissue. Some nuclei of Kupffer cells were also observed (liver macrophages).

Treated group I – (30 mg/kg): The integrity of the liver tissue was increasingly lost as the açaí oil doses increased. In this sense, it can be noted that the occurrence of intercellular spaces that arose between the cells, and also between the cords of hepatocytes, featuring the start of tissue disorganization already at the 30 mg/kg oil dose. At this dosage, the hepatocytes began to present mild eosinophilic granulation in their cytoplasm (Fig. 1C and D).

Treated group II – (100 mg/kg): Although this was an intermediary tested concentration, the histochemical analysis revealed that it was the more toxic to the liver tissue, being observed great tissue disorganization, including in the typical cords arrangement of the liver. Hepatocytes have undergone serious changes since large vacuolation was observed, and the cells changed from eosinophilic to basophilic, meaning that the morphological changes caused, consequently, significant physiological changes. Kupffer cells were found in major frequency in this treatment, probably due to the great toxicity of the oil at this dosage. Nuclear pyknosis was observed in several hepatocytes, evidencing the occurrence of cell death (Fig. 1E and F).

Treated group III – (300 mg/kg): Although this was the highest dose tested, the histological changes of the hepatic tissue were significantly lower than the observed in the 100 mg/kg dose.

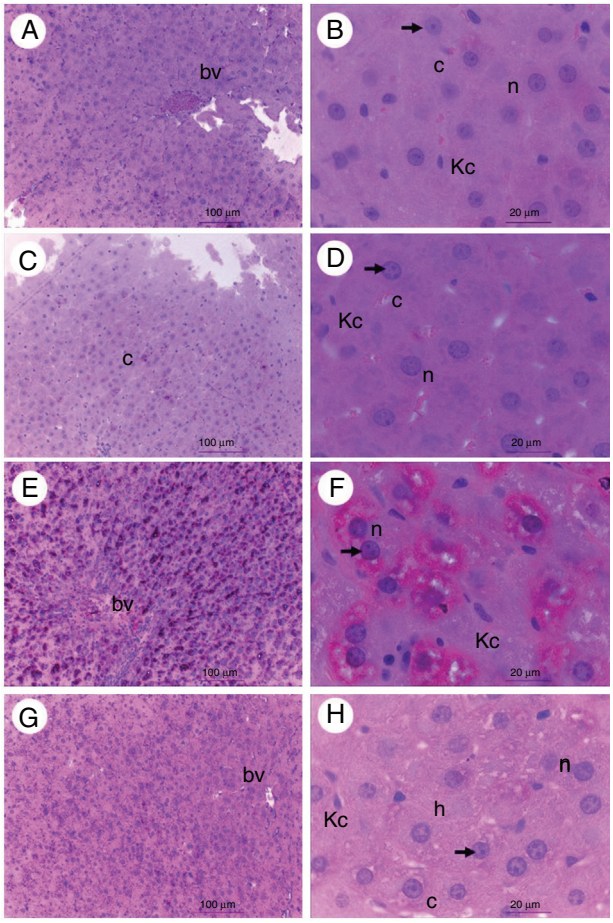

Fig. 1. Histological sections of the liver of *Euterpe oleracea* oil-treated rats. (A–H) Hematoxylin and eosin (HE) staining. (A and B) Control group treated with Tween 80 at 1%. (C and D) Group treated with 30 mg/kg, (E and F) Group treated with 100 mg/kg. (G and H) Group treated with 300 mg/kg. c, hepatic sinusoidal capillaries; h, hepatocytes; Kc, Kupffer cells; n, nucleus of hepatocytes; bv, blood vessels; arrow, nucleoli. All sections had a thickness of 3 µm.

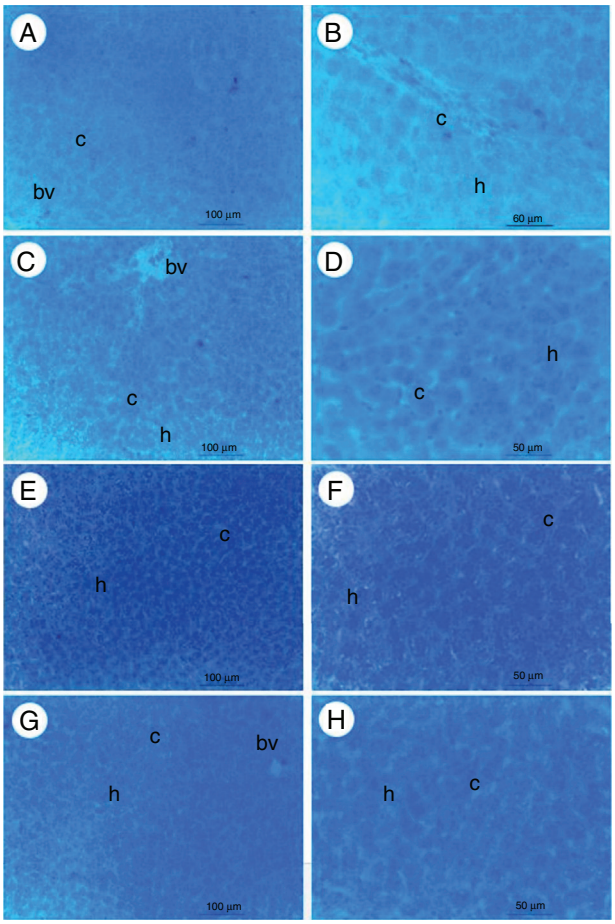

Fig. 2. Histological sections of the liver of *Euterpe oleracea* oil-treated rats. Blue Nilo staining to detect lipids. (A and B) Control group treated with Tween 80 at 1%). (C and D) Group treated with 30 mg/kg. (E and F) Group treated with 100 mg/kg. (G and H) Group treated with 300 mg/kg. c, hepatic sinusoidal capillaries; h, hepatocytes; Kc, Kupffer cells; n, nucleus of hepatocytes; bv, blood vessels; arrow, nucleoli. All sections had a thickness of 3 µm.

The presence of cytoplasmic vacuolization was observed in hepatocytes, as well as the appearance of spaces between cordonal arrangements of the hepatocytes. Some cells showed irregularities in their forms. However, the hepatocytes nuclei apparently demonstrated to be intact, presenting one or two nucleoli. The observation of the Kupffer cells nuclei was not so frequent in this dosage in comparison with the previous one (Fig. 1G and H).

Liver histochemistry

Nilo blue staining for lipid detection

Control group: The histochemical technique applied in the liver to detect total lipids showed a clear presence of this element in the liver tissue. The nuclei of both liver and Kupffer cells were also evident due to simultaneous staining with hematoxylin (Fig. 2A and B).

Treated groups: Differences were detected in the intensity of lipid marking which ranged from moderately positive in control subjects to strongly positive in individuals subjected to the treatments proposed (Fig. 2C–H). At the dosages of 100 and 300 mg/kg, lipid marking intensity was stronger, being strongest in the hepatic cells of the animals treated with 100 mg/kg (Fig. 2E–H).

Baker's method for lipid detection

Control group: The application of the Baker's test reveals the presence of lipids in the liver of control animals, which is demonstrated by the positive reaction of the liver parenchyma. The nuclei of all cells, including hepatocytes, Kupffer cells and nucleoli (hepatocytes), are coloured due to the use of hematein, solution obtained by hematoxylin oxidation (Fig. 3A).

Treated groups: An enhancement of the lipid marking in the groups of animals treated with 30 and 300 mg/kg of the açaí oil was evident, more than in animals treated with 100 mg/kg, confirming an increase in the synthesis of this element when applying these two dosages (Fig. 3B–D). Despite the results of the other techniques previously used, the liver tissue did not present large vacuolization. On the other hand, the images allow to infer that hepatocytes hypertrophy may have occurred due to the large presence of lipids in the cytoplasm, which did not leave empty spaces between the cell strings neither promoted extensive hepatocytes vacuolization (Fig. 3B–D).

Bromophenol blue technique for total protein detection

Control group: A strong mark in the liver of the control group was revealed, as expected, since this is responsible for the synthesis of a vast array of proteins, which are used in various metabolic processes in the body (Fig. 4A and B).

Treated groups: There was an increase in both, cytoplasmic vacuolization (hepatocytes) and the own liver tissue (intercordonais

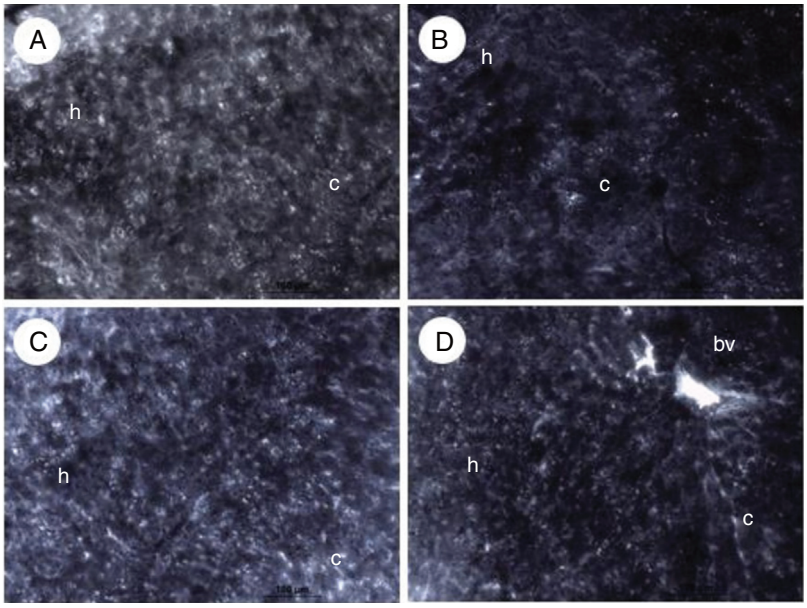

Fig. 3. Histological sections of the liver of *Euterpe oleracea* oil-treated rats. Baker's technique for phospholipid detection. (A) Control group treated with Tween 80 at 1%), (B) Group treated with 30 mg/kg, (C) Group treated with 100 mg/kg, (D) Group treated with 300 mg/kg. c, hepatic sinusoidal capillaries; h, hepatocytes; bv, blood vessels. All sections had a thickness of 3 μm.

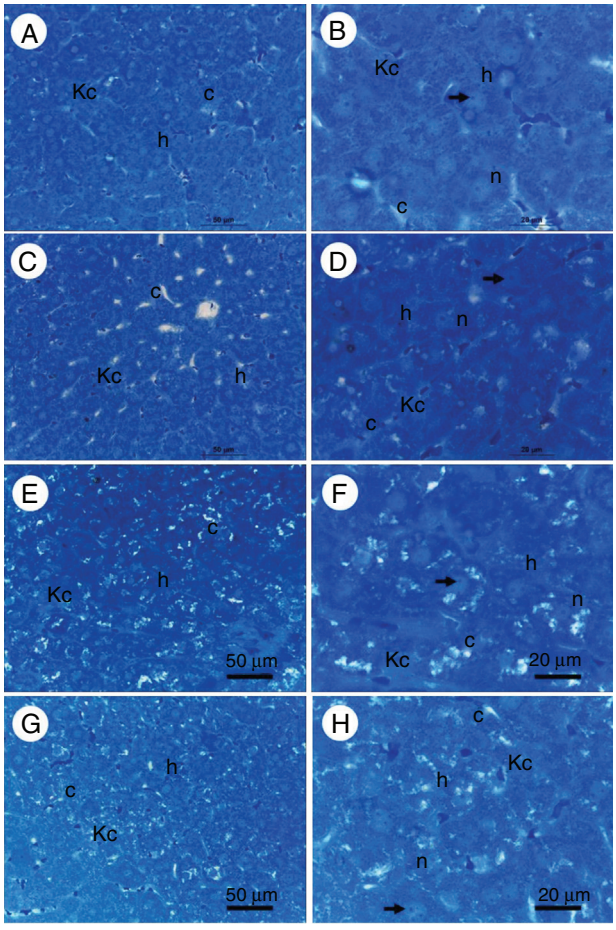

Fig. 4. Histological sections of the liver of *Euterpe oleracea* oil-treated rats. Bromophenol blue staining for protein detection. (A and B) Control group treated with Tween 80 at 1%). (C and D) Group treated with 30 mg/kg. (E and F) Group treated with 100 mg/kg. (G and H) Group treated with 300 mg/kg. All sections had a thickness of 3 μm.

regions), showing the presence of many blanks, indicating that morphological changes have occurred. The vacuolization became more evident in the liver of animals exposed to 100 and 300 mg/kg in contrast with the ones exposed only to the 30 mg/kg oil concentration. Hence, even at this lower dose, it was evident that the appearance of vacuolated regions in the cytoplasm, and therefore, morphological alterations (Fig. 4C–H).

Periodic acid Schiff for polysaccharides detection

This histochemical technique allowed us to evaluate the presence of polysaccharides in the histological liver sections of rats subjected to treatments with açaí oil and the control group.

Control group: The results obtained clearly show the absence of PAS reaction in the nuclei regions of both hepatocytes and Kupffer cells, since such regions are composed of other elements (proteins) and not polysaccharides (Fig. 5A–H).

Treated groups: In the cytoplasmic regions of hepatocytes from the animals treated with 100 mg/kg, it was observed increased polysaccharide marking, where the cells are filled with glycogen natural granules (Fig. 5E and F).

As for the control group (Fig. 5A and B), in the individuals exposed to 30 mg/kg of açaí oil there were no increases in the polysaccharides (Fig. 5C and D). However, in the individuals exposed to 300 mg/kg, increases were observed in the hepatic tissue areas which are negative to the PAS reaction, thus demonstrating the absence of glycogen in their constitution. This characteristic is more evident when the adjacent regions of the centrolobular veins are observed (Fig. 5G–H).

Thyroid histology

Control group: The histological analysis of control subjects showed the preservation of the thyroid. Sections of thyroidean follicles containing colloid (pre-hormone) can be observed. These follicles are constituted by a simple columnar epithelium, with cell morphology varying from squamous to cubic, according to its physiological stage (active follicles have cuboidal epithelium and those with less activity or inactive have squamous epithelium).

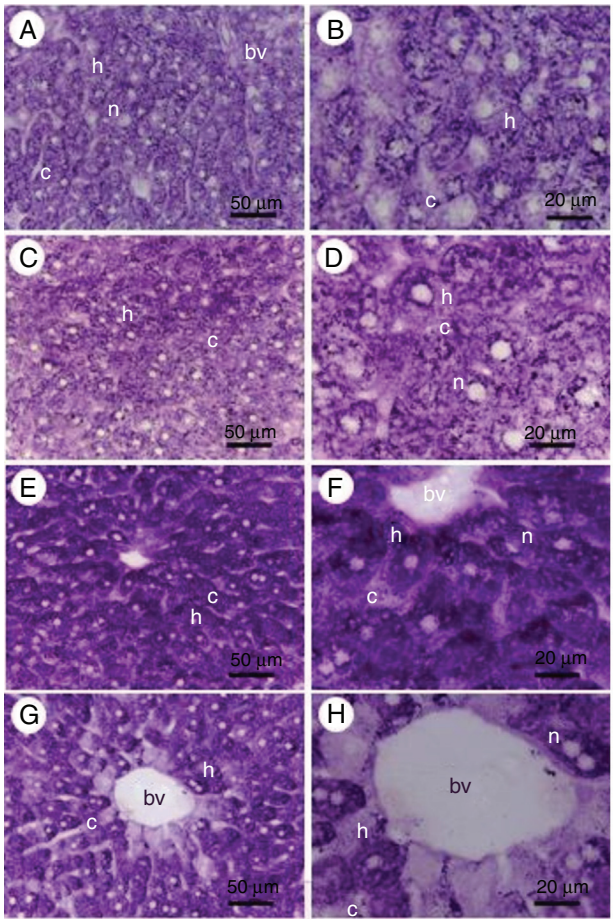

Fig. 5. Histological sections of the liver of *Euterpe oleracea* oil-treated rats. PAS staining for neutral polysaccharide detection. (A and B) Control group treated with Tween 80 at 1%). (C and D) Group treated with 30 mg/kg. (E and F) Group treated with 100 mg/kg. (G and H) Group treated with 300 mg/kg. c, hepatic sinusoidal capillaries; h, hepatocytes; Kc, Kupffer cells; n, nucleus of hepatocytes; bv, blood vessels; arrow, nucleoli. All sections had a thickness of 3 µm.

Parafollicular cells with calcium regulation function can also be observed among the follicles (Fig. 6A).

Treated groups: Changes occurred in the follicular morphology of individuals treated with oil, particularly in the size of the follicular lumen, which has undergone reduction and/or a marked increase in the treatments of 30 and 100 mg/kg (Fig. 6B–D). The reduction may be due to a follicular cell hypertrophy (which constitute the follicles), since cells appear with a cubic morphology much more frequently than those observed in the control.

A remarkable change in the connective tissue between the follicles of the glands from oil-treated individuals (100 and 300 mg/kg) was observed. This change is better observed in the tissue of animals treated with 300 mg/kg, being possible to observe more severe colloid reduction than observed in the animals treated with the lower dose (Fig. 6D). An alteration in the colloid staining intensity was also observed.

Thyroid histochemistry

Bromophenol blue technique for total protein detection

Histological sections show that the thyroid cells (follicular and parafollicular) responded to the applied test, demonstrating the presence of these protein elements. However, in the region where the parafollicular cells are located, in the interstices between the thyroid follicles, it was found a weak cytoplasmic reaction. An exception was observed in the individuals treated with 100 mg/kg, where a group of parafollicular cells had an intermediate reaction to the staining (Fig. 7A–D).

Considering the colloid within the follicles, a weak response indicated little presence of protein elements. For the rest of the treatments (and the control), no changes in the colloid of the follicles were observed.

For the individuals subjected to the oil treatments, large spaces indicating the lack of response to the protein detection were found in the interfollicular regions (Fig. 7B–D), specially in the thyroids of animals treated with 30 and 300 mg/kg (Fig. 7B and D).

Periodic acid Schiff for polysaccharides detection

This histochemical test revealed the biggest changes in the thyroid glands from açaí oil treated animals. Histological sections, for

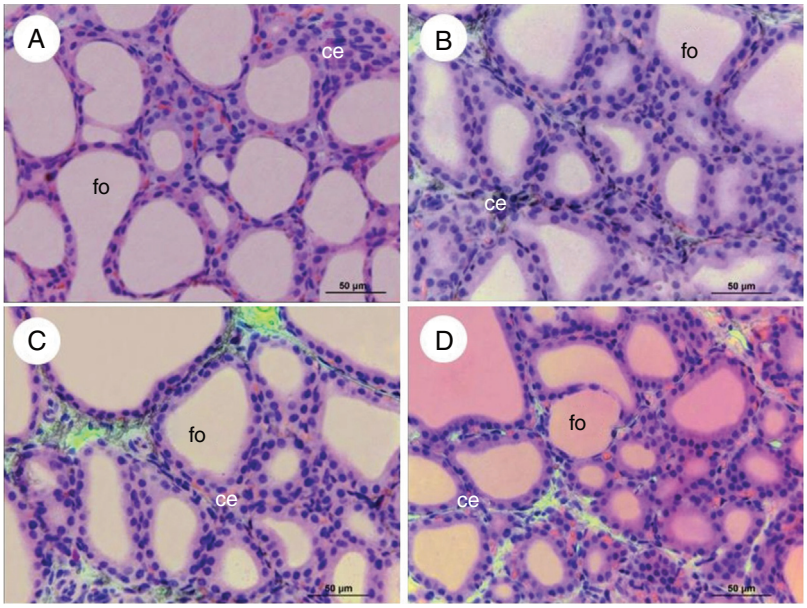

Fig. 6. Photomicrographs of the thyroid glands of *Euterpe oleracea* oil-treated rats. (A–D) Hematoxylin and eosin (HE) staining. (A) Control group treated with Tween 80 at 1%), (B) Group treated with 30 mg/kg, (C) Group treated with 100 mg/kg, (D) Group treated with 300 mg/kg. fo, follicles; ce, cubic epithelium. All sections had a diameter of 3 µm.

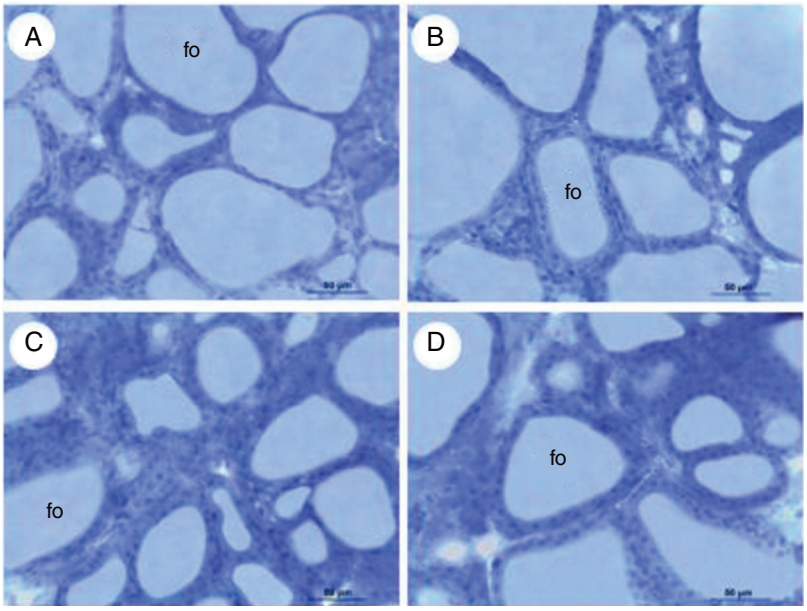

Fig. 7. Photomicrographs of the thyroid glands of *Euterpe oleracea* oil-treated rats. Bromophenol blue technique for total protein detection. (A) Control group treated with Tween 80 at 1%), (B) Group treated with 30 mg/kg, (C) Group treated with 100 mg/kg, (D) Group treated with 300 mg/kg. fo, follicles. All sections had a thickness of 3 μm.

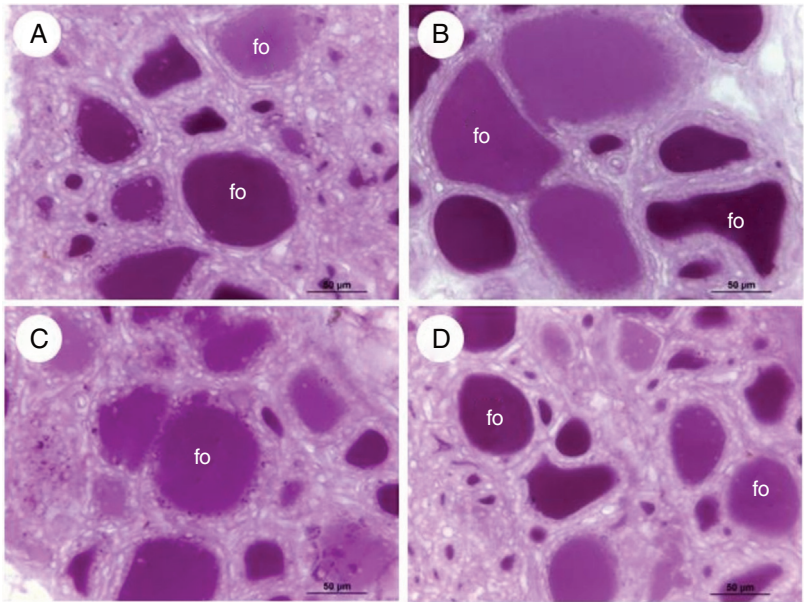

Fig. 8. Photomicrographs of the thyroid glands of *Euterpe oleracea* oil-treated rats. Periodic acid Schiff (PAS) staining to detect polysaccharides. (A) Control group treated with Tween 80 at 1%, (B) Group treated with 30 mg/kg, (C) Group treated with 100 mg/kg, (D) Group treated with 300 mg/kg. fo, follicles. All sections had a thickness of 3 μm.

the control group, showed preservation of the thyroid follicles, surrounded by cell epithelium with squamous or cubic morphology, where nuclei cannot be observed due to the technique (Fig. 8A). The cytoplasm of follicular cells showed an intermediate reaction to the test, as well as the parafollicular cells. The colloid inside the follicles was strongly marked, indicating a high concentration of polysaccharides in it. For animals treated with 30 mg/kg, most of the thyroid follicles showed less intense reaction to PAS than in the animals treated with higher doses. In addition, for animals treated with 30 mg/kg, large spaces not responding to the test occurred between the follicles (Fig. 8B).

In the animals treated with 100 mg/kg, morphological changes were more evident when focused on the peripheral regions of the follicles, where there was the same gnawed aspect indicating increased activity of follicular cells in order to be turning colloid in thyroid hormone (Fig. 8C). The same behaviour was observed in the cells exposed to 300 mg/kg and, furthermore, vacuoles were present inside the follicles, indicating the use of the colloid in the transformation into a thyroid hormone (Fig. 8D).

Discussion

The liver is the second largest organ in the body and its main function is to process the nutrients that are absorbed by the digestive tract, for later use by other organs of the body. The position of the liver in the circulatory system is strategic, in the sense that its location allows you to capture, transform and accumulate

metabolites, as well as neutralize and eliminate toxic substances (Junqueira and Carneiro, 2013).

This study is showing the results obtained by the analysis of liver and thyroid glands of Wistar rats treated for 14 consecutive days with the fruit oil of *Euterpe oleracea* (açaí), at doses of 30, 100 and 300 mg/kg body weight.

Thus, the results demonstrate that the ingestion of 100 mg/kg of açaí oil is capable of causing great morphophysiological changes in the liver, as can be confirmed by the histological sections. Although this dosage was not the highest tested, it was the most harmful (from the concentrations tested) to the liver tissue. This may have occurred due to a possible saturation in the processing capacity of the oil by the liver. The most common damage was represented by the disorganization of the liver tissue, which causes great damage to the hepatic system, affecting the circulation and functions of the organ (Junqueira and Carneiro, 2013).

Another change that was observed in the liver of individuals exposed to açaí oil was the large storage of glycogen in the cytoplasm of hepatocytes, compared with the control animals, indicating that the system is receiving toxic stimuli, which certainly causes a change or deficiency in enzymes produced in the liver responsible for the degradation of this polysaccharide. It should also be taken into consideration the fact that the liver is the organ of vertebrates that, due to its location, is the first to be exposed to foreign products to the body, especially the toxic ones.

Still regarding the observed liver changes, the cytoplasmic vacuolization is a morphological condition which indicates that the cells are undergoing changes which will have consequences on cellular and tissue physiology. Changes in the nuclei of hepatocytes were also observed, and many of them showed pyknosis/chromatin marginalization changes, typically seen in cells that are in death process (Roma et al., 2012).

Changes in form of spaces between the hepatic strands were also observed, and may suggest the beginning of an edema in the organ. The presence of edema is a morphological change of the tissue caused mainly due to pathological situations, where the amount of liquid present in the interstitial tissue (connective) is considerably increased. In histologic sections this change is often observed as increased spaces in the organ (Junqueira and Carneiro, 2013).

The hepatic tissue toxicity observed in the present study may be due to the action of some of the major constituents of açai oil, as palmitic and oleic acid. Literature data reported the induction of steatosis on human hepatocytes exposed to palmitic acid, as well as in primary cultures and immortalized hepatocyte cell lines (Joshi-Barve et al., 2007; Gómez-Lechón et al., 2007; Ricchi et al., 2009). Luo et al. (2012) observed that cyclosporine A (CsA) by itself at therapeutic exposure levels did not induce detectible cytotoxicity in HepG2 cells (human hepatoma cells). However, co-treatment of palmitic acid and CsA resulted in a dose dependent increase in cytotoxicity. Park et al. (2014) reported that palmitic acid induces apoptosis accompanied by autophagy through mitochondrial dysfunction and ER stress, which are triggered by oxidative stress in Chang liver cells. Regarding oleic acid, studies reveal the induction of hepatic steatosis in mice (Malhi et al., 2006; Tang et al., 2011), in human (Araya et al., 2004), and also in human cultured liver cells (Gómez-Lechón et al., 2007; Ricchi et al., 2009).

Regarding the thyroid, this is an endocrine gland (vesicular type) present in vertebrates and is the endocrine gland which, due to its morphology, has the ability to store its secretion products. In the thyroid, the pre-hormones (or colloids), after the occurrence of an adequate stimulus, will be transformed into thyroid hormones (T3 and T4) for being used by the body when needed (Junqueira and Carneiro, 2013).

The thyroid gland is composed of globular structures called thyroid follicles, which are surrounded by simple epithelial cells that can be squamous or cubic, depending of the activity of each follicle stage. Active follicles have its epithelium composed of cubic cells and less active or inactive follicles (colloid storage) are composed of squamous epithelial cells (Junqueira and Carneiro, 2013).

In the present work, the changes in the thyroid gland were directly related to the thyroid follicles, or follicular cells, as well as the hypertrophy associated with disorganization. These changes caused alterations in the epithelium overlying each follicle, reducing its storage capacity of the pre-hormone, probably due to an excessive stimulation, as a result of hormonal changes caused by the test substance in the treated animals. Our review of the literature showed that there are no studies in the literature evaluating the effects of açai oil, or its major components on the thyroid. However, there are a few other studies with substances not related to acai that have shown that continuous stimuli on thyroid follicular cells can cause a hormonal imbalance which causes a decrease in the movement of T3 and T4 hormones, increasing TSH circulation which could prove to be a stimulus for the emergence of thyroid tumors (Ferreira et al., 2012; Hurley et al., 1998).

Thus, the data presented here demonstrates that the exposure of the rat's thyroid gland to the açaí oil not only disrupts the follicular tissue but also alters the chemical composition of the colloid mainly due to the polysaccharides, as it is composed by thyroglobulin, a glycoprotein containing phosphorus residues and covalently linked sulfate. In contrast, Ferreira et al. (2012) observed that this change was mainly due to protein-based components.

According to our literature review, this work represents the first study evaluating the cytotoxic potential of açaí oil *in vivo* by histological and histochemical analysis. The other toxicological analysis of this plant is related to its genotoxic potential. Marques et al. (2016), evaluating the genotoxic potential of the same sample of açaí oil used in our work, and on the same animals, observed absence of genotoxic effects on leukocytes, liver, bone marrow and testicular cells, assessed by the comet assay; and also absence of clastogenic/aneugenic effects in the bone marrow cells, by the micronucleus test. The results obtained by these authors corroborate the results previously obtained by Ribeiro et al. (2010) with açaí pulp, reporting the absence of genotoxic effects in the bone marrow, liver, kidney and peripheral blood cells of mice.

In the present study, the histological and histochemical analysis of the liver and thyroid tissue of rat exposed to different doses of açaí oil during 14 consecutive days revealed damage in cells and tissues of both organs. In the liver, characterized by disorganization of the hepatic tissue, alteration in the amount of lipids, polysaccharides, vacuoles in the cytoplasm, and proliferation of Kupffer cells; and in the thyroid, characterized by alterations in the size of the follicular lumen and also in the connective tissue found between the follicles. Despite the therapeutic potential of the *Euterpe oleracea* fruit oil (or açaí oil), the cytotoxicity observed in this work is worrying, especially considering the liver, since frequent or continuous damage leads to the considerable increase in the amount of connective tissue. The excessive production of connective tissue causes hepatic circulation disorganization, leading to serious pathological disorders in the liver.

Author's contributions

ESM, JGF, PRO contributed in to perform the hystological and histochemical analysis. FFP, PCPR contributed in to obtention and chemical description of the oil extracted of the fruits. ELM, MICM designed the experiments. ELM obtained the financial suport for the research. ELM, MICM, IOMG contributed to critical reading and final editing of the manuscript. All the authors approved the submission of the manuscript.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by FAPESP (Grant: 2012/17241-8). E.S. Marques thanks to CAPES for the PhD's scholarship.

References

Araya, J., Rodrigo, R., Videla, L.A., Thielemann, L., Orellana, M., Pettinelli, P., Poniachik, J., 2004. Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. Clin. Sci. 106, 635–643.

Baker, J.R., 1946. The histochemical regognition of lipine. Q. J. Microsc. Sci. 87, 441–470.

Bonomo Lde, F., Silva, D.N., Boasquivis, P.F., Paiva, F.A., Guerra, J.F., Martins, T.A., de Jesus Torres, A.G., de Paula, I.T., Caneschi, W.L., Jacolot, P., Grossin, N., Tessier, F.J., Boulanger, E., Silva, M.E., Pedrosa, M.L., Oliveira Rde, P., 2014. Acai (*Euterpe oleracea* Mart.) modulates oxidative stress resistance in *Caenorhabditis elegans* by direct and indirect mechanisms. PLoS One 9, e89933.

Caetano, R.S., de Souza, A.C.R., Feitozao, L.F., 2014. O uso de plantas medicinais utilizadas por frequentadores dos ambulatórios Santa Marcelina, Porto Velho - RO. Rev. Saúde Pesq. 7, 55–63.

de Bem, G.F., da Costa, C.A., de Oliveira, P.R., Cordeiro, V.S., Santos, I.B., de Carvalho, L.C., Souza, M.A., Ognibene, D.T., Daleprane, J.B., Sousa, P.J., Resende, A.C., de Moura, R.S., 2014. Protective effect of *Euterpe oleracea* Mart. (acai) extract on programmed changes in the adult rat offspring caused by maternal protein restriction during pregnancy. J. Pharm. Pharmacol. 66, 1328–1338.

Favacho, H.A.S., Oliveira, B.R., Santos, K.C., Medeiros, B.J.L., Sousa, P.J.C., Perazzo, F.F., Carvalho, J.C.T., 2011. Anti-inflammatory and antinociceptive activities of *Euterpe oleracea* oil. Rev. Bra. Farmacogn. 21, 105–114.

Ferreira, M., De Oliveira, P.R., Denardi, S.E., Bechara, G.H., Mathias, M.I., 2012. Fipronil (active ingredient of acaricide Frontline(R)) acting on the mice thyroid. Microsc. Res. Tech. 75, 265–270.

Gómez-Lechón, M.J., Donato, M.T., Martinez-Romero, A., Jiménez, N., Castell, J.V., O'Connor, J.E., 2007. A human hepatocellular in vitro model to investigate steatosis. Chem. Biol. Interact. 165, 106–116.

Hurley, P.M., Hill, R.N., Whiting, R.J., 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. Environ. Health Perspect. 106, 437–445.

Joshi-Barve, S., Barve, S.S., Amancherla, K., Gobejishvili, L., Hill, D., Cave, M., Hote, P., McClain, C.J., 2007. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology 46, 823–830.

Junqueira, L.C.U., Junqueira, L.M.M.S., 1983. Técnicas básicas de citologia e histologia. Livraria Editora Santos, São Paulo, pp. 48–81.

Junqueira, L.C., Carneiro, J., 2013. Histologia Básica. 12ª ed. Guanabara Koogan S.A., 556p.

Leão, R.B.A., Ferreira, M.R.C., Jardim, M.A.G., 2007. Study of therapeutical use plants in municipality of Santa Bárbara do Pará, State of Pará. Brazil. Rev. Bras. Farm. 88, 21–25.

Lison, L., 1960. Histochimie et Cytochimie Animales, Gauthier-Villars, Editeur, Paris.

Luo, Y., Rana, P., Will, Y., 2012. Cyclosporine A and palmitic acid treatment synergistically induce cytotoxicity in HepG2 cells. Toxicol. Appl. Pharmacol. 261, 172–180.

Malhi, H., Bronk, S.F., Werneburg, N.W., Gores, G.J., 2006. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. J. Biol. Chem. 281, 12093–12101.

Marques, E.S., Froder, J.G., Carvalho, J.C.T., Rosa, P.C.P., Perazzo, F.F., Maistro, E.L., 2016. Evaluation of the genotoxicity of *Euterpe oleraceae* Mart. (Aracaceae) fruit oil (açaí), in mammalian cells in vivo. Food Chem. Toxicol. 93, 13–19.

Neergheen-Bhun, V.S., 2013. Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. Biomed. Res. Int., http://dx.doi.org/10.1155/2013/804086.

Park, E.J., Lee, A.Y., Park, S., Kim, J.H., Cho, M.H., 2014. Multiple pathways are involved in palmitic acid-induced toxicity. Food Chem. Toxicol. 67, 26–34.

Pearse, A.G.E., 1985. Histochemistry theoretical and applied. Churchill Livingstone, 2nd ed., 449 p.

Plotkin, M.J., Balick, M., 1984. Medicinal uses of South American palms. J. Ethnopharmacol. 10, 157–179.

Ribeiro, J.C., Antunes, L.M.G., Aissa, A.F., Darin, J.D.C., De-Rosso, V.V., Mercadante, A.Z., Bianchi, M.L.P., 2010. Evaluation of the genotoxic and antigenotoxic effects after acute and subacute treatments with açaí pulp (*Euterpe oleracea* Mart.) on mice using erythrocytes micronucleus test and comet assay. Mutat. Res. 695, 22–28.

Ricchi, M., Odoardi, M.R., Carulli, L., Anzivino, C., Ballestri, S., Pinetti, A., Fantoni, L.I., Marra, F., Bertolotti, M., Banni, S., Lonardo, A., Carulli, N., Loria, P., 2009. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. J. Gastroenterol. Hepatol. 24, 830–840.

Richardson, M.A., Sanders, T., Palmer, J.L., Greisinger, A., Singletary, S.E., 2000. Complementary/alternative medicine use in a comprehensive cancer center and the implications for oncology. J. Clin. Oncol. 18, 2505–2514.

Roma, G.C., Oliveira, P.R., Bechara, G.H., Camargo-Mathias, M.I., 2012. Cytotoxic effects of permethrin on mouse liver and spleen cells. Microsc. Res. Tech. 75, 229–238.

Ross, M.H., Pawlina, W., 2008. Histologia em correlação com Biologia Celular e Molecular. Guanabara Koogan, Rio de Janeiro, pp. 908.

Sampieri, B.R., 2012. Ação dos ésteres do ácido ricinoléico do óleo de mamona (*Ricinus communis*) sobre as células dos ovários de *Rhipicephalus sanguineus* (Acari: Ixodidae): dinâmica da vitelogênese. 109f. Dissertação (mestrado) – Universidade Estadual Paulista (UNESP) – Instituto de Biociências de Rio Claro/SP. (Baker modificada).

Souza, M.O., Santos, R.C., Silva, M.E., Pedrosa, M.L., 2011. Açaí (*Euterpe oleraceae* Martius): chemical composition and bioactivity. J. Braz. Soc. Food Nutr. 36, 161–169.

Sreelatha, S., Padma, P.R., Umadevi, M., 2009. Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. Food Chem. Toxicol. 47, 702–708.

Tang, Y., Bian, Z., Zhao, L., Liu, Y., Liang, S., Wang, Q., Han, X., Peng, Y., Chen, X., Shen, L., Qiu, D., Li, Z., Ma, X., 2011. Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. Clin. Exp. Immunol. 166, 281–290.

Vásquez, S.P.F., de Mendonça, M.S., Noda, S.N., 2014. Ethnobotany of medicinal plants in riverine communities of the Municipality of Manacapuru, Amazonas, Brasil. Acta Amazonica 44, 457–472.

Zapata-Sudo, G., da Silva, J.S., Pereira, S.L., Souza, P.J., de Moura, R.S., Sudo, R.T., 2014. Oral treatment with *Euterpe oleracea* Mart. (acai) extract improves cardiac dysfunction and exercise intolerance in rats subjected to myocardial infarction. BMC Complement. Alter. Med. 14, http://dx.doi.org/10.1186/1472-6882-14-227.