Journal of Food and Nutrition Research, 2019, Vol. 7, No. 2, 132-140
Available online at http://pubs.sciepub.com/jfnr/7/2/5
Published by Science and Education Publishing
DOI:10.12691/jfnr-7-2-5

Araçá (*Psidium cattleianum* Sabine) Ameliorates Liver Damage and Reduces Hepatic Steatosis in Rats Fed with a High-fat Diet

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Received November 12, 2018; Revised January 03, 2019; Accepted February 14, 2019

Abstract Bioactive compounds, present in some foods, act enhancing the endogenous antioxidant system and are proposed as an effective strategy in preventing the changes induced by free radicals in some diseases, such as nonalcoholic fatty liver disease (NAFLD). There has been an increase in the number of studies carried out with the aim of finding natural antioxidant compounds present in fruits, mainly the native fruits of Brazil, because they contain a high content of these compounds. Araçá (*Psidium cattleianum* Sabine) is a fruit that is rich in polyphenols and exhibits strong antioxidant, antiproliferative, and anti-inflammatory activities. Therefore, the present study investigated the effects of araçá flour on oxidative stress, liver injury, and antioxidant defenses in high-fat diet-induced hepatic steatosis in rats. *In vitro* experiments showed that araçá contains high concentrations of total polyphenols and exhibits strong antioxidant activity with no cytotoxicity. *In vivo* experiments indicated that the addition of araçá to a high-fat diet inhibited the activities of alanine aminotransferase and aspartate enzymes, reduced macrovesicular steatosis, increased the paraoxonase activity, and increased the concentration of the total and reduced forms of glutathione. Therefore, our findings suggested the hepatoprotective role of araçá against the progression of steatosis.

Keywords: *Psidium cattleianum Sabine, araçá, phenolic compounds, hepatic steatosis, antioxidants defenses*

Cite This Article: Alice Helena S. PAULINO, Ana Maria F. VIANA, Larissa F. BONOMO, Joyce Ferreira C. GUERRA, Juliana Márcia M. LOPES, Ana Carolina S. RABELO, Miliane Martins A. FAGUNDES, Ana Lúcia Rissoni S. RÉGIS, Wanderson G. LIMA, Maria Lúcia PEDROSA, and Marcelo Eustáquio SILVA, "Araçá (*Psidium cattleianum* Sabine) Ameliorates Liver Damage and Reduces Hepatic Steatosis in Rats Fed with a High-fat Diet." *Journal of Food and Nutrition Research*, vol. 7, no. 2 (2019): 132-140. doi: 10.12691/jfnr-7-2-5.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by abnormal fat accumulation, this deposit being greater than 55 mg/g of liver or affecting more than 5% of hepatocytes in the form of vesicles, which can in turn lead to more severe damage (steatohepatitis, fibrosis, cirrhosis, and sporadic hepatic carcinoma) [1]. In general, NAFLD prevalence in western countries ranges from 20% to 35% and affects most ethnic groups and age [2,3]. NAFLD is strongly associated with other diseases, such as obesity, type 2 diabetes mellitus, hypertension, and hyperlipidemia and is considered a hepatic manifestation of metabolic syndrome [2,3,4].

NAFLD is a multifactorial disease that is characterized by changes that are mainly related to increased oxidative stress caused by imbalanced redox control [5]. Oxidative stress is characterized by increased free radical production and consequent increase in lipid and protein oxidation, which could in turn promote hepatic inflammation and aggravation of steatosis [6].

The strong antioxidant effects of certain bioactive compounds that are present in the diet, such as phenolic compounds, flavonoids, anthocyanins, and carotenoids, aids the endogenous enzymatic antioxidant defense system (catalase, glutathione peroxidase, and superoxide dismutase) and reduces oxidative stress-induced damage [7,8]. As a result, foods containing bioactive compounds, especially fruits, have been widely used in the prevention and

treatment of chronic diseases, including NAFLD [9,10]. Consistent with the above findings, previous *in vitro* and *in vivo* studies have demonstrated that fruits with high phenolic content act as therapeutic agents against hepatic damage during the initial stages of NAFLD [10,11,12].

Brazil is one of the major centers of genetic diversity. Native fruits of the Cerrado Biome have received great interest because of nutritional potential. The Araçá (*Psidium cattleianum* Sabine), also known as yellow guava, is a fruit that belongs to the family Myrtaceae. *In vitro* studies revealed that araçá contains high concentrations of polyphenols and exerts strong antioxidant, anti-inflammatory, and antiproliferative effects [4,13,14]. Given the above findings and the fact that no previous studies have investigated the effects of araçá in high-fat diet-induced hepatic steatosis, we aimed to characterize araçá flour and investigate the effects of araçá on oxidative stress, liver injury, and antioxidant status in rats.

2. Material and Methods

2.1. Plant Material

Yellow araçá fruits were collected from trees located in the Federal University of Ouro Preto, Ouro Preto (State of Minas Gerais, Brazil). Fully mature fruits were harvested in May and June. The fruits were pre-selected and those with any type of injury were discarded. Samples were washed in filtered water, dried in an oven at 40 ℃ (for three days), and powdered. The flour was stored in a special plastic bag at 4 ℃. The resulting araçá flour was mixed with the experimental diet (2%) and administered to the animals. Exsiccates were prepared, and the voucher specimen was deposited in the collection of the Herbarium Professor José Badini (OUPR) under the registration number OUPR 28376. The composition of araçá (per 100 g) was: 7.82 ± 0.33 g of moisture, 9.20 ± 1.07 g of lipids, 3.36 ± 0.08 g of protein, 3.69 ± 0.06 g of total ash determined according to the guidelines [15], 37.20 ± 0.01 g of insoluble fiber, determined based on the neutral detergent fiber method [16], and 38.73 g of carbohydrate (determined by calculating the difference in relation to the other components).

2.2. Total Phenolics, Antioxidant Activity, and Cytotoxicity *in vitro*

Total polyphenol content was determined based on the Folin-Ciocalteu method [17]; gallic acid was used as a standard for generating the calibration curve. The total amount of phenolic compounds was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of araçá

Antioxidant capacity was determined based on the modified DPPH (2,2-diphenyl-1-picryl-hydrazil) method [18], which measures the ability to scavenge free radicals. Briefly, a methanol solution containing 0.06 mM DPPH was prepared. After adjusting the blank with methanol, a 100 µL aliquot of the araçá solution was added to 3.9 mL of the methanol solution. The mixture was homogenized and placed in the dark for 30 min at room temperature. Antioxidant activity was determined by measuring the reduction in absorbance of the DPPH radicals at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as an antioxidant standard. Data were expressed as trolox equivalent antioxidant capacity (µM TEAC/g).

Cell viability was evaluated by conducting the MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, which measures mitochondrial function based on the reduction of MTT to formazan crystals. The amount of formazan crystals formed is directly proportional to the number of viable cells [19]. HepG2 cells were plated (1×10^5 cells) in 96-well microplates with DMEM supplemented with 10% fetal bovine serum (FBS), with a total final volume of 160 µL and subsequently incubated for 24 h in a humidified oven with 5% CO_2 atmosphere at 37 ℃ for adhesion of cells. After 24 h, the culture medium was removed, and the cells were added with DMEM and varying concentrations of araçá; the controls were added with medium alone. After 24 h of incubation, the supernatant was removed, and the wells were washed with PBS. Subsequently, each well was added with 200 µL of a solution containing 5 mg/mL MTT in DMEM were added and incubated for 1 h at 37 ℃. Afterwards, MTT solution was removed, and 100 µL of DMSO was added to each well. The absorbance at 570 nm was read on an ELISA reader. Cell viability was calculated based on the following formula: (absorbance of treated cells/absorbance control) × 100. The control was assigned 100% viability.

2.3. Animals and Experimental Design

Forty-day-old female Fischer rats weighing 125 g each were acquired from the Laboratory of Experimental Nutrition at the School of Nutrition, Federal University of Ouro Preto (UFOP), Ouro Preto, MG, Brazil. Animals were individually kept in metabolic cages with controlled temperature, ventilation, and humidity. Food and water were provided *ad libitum*. All procedures were approved by the university's Ethics Committee in Animal Research (Protocol No. 2013/40). Animals were divided into the following four treatment groups according to their diet: control (C), AIN-93M standard diet [20]; high-fat (HF), high-fat diet containing 25% soya oil and 2% cholesterol [21]; control araçá (CAF), AIN-93M standard diet containing 2% araçá flour; and high-fat araçá (HFAF), high-fat diet containing 2% araçá flour. The compositions of the experimental diets are described in Table 1. After eight weeks of treatment, rats were fasted for 12 h, anesthetized with isoflurane, and subsequently euthanized by exsanguination.

Table 1. Compositions of the experimental diets (g/1000g diet)

Components	Diets			
	C	HF	CA	HFA
Choline	2.5	2.5	2.5	2.5
Methionine	3.2	3.2	3.2	3.2
Cholesterol	0	20	0	20
Vitamin mix [1]	10	10	10	10
Araçá	0	0	20	20
Mineral mix [1]	35	35	35	35
Cellulose	50	50	50	50
Sucrose	100	100	100	100
Casein	140	140	140	140
Soy bean oil	40	250	40	250
Cornstarch	619.3	389.3	599.3	369.3

[1] Vitamin and mineral mixture as recommended by the AIN-93M rodent diet [20].

2.4. Biochemical Serum Analysis

The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum lipid profiles (HDL-cholesterol, total cholesterol, and triacylglycerols) were determined using Labtest kits (Lagoa Santa, MG, Brazil).

2.5. Oxidative Stress Markers

Lipid peroxidation was determined following the method of [22]. Tissues (100 mg) were homogenized in 1 mL of Tris-HCl buffer (20 mM) and subsequently centrifuged at 4 ℃ for 10 min. The supernatant was mixed with trichloroacetic acid (TCA - 28% w/v in 0.25 N HCL) and centrifuged at 25 ℃ for 1 min. Afterwards, samples were added with 500 µL of thiobarbituric acid (TBA - 1% in 0.25 N acetic acid) and 125 µL of butylated hydroxytoluene (BHT - 5 mM in ethanol). The resulting mixture was vortexed, heated for 15 min at 95 ℃, and placed in an ice bath for 5 min. The absorbance was determined at 535 nm. Thiobarbituric acid reactive substances (TBARS) levels were determined by linear regression based on the tetrahydroxypropane curve straight line equation (used as the standard solution) and the absorbance values of the samples.

Carbonyl protein levels were determined according to the method of [23]. Tissues (400 mg) were homogenized in 1 mL of phosphate buffer (50 mM, pH 6.7) containing 1 mM EDTA and then centrifuged for 10 min at 4 ℃. Each sample was precipitated with 10% (w/v) TCA. After centrifugation, the precipitate was treated with 10 mmol 2,4-dinitrophenylhydrazine (DNPH), incubated in the dark for 30 min, and subsequently treated with 10% TCA. After centrifugation, the precipitates were washed twice with ethanol/ethyl acetate (1:1) and then dissolved in 6% SDS. The absorbance was measured at 370 nm. Data were expressed as nmol of DNPH incorporated/mg of protein. The carbonylated protein content was calculated using the molar extinction coefficient of hydrazone (22000 M^{-1} cm^{-1}) and expressed as nmol of carbonylated protein formed per mg of protein.

2.6. Measurement of Antioxidant Defenses

Catalase (CAT) activity was measured according to the method described by [24], which is based on the decomposition of H_2O_2 by the enzyme and is measured by spectrophotometry at 240 nm. The homogenate supernatant (10 µL) was added to a cuvette containing 100 mM phosphate buffer (pH 7.2), after which the reaction was initiated by the addition of 10 mM H_2O_2. Hydrogen peroxide decomposition was calculated using the molar absorption coefficient 39.4 M^{-1} cm^{-1}. Data were expressed as activity per mg of protein. One unit of CAT is equivalent to the hydrolysis of 1 µmol of H_2O_2 per min. Tissue protein content was determined according to the method developed by [25] using bovine serum albumin as a standard.

Liver superoxide dismutase (SOD) activity was determined according to the method of [26]. Briefly, 100 mg of tissue was homogenized in 1 mL of phosphate buffer (pH 7.2). The mixture was centrifuged at 12,000 g for 10 min at 4 ℃, and the supernatant was collected. Thirty microliters of the sample (1.5 µL of supernatant + 28.5 µL of buffer), 99 µL of buffer, 6 µL of MTT, and 15 µL pyrogallol were added to each well of the 96-well plate. Afterwards, the plate was incubated at 37 ℃ for 5 min. The reaction was stopped by the addition of 150 µL of DMSO, and measurements were obtained at 570 nm. Data were expressed as activity per mg of protein.

Total glutathione content was determined as proposed by [27]. Briefly, 10 µL of the supernatant homogenate was mixed with 150 µL of working mixture (100 mM potassium phosphate buffer, 6 U/mL glutathione reductase enzyme solution, and 1.5 mg/mL DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid)), and subsequently added with 50 µL of 0.16 mg/mL NADPH after 5 min of incubation at room temperature. To generate the standard curve, 10 mM glutathione (GSH) was diluted in 5% sulfosalicylic acid. The reaction proceeds by the reduction of DTNB in TNB. Sample absorbance was measured at 412 nm. For the determination of oxidized glutathione levels, 100 µL of the supernatant and 2 µL of 2-vinylpyridine was added to the samples. The samples were then incubated at room temperature for 1 h. The same procedure was conducted for determination of total glutathione levels. Reduced glutathione levels were determined by subtracting the amount of oxidized glutathione from the total amount of glutathione.

Arylesterase activity of the paraoxonase enzyme was determined using phenylacetate as the substrate following the method of [28]. Briefly, 2 mL of 9 mM Tris-HCl buffer (pH 8.0) containing 2 mM calcium chloride and 5 µL of each sample were added to a tube. The mixture was vortexed and incubated in a water bath for 2 min. Afterwards, the samples were added with 0.5 mL of Tris-phenylacetate solution (1 µL of phenylacetate for every 1.5 mL of 9 mM Tris-HCl, pH 8.0). The absorbance was measured at 270 nm. Hydrolysis of phenylacetate was calculated using the molar absorption coefficient 1310 L mol^{-1} cm^{-1}. Data were expressed as U/mL, where 1 U of paraoxonase is equivalent to the hydrolysis of 1 mmol of phenylacetate per minute.

2.7. Histological Analyses

For each rat, the smaller lobe of the liver was removed at the end of the experiment and subsequently fixed in 10% buffered formalin. For histological analysis, livers were cut transversely, incubated in an increasing alcohol gradient and embedded in paraffin. Tissue sections were cut using a microtome (Leica, Germany) and mounted on microscope slides. Slides were stained with hematoxylin and eosin (H&E) and photographed at 40× magnification. A semiquantitative scoring system was used to assess the severity of steatosis in ten microscopic fields as previously described by [29]. Briefly, steatosis was graded from 1 to 3 depending on the percentage of hepatocytes that contained fat. Samples were graded as follows: grade 1, <33% of hepatocytes contained fat; grade 2, 33% to 66% of hepatocytes contained fat; and grade 3, >66% of hepatocytes contained fat.

2.8. Statistical Analysis

Statistical analysis was performed using the GraphPad PRISM® v.5 software for Windows (San Diego, CA, USA).

Data were first analyzed by performing the Kolmogorov-Smirnov normality test. Data that followed a parametric distribution were analyzed by two-way or one-way analysis of variance (ANOVA). Values were expressed as mean ± standard deviation. Non-parametric Kruskal-Wallis test and Dunn's post-test were used to analyze data that did not follow a normal distribution. Results were considered statistically significant for p values < 0.05.

3. Results

3.1. *In vitro* Assessment of Polyphenol Content, Antioxidant Activity, and Cytotoxicity

Araçá was found to have high phenolic content (697.10 ± 10.68 mg GAE/100 g), which corresponded to a strong capacity to scavenge free radicals based on the reduction in absorbance of the DPPH radicals (88.77 ± 2.22 µM TEAC/g). Results indicated that araçá effectively neutralizes the oxidant molecules produced in NAFLD. To evaluate the araçá toxicity, HepG2 cells were incubated for 24 h with varying concentrations of araçá flour (10, 50 100, 200, 400, 500, 600, 800, and 900 µg/mL). The viability of the cultures was expressed as the percentage of live cells (Figure 1). Cell viability was greater than 70% at all the concentrations tested, indicating that araçá is not cytotoxic (ISO 2009-10993-5).

3.2. Effect of Araçá on Body Weight, Food Intake, Fecal Excretion, and Serum Lipid Profile in Rats

The animals fed with HF diet exhibited more weight gain than the animals in the C group, despite the fact that the food intake was lower, which may be justified by the higher calorical density of the high-fat diet. The addition of araçá to the diet did not affect weight gain and food intake. However, animals fed with the high-fat diet showed increased excretion, which was proportional to the amount of araçá added to the diet (Table 2).

The high-fat diet increased total cholesterol levels and lowered triacylglycerols and HDL-cholesterol levels in the sera of the animals. Araçá effectively reduced the concentration of triacylglycerols both in the control group (CAF) and the high-fat diet (HFAF) group (Table 2).

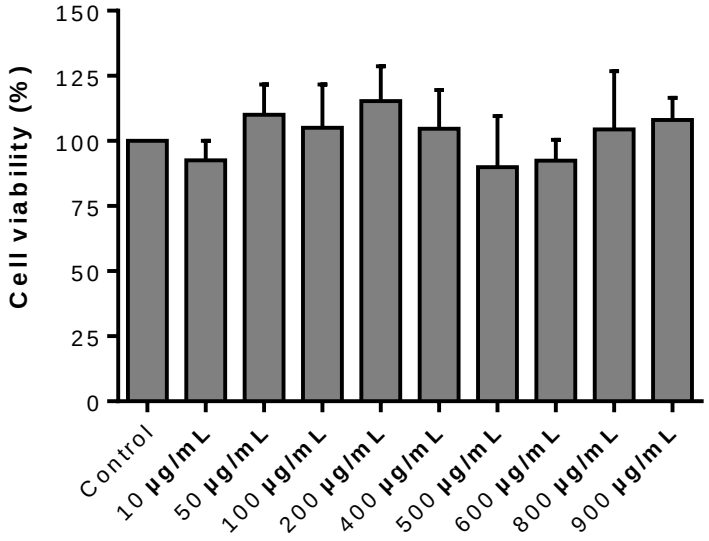

Figure 1. Cell viability of Araçá (10–900 µg/mL) for 24 h measured by MTT assay. HepG2 cells were incubated for 24 hours with the araçá using the MTT test. The assay was carried out in six-fold, using a control (untreated cells) and assigned 100% cell viability. Data are presented as mean = standard deviation by One-way ANOVA, followed by the Bonferroni post-test. No significant differences were observed

Table 2. Initial body weight, final body weight, weight gain, food intake and fecal excretion of groups of animals fed a control diet (C) or high-fat diet (HF) or araçá (AF)

	Experimental Groups				*p Value*		
Biometry	**C**	**CA**	**H**	**HA**	***Interação***	***Dieta***	***Araçá***
IBW (g)	143.6 ±6.6	142.4 ±12.6	143.0 ±13.2	143.5 ±12.6	0.8177	0.9144	0.9459
FBW (g)	200.2 ±12.1	202.7 ±12.5	217.3 ±17.1	224.9 ±15.5	0.5802	0.0001	0.2763
MG (g)	56.6 ±11.9	60.3 ±6.6	74.3 ±12.3	81.4 ±9.9	0.6104	<0.0001	0.1113
FI (g)	119.5 ±8.1	127.1 ±5.3	98.2 ±9.9	96.9 ±7.2	0.0886	<0.0001	0.2220
FE (g)	5.1 ±0.3	7.4 ±0.4	6.70 ±0.7	8.27 ±0.8	NS	<0.0001	<0.0001
Lipid profile							
TC (mg/dL)	81.4 ±13.7	79.3 ±9.9	106.7 ±55.1	138.7 ±63.7	0.1085	0.0014	0.1429
TG (mg/dL)	59.5 ±11.7	44.4 ±6.8	34.51 ±7.9	31.28 ±9.9	0.0529	<0.0001	0.0039
HDL-c (mg/dL)	70.3 ±15.5	59.6 ±16.2	18.3 ±9.2	19.2 ±6.9	0.1130	<0.0001	0.3375

IBW: Initial body weight. FBW: Final body weight. GW: Gain weight FI: Food intake. FE: Fecal excretion. TC: Total cholesterol. TG: Tryacyglicerols. HDL-c: HDL cholesterol. C: Control. CAF: Control + Araçá HF: High-fat. HFAF: High-fat + Aracá Data are presented as mean ± standard deviation. Two-way ANOVA test followed Tukey's posttest. Significant differences were considered for $p < 0.05$.

3.3. Araçá Ameliorates Liver Injury and Macrovesicular Steatosis

Histological analysis revealed that animals fed with the HF diet exhibited hepatomegaly (Figure 2B), as evidenced by increased lipid deposition in the liver (Figure 2A, HF and HFAF). Animals in the high-fat diet group presented abnormalities in hepatocytes, which were characterized by accentuated micro- and macrovesicular fat droplets. The addition of araçá improved macrovesicular steatosis in HFAF rats, which presented values similar to those of groups C and CAF (Figure 2C).

Furthermore, the high-fat diet treatment upregulated the activities of the serum enzymes ALT and AST, which are used as markers for liver injury (Figure 2D). The addition of araçá ameliorated the increase in both ALT and AST activities.

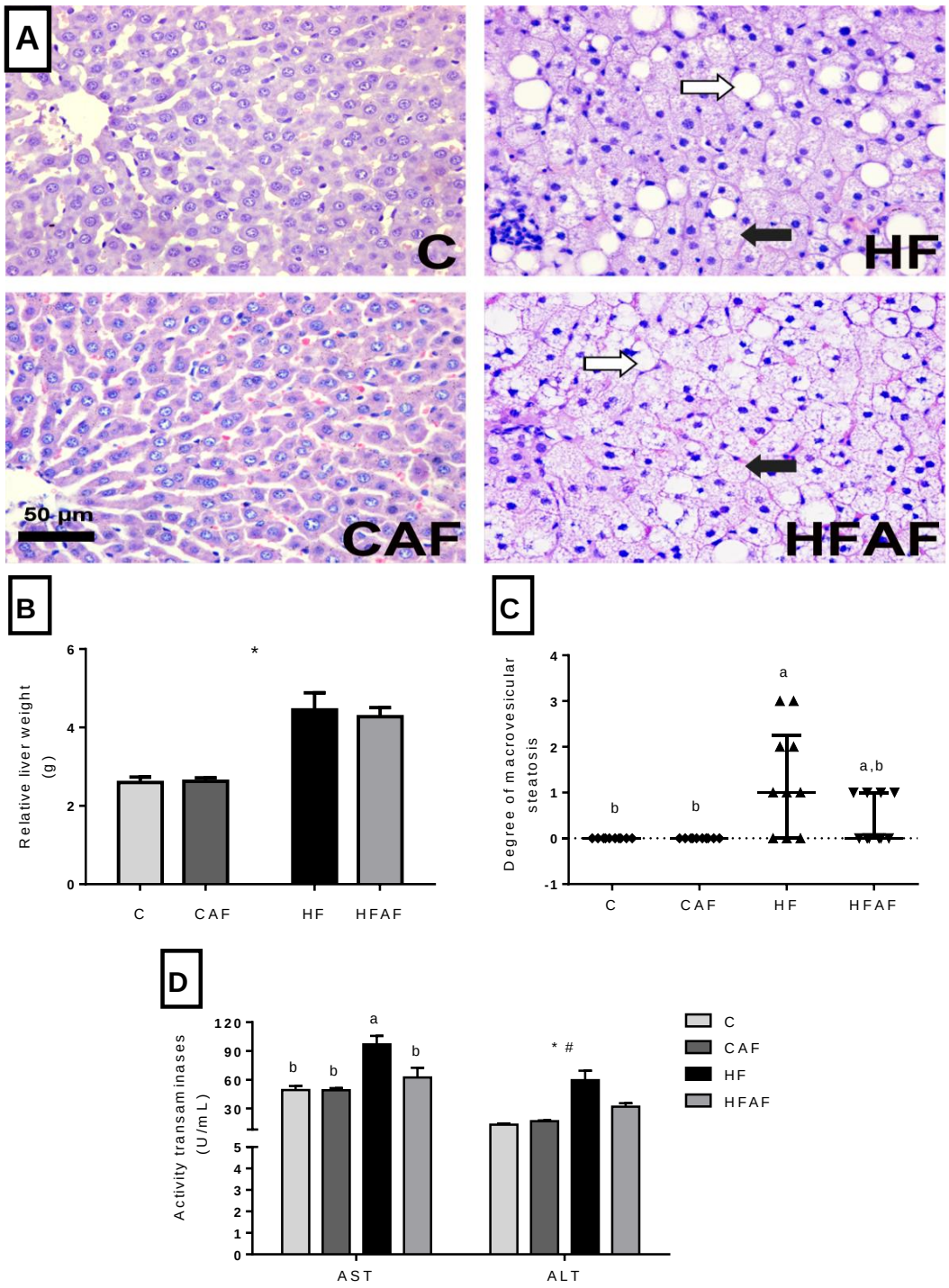

Figure 2. Lipid deposition and indicators of liver function of rats fed either a control or high-fat diet with addition of araçá or not. Representative hematoxylin and eosin-stained histological sections of livers from rats fed a control or high-fat diet treated with araçá (A). Normal hepatocytes in C and CAF groups. Presence of macro (white arrow) and microvesicular (black arrow) steatosis in HF and HFAF groups. C: Control. CAF: Control + Araçá. HF: High-fat. HFAF: High-fat + Araçá. Images photographed at 400× magnification. Relative liver weight (B), degree of liver steatosis (C) and activity of the enzymes ALT and AST (D). Data in (B and D) are represented as mean ± standard deviation. (*) Indicates significant difference of diet, (#) indicates significant difference of araçá and different letters indicate interaction between diet and araçá, by two-way ANOVA test followed Tukey's post-test. Data in (C) are represented as median and interquatile range. Different letters indicate interaction between diet and araçá, by Kruskal-Wallis test followed by Dunn's post-test. Significant differences were considered for $p < 0.05$

Table 3. Effect of high-fat diet and araçá on oxidative damage markers in rats

Variáveis	Experimental Groups				*p Value*		
	C	**CA**	**H**	**HA**	*Interação*	*Dieta*	*Araçá*
TBARS (nmol/mg protein)	0.62 ±0.3	0.66 ±0.2	1.16 ±0.5	0.80 ±0.3	0.1061	0.0060	0,1762
PCO (nmol/mg protein)	1.29 ±0.2	1.41 ±0.2	1.03 ±0.4	1.12 ±0.4	0.7923	<0.0001	0.3839

TBARS: Substances reactive to thiobarbituric acid. PCO: carbonylated protein. C: Control. CAF: Control + Araçá HF: High-fat. HFAF: High-fat + Araçá Data are presented as mean ±standard deviation. Two-way ANOVA test followed Tukey's posttest. Significant differences were considered for $p < 0.05$.

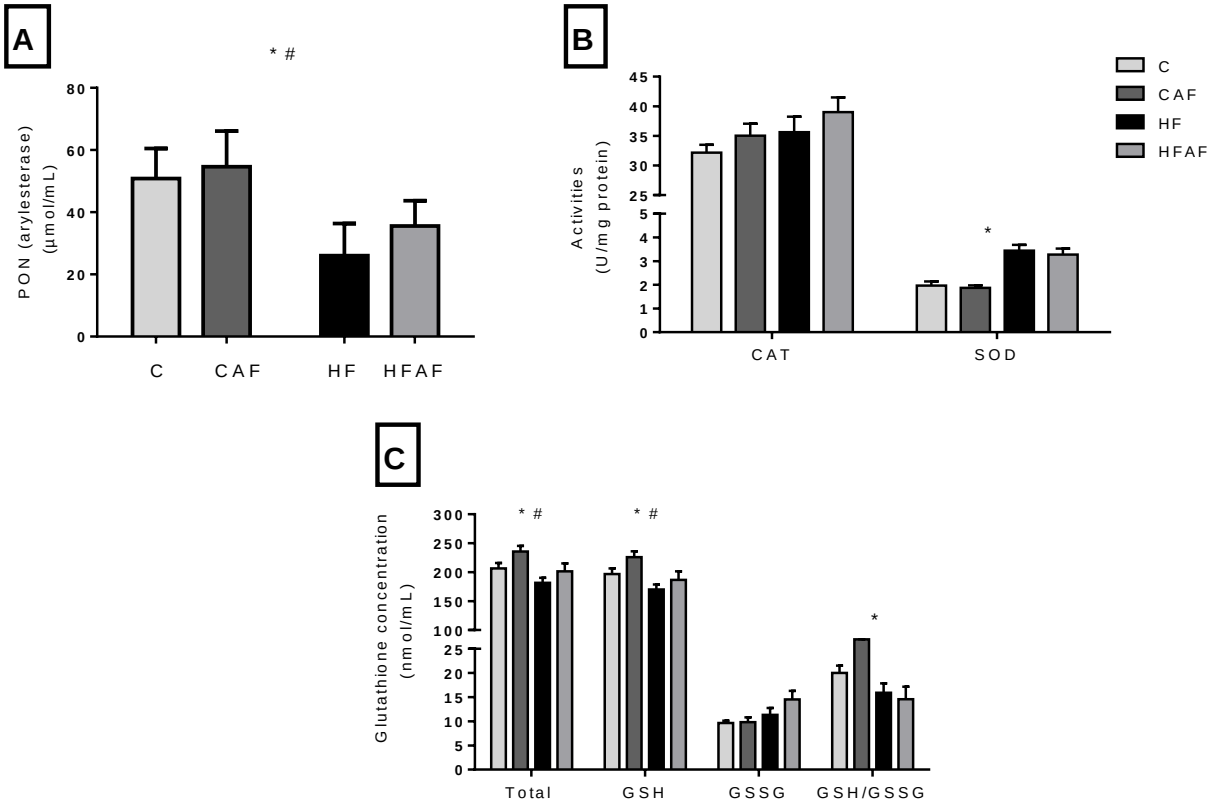

Figure 3. Effect of araçá on antioxidant defenses in rats. PON (A), CAT and SOD activity (B) and glutathione concentration (C). PON: Paraoxonase enzyme; CAT: Catalase enzyme; SOD: Superoxide dismutase enzyme; GSH: Reduced glutathione; GSSG: Oxidized glutathione. C: Control. CAF: Control + Araçá HF: High-fat. HFAF: High-fat + Araçá Data are presented as mean ±standard deviation. (*) Indicates significant difference of diet and (#) indicates significant difference of araçá by two-way ANOVA test followed Tukey's post-test. Significant differences were considered for $p < 0.05$

3.4. Araçá Improves Antioxidant Defenses but does not increase the Levels of Oxidative Damage Markers

TBARS and carbonyl protein levels were measured to estimate the extent of oxidative damage in the liver. Animals fed with high-fat diet showed higher levels of TBARS and carbonyl protein levels compared to those of control animals. Therefore, araçá treatment did not alter these variables (Table 3).

Rats fed with the high-fat diet showed lower serum arylesterase activity of PON decreased, and the addition of araçá increased PON activity in the HFAF group (Figure 3A). The two groups showed similar catalase activities. Rats fed with the high-fat diet showed elevated SOD activity relative to those in the control groups, but the addition of araçá did not influence SOD activity (Figure 3B).

The high-fat group presented lower concentrations of total and reduced glutathione relative to the control group. The addition of araçá increased total and reduced glutathione levels in the CAF and HFAF groups relative to those in the control group. We observed no significant differences in the concentration of oxidized glutathione among the different treatment groups (Figure 3C).

4. Discussion

The present study investigated the effects of araçá on a rat model of NAFLD induced by a high-fat diet. The addition of araçá to the high-fat diet ameliorated the severity of steatosis, reduced the degree of macrovesicles, and reduced the activities of the ALT and AST enzymes. Furthermore, the addition of araçá increased the concentrations of total and reduced glutathione and increased PON activity in the liver tissues of animals fed with the high-fat diet.

Consistent with the results of other studies, our findings revealed that araçá has higher total phenolic content compared to other fruits common in the Brazilian Cerrado. In particular, the total phenolic content in araçá is approximately three times higher than those of pequi and gabiroba and six times higher than that of cagaita [30,31]. In addition, araçá has been shown to contain to six to eight

times higher polyphenol content than standard fruits, such as grape, blackberry, pineapple, orange, and even guava, which belongs to the same family and genus as that of araçá [32,33]. Considering that antioxidant capacity is positively correlated with the total polyphenol content, it is expected that araçá exhibits strong antioxidant capacity. Araçá exhibited an antioxidant capacity approximately 14 times stronger than açaí a Brazilian fruit that has been widely studied because of its phytochemical composition and its beneficial effects against chronic non-communicable diseases [12-34].

Polyphenols in the diet make up one of the most important class of natural antioxidants. In addition to their antioxidant activities, polyphenols exhibit anti-inflammatory activities and thus help prevent the development of chronic-degenerative diseases and metabolic disorders [35,36]. A study performed HPLC to analyze individual polyphenols that are present in araçá and showed that the most abundant compound is (-)-epicatechin, followed by gallic acid, which represent 69.2% and 29.5% of total phenolic compounds in araçá, respectively. In particular, (-)-epicatechin is known to play a role in antioxidant defense, vasodilation, in addition to its neuroprotective role, which is mediated by the upregulation of Nrf2 cascade and heme oxygenase-1 (HO1) enzyme [37].

The addition of fruits in the diet has been increasingly associated with reduced risk factors for various diseases, including the reduction of total cholesterol levels, triacylglycerol levels, and non-HDL fractions, which could be attributed to the antioxidant-rich fibers of fruits. Our current results showed that araçá contains higher amounts of fibers than those reported in [38]. Furthermore, araçá is considered a rich source of polyphenols. Reference [39] reported that long-term consumption of different fruits lowers cholesterol and triglyceride levels and reduces lipid deposition in the livers of rats fed with a high-fat diet. Similarly, [40] demonstrated that hypercholesterolemic rats fed with guava concentrate pulp in the form of puree (mixed with strawberry, passion fruit, soursop, or blackberry) presented significantly lower concentrations of total cholesterol and serum triacylglycerols, as well as lower hepatic damage related to reduced lipid deposition in the tissues. Although the normal serum lipid profiles were not completely restored, the addition of araçá to the high-fat diet significantly reduced the levels of triacylglycerols, which comprise the majority of the lipid droplets. The above findings could explain the observed reduction of macrovesicles in the groups treated with araçá

It has commonly been observed in NAFLD patients that the activities of serum transaminases (ALT and AST) increase up to four times the upper limit of normality. Similar observations have been reported based on animal experiments, and changes in ALT and AST activities have been associated with lipid accumulation in the liver [41,42]. Our current results were consistent with the abovementioned findings, since the induction of hepatic steatosis was reported to significantly increase ALT and AST activities. Addition of araçá ameliorated the effects of the high-fat diet and was accompanied by the restoration of normal ALT and AST activities and decreased fat deposition.

Hepatocytes increase β-oxidation to counteract excessive lipid production in the liver. However, the increase in β-oxidation involves increased generation of free radicals and consequent lipid peroxidation and oxidative stress, which further promote hepatic inflammation and steatosis [6]. Consistent with our current findings, previous studies have reported that a high-fat diet significantly increased malondialdehyde and carbonylated protein levels in the livers of animals with hepatic steatosis. Furthermore, ingestion of functional foods that are rich in polyphenols, tannins, and lycopene or isolated bioactive compounds with recognized biological effects are known to exert a positive effect on antioxidant status and to reduce lipid peroxidation [43,45]. Araçá contains active ingredients that exhibit antioxidant and anti-inflammatory effects [13,14,46]. Therefore, the addition of araçá to the diet was expected to inhibit the increase in oxidation of lipids and proteins. The observed differences in the effects of araçá could be attributed to differences in the duration of the experiment, the amount of each compound present in the diet, the method of administration of the diet, the lineage of the animal, or the animal model used.

Increased oxidative stress and the consequent increase in lipid peroxidation and carbonylation of proteins can interfere with the activities of PON and antioxidant enzymes and alter the concentrations of sulfhydryl groups. The paraoxonase family includes three members, namely, PON1, PON2, and PON3. PON1, which was analyzed in the present study, is an enzyme that is primarily synthesized in the liver and is present in the circulation and linked to high density lipoproteins (HDL) levels. The primary function of PON1 is the degradation of oxidized lipids, which plays an important role in maintaining the body's antioxidant system. Changes in the circulating levels of PON1 are associated with various diseases that are characterized by oxidative stress [47]. In the present study, we observed that PON activity was significantly reduced by the high-fat diet but was effectively restored by the addition of araçá

Therefore, the araçá-induced increase in PON activity is likely to be related to the high content of total polyphenols of the fruit. Reference [48] demonstrated that mice that are deficient in apolipoprotein E (E^0 mice), the ascorbic acid and polyphenols-rich pomegranate juice, led to increased PON activity, which was also measured by arylesterase activity, as well as oxidative stress reduction, atherogenic LDL modification and platelet aggregation. Reference [49] evaluated the effects of açaí pulp, which contains high levels of total phenolic compounds and monomeric anthocyanins, in rats and similarly observed that açaípulp promoted an increase in serum and hepatic activity of PON1 and upregulated PON1 mRNA expression.

In addition to the increase in PON activity, araçá induced an increase in total and reduced glutathione concentrations. Glutathione is a low molecular weight thiol and many of its reactions involve the highly polarizable sulfhydryl (SH) group. Glutathione is an important endogenous antioxidant that reduces H_2O_2 levels and thus controls the redox state and several cascades of cellular signaling. For example, the GSH/GSSG ratio is used as a reliable indicator of oxidative stress. Disruption in GSH homeostasis leads to

an increase in the production of reactive oxygen species (ROS), which impairs cellular function and interferes with pathways involved in intermediate metabolism, thereby contributing to the development of several diseases [50,51]. GSH levels were found to be upregulated in the groups treated with araçá, including those who received the high-fat diet, thereby demonstrating the antioxidant effects of araçá.

However, araçá treatment did not affect the activities of the SOD and CAT enzymes, which play critical roles in cellular antioxidant defenses. Basal decreases in the concentrations of these enzymes could severely affect oxidative status [43]. The observed increase in catalase activity could be explained by the increase in the concentration of GSH, which could serve as first line of defense against oxidative stress.

5. Conclusion

The present study demonstrated that araçá reduced ALT and AST activities and steatosis, increased PON activity, and increased glutathione concentrations. The abovementioned effects are potentially mediated by the high phenolic content of the fruit. Results indicated that araçá exerts a hepatoprotective effect by modulating the antioxidant response in rats fed with a high-fat diet. Therefore, the current findings could serve as the basis for the development of potential treatment strategies for the treatment of metabolic diseases. In addition, the results provided insights into the metabolic mechanisms underlying the effects of araçá, thereby increasing our understanding of the fruit and highlighting its potential use as a functional food.

Conflicts of Interest

All authors declare no competing interests.

Acknowledgments

The authors thank the Federal University of Ouro Preto and acknowledge the financial support provided by the following Brazilian government agencies: Foundation for Research Support of the State of Minas Gerais (FAPEMIG), National Council of Scientific and Technological Development (CNPq), and Higher Education Personnel Training Foundation (CAPES/Brazil).

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