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RESEARCH

Preventive effect of the bark of *Passiflora edulis* on obesity-related disorders and oxidative stress in db/db mice

Marielle Fernanda Panelli[1] · Jéssica Leite Garcia[1] · Sérgio Luiz Borges de Souza[1] · Mariane Róvero Costa[1] · Artur Junio Togneri Ferron[1] · Cristina Schmitt Gregolin[1] · Igor Otávio Minatel[1,2] · Ana Paula Costa Rodrigues Ferraz[1] · Damiana Tortolero Pierine[1] · Fabiane Valentini Francisqueti- Ferron[1] · Camila Renata Corrêa[1]

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Abstract

Purpose To verify if the bark of *Passiflora edulis* prevents obesity-related disorders and oxidative stress in *db/db* mice.
Methods Obese male *db/db* mice ($n = 14$ animals) were randomly divided into two groups to receive standard chow and water (obese, $n = 7$ (OB)) or standard chow with bark of *Passiflora edulis* (BPe) (obese + BPe, $n = 7$ (OB + BPe)) for 16 weeks. The evaluated parameters in animals included food and caloric intake, body weight, total body fat and fat deposits, serum glucose, triglycerides, and total cholesterol. Malondialdehyde (MDA) and antioxidant capacity were evaluated in serum and organs (adipose tissue, kidney, liver, and heart). All groups were compared by Student t test, with $p < 0.05$.
Results The results showed the benefits from BPe by preventing abdominal fat deposition and by reducing the total cholesterol. Moreover, the compound increased the antioxidant capacity from the organs analyzed and reduced the MDA levels in the liver.
Conclusion It is possible to conclude that the consumption of the BPe prevents obesity-related disorders and oxidative stress in db/db mice.

Keywords Obesity · Db/db mice · Passion fruit

Introduction

The prevalence of obesity and chronic diseases, such as hypertension, hyperglycemia, and dyslipidemia, has increased around the world. The literature reports that fat accumulation, especially abdominal obesity, is related to the development of obesity-related disorders [1–6]. Several mechanisms try to explain the association between obesity and diseases, among them, higher adiposity, which would induce a redox system imbalance, characterized by the excess of oxygen reactive species (ROS) [7]. This condition leads to oxidation of different molecules such as lipids, carbohydrates, proteins, and DNA, which are involved in the development of different disorders [8, 9].

Antioxidants are substances able to protect the organism against diseases by avoiding molecule oxidation [10, 11]. They are obtained from both endogenous and exogenous sources; however, the latter is considered the most important since the nutrients from the diet are indispensable for the endogenous antioxidant synthesis [12]. Based on this, the early introduction of fruits and vegetables in the diet, which are rich in antioxidants and bioactive compounds, could be an effective strategy to prevent or delay the obesity-related disorders [13–15].

Passiflora edulis, popularly known as "passion fruit" or "maracujá," is a fruit that contains several antioxidants in the pulp, leaves, seeds, and bark, such as phenolics, carotenoids, vitamin C, and polyamines [16]. In a previous study published by our group [17], it was demonstrated that the treatment of obese *db/db* mice with the BPe reduced the body fat and improved metabolic and antioxidant parameters. However, there is a lack of studies regarding the preventive effect of the BPe. So, the aim of this study was to verify if the BPe prevents obesity-related disorders and oxidative stress in *db/db* mice.

✉ Fabiane Valentini Francisqueti- Ferron
fabiane_vf@yahoo.com.br

[1] São Paulo State University (UNESP), Medical School, Botucatu, Brazil

[2] São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil

Materials and methods

Animals and experimental protocol

In this study, *db/db* mice were used, which are animals genetically deficient for the leptin receptor, and considered by the literature an established model for obesity and type 2 diabetes [18]. All the animals were acquired from Universidade de São Paulo—USP, Brazil. After weaning (21 days of age), male *db/db* mice ($n = 14$ animals) were randomly divided into two groups to receive standard chow (Presence for rats and mice, Presence Nutrição Animal, Brazil) and water (obese, $n = 7$ animals (OB)) or standard chow with BPe ((obese + BPe, $n = 7$ animals (OB + BPe)) during 16 weeks.

For all the animals, chow and water were offered ad libitum. Feed and water consumption were measured daily, and body weight was measured weekly. Two animals per cage were kept in an environment with controlled temperature (24 ± 2 °C), humidity (55 ± 5%), and light-dark cycle (12–12 h). The study protocol was approved by the Ethics Committee on Animal Experimentation of the Botucatu Medical School, Universidade Estadual Paulista-UNESP, (1104/2017) in São Paulo, Brazil, and followed the recommendations of the *Guide for the Care and Use of Experimental Animals* [19]. At the end of the experiment, the animals were anesthetized with ketamine and xylazine and then euthanized by cardiac puncture, and the organs and blood were collected for analysis.

Preparation of chow with bark of Passiflora edulis

The *Passiflora edulis* fruits were obtained from a rural producer in Presidente Prudente city, São Paulo, Brazil, at the ripening stage and submitted to selection, washed, cut into small pieces, and oven-dried at 60 °C for 48 h. After this process, the dried barks were milled (Logen Scientific, Diadema São Paulo, Brazil) and added to grounded commercial chow (Presence, Paulínea, São Paulo, Brazil), to reach the proportion of 7 g of *Passiflora edulis* bark/kg of chow (correspondent to 1.5 g/kg of body weight per day) [20]. Following this, the mixture was pelleted again for consumption.

Nutritional parameters

In order to characterize the nutritional profile, the initial body weight (IBW), final body weight (FBW), total body fat (TF—sum of fat deposits: epididymal, retroperitoneal, visceral, and subcutaneous), adiposity index (AI—total body fat/final body weight × 100), and feed intake (g/day) were considered.

Metabolic parameters

After 8-h fasting, serum glucose, triglycerides, and cholesterol levels (kits from BioClin®, Belo Horizonte, MG, Brazil) were determined by an automatic enzymatic analyzer system (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China).

Preparation of tissues for oxidative stress analysis

Increased reactive oxygen species (ROS) are able to oxidize biomolecules and to affect the antioxidant capacity, so it was analyzed in this study: MDA levels, an important lipid peroxidation biomarker, and the antioxidant capacity in the serum and tissues (adipose, hepatic, cardiac, and renal). Tissues (100 mg) were homogenized in 1 mL of cold phosphate saline buffer (PBS), pH = 7.4, and centrifuged (800×*g*, 4 °C, 10 min). The supernatant was used in the following analyses:

Malondialdehyde

One hundred microliters of the homogenate was used for malondialdehyde (MDA) analysis. Briefly, 700 μL of 1% orthophosphoric acid and 200 μL of thiobarbituric acid (42 mM) were added to the samples. Then, the mixture was boiled for 60 min in a water bath, and afterward, it was immediately cooled on ice. A total of 200 μL was transferred to a 2-mL tube containing 200-μL sodium hydroxide/methanol (1:12 *v/v*). The sample was vortex-mixed for 10 s and centrifuged for 3 min at 13,000*g*. The supernatant (200 μL) was transferred to a 300-μL glass vial and 50 μL was injected onto the column. The HPLC was a Shimadzu LC-10AD system (Kyoto, Japan) equipped with a C18 Luna column (5 μm, 150 × 4.60 mm, Phenomenex Inc., Torrance, CA, USA), a Shimadzu RF-535 fluorescence detector (excitation 525 nm, emission 551 nm), and 0.5 mL/min flow of phosphate buffer (KH2PO4 1 mM, pH 6.8) [10]. The MDA was quantified by the determination of the peak area in the chromatograms relative to a standard curve of known concentrations. A calibration curve was obtained by tetra-ethoxypropane (TEP) solutions [21].

Antioxidant capacity

The hydrophilic antioxidant capacity was determined fluorometrically as described by Beretta et al. [22] using a microplate reader (VICTOR X2, Perkin Elmer-Boston, MA, USA). The antioxidant capacity was quantified by the comparison between the areas under the curve relative to the oxidation kinetics of the phosphatidylcholine (PC) suspension, used as a reference for the biological matrix. The compound 2,2′-Azobis (2-aminopropano)-dihydrochloride (AAPH) was used as the peroxyl radical initiator. The results represent the percentage of the inhibition of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (BODIPY, 581/591) in serum relative to that occurring in the control sample of BODIPY 581/591 in PC liposome. All

Table 1 Nutritional parameters

	Obese	Obese + passiflora
Feed intake (g/day	6.53 ± 0.27	10.8 ± 0.5*
IBW (g)	15.8 ± 2.23	14.3 ± 1.8
FBW (g)	53.8 ± 3.6	49.3 ± 5.4
RAT (g)	1.93 ± 0.29	1.50 ± 0.28*
EAT (g)	3.00 (0.19)	2.93 (0.16)
VAT (g)	1.76 ± 0.13	1.42 ± 0.33*
TAS (g)	9.50 ± 0.89	8.07 ± 1.68
TF (g)	16.3 (1.3)	14.5 (1.6)*
AI (%)	31.1 (2.5)	28.9 (2.4)*

BPe, bark of *Passiflora edulis*; *IBW*, initial body weight; *FBW*, final body weight; *RAT*, retroperitoneal adipose tissue; *EAT*, epididymal adipose tissue; *VAT*, visceral adipose tissue; *SAT*, subcutaneous adipose tissue; *TF*, total fat; *AI*, adiposity index. Data are expressed in mean ± standard deviation, or median and interquartile range (FBW, RAT, TF, AI). *Indicates $P < 0.05$

analyses were performed in triplicate, and the results represent the percentual of protection.

Statistical analysis

Data were analyzed by the Student *t* test or the Mann–Whitney and the results are presented as means ± standard deviation (SD) or medians (interquartile range). Statistical analyses were performed using Sigma Stat for Windows version 3.5. (Systat Software, Inc., San Jose, CA, USA), and a *p* value of 0.05 was considered as statistically significant.

Results

The nutritional parameters are presented in Table 1 and it is possible to verify that BPe was able to prevent the increase in both visceral and retroperitoneal adipose tissues. Moreover, the treated group (obese + BPe) showed

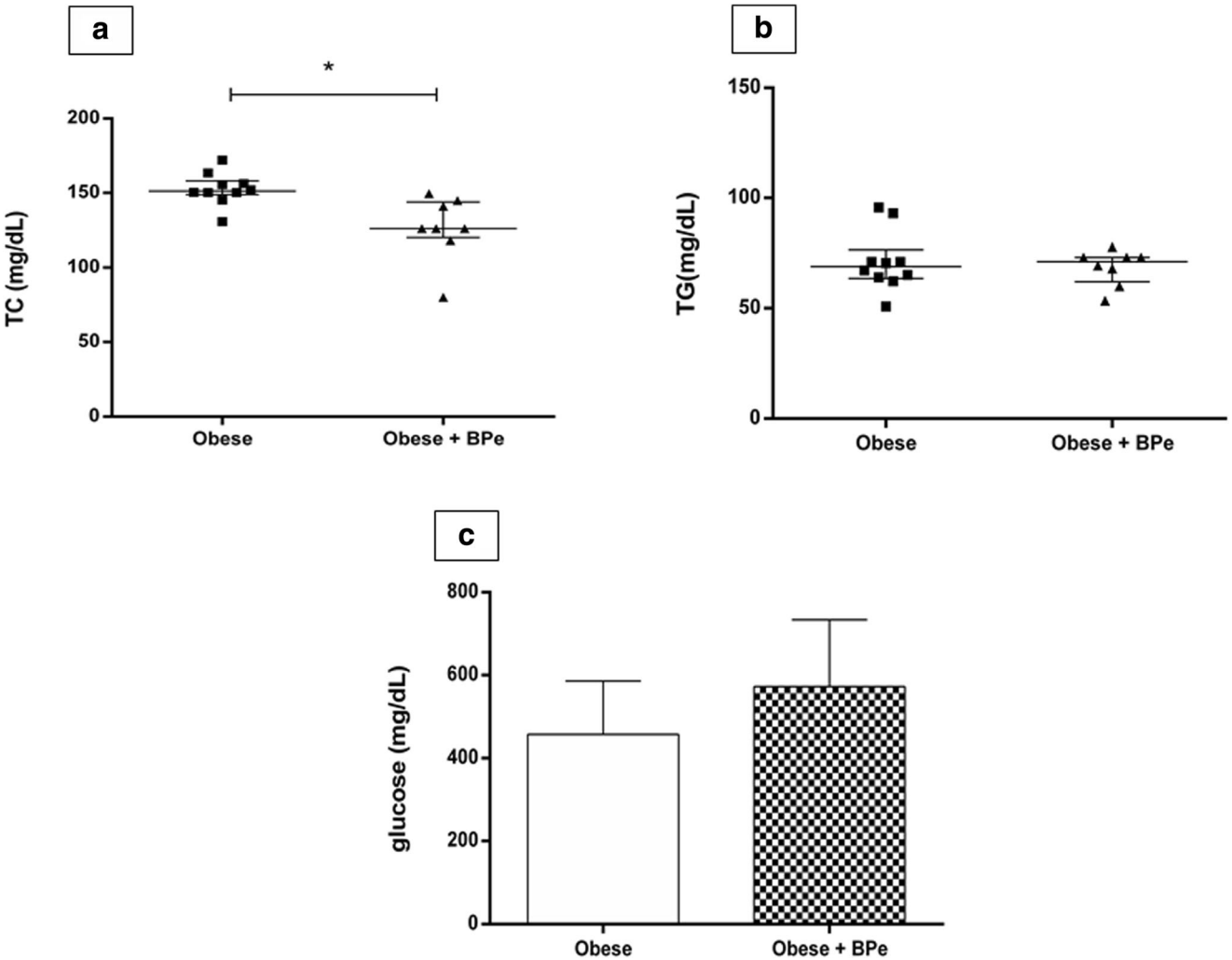

Fig. 1 Serum biochemical parameters for total cholesterol (**a**), triglycerides (**b**), and glucose (**c**). Data are expressed in mean ± standard deviation, or median. Asterisk indicates $p < 0.05$. BPe, bark of *Passiflora edulis*

lower total body fat and adiposity index than the obese group.

Figure 1 presents the serum biochemical parameters. It is possible to verify the positive effect of the BPe preventing the increase in the total cholesterol in the treated group. No effect was observed on glucose and triglycerides levels.

The antioxidant capacity in serum, liver, kidney, left ventricle, and adipose tissue was analyzed an is presented in Fig. 2. The BPe was able to increase the antioxidant capacity in serum, kidney, liver, and adipose tissue. Regarding the effect on the left ventricle, no change was observed.

Figure 3 presents the MDA levels in the kidney, liver, left ventricle, and adipose tissue. It is possible to verify a lower level of this marker in the liver. There was no effect on MDA levels in the kidney, left ventricle, and adipose tissue.

Discussion

The principal findings of this study are that BPe treatment conferred protection against obesity, reduced levels of total cholesterol, increased antioxidant defense, and reduced MDA in the liver. These are important findings since the BPe was effective to modulate the genome-wide expression profiling in a classic obesity experimental model [23].

The expansion of the adipose tissue, especially the visceral fat, is indicated as the central dogma regarding the physiopathology of obesity disorders [24]. Our results showed a positive effect from the BPe by preventing visceral and total fat accumulation in the treatment group. Studies show that natural bioactive compounds can prevent obesity development by the modulation of several pathways [25], among them the stimulation of the *peroxisome proliferator–activated receptor gamma* (PPAR-γ) synthesis. The increased levels of PPAR-γ induced by some bioactive compounds would be responsible for the adipose tissue browning, making it more metabolic and decreasing its lipid deposition [26]. Once the bark of *Passiflora edulis* is rich in carotenoids and flavonoids [17], the compound used in this study may be able to modulate some of these mechanisms involved in fat accumulation.

According to the literature, the reduction in body fat is responsible for significant metabolic benefits, as the prevention of insulin resistance, glucose intolerance, type 2 diabetes, and dyslipidemia [27]. In this study, the treated group presented only lower levels of total cholesterol compared with the non-treated group. This finding can be attributed to the pectin, a soluble fiber presented in the BPe, able to form gels and prevents cholesterol absorption [28]. No effect was observed in glucose level, which is an expected result once *db/db* mice are a genetic rodent model for type 2 diabetes [23]. The absence of effect on triglycerides levels can be related to insulin resistance. Increased secretion of triglyceride-enriched VLDL (very-low-density protein) is the commonest cause of elevated plasma triglycerides in insulin resistance and diabetes conditions [29].

The imbalance of the redox system is considered one of the main causes of obesity-related disorder development [30]. In

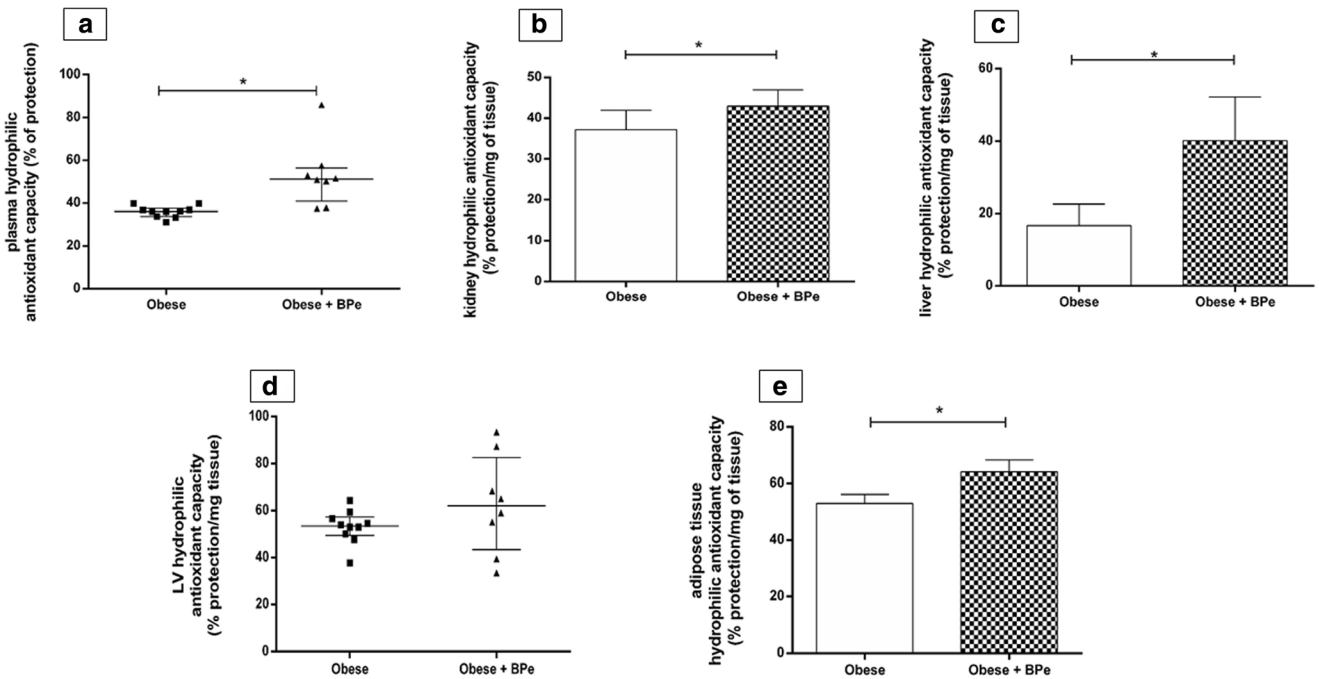

Fig. 2 Antioxidant capacity in serum (**a**), kidney (**b**), liver (**c**), left ventricle (**d**), and adipose tissue (**e**). Data are expressed in mean ± standard deviation, or median. Asterisk indicates $P < 0.05$. BPe, bark of *Passiflora edulis*

the present study, the BPe increased both systemic and tissues antioxidant capacity, which can be attributed to the antioxidant activity from the BPe, already described in our previous study [17]. This property is an important finding that demonstrates the effectiveness to prevent diseases and modulates antioxidant response by the bioactive compounds present in discarded parts from foods.

One consequence of the oxidative stress is the lipid peroxidation, a free radical–mediated chain of reactions that result in an oxidative deterioration of polyunsaturated lipids, which are components of biological membranes and common targets of reactive species [31]. Lipid peroxidation results in MDA production, the most frequently used biomarker of the oxidative stress in many health problems such as cancer, chronic obstructive pulmonary disease, and cardiovascular diseases [32]. Our results showed that the BPe prevented lipid peroxidation (MDA production) only in the liver. There are several potential mechanisms that explain the increased lipid peroxidation in hepatic tissue. Higher levels of triglycerides are deposited inside the hepatocytes, which favors the occurrence of oxidative stress and the progression of steatosis to steatohepatitis and fibrosis. Reactive oxygen species and lipid peroxidation products impair the respiratory chain in hepatocytes, either directly or indirectly, exposing the mitochondrial genome to oxidative damage. These features, in turn, lead to the generation of more ROS, and a vicious cycle may ensue. All groups present higher levels of TG, and the BPe was able to prevent the hepatic lipid peroxidation [33].

The absence of effect on MDA in the other organs can be explained by the duration of the experiment. The occurrence of free radical production and consequently lipid peroxidation in the heart is associated with the elevated myocardial work and mechanical overload. In kidneys, the lipid peroxidation is responsible for the accumulation of adipose tissue around the kidneys of obese rats [33]. In adipose tissue, oxidative stress occurs especially in hypertrophied cells [34]. Probably the obese group did not develop a level of obesity which is able to lead to oxidative stress in all organs. However, it demonstrates that the liver is the primary organ affected

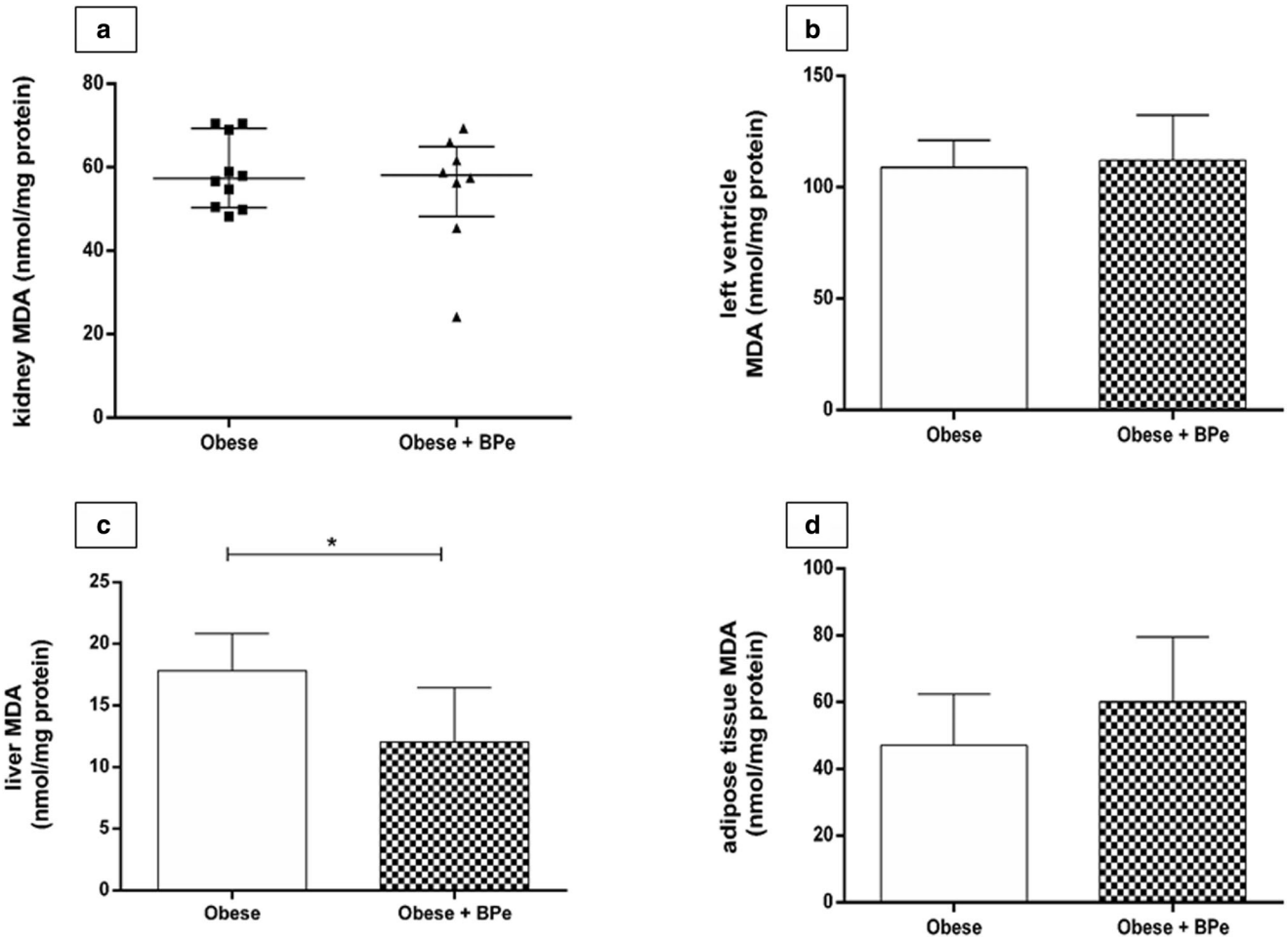

Fig. 3 Malondialdehyde levels (MDA) in the kidney (**a**), left ventricle (**b**), liver (**c**), and adipose tissue (**d**). Data are expressed in mean ± standard deviation, or median. Asterisk indicates $P < 0.05$. BPe, bark of *Passiflora edulis*

by the reactive species in obesity condition and the BPe prevents the lipid peroxidation.

Conclusion

In summary, the results showed that the BPe is a good source of fiber and phytochemicals and have a wide variety of health benefits as a protection against obesity. In addition, it is noteworthy that BPe reduced the total cholesterol and increased the antioxidant capacity of treated animals. Based on these results, it is possible to conclude that the BPe consumption prevents obesity-related disorders and oxidative stress in db/db mice.

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Compliance with ethical standards

The study protocol was approved by the Ethics Committee on Animal Experimentation of the Botucatu Medical School, Universidade Estadual Paulista-UNESP, (1104/2017) in São Paulo, Brazil, and followed the recommendations of the Guide for the Care and Use of Experimental Animals [19].

Conflict of interest The authors declare that they have no conflict of interest.

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