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To cite this article: R. B. Freitas, D. N. Rômulo, G. M. Bianca, C. S. Eliziária, S. A. Murilo, G. F. Luciano, M. L. Luciana, P. Maria do Carmo, V. G. Reggiani & V. L. João Paulo (2017): Euterpe edulis extracts positively modulates the redox status and expression of inflammatory mediators, Food and Agricultural Immunology

To link to this article: <http://dx.doi.org/10.1080/09540105.2017.1360255>



Published online: 11 Aug 2017.



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Euterpe edulis extracts positively modulates the redox status and expression of inflammatory mediators

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ABSTRACT

Fruits of *Euterpe* spp. are rich in phenolic compounds, mainly anthocyanins, which are endowed with a high antioxidant capacity. The objective of the study was to evaluate the effects of derivatives from *Euterpe* spp. fruits on oxidative metabolism and inflammatory mediators. The oil (OE), total lyophilized pulp (LEE) and defatted pulp (LEDE) were obtained from the fruits of *Euterpe edulis*. Thirty-six animals were divided into four experimental groups: G1: Control; G2: OE (4%), G3: LEE (10%), G4: LEDE (10%), each of which received a particular extract in their diet for 50 days. The activities of catalase, glutathione-S-transferase, superoxide dismutase, malondialdehyde produced in liver and expression of pro-inflammatory cytokines tissue were lower in G4 than in the other groups. The study indicates that dietary supplementation with extracts of *E. edulis* has no deleterious effects and may be beneficial, especially for LEDE extracts containing high concentrations of anthocyanin.

ARTICLE HISTORY

Received 23 June 2017

Accepted 24 July 2017

KEYWORDS

Euterpe edulis; antioxidant enzymes; peroxidation markers; inflammatory cytokines

Introduction

The use of fruits and medicinal plants is an ancient practice that has shown worldwide growth (Ferreira & Pinto, 2010; Rufino et al., 2010). However, to demonstrate the curative or preventive effects of a compound it is necessary determine its potential, biochemical, oxidative and inflammatory aspects. There is increasing interest in naturally occurring antioxidants for use in foods to replace synthetic antioxidants (Kanyaiya, Digambar, Arora, Kapila, & Singh, 2014). In this context, although the fruit of the jussara palm tree (*Euterpe edulis*) is widely distributed in the Brazilian Atlantic Forest, its consumption and processing is not as well explored in the different regions as that of another palm tree fruit known as açai (*Euterpe oleraceae*) (Sousa De Brito et al., 2007). The final product of jussara fruit processing is a thick purple pulp with considerable nutritional value that can be used in the manufacturing of a variety

of beverages, sweets and ice cream (Borges et al., 2011). The nutritive potential of this fruit is well justified due to the high content of polyphenols, especially anthocyanins and polyunsaturated fatty acids (Rufino et al., 2010). Anthocyanins can stimulate the activity of immunocompetent cells, capture free radicals directly, repair DNA and repair damaged cells (Hartati, Widjanarko, Widyaningsih, & Rifa'i, 2017). Studies have shown that anthocyanins have a high antioxidant capacity and activate important pathways in the fight against metabolic reactors, which highlights its importance in the fight against cellular oxidation and consequently in the pathogenesis of cardiovascular diseases, hepatic diseases, diabetes and cancer. Thus, the analysis of foods rich in these compounds has been steadily growing because dietary supplementation with tropical fruit pulp rich in antioxidants has been presented as an effective curative and preventive therapy for the treatment of several conditions associated with pathological conditions (Felzenszwalb, da Costa Marques, Mazzei, & Aiub, 2013; Freitas et al., 2016; Pereira et al., 2014; Prior & Wu, 2006). The fruits of jussara contain two main anthocyanins identified as cyanidin 3-glucoside and cyanidin 3-rutinoside (Harborne, Saito, & Detoni, 1994), in addition to minor compounds such as cyanidin 3-sambubioside, pelargonidine 3-glycoside, cyanidin 3-rhamnoside and pelargonidine 3 rutinoside identified by LC-DAD-ESI/MS (Rufino et al., 2010). The lipid fraction obtained from the pulp of jussara (although little used) is also suitable for consumption due to its high content of polyunsaturated fatty acids, remarkable concentration of oleic acid, palmitic and linoleic acids, similar to *E. oleraceae*, and lower saturated lipid concentration compared to other oils (Freitas et al., 2016). The saturated fatty acids present in this fruit represent 24.32–28.89% of the total lipid content (Neida & Elba, 2007; Schauss et al., 2006). Considering the increased consumption of *E. edulis* fruit, it is prudent to carry out a toxicological analysis of the extracts to guarantee their safe consumption. In this context, hepatic toxicity analysis is an important tool because the bioactivation of the metabolites present in food extracts is able to result in interactions with the hepatocytes leading to DNA damage, loss of protein function, lipid peroxidation and consequently to tissue oxidative stress (Wang, Lee, Chang, Liou, & Ho, 2001). In addition, liver dysfunction also has the capacity to initiate immune reactions, which contribute to the progression of liver injury through the production of inflammatory cytokines (Lacour, Gautier, Pallardy, & Roberts, 2005). According to Schauss et al. (2010), the lyophilized pulp of *E. oleraceae* does not present mutagenic, clastogenic, cytotoxic or genotoxic effects. However, the biosafety of the pulp of *E. edulis* requires analysis to determine its effects on biochemical, oxidative and cellular parameters and, consequently, to observe its action on energy metabolism. The implications highlighted in this study on the potential of *E. edulis* extracts may open new avenues for dietary supplementation and represent a promising path to be explored by the food and pharmaceutical industry. In addition, health professionals may associate conventional treatments for several pathologies with diets rich in antioxidant molecules obtained inexpensively and that are accessible to the general population. Therefore, we evaluated the safety of the administration of OE, lyophilized extract (LEE) and lyophilized and defatted extract (LEDE) of *E. edulis* supplemented with the standardized diet of male Wistar rats following the guidelines recommended by ANVISA Resolution 16 e 17/99, which establishes basic guidelines for risk assessment and food safety (BRASIL, 1999).

Material and methods

Plant material and obtaining OE, LEE and LEDE

The fruits of *E. edulis* were collected in a remnant area of the Atlantic forest, located in the “Zona da Mata” (Latitude: -20.97337439 ; Longitude: -42.52883943 ; Height: 744661) in the State of Minas Gerais, Brazil. The ripe fruits were selected, washed, weighed, disinfected with chlorinated water, cooked in water, pulped (Itametal® Depolpadeira Bonina 0.25DF) and refined. The pulp was passed through a fine mesh screen and then lyophilized (Lyophilizer Liotop LP510, Liobras®), and the LEE extract was obtained. This extract served as raw material for the production of the defatted extract (LEDE) and the oil (OE) of *E. edulis*. The LEDE was obtained from Soxhlet Quimis® (Model Q308-16B) for 12 h, using ethyl ether as the separation solvent. The three extracts were conditioned in nitrogen atmosphere and stored at -20°C .

Identification of anthocyanins by UPLC-DAD-ESI/MS

The chemical composition of LEE and LEDE was analyzed using ACQUITY Ultra Performance LC equipment (Waters, Milford, MA, USA) simultaneously coupled to PDA 2996 photodiode detector (Waters, Milford, MA, USA) and ACQUITY TQ detector (Waters MS Technologies, Manchester, UK), equipped with electrospray Z-spray (ESI) ionization with source operating in positive mode. MassLynx software (version 4.1, Waters, Milford, MA, USA) was used. Sample solutions ($3\ \mu\text{L}$; $0.5\ \text{mg mL}^{-1}$) were injected into a reversed phase column using a Waters Acquity SDS C-18 column (50 mm, 2.1 mm diameter, $4.6\ \mu\text{m}$ particle size). Chromatographic analysis was performed using a mobile phase gradient consisting of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid with the initial condition set at 95% A. The mobile phase was then linearly increased to 95% B in 10 min, maintained at this concentration for 1 min, and then returned to initial conditions at 13 min.

Animals and treatment

The method used to evaluate *in vivo* toxicity was authorized by the Ethics Committee for the Use of Animals (CEUA)/UFV through process no 60/2012. Thirty-two Wistar rats (*Rattus norvegicus*) at 4 weeks of age were randomly divided into four experimental groups: G1: animals that received a commercial ration (Control), G2: commercial ration + *E. edulis* oil (OE), G3: commercial ration + freeze-dried extract of *E. edulis* (LEE 10%) and G4: commercial ration + lyophilized and defatted extract (LEDE 10%). The animals received the extracts mixed with commercial feed for 50 days. All diets and water were provided *ad libitum*. All procedures took place under controlled conditions of brightness (cycles of 12 h clarity/darkness), temperature ($21 \pm 2^{\circ}\text{C}$) and relative humidity (60%).

Serum biochemistry and organ weight

After 50 days, the animals were anesthetised in a halothane chamber followed by euthanasia by hypovolemia with collection of blood by puncture of the abdominal aorta. Blood

was centrifuged at 1750g for 10 min at 4°C for the determination of serum levels: ultra-sensitive C-reactive protein, glucose, total cholesterol (TC), triglycerides, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were processed according to the manufacturer's instructions. The animals were weighed before euthanasia to evaluate possible differences between the groups that received *E. edulis* extracts and the control group. Immediately after euthanasia, the organs (liver, adipose tissue, kidneys, brain, gonads and heart) were removed and weighed.

Analysis of redox status

Aliquots (100 mg) of frozen liver fragments were homogenized in phosphate-buffered saline, centrifuged at 3500g under refrigeration (5°C), and the supernatant was used for analysis of catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD). CAT activity was evaluated by measuring the rate of decomposition of hydrogen peroxide according to the method described by Aebi (1984). SOD activity was evaluated by the xanthine oxidase method based on the production of hydrogen peroxide and the reduction of nitroblue tetrazolium (Sarban, Kocyigit, Yazar, & Isikan, 2005). GST activity was followed by spectrophotometry to measure the product formed from the complexation of reduced glutathione with 1-chloro-2,4-dinitrobenzene according to Keen, Habig, and Jakoby (1976). Total protein levels in hepatic tissue were measured by the method of Bradford (1976). The determination of lipid peroxidation in the liver homogenates was performed by the detection of malondialdehyde (MDA) (Buege & Aust, 1978).

Inflammatory mediators

Total RNA was extracted from hepatic tissue using the Trizol reagent (Life Technologies). Fractions of 40 mg of hepatic tissue from each animal were macerated in liquid nitrogen. Total RNA was treated with RNase-free DNase (Life Technologies) and used for cDNA synthesis, using the M-MLV Reverse transcriptase kit (Invitrogen Brazil Ltd.). Real-time PCR reactions were performed using the 7500 Real Time PCR Systems (Applied Biosystems) apparatus, specific oligonucleotides (Table 1), cDNAs from the treatments and SYBR Green PCR Master Mix (Applied Biosystems). The amplification conditions were: 95°C for 10 min, and 40 cycles of 94°C for 15 s and 60°C for 1 min. For the quantification of gene expression, the comparative methods of C_t : $2^{-\Delta C_t}$ and $2^{-\Delta\Delta C_t}$ were used. As endogenous control for the normalization of qRT-PCR data, primers specific for *Rattus norvegicus* var. *albinus* β -actin were used (Table 1).

Table 1. List of primers that were used in real-time PCR reactions.

Protein	Primer 1 (Forward)	Primer 2 (Reverse)
IL6	GGA ACG AAA GTC AAC TCC ATC TG	AAG GCA ACT GGC TGG AAG TCT
IL 10	GCC AAG CCT TGT CAG AAA TGA	CTT GAT TTC TGG GCC ATG GT
IL4	AGG GCT TCC AGG GTG CTT	CCG AGA ACC CCA GAC TTG TTC
IL17	GAC GGA ACG TCT GGT CAT CA	CAA GGA ATG AGC GCC ACA CT
TNF- α	CCC AGA AAA GCA AGC AAC CA	TTT CCG GAG GGA GAT GTG TT
INT- γ	TCA TCG AAT CGC ACC TGA TC	GAA CTT GGC GAT GCT CAT GAA
β - Actin	AGG CCA ACC GTG AAA AGA TG	CAC AGC CTG GAT GGC TAC GT

Note: IL6: interleukin 6; IL4: interleukin 4; IL10: interleukin 10; IL17: interleukin 17; TNF- α : tumor necrosis factor alpha; INT- γ : interferon gamma; β -actin: beta actin.

Stereometry and hepatic karyometry

The volume density of hepatocytes (V_v [hep],%), interstitium (V_v [int],%), sinusoidal capillaries (V_v [inf],%), and inflammatory cells of lipids (V_v [lipid],%) were estimated using 4- μm thick histological sections stained with hematoxylin and eosin (H&E) (Gonçalves et al., 2012). Fifty histological fields of each group (40 \times objective lens) were sampled randomly, and a total of $3.65 \times 104 \mu\text{m}^2$ liver areas were analyzed. To avoid repeated analysis of the same histological site, the sections were evaluated in semi-serial sections using one in every 20 cuts. For stereology analysis, a 300-point test system was used in a standard test area (A_t) of $73 \times 103 \mu\text{m}^2$. The volume densities (v_v) were estimated by counting points using the following formula: $v_v = PP[\text{structure}]/PT$; where PP is the number of points located along the structure in question and PT are the total test points (Gonçalves et al., 2012; Novaes et al., 2013). The volume of 50 hepatocyte nuclei for each animal was determined according to Novaes et al. (2013). All morphological analyses were performed using the Pro-Plus image analysis software 4.5 (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

Data were expressed as the mean \pm standard deviation (mean \pm SD). The normality of the data distribution was verified by D'Agostino–Pearson's test. Morphological data were subjected to the Kruskal–Wallis test, and the biochemical and molecular data were analyzed by the unidirectional analysis of variance, followed by Student–Newman–Keuls *post hoc* test for multiple comparisons. All tests were performed using the GraphPad Prism 5.01 statistical software (GraphPad Software, Inc., CA, USA), and statistical significance was set at $p < .05$.

Result

Identification of anthocyanins

The UPLC-DAD-ESI/MS chromatogram analysis of the LEE and LEDE extracts showed the presence of two major peaks when analyzed in the absorption region at 254 nm. The UV/vis absorption spectra of these peaks showed two bands with λ_{max} between 240 and 280 nm (band II) and other between 500 and 540 nm (band I), characteristic of anthocyanin compounds. The ESI/MS full scan data showed for the two major anthocyanins molecular ion $[M + H]^+$ at m/z 449 and $[M + H]^+$ ion at m/z 595 MS/MS, both with fragments at $[M + H - 162]^+$, can be assigned to cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively. Anthocyanin standards were used to confirm these compounds.

Analysis of organ weights

There was no significant difference between weight gain and absolute body weight ($p < .05$) (Table 2).

Table 2. Weight variation and absolute weight of organs of Wistar rats fed various extracts (OE, LEE and LEDE) of *E. edulis*.

Ponderal variation	Absolute weight of organs						
	Weight gain	Liver	Adipose tissue	Kidneys	Brain	Gonads	Heart
G1	239.45 ± 16.25 ^a	9.56 ± 0.67 ^a	6.55 ± 1.06 ^a	1.94 ± 0.15 ^a	1.44 ± 0.15 ^a	5.30 ± 0.35 ^a	1.00 ± 0.07 ^a
G2	260.10 ± 4.86 ^{a,b}	10.02 ± 0.76 ^a	9.15 ± 2.18 ^a	2.01 ± 0.08 ^a	1.46 ± 0.21 ^a	5.02 ± 0.14 ^a	1.05 ± 0.11 ^a
G3	270.00 ± 7.18 ^b	9.98 ± 0.78 ^a	9.06 ± 2.21 ^a	2.09 ± 0.24 ^a	1.38 ± 0.18 ^a	5.05 ± 0.49 ^a	1.10 ± 0.07 ^a
G4	239.45 ± 20.91 ^{a,b}	9.25 ± 0.85 ^a	8.83 ± 2.76 ^a	2.20 ± 0.70 ^a	1.38 ± 0.15 ^a	4.91 ± 0.56 ^a	1.11 ± 0.15 ^a

Note: G1: commercial ration; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE defatted.

^{a,b}Different letters in the columns denote statistical difference between groups ($p > .05$).

Table 3. Biochemical parameters of serum (mg/dL) of control animals (G1) and animals receiving three different *Euterpe edulis* extracts incorporated into the diet (G2, G3 and G4).

	US-PCR	Glucose	TC	TG	HDL	AST	ALT
G1	0.36 ± 0.18 ^a	139.66 ± 18.47 ^a	69.50 ± 8.82 ^a	75.66 ± 11.41 ^a	37.16 ± 8.97 ^a	137.33 ± 38.95 ^a	51.33 ± 14.47 ^a
G2	0.38 ± 0.21 ^a	168.16 ± 26.31 ^a	73.16 ± 10.79 ^a	74.16 ± 32.77 ^a	37.00 ± 2.68 ^a	122.00 ± 39.14 ^a	52.00 ± 19.96 ^a
G3	0.36 ± 0.22 ^a	143.66 ± 13.07 ^a	66.66 ± 8.16 ^a	62.50 ± 19.33 ^a	36.33 ± 2.58 ^a	100.16 ± 21.94 ^a	40.33 ± 4.36 ^a
G4	0.27 ± 0.15 ^a	155.33 ± 12.38 ^a	51.66 ± 5.50 ^b	63.50 ± 11.36 ^a	31.00 ± 1.54 ^a	117.33 ± 21.63 ^a	41.66 ± 3.88 ^a

Note: US-PCR: ultra-sensitive c-reactive protein; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase. G1: commercial ration; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE defatted.

^{a,b}Different letters in the columns denote a statistical difference between groups at $p > .05$.

Serum biochemistry

The values of TC were lower in the groups that received extract of LEDE (G4) incorporated into the diet ($p < .05$). Analysis of C-reactive protein, glucose, triglycerides, HDL, AST and ALT did not show significant differences between the different groups ($p < .05$) (Table 3).

Redox status

The analysis of the redox potential showed that LEDE (G4) was effective in reducing the production of malondialdehyde (MDA) and consequently decreased the activity of the anti-oxidant enzymes CAT, GST and SOD when compared to the other treatment groups. LEE (G3) was also effective in reducing MDA levels when compared to G1 and G2 (Figure 1).

Inflammatory mediators

Analyses of the expression of proinflammatory mediators (IL-17, IFN- γ and TNF- α) revealed a decrease of all the mediators in animals that received the LEDE-supplemented diet (G4) when compared to the other treatment groups. The LEDE-supplemented group was also associated with a decrease in expression of the anti-inflammatory mediators (IL-4 and IL-10) compared to that of the other treatment groups ($p < .05$) (Figure 2).

Stereological parameters

Stereological analysis of hepatic tissue showed that the fat volume density in hepatocytes was higher in the G2 and G3 groups, which received commercial feed + *E. edulis* oil and

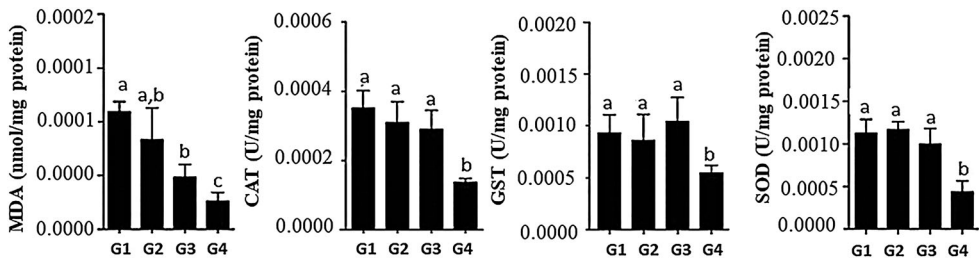


Figure 1. Redox status of the hepatic tissue of rats receiving the *Euterpe edulis* extract-supplemented diet. MDA: malondialdehyde; CAT: catalase; GST: glutathione-S-transferase; SOD: superoxide dismutase. G1: commercial ration; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE defatted. a, b, c Different letters in the columns denote a statistical difference between groups at $p < .05$.

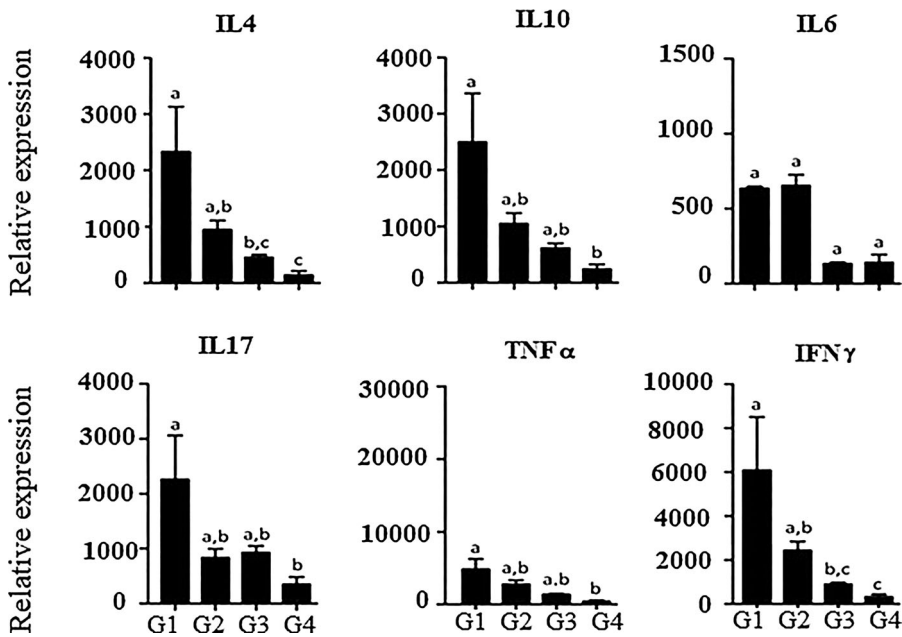


Figure 2. Expression of cytokine mRNA in the liver tissue of rats treated with *Euterpe edulis* in the IL diet, interleukin; TNF, tumor necrosis factor alpha; IFN- γ , or gamma interferon. G1: commercial ration; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE defatted. a, b, c Different letters in the columns denote a statistical difference between groups at $p < .05$.

commercial ration + *E. edulis* extract lyophilized, respectively. The fat deposition of the LEDE group (G4), which received the defatted extract, was similar to that of the control group ($p < .05$). The other analyzed parameters did not present a significant difference between the groups (Table 4 and Figure 3).

Table 4. Stereological parameters of the liver tissue of rats treated with *Euterpe edulis* extract in their diets.

	Vv[hep], %	Vv[int], %	Vv[cap], %	Vv[Inf], %	Vv[LD], %	V[HN], mm ³
G1	83.37 ± 1.89 ^a	17.63 ± 1.89 ^a	10.40 ± 1.14 ^a	3.11 ± 1.03 ^a	7.38 ± 2.71 ^a	573.81 ± 20.13 ^a
G2	83.25 ± 2.21 ^a	17.75 ± 2.21 ^a	9.93 ± 1.66 ^a	3.74 ± 1.31 ^a	12.71 ± 2.59 ^b	560.19 ± 23.81 ^a
G3	84.71 ± 2.34 ^a	16.29 ± 2.34 ^a	9.35 ± 1.50 ^a	3.25 ± 1.15 ^a	10.27 ± 2.67 ^b	563.21 ± 22.65 ^a
G4	84.54 ± 2.15 ^a	17.46 ± 2.15 ^a	9.85 ± 1.41 ^a	2.97 ± 1.0 ^a	8.52 ± 2.19 ^{a,b}	579.11 ± 25.40 ^a

Note: Vv: volume density; Hep: hepatocytes; Int: interstitial; Cap: capillaries; Inflammatory cells; LD: lipid droplets; HN: hepatocyte nuclei. G1: commercial ration + 4% OE; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE defatted.

^{a,b}Different letters in the columns denote statistical difference between the groups, $p > .05$.

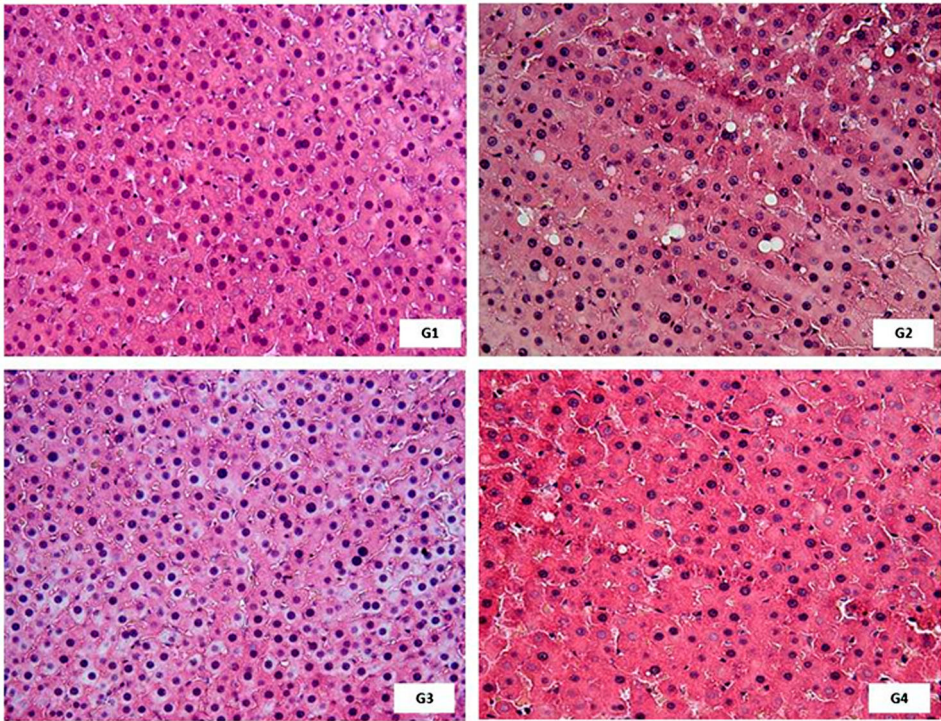


Figure 3. Effect of the extract of *Euterpe edulis* on liver tissue of Wistar rats. G1: commercial ration; G2: commercial ration + 4% OE; G3: commercial feed + 10% LEE; G4: commercial ration + 10% LEDE. Color: hematoxylin and eosin. Magnification: 400 \times .

Discussion

Our results showed that the defatted extract of *E. edulis* presents a great potential for dietary supplementation due to its capacity to positively modulate the redox potential and to induce the release of inflammatory mediators. These findings are important because they show that the consumption of *E. edulis* extract does not induce toxicity to the organism and can be safely consumed. In addition, the beneficial potential of the consumption of this extract on the body is evident from its modulation of important metabolic pathways involved in oxidative stress and tissue inflammation. It is interesting to observe that dietary supplementation with LEDE was able to reduce the serum levels of

TC in the blood and in this way, decrease the possibility of endothelial damage and consequently its deposition in cells that normally do not store this molecule. These results corroborate the findings of Basu et al. (2010), which demonstrated that the increase of dietary flavonoid content decreases the markers of atherosclerotic lesions in the metabolic syndrome. Furthermore, it has been observed that anthocyanin-rich foods are capable of promoting an improvement in the lipid profile (Basu et al., 2016; Hwang et al., 2011).

Analysis of the oxidative status of hepatic tissue showed that there was a decrease in the production of MDA, an important marker of lipid peroxidation and consequently a decrease in the activity of antioxidant defense enzymes (CAT, GST and SOD) in the groups that received LEDE dietary supplementation. Lipid peroxidation is a process generally associated with a significant production of free radicals within the cell, which can culminate in the destruction of membranes, proteins and DNA damage. The antioxidant enzymes act to accelerate the process of passage of electrons and consequently neutralize the action of free radicals. Therefore, a decrease in free radical production may correlate with the reduced expression of antioxidant enzymes (Lei et al., 2016). These data are supported by the findings of some studies that have demonstrated that flavonoid-rich extracts have a high antioxidant effect, promoting a reduction of the chain peroxidation process, reducing the formation of lipoperoxyl radicals and consequently causing less damage to cellular structures (Novaes et al., 2012; Virgili & Marino, 2008; Zhou et al., 2009). In addition, anthocyanins have been shown to exert a positive effect on the inhibition of free radical synthesis, thereby contributing to the maintenance of cellular function. Anthocyanin being one of the most popular antioxidants (Afanas'ev, Dcrozsko, Brodskii, Kostyuk, & Potapovitch, 1989; Dwijayanti, Widodo, Ibrahim, & Rifa'i, 2016; Fukumoto & Mazza, 2000; Gonçalves et al., 2012).

In addition to the oxidative status, the action of *E. edulis* on the release of inflammatory mediators was evaluated, because the pathways related to the inflammatory process are closely related to the cellular redox status (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010). Our results showed that the consumption of an LEDE-supplemented diet was associated with a reduction in the release of pro-inflammatory mediators, and there was a consequent decrease in the release of anti-inflammatory mediators. These mediators are released in small amounts and in a similar proportion in order to maintain tissue homeostasis (Zhang, Liu, & Tsao, 2016).

The release of pro-inflammatory mediators occurs at the beginning of the inflammatory process, and as the inflammation is resolved the production of anti-inflammatory mediators outweighs the production of pro-inflammatory molecules (Tilg, Kaser, & Moschen, 2006). Thus, a reduction in pro-inflammatory mediator production generally indicates no inflammation and results in a decrease in anti-inflammatory mediators (Jiang et al., 2016). During the common cellular migration of phagocytes a process known as respiratory explosion occurs in which the cells produce free radicals to aid in the destruction of aggressive agents and apoptotic remnants of cells derived from their own tissue (Hussain, Hofseth, & Harris, 2003). This may result in the significant association between the production of ROS and the inflammatory process (Pohl et al., 2002). In our study, although no inflammation was induced, we observed that the LEDE extract was effective in inhibiting the release of inflammatory mediators. These results suggest that the consumption of this extract as a food supplement should positively regulate the release of these mediators and consequently modulate the inflammatory response.

We believe that this potential is due to the high concentration of the anthocyanin flavonoid in the fruits of *E. edulis*. The modulating effect of anthocyanins on the inflammatory process has been previously demonstrated to occur through the control of cell migration and proliferation, as well as by inhibiting the production of inflammatory mediators and effectively improve liver damage (Youdim, Martin, & Joseph, 2000; Zhang, Liu, et al., 2016; Zhang, Pan, Jiang, & Mo, 2016). These data corroborate the results shown in the histopathological analysis of the liver in which the groups that received fat-rich extract had a high concentration in the amount of fat droplets in the hepatic tissue when compared to the control and the group supplemented with degreased LEDE. The accumulation of liver fat may be indicative of a reversible degenerative process called steatosis, which is closely related to the production of ROS and tissue inflammation (Gonçalves et al., 2012; Novaes et al., 2012). The more inflammatory mediators are released, the greater the production of ROS within phagocytes, the greater the chance of extravasation of these reactive molecules, which increases the probability of lipid peroxidation and consequently the development of a degenerative process (Gu, Wu, & Li, 2013; Hammerich & Tacke, 2014; Mourtzikou et al., 2014; Zhang, Miao, Han, & Dou, 2011). In the present study, the defatted extract showed morphological characteristics similar to those of the control, indicating that the consumption of this fruit is not toxic and can be safely consumed. It is interesting to note that the maintenance of the normal hepatic architecture in the groups treated with LEDE can be justified by the positive regulation in the redox status and in the release of inflammatory mediators.

This study includes novel data on the hepatic toxicology standard of three *E. edulis* extracts (LEE, LEDE and OE). Dietary supplementation with the extracts revealed no change in weight and absolute body weight as well as biochemical abnormalities in the blood of the animals. The oxidative status and inflammatory mediators generally had a positive modulation in the groups treated with the different extracts. However, the OE and LEE groups showed an increase in the amount of fat droplets when compared to the control and the degreased LEDE group. Based on these findings, we believe that among the extracts tested, the best results were observed in the animals treated with LEDE because there was a significant modulation of the oxidative and inflammatory status in this group compared to that of the other groups. In addition, no morphological changes were observed in the hepatic tissue of these animals. Therefore, our results demonstrate that extract of LEDE is a safe and promising compound with possible antioxidant and anti-inflammatory activities. However, additional studies are required to associate this extract with pathologies associated with an inflammatory process to elucidate the mechanism of action of this extract.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Rodrigo de Barros Freitas is D.S. in Agricultural Biochemistry. Currently, he works as a professor and researcher at the College Governador Ozanam Coelho in Brazil. The interest of their research includes Immunological processes and oxidative stress.

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