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ARÁN

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Trabajo Original

Obesidad y síndrome metabólico

Açai improves non-alcoholic fatty liver disease (NAFLD) induced by fructose

El açai mejora la enfermedad de hígado graso no alcohólico (NAFLD) inducida por la fructosa

Mayara Medeiros de Freitas Carvalho[1,2], Larissa Lélis Teixeira Reis[2], Juliana Márcia Macedo Lopes[2], Nara Nunes Lage[2], Joyce Ferreira da Costa Guerra[3], Helena Porto Zago[4], Larissa de Freitas Bonomo[5], Renata Rebeca Pereira[2,6], Wanderson Geraldo de Lima[1,2,6], Marcelo Eustáquio Silva[1,2,7] and Maria Lucia Pedrosa[1,2,6]

[1]Program in Health and Nutrition. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [2]Research Center in Biological Sciences. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [3]Institute of Genetics and Biochemistry. Federal University of Uberlândia. Patos de Minas Campus. Patos de Minas, MG. Brazil. [4]School of Nutrition. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [5]Department of Pharmacy. Federal University of Juiz de Fora. Governador Valadares Campus. Governador Valadares, MG. Brazil. [6]Department of Biological Sciences. Federal University of Ouro Preto. Ouro Preto, MG. Brazil. [7]Department of Foods. Federal University of Ouro Preto. Ouro Preto, Minas Gerais. Brazil

Abstract

Introduction: the excessive consumption of fructose can cause liver damage, characteristic of non-alcoholic fatty liver disease (NAFLD) associated with changes in lipid metabolism and antioxidant defenses. Açai, the fruit of *Euterpe oleracea* Mart., has demonstrated numerous biological activities, including anti-inflammatory, antioxidant, and lipid metabolism modulating action.

Objective: we evaluated the benefits of açai supplementation on liver damage caused by replacing starch with fructose in rats.

Methods: thirty male *Fischer* rats were divided into two groups, the control group (C, 10 animals), which consumed a standard diet (AIN-93M), and the fructose (F, 20 animals) group, which consumed a diet containing 60% of fructose. After eight weeks, 10 animals from the fructose group received 2% of lyophilized açai, and were called the açai fructose group (FA). The animals were fed *ad libitum* with these diets for another ten weeks. Serum, hepatic and fecal lipid profile, antioxidant enzymes and carbonylated protein were assessed and histopathological characterization of the liver was performed.

Results: açai promoted the reduction of ALT activity in relation to the fructose group (F), reduced alkaline phosphatase to a level similar to that of the control group (C) in relation to the fructose group (F), and reduced catalase activity. The fruit also increased the ratio of total/oxidized glutathione (GSH/GSSG) and reduced the degree of macrovesicular steatosis and the number of inflammatory cells.

Conclusion: the replacement of starch by fructose during this period was effective in promoting NAFLD. Açai showed attenuating effects on some markers of hepatic steatosis and inflammation.

Key words:

Euterpe oleracea Mart. Fructose-diet. Hepatic steatosis. Antioxidant enzymes. Inflammation.

Resumen

Introducción: el consumo excesivo de fructosa puede causar daño hepático, característico de la enfermedad hepática grasa no alcohólica (EHGNA), asociada con cambios en el metabolismo de los lípidos y defensas antioxidantes. El açai, fruto del *Euterpe oleracea* Mart., ha demostrado desempeñar numerosas actividades biológicas, incluidas acciones antiinflamatorias, antioxidantes y moduladoras del metabolismo lipídico.

Objetivo: se evaluaron los beneficios de la suplementación con açai en el daño hepático causado por la sustitución del almidón por fructosa en ratas.

Métodos: se distribuyeron 30 ratas Fischer macho en dos grupos: 10 ratas en el grupo control (C), que consumía una dieta estándar (AIN-93M), y 20 ratas en el grupo fructosa (F), que consumía una dieta que contenía un 60% de fructosa. Después de ocho semanas, diez animales del grupo fructosa recibieron un 2% de açai liofilizado, por lo que pasaron a integrar el grupo açai fructosa (FA). Los animales fueron alimentados *ad libitum* con estas dietas durante otras diez semanas. Se analizaron el perfil lipídico hepático y fecal, las enzimas antioxidantes y la proteína carbonilada, y se realizó la caracterización histopatológica del hígado.

Resultados: el açai promovió la reducción de la actividad de ALT en relación al grupo de fructosa (F) y la reducción de la fosfatasa alcalina a niveles similares a los hallados en el grupo control (C) en relación con el grupo de fructosa (F). El fruto también aumentó la proporción de glutatión total/oxidado (GSH/GSSG) y redujo el grado de esteatosis macrovesicular y el número de células inflamatorias.

Conclusión: la sustitución de almidón por fructosa durante este periodo fue eficaz en la promoción de NAFLD. El açai mostró efectos atenuantes en algunos marcadores de esteatosis hepática y de inflamación.

Palabras clave:

Euterpe oleracea Mart. Dieta rica en fructosa. Esteatosis hepática. Enzimas antioxidantes. Inflamación.

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Correspondence:

M. L. Pedrosa. Biological Research Center. Federal University of Ouro Preto. Ouro Preto, MG. Brazil
e-mail: mlpedrosa@gmail.com

INTRODUCTION

The non-alcoholic fatty liver disease (NAFLD) includes isolated hepatic steatosis and non-alcoholic steatohepatitis (NASH) and their prevalence has doubled in the last 20 years. It has been considered as the primary cause of liver disease (1). The prevalence of this disease is estimated to be 20-30% of the general population in Europe and the USA (2). NAFLD was confirmed as the etiology that most involves hepatocellular carcinoma in the United States (3). Mechanisms involved in NAFLD pathogenesis have been elucidated and it is known that dietary components may aggravate or reduce the risk of development of steatosis. Insulin resistance and obesity increase caloric intake, *de novo* lipogenesis (DNL) and free fatty acid (FFA) flux from adipose tissue to the liver, and impaired VLDL secretion leads to fat accumulation in the liver. The accumulation of lipids and multiple hits are involved in the development of NASH, including mitochondrial impairment, the role of microbiota, iron accumulation, genetic factors, and the release of reactive oxygen species (4).

Fructose has been a key player in the development of NAFLD; its hepatic extraction and metabolism are especially high, as compared to glucose. This happens because of the extensive amount of fructokinase that phosphorylates fructose to fructose 1-phosphate in the liver and to the subsequent metabolism of fructose 1-phosphate at the triose phosphate level, which bypass flux control at phosphofructokinase (5).

The high intake of fructose results in substrate for *de novo* lipogenesis, the FFA are incorporate into triacylglycerols (TAG) or other lipids are connected with increased VLDL synthesis, which has a player in non-alcoholic fatty liver disease. Whenever hepatic DNL is induced, new lipids are synthesized, nonesterified FA are re-esterified, and hepatic lipid oxidation is downregulated. These facts cause an imbalance between hepatic lipids, induce in intrahepatic fat accumulation (4,6).

Excessive accumulation and deposition of fat in the liver predisposes for the devolvement of NASH, increasing the vulnerability for cirrhosis and hepatocellular carcinoma. The presence of oxidative stress and proinflammatory mediators accelerates the deterioration of the liver, because of reactive oxygen species (ROS) overproduction and excessive fatty acids oxidation, which increases lipid peroxides. The presence of toxic metabolites activates the lysosomal cell death pathway and it results in cytotoxicity, collaborating with hepatic inflammation, ROS and the possibility of hepatic fibrosis and cirrhosis by activating hepatic stellate cells. However, these indications can be relieved as activating hepatic antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and antioxidants compounds (4,7).

Antioxidant therapy for the treatment of NAFLD, and also including NASH, has the potential to alleviate oxidative stress and cell death, which promote diseases (8). Anthocyanins have shown beneficial effects in reducing steatosis and liver damage (9).

The *Euterpe oleracea* Mart., popularly known as açai, is native to Brazilian Amazon, and has gained new markets by the presence of beneficial antioxidants which are beneficial to health, such as phenolic compounds, mainly anthocyanins and vitamin E (10). *In vivo* studies demonstrate the action of açai in improving the lipid profile, different parameters related to oxidative stress, antioxidant enzymes and the reduction of non-alcoholic liver steatosis (11-14). In addition to acting as an antioxidant, anthocyanins also regulate the lipid metabolism, and we have previously shown that açai attenuates the development of hepatic steatosis in mice on a high-fat diet, downregulating the expression of genes involved in lipogenesis (15).

In this context, this study aimed to evaluate the effect of açai consumption on the metabolism of rats under NAFLD induced by the isocaloric replacement of starch by fructose. In this study, the administration of lyophilized açai improved liver function and antioxidant enzymes, increased the ratio of the total glutathione per oxidized (GSH/GSSG), and reduced the degree of macrovesicular steatosis and inflammation. The clarification of the protective effects of açai may expand the range of dietary options in the treatment and prevention of this disease, and contributes to the hypothesis that adding bioactive compounds sources to the diet can alleviate NAFLD progression (8). We expect our results may subsidize future efforts researching açai and other potential sources to be used in the NAFLD treatment.

MATERIALS AND METHODS

LYOPHILIZED AÇAI PURCHASE AND COMPOSITION

Lyophilized açai pulp was kindly provided by the Company Liotécnica Tecnologia em Alimentos (São Paulo-SP/Brazil). Each 100 g of freeze-dried açai contained had 541 kcal, 5 g of total carbohydrate, 9.8 g of protein, 54 g of total fat and 27 g of dietary fiber, according to the provider.

The Folin-Ciocalteu method was used to determine total phenolic content, as described by Georgé et al. (16). The total amount of phenolic compounds was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of açai.

The antioxidant capacity was determined by the modified 2.2-diphenyl-1-picrylhydrazyl (DPPH) method (17), which is based on the quantification of free radical-scavenging. A methanol solution containing DPPH was prepared, and an aliquot of açai was added, homogenized and kept in the dark for 30 min at room temperature. Antioxidant activity was determined by the reduction in absorbance of the DPPH radical at 515 nm. Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) was used as an antioxidant standard and the results were expressed as trolox equivalent antioxidant capacity (TEAC) per 100 g of açai.

ANIMALS AND EXPERIMENTAL DESIGN

Eleven-week-old male Fischer rats weighing approximately 200 g were obtained from the Laboratory of Experimental Nutrition at the School of Nutrition of the Federal University of Ouro Preto (UFOP).

The Ethics Committee in Animal Research of the UFOP (protocol no. 2013/46) approved animal procedures. The rats were housed individually under a 12-h light/12-h dark cycle and temperature-controlled conditions, with food and water *ad libitum*.

Initially, 30 rats were distributed into two experimental groups: 10 animals in the control (C) group were fed the AIN-93 M standard diet (18), and 20 animals in the fructose (F) group received a fructose-rich diet (containing 60% fructose) (19,20). The composition of these experimental diets is described in table I. After eight weeks, group F was further divided and ten animals started receiving the same diet but now containing 2% of lyophilized açai, and was called the group FA (fructose açai). Rats were fed these diets *ad libitum* for ten weeks. After all 18 weeks, the rats were euthanized by total blood collection from adjacent vessels to the brachial plexus under isoflurane anesthesia. Blood was collected in polypropylene tubes and centrifuged at 3,000×g for 15 min. Serum was then removed and stored at -80 °C. The liver was collected and weighed during the experiment, and body weight and food intake were weekly monitored.

LIPIDS EXTRACTION AND SERUM ANALYSIS

The lipid extraction of the liver was performed according to the method described by Folch et al. (21) and the total hepatic lipids obtained by solvent evaporation. These lipids were dissolved in 1 ml of isopropanol, and triglycerides were measured using Labtest® kits (Lagoa Santa, MG, Brazil). In serum, the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, as well as cholesterol, triglycerides, high density lipoprotein (HDL), and glucose were measured using Labtest® (Lagoa Santa, MG, Brazil) kits. Measurements were performed according to the manufacturer's instructions.

ANTIOXIDANT PROFILE AND PROTEIN CARBONYL LEVEL

Superoxide dismutase (SOD) was assayed using Superoxide Dismutase Assay Kit (Cayman Chemical Company, no. 706002; lot 0450282). This essay utilizes tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxantine. One unit of SOD was defined as the amount of enzyme needed to cause 50% dismutation of the superoxide radical; only Cu/Zn-SOD is measured.

Catalase (CAT) activity was determined according to Aebi (22), a method that is based on the enzymatic decomposition of H_2O_2 observed spectrophotometrically at 240 nm for 3 min, using 50 mM phosphate buffer, pH 7.0, containing 5 mM H_2O_2. Hydrogen peroxide decomposition was calculated using the molar absorption coefficient 0.0394 l/mmol/l/cm of peroxide. One unit of CAT is equivalent to the hydrolysis of 1 µmol of H_2O_2 per min and the results were expressed as activity per milligram of protein.

The total glutathione content was determined by a kinetic assay which utilizes a method based on the reduction of DTNB (5,5'ditiobis [2-nitrobenzoic] acid) in TNB (5-thio-2-nitrobenzoic acid) proposed by Griffith (23), which can be detected spectrophotometrically at 412 nm. Oxidized glutathione (GSSG) was determined after derivatization of total GSH with 2-vinylpiridine. Oxidative stress index was calculated from the GSH/GSSG ratio.

The activity of glutathione peroxidase (GPx) was determined according to the method proposed by Paglia and Valentine (24) with modifications. The method is based on the oxidation of reduced glutathione (GSH), catalyzed by glutathione peroxidase, coupled to the recycling of GSSG through the reaction catalyzed by the enzyme glutathione reductase that uses NADPH as cofactor. The decrease in absorbance measured at 340 nm during the oxidation of NADPH is indicative of the activity of glutathione

Table I. Composition of experimental diets

Components	Control (g/kg)	Fructose (g/kg)	Fructose açai (g/kg)
Choline	2.5	2.5	2.5
Methionine	2.5	2.5	2.5
Vitamins mixture	10.0	10.0	10.0
Mineral mixture	35.0	35.0	35.0
Cellulose	50.0	50.0	50.0
Sucrose	100.0	100.0	100.0
Soybean oil	40.0	40.0	40.0
Casein	140.0	140.0	140.0
Corn starch	620.0	20.0	0.0
Fructose*	0.0	600.0	600.0
Lyophilized açai†	0.0	0.0	20.0

*Vitamin mixture (IU or g/kg of mixture): retinol acetate, 2 000 000 IU; cholecalciferol, 200,000 IU; p-aminobenzoic acid, 10.00; inositol, 10.00; niacin, 4.00; calcium pantothenate, 4.00; riboflavin, 0.80; thiamin HCl, 0.50; pyridoxine HCl, 0.50; folic acid, 0.20; biotin, 0.04; vitamin B12, 0.003; sucrose, quantity sufficient to 1 kg; choline, 200.0; α-tocopherol, 10,000 IU (AOAC, 1980). Salt mixture (g/kg of mixture): NaCl, 139.3; KI, 0.79; MgSO4.7H2O, 57.3; aCO3, 381.4; MnSO4.H2O, 4.01; FeSO4.7H2O, 0.548; CuSO4.5H2O, 0.477; CoCl2.6H2O, 0.023; KH2PO4, 389.0. (AOAC, 1980). *Fructose (Sythn); †Lyophilized açai (Liotécnica/Brazil).*

peroxidase. The determination of the enzymatic activity of glutathione reductase (GR) was performed according to the method proposed by Carlberg and Mannervik (25). The assay is based on the reduction of glutathione oxidized by NADPH in the presence of glutathione reductase. The decrease in absorbance measured at 340 nm during the oxidation of NADPH is indicative of the activity of glutathione reductase.

Carbonyl protein (PC) levels were determined according to the method described by Levine et al. (26). Each sample was precipitated with 10% (w/v) TCA (tichloroacetic acid). After centrifugation, the precipitate was treated with 10 mmol of DNPH in 2N HCl, incubated in the dark for 30 min and then treated with 10% TCA. After centrifuging, the precipitate was washed twice with ethanol/ethyl acetate (1:1) and dissolved in 6% SDS. Absorbance was determined at 370 nm. The results were expressed in nmol of DNPH incorporated/mg of protein. The content of DNPH incorporated was calculated using the molar absorption coefficient of DNPH (22,000 $M^{-1}cm^{-1}$).

Total protein content was determined according to the method described by Lowry et al. (27) using bovine serum albumin (BSA) as the standard. This test was used only for correction of previous trials, such as SOD, CAT, PC, glutathione peroxidase and reductase.

HEPATIC HISTOLOGY

Livers were removed at the end of the experiment and fixed in 10% buffered formalin, subsequently embedded in paraffin, for cutting in sections of about 4 µm in a semi-automatic microtome, mounted and stained by hematoxylin and eosin (H&E). Photomicrographs were taken on a Leica DM5000 microscope coupled to a digital camera at 400× magnification. A semiquantitative scoring system was used to assess the severity of hepatic steatosis in ten microscopic fields examined as described previously (28). In brief, macrovesicular steatosis was graded from 1 to 3 depending on the percentage of hepatocytes that contained fat; grade 1 was assigned if < 33% of hepatocytes contained fat; grade 2, if 33-66% contained fat; and grade 3, if > 66% contained fat.

Inflammation was assessed with the Leica QWin software (Leica Microsystems, Germany) using 15 images of randomly-selected fields (total area 1.15 × 106 μm^2) of tissue sections for a single slide per animal. The inflammatory process was determined by the difference ($p < 0.05$) between the number of inflammatory cells present in the liver of the animals.

STATISTICAL ANALYSIS

Data were subjected to the Kolmogorov-Smirnov test for normality, expressed as mean ± standard deviation (SD) in cases of normal distribution, and expressed as median and interquartile ranges in cases of non-parametric distribution. The data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test, for parametric data or the Kruskal-Wallis test followed by Dunn post-test for non-parametric data. Differences were considered as significant when $p < 0.05$. All analyses were conducted using the software GraphPad Prism version 6.00 for Windows (San Diego, CA).

RESULTS

ANALYSIS OF LYOPHILIZED AÇAI

Freeze-dried açai showed a high content of total polyphenols (1,619.03 mg GAE/g) and antioxidant capacity (104.80 µMTEAC/g).

EFFECTS ON BODY MASS AND FOOD INTAKE

At the end of the experimental period there was no difference between groups with respect to food intake, weight gain, feed efficiency coefficient and fecal excretion (Table II).

EFFECTS ON BLOOD GLUCOSE AND LIPID PROFILE IN SERUM AND THE LIVER

The fructose-rich diet promoted changes in the lipid profile of the animals, increasing serum and liver TAG levels. The administration of açai did not change theses parameters. The other serum fractions, total cholesterol, HDL cholesterol and non-HDL showed no significant differences between groups (Table III).

EFFECTS ON ANTIOXIDANT ENZYMES

Superoxide dismutase (SOD) showed reduced activity in the F and FA groups. On the other hand, catalase activity (CAT) was higher in group F and the group FA showed a partial reduction of this activity when compared to group F.

The concentration of GSSG in group F was not changed and treatment with açai reduced this parameter compared to controls. The ratio between reduced and oxidized glutathione (GSH/GSSG)

Table II. Food intake, weight gain and fecal excretion in rats fed a control diet or a fructose rich-diet supplemented (FA) or not (F) with açai

Parameter	Experimental groups		
	C	F	FA
Food intake (g/day)	19.8 ± 1.2	19.5 ± 0.8	19.3 ± 0.9
Weight gain (g)	171.3 ± 30.1	153.5 ± 19.5	157.4 ± 31.4
Fecal excretion (g/week)[1]	5.1/4.2-6.3	5.0/4.1-6.6	5.7/4.5-5.8

Values are expressed as the mean ± SD or [1]median and interquartile ranges (n = 10).

Table III. Serum lipid profile and liver triacylglycerols of rats fed a control diet or a fructose rich-diet supplemented (FA) or not (F) with açai

Parameter	Experimental groups		
	C	F	FA
Glucose (mg/dL)	96.9 ± 15.6‡	137.1 ± 29.2†	138.8 ± 13.9†
TAG (mg/dL)	116.6 ± 34.5‡	180.4 ± 36.1†	185.8 ± 50.0†
CT (mg/dL)	115.0 ± 17.6	103.4 ± 14.9	104.4 ± 16.7
HDL (mg/dL)	79.9 ± 13.9	70.3 ± 10.6	69.7 ± 12.6
Non-HDL (mg/dL)	35.0 ± 7.2	33.0 ± 6.6	34.8 ± 6.5
TAG (mg/g of liver) *	8.5/7.4-10.7‡	16.0/11.9-29.4†	14.8/8.9-31.4†

*TAG: triacylglycerols; CT: total cholesterol; Non-HDL: VLDL + LDL. Values are expressed as the mean ± SD or *median and interquartile ranges (n = 10). Within a row, significantly different values are marked with different superscript signs.*

was higher in group FA. Total glutathione, reduced glutathione, GR and GPx showed no statistical difference between the experimental groups. There were no statistical differences in the PC level (Table IV).

EFFECTS ON STEATOSIS AND LIVER INJURY

As shown in table V, the fructose-rich diet increased liver weight and glucose levels as compared to group C. ALT and alkaline phosphatase activities in group F were increased, as compared to the other groups. The group FA showed reduction in ALT and alkaline phosphatase activity to similar levels as the control group. There was no difference in AST activity between the experimental groups (Table V).

According to histological analyses after processing and staining with H&E, we observed the high presence of macrovesicular steatosis and the number of inflammatory cells (Fig. 1A-C). The addition of açai reduced the degree of steatosis, since 90% of the animals were classified as grade 1 steatosis (Fig. 1D) and reduced the number of inflammatory cells to intermediate levels (Fig. 1E).

Table IV. Antioxidant enzymes and protein carbonyl levels in the liver of rats fed a control diet or a fructose rich-diet supplemented (FA) or not (F) with açai

Parameter	Experimental groups		
	C	F	FA
SOD (U/mg protein)	0.11 ± 0.03†	0.06 ± 0.03‡	0.08 ± 0.03‡
CAT (U/mg protein)	83.2 ± 11.0‡	99.6 ± 16.5†	85.1 ± 13.6†,‡
Total glutathione (nmoles/mL)	69.2 ± 27.2	68.9 ± 32.5	94.0 ± 15.3
GSSG* (nmoles/mL)	5.5/4.2-8.4†	7.01/5.1-7.7†	4.5/3.2-5.4‡
GSH (nmoles/mL)	63.1 ± 27.6	62.6 ± 31.0	98.2 ± 14.8
GSH/GSSG ratio	10.9 ± 6.1‡	9.6 ± 4.9‡	19.7 ± 5.1†
GPx (U/mg protein)	0.02 ±0.00	0.01 ± 0.00	0.01 ±0.00
GR (U/mg protein)	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0,02
PC (nmoles/ mg protein)	1.8 ± 0.5	1.9 ± 0.4	1.9 ± 0.3

*SOD: superoxide dismutase; CAT: catalase; PC: protein carbonyl; GSSG: oxidized glutathione; GSH: reduced glutathione; GSH/GSSG: ratio of reduced glutathione and oxidized glutathione; GPX: glutathione peroxidase; GR: glutathione reductase. Values are expressed as the mean ± SD or *median and interquartile ranges (n = 10). Within a row, significantly different values are marked with different superscript signs.*

DISCUSSION

We examined whether açai improves liver damage, antioxidant enzymes and inflammation in fructose rich diet-induced NAFLD in rats. Although there are other reports on positive effects of açai on steatosis induced by a high-fat diet as well as its antioxidant capacity (11-15), studies using açai on steatosis induced by fructose were not found. We provide evidence that the açai fruit reduced lipid accumulation, inflammation and oxidative stress in rats with NAFLD induced by a fructose-rich diet.

The lyophilized açai used in this study showed a total phenolic content of 1,619.03 mg GAE/g. This value is higher than that in commercial açai pulps, ranging between 182.95 and 598.55 mg GAE/g (29) and a fresh fruit extract, which had 31.20 mg GAE/g of polyphenols (30). In the present study, the antioxidant capacity (TEAC) of lyophilized açai was 104.80 μM/g, which corresponds to higher values of commercial pulp studies which presented between 10.21 and 52.47 uM/g (29). The addition of 2% lyophilized açai to the fructose-rich diet corresponded to an increase of 28.3 kcal/kg, which did not alter significantly the calorie content; previous studies using 2% pulp açai showed protective effect against metabolic disorders (12,14).

In the present study, increased food consumption, body weight gain and fecal excretion in group F is in accordance with findings of previous studies, in which a fructose-rich diet was used (31-33).

Table V. Relative weight of the liver and enzyme activity in serum of rats fed a control diet or a fructose rich-diet supplemented (FA) or not (F) with açai

Parameter	Experimental groups		
	C	F	FA
Liver (%)	2.3 ± 1.1‡	3.05 ± 0.3†	3.10 ± 0.2†
AST (U/mL)	52.9 ± 9.9	54.4 ± 12.1	54.3 ± 12.7
ALT (U/mL)	16.9 ± 2.7†,‡	20.1 ± 5.4†	15.7 ± 1.9‡
Alkaline phosphatase (U/L)	79.3 ± 19.0‡	107.2 ± 18.9†	98.9 ± 22.9†,‡

AST: aspartate-aminotransferase; ALT: alanine-aminotransferase. Values are expressed as the mean ± SD (n = 10). Within a row, significantly different values are marked with different superscript signs.

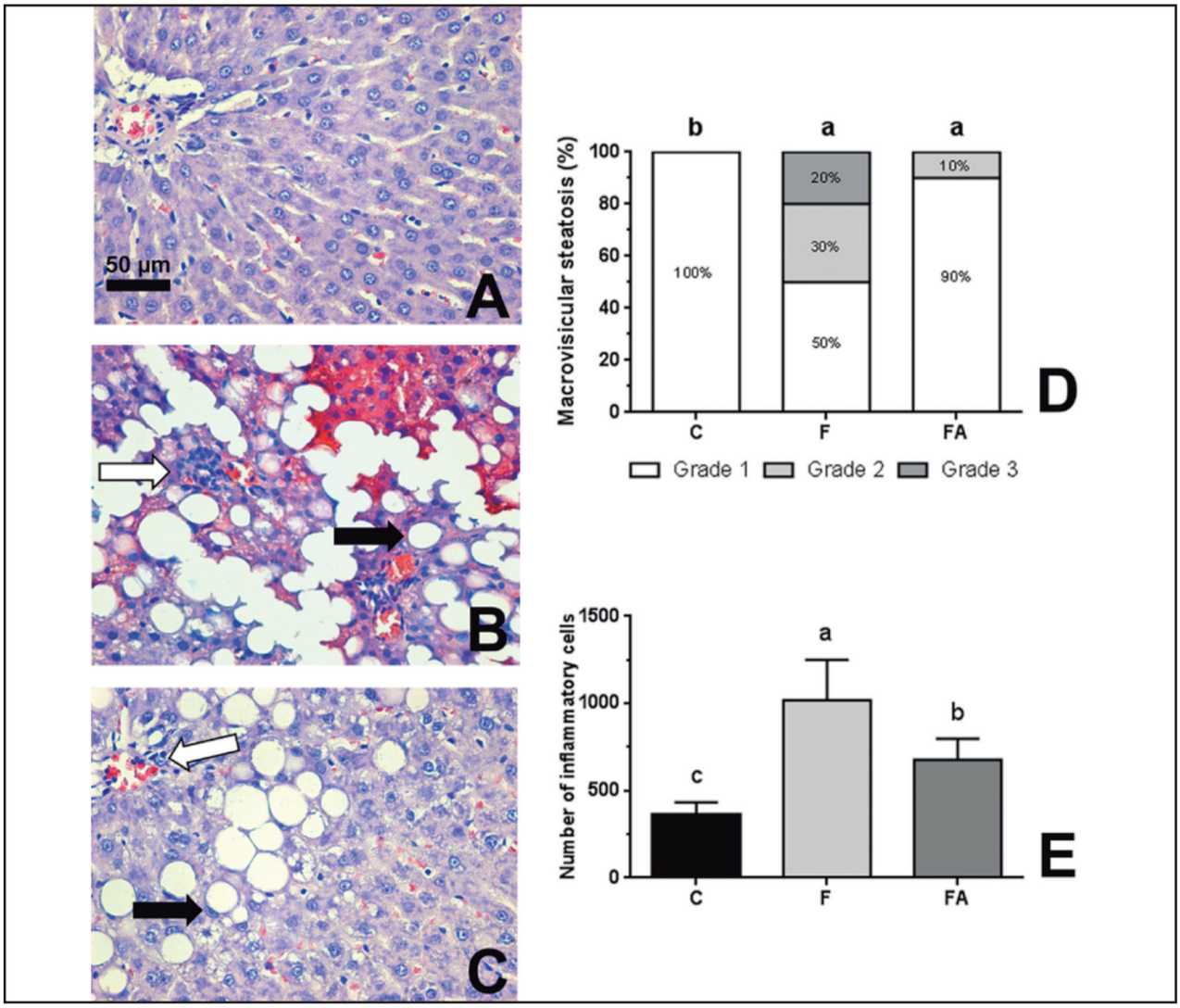

Figure 1.

Histological micrographs of liver tissue stained with H&E. A. Group C presenting normal histological picture. B. Group F presenting intense macrovesicular steatosis (black arrow). C. Group FA presenting mild macrovesicular steatosis (black arrow). D. Classification of the degree of macrovesicular steatosis. E. Number of inflammatory cells between experimental groups. The white arrow in panels B and C indicates inflammatory cells. Steatosis rating: grade 1, < 33%; grade 2, 33-66%; and grade 3, more than 66%. Different letters represent statistical differences using the Kruskal-Wallis test followed by Dunn's post-test test. Bar = 50 uµ. Images were shot with 400x magnification.

The presence of açai in the FA group did not change body weight gain and fecal excretion; the same behavior was observed in studies using açai in high-fat model (11,15). In addition, the açai treatment did not modify food consumption.

Fructose is primarily metabolized in the liver and its your hepatic load increases gluconeogenesis and reduces the ability of insulin to suppress glucose production. In addition, enhanced lipogenesis and decreased peripheral lipid catabolism play a role in hyperinsulinemia, hypertriglyceridemia and insulin resistance induced by fructose-rich diet, as demonstrated in studies using rat models (32,33). Here, we reinforce the previous findings, but in a model that may best portray the pathogenesis of NAFLD in humans, since it has been previously observed that fructose supplementation also induces hyperinsulinemia, hypertension, hypertriglyceridemia and insulin resistance (34), characteristics associated with NAFLD and findings observed in the present report. The administration of açai did not change glucose and TAG in serum and the liver. Previously, Guerra et al. (15) observed reduced hepatic lipid content and downregulation in the expression of genes involved in lipogenesis. This may be due to the different models of NAFLD induction, and therefore, different metabolic pathways are involved in the accumulation of TAG, suggesting that the pathways involved in the accumulation of TAG from fructose were not influenced by açai components.

NAFLD pathogenesis involves multiple *hits*, and oxidative stress plays a central role and correlates with severity and disease states

such as cell death and tissue damage. We investigated antioxidant defense and oxidative damage to macromolecules. Oxidative damage was evaluated using protein carbonyls, a generic marker of protein oxidation for examining the extent of protein oxidation *in vivo* (35). No differences were found amongst the experimental groups regarding these analyses in our study, possibly the longest experimental period, as compared to other studies in the literature (36,37).

However, fructose addition effectively improved the GSH/GSSG ratio. Glutathione is synthesized in the liver and is the first line of defense against oxidative stress (38). The addition of açai promoted a reduction in the levels of GSSG and an increase in GSH/GSSG ratio, showing its beneficial action on the main antioxidant system. Chronic insults increased the GSSG levels and the application of the GSH/GSSG ratio is used to measure the oxidative status (39). In fact, the formation and accumulation of GSSG were effectively inhibited by açai feeding under the present experimental conditions, and it suggests that the endogenous glutathione pool slowly shifted toward the reduced state in the açai group when compared to the fructose group, developing an antioxidative *status* in the açai-fed rats.

The results obtained in this study suggest that açai may improve oxidative stress and NAFLD by regulating glutathione metabolism. Similar results were observed in other models with different polyphenols (13,36,37). This suggests that polyphenols and other nutrient fractions in açai pulp could function to reduce the stressful environment caused by the fructose-rich diet.

Similar to the antioxidant defense system GSH/GSSG, an improvement in the activity of CAT was observed. CAT is an important enzyme which is responsible for the removal of H_2O_2 produced under various stress conditions, while SOD plays an important role in protecting against the toxic effects of superoxide radicals by catalyzing radical dismutation reactions, protecting cells from oxidative-stress-related damage. In view of these findings, it was observed that CAT activity was partially reduced in the acai-fed group, what can be justified by the fact that flavonoids reduce the activity of antioxidant enzymes, particularly CAT, due to an improvement in the redox state.

The administration of a fructose-rich diet resulted in a higher relative mass of the liver in the animals and this can be explained by the fact that the monosaccharide is primarily metabolized in the liver, resulting in increased *de novo* lipogenesis, lipogenic enzymes and gluconeogenesis (31-33). The presence of açai in this model reduced the liver mass and the number of inflammatory cells, and improved ALT activity; in line with this, serum alanine and aspartate aminotransferase (ALT and AST) activities have been associated with the improvement of hepatic steatosis, and lobular inflammation, so their serum activity has been used as a biomarker of liver damage and/or treatment benefits (4).

The liver changes found in our study and described to date were confirmed by histological analysis, since there was a prevalence of fatty macrovesicles in the model group, which are characteristic of NAFLD, as demonstrated elsewhere (31-33,40). The addition of açai also reduced the macrovesicular steatosis levels resulting in the presence of 90% of steatosis grade 1, against 50% in the presence of açai. This improvement in the severity of macrovesicular steatosis stages was also observed in NAFLD induced by a high-fat diet (14).

Taken together, our results showed that an isocaloric substitution of fructose for starch in rats causes steatosis and inflammation and reduces antioxidant systems in the liver. The açai treatment attenuated the degree of steatosis and inflammation, and improved the response to oxidative stress by increasing the activity of catalase and the GSH/GSSG ratio. Therefore, the present study adds knowledge about açai feasible therapeutic strategy for prevention of NAFLD induced by a fructose-rich diet.

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