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Overview of bergamot leaves extract (*Citrus bergamia*) effect on the RedOx/Inflammatory scenario in obesity target organs in an animal model of metabolic syndrome

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ABSTRACT

Bergamot (*Citrus bergamia*) is a fruit with anti-inflammatory and antioxidant properties that contains similar bioactive composition to its leaf, evidencing its therapeutic potential in the treatment of chronic diseases. The aim was to evaluate the treatment effect of bergamot leaf extract (BLE) on the redox/inflammatory scenario in different organs of obese rats with metabolic syndrome. After detection of metabolic syndrome, male Wistar rats were allocated (n = 10/group) for the treatment with BLE by gavage (50 mg/kg): Control, Control+BLE, High Sugar fat (HSF), and HSF+BLE. Evaluations included: metabolic-nutritional profile; tissues function and redox/inflammatory parameters. The HSF group presented metabolic syndrome, cardiac, hepatic and renal dysfunction; inflammation; and oxidative stress. BLE decreased oxidative stress and inflammation levels in adipose tissue, heart, liver and kidneys, as well as decreased levels of triglycerides, insulin and insulin resistance. BLE act in all target organs of obesity, improving the redox/inflammatory scenario in a diet-induced metabolic syndrome.

1. Introduction

Obesity is a chronic complex disease defined by the World Health Organization as an excessive adiposity that may impair health. The disease contributes to the public health emergency, and it is estimated that, in 2030, over one billion adults will be obese (Lobstein, Brinsden, Neveux, Cavalcanti, Barquera, Baur, & Wilding, 2022; Organization, 2023). The adipose tissue (AT) is an autocrine, paracrine and endocrine organ with action on energy homeostasis (Choe, Huh, Hwang, Kim, & Kim, 2016). However, in conditions of positive energy balance, commonly characterized by an excessive caloric consumption, especially from simple sugars and saturated fats, the AT becomes dysfunctional, presenting exceeded stock capacity, promoting pro-inflammatory response and oxidative stress, characterized by high reactive oxygen species (ROS) generation (Longo et al., 2019) which is associated with the development of a constellation of comorbidities, such as insulin resistance (IR), dyslipidemia, cardiovascular diseases and obesity, known as metabolic syndrome, reaching and compromising tissues and organs (Costa, Garcia, & de Silva, 2019; de Heredia, Gómez-Martínez, & Marcos, 2012; Ferron et al., 2019; Francisqueti et al., 2017; Jung & Choi, 2014; Lo et al., 2018; Popkin & Adair, 2012).

Although the clinical outcomes associated with obesity and metabolic syndrome is quite extensive, the oxidative and pro-inflammatory scenario associated with ectopic fat deposition and insulin resistance can lead to negative repercussions on the health of vital organs, such as the heart, kidneys, and livers, contributing to a cascade of detrimental effects (Priest & Tontonoz, 2019). The imbalance in these parameters contributes to higher oxidation of lipids and proteins, impairment of blood flow, glomerular filtration, and detoxification, contributing to damage to cellular integrity and function and, consequently, the development of cardiovascular disfunction, chronic kidney disease and liver fibrosis, highlighting the need for therapeutic strategies to address

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the complications generated by the high consumption of high sugar-fat diets(Jung & Choi, 2014; Priest & Tontonoz, 2019). On the other hand, bioactive compounds supplementation in the diet, with anti-inflammatory and antioxidant characteristics, has been increasingly in evidence, and may be an effective non-drug therapeutic strategy in the face of metabolic diseases (Jayarathne et al., 2017).

The polyphenolic fraction (BPF) of Bergamot (*Citrus bergamia*) fruit juice has been highlighted in recent years, evidencing its hypolipidemic, hypoglycemic, anti-inflammatory and antioxidant action (Di Donna et al., 2009; Formisano et al., 2019; Mollace et al., 2011; Moufida & Marzouk, 2003; Risitano et al., 2014). However, as demonstrated by Baron et al. (2021), the leaves have a higher amount of polyphenols, evidencing a promising and sustainable therapeutic alternative with parts that are usually discard (Baron et al., 2021). Based on Baron's research, only three pre-clinical studies were conducted, demonstrating that the compounds present in the leaves are capable of promoting antioxidant and anti-inflammatory activity in the hypothalamus, muscle tissue and liver (Nakandakare-Maia et al., 2023; Palacio et al., 2022; Siqueira et al., 2022), Therefore, it becomes relevant to continue investigations to evaluate the other organs that are normally affected in obese people with metabolic syndrome. This present investigation, involving several organs, will be an initial step for future studies to assess the mechanisms involved in the treatment of disorders associated with oxidative stress and inflammation. Therefore, the purpose of this study was to evaluate the treatment effect of bergamot leaf extract (BLE) on the redox/inflammatory scenario in different organs of obese rats with metabolic syndrome.

2. Materials and methods

2.1. Experimental protocol

The study protocol followed the guidelines from the Guide for the Care and Use of Experimental Animals and received approval from the Ethics Committee for Animal Experimentation of the Botucatu Medical School, São Paulo State University (UNESP), São Paulo, Brazil (1393/2021). Over a period of 20 weeks, 48 male Wistar rats were randomly allocated into 2 groups: Control diet (C, n = 24) and HSF diet plus 25 % of sucrose in the drinking water (HSF, n = 24). The diets composition was previously published (F. Francisqueti et al., 2017).

2.1.1. Inclusion criteria

Animals submitted to different diet models do not always behave as expected, which may generate misunderstandings results. Therefore, a separation point based in metabolic syndrome parameters as: body weight, plasma triglycerides, and systolic blood pressure; cardiac (echocardiogram) and renal disfunction was adopted as an inclusion criterion at the 20th week (Siqueira et al., 2022). Animals in the HSF group below the cut-off point that did not show weight gain, elevated plasma triglyceride levels, hypertension or cardiac and renal dysfunction were excluded from the study, as well as animals in the control group below the cut-off point.

Then, the animals, selected for the experiment, were reallocated receiving control diet (C, n = 10), control diet with bergamot leaves extract (C+BLE, n = 10), HSF diet (HSF, n = 10) and HSF diet with bergamot leaves extract (HSF+BLE, n = 10) for 10 weeks, totaling 30 weeks. The animals had food and water *ad libitum*. Body weight, food and water intake were monitored weekly; caloric intake was determined by the consumption and the amount of energy content of the diets. The animals were kept in individual cages enriched with paper towels crumpled in the shape of balls to reduce stress. At week 30th, the animals were exposed to an 8-hour fast and anesthetized with Thiopental (120 mg/kg/IP). After checking palpebral, foot, interdigital and caudal reflexes, the animals were euthanized by decapitation for tissue and blood collection.

2.1.2. Bergamot leaves extract (BLE)

The leaves were harvested at a farm in the Reggio Calabria, Italy. The dry extract was obtained by H&AD (Herbal & Antioxidant Derivatives S. r.l.) located at Località Chiusi, 89032 Bianco (RC), Italy (https://www.head-sa.com). The descriptions of the extraction procedures were previously published by our research group (Siqueira et al., 2022). The extract was administered daily by gavage at a dose of 50 mg/kg of animal weight diluted in drinking water. As a vehicle, the C and HSF groups received drinking water by gavage. The dosage was determined according to published data using fruit juice (Musolino et al., 2020).

2.2. Nutritional profile

Caloric intake, body weight, and adiposity index were used to analyze the nutritional profile. For the HSF diet, calories from water containing 25 % sucrose were also included (0.25 × 4 × mL consumed). Obesity was measured using the adiposity index by the total amount of fat deposits (epididymal, visceral and retroperitoneal) normalized by body weight (Luvizotto, Nascimento, Imaizumi, Pierine, Conde, Correa, & Ferreira, 2013).

2.3. Comorbidities

The following parameters were examined since obesity is linked to modifications in glycemic, lipid, hormone, cardiovascular, hepatic, and renal profiles:

2.3.1. Plasma parameters

Before euthanasia, glucose levels (mg/dL) were assessed using a glucometer (Accu-Chek Performa; Roche Diagnostics, Indianapolis, IN, USA). The plasmatic levels of triacylglycerol and high-density lipoprotein (HDL) were measured by an automated device (Technicon, RA-XTTM System, Global Medical Instrumentation, Minnesota, USA), using CELM® kits from Barueri, São Paulo, Brazil. Insulin quantification was obtained in plasma by ELISA (Leinco Research Inc.). The reading was performed on a Spectra Max 190 microplate spectrophotometer (Molecular Devices®, Sunnyvale, CA, USA). The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was considered a marker of insulin resistance (Matthews, Hosker, Rudenski, Naylor, & Treacher, 1985).

2.3.2. Systolic blood pressure

Systolic blood pressure analysis was performed by tail-cuff plethysmography using a Narco Bio-System® PE 300 electro-sphygmomanometer, model 709–0610 (International Biomedical, Inc, Houston, TX, USA) (Gonc et al., 2014). This method does not allow the assessment of diastolic blood pressure. The data was gathered with the polygraph Gould RS 3200(Gould Instrumenta Valley View, Ohio, USA).

2.3.3. Cardiac structure and function

A qualified examiner conducted a Doppler echocardiographic evaluation. Ketamine (50 mg/kg) and Xylazine (1 mg/kg) were administered intraperitoneally to the animals. The anterior portion of the chest was trichotomized and the animals were gently placed in the left lateral decubitus for examination. Model Vivid S6 (General Electric Medical Systems, Israel) with a multifrequency ultrasonic transducer from 5.0 to 11.5 MHz was employed. The steps have were previously been published (Ferron et al., 2018).

The following structures were used to analyze the cardiac morphology: posterior wall diastolic thickness (PWT), interventricular septal diastolic thickness (IST), LV mass index (LVMI) and relative thickness (LVRT). The systolic function of the left ventricle was determined by the following parameters: endocardial fractional shortening (EFS), posterior wall shortening velocity (PWSV) and ejection fraction. Left ventricular diastolic function was assessed by E-wave deceleration time (EWDT) and E/E' wave ratio (Nagueh et al., 2009).

2.3.4. Hepatic function

An automated system (Technicon, RA-XTTM System, Global Medical Instrumentation, Minnesota, USA) was used to assess the levels of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using CELM kits® from Barueri, Sao Paulo, Brazil. The Ritis ratio (AST/ALT) was also used since it marks liver impairment progression (Giannini, 2005).

2.3.5. Renal function

Urine was collected for 12 h (from animals in individual metabolic cages) in conical tubes containing 1 mL of the antioxidant butylated toluene hydroxide (BHT 360 mM – Butylated Hydroxytoluene) and approximately 5 mL of these aliquots were stored at −80 °C until analysis. Subsequently, creatinine and total protein concentrations were measured using commercial colorimetric kits (CELM® kits, Barueri, São Paulo, Brazil). Renal function was evaluated according to proteinuria and glomerular filtration rate (GFR) (Anna, Mátyus, Sárkány, Horváth, & Fodor, 2010).

2.4. Redox/inflammatory state in obesity target organs

Inflammation and oxidative stress resulting from the consumption of high sugar-fat diets and obesity have effects on several target organs (Martı, Felix-soriano, & Escote, 2018). Thus, the effect of BLE on inflammatory parameters and RedOx system described below were analyzed in epididymal adipose tissue (AT), heart, liver and kidneys of animals. Tissues were homogenized with ice-cold Phosphate Buffered Saline solution (PBS, 1 mL, pH 7.4) or potassium chloride solution (KCl 1.15 %) using the ULTRA-TURRAX® T25 basic IKA® Werke Staufen/Germany, and centrifuged at 3500 rpm, at 4 °C for 10 min. A Spectra Max 190 microplate spectrophotometer (Molecular Devices®, Sunnyvale, CA, USA) was used to take the readings.

2.4.1. Inflammatory parameters

Tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and interleukin-10 (IL-10) levels were measured in the target organs. Analyzes were performed using the ELISA technique, using a commercial kit (R&D Systems, Minnesota, US, #DY510 #DY506 #DY417 #DY522).

2.4.2. RedOx system parameters

The effect of bergamot on RedOx system parameters was evaluated by the plasmatic levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and cardiac, hepatic and renal levels of malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), carbonyl proteins, advanced oxidation protein products (AOPP), Ferric Reducing Antioxidant Power (FRAP) and activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT).

As a marker of systemic genotoxicity, the levels of 8-OHdG (pg/mL) marker in the urine was used through the commercial kit (Fine Biotech Co., Ltd., Wuhan, China, #ER1487-HS).

To assess MDA levels (nmol/mg of protein), Thiobarbituric acid (TBA) at 0.67 % was added to the supernatant (2:5; TBA:Supernatant) and followed by centrifugation at 3500 rpm for 10 min. Afterwards, the samples were heated for 45 min in a water bath and, after cooling, transferred to a 96-well plate for reading at 532 and 600 nm to calculate de MDA concentration from the molar extinction coefficient ($1.56 \times 105\ M^{-1}\ cm^{-1}$) (Uchiyama & Mihara, 1978).

4-HNE (pg/g of protein) was measured following the instructions of the commercial ELISA kit (Elabscience Biotechnology Co., Ltd, USA, #E-EL-0128).

Protein carbonylation (nmol of DNPH/mg of protein) was performed using 2,4-dinitrophenylhydrazine (DNPH) to detect by photometry proteins modified by carbonylation (Mesquita et al., 2014).

Values of AOPP (μmol/U chloramine T/g protein) were determined by spectrophotometry, according to Kalousová et al. (Kalousová, Skrha, & Zima, 2002), by diluting tissues 1:5 with PBS, preparing a Chloramin T calibration curve (10-400umol/L), adding potassium iodide (KI, 1.16 M) and glacial acetic acid. The reading was immediately performed at 340 nm.

To determine the antioxidant activity of tissues by reducing iron, the FRAP assay (uM FeSO4·H2O/ug protein) was adapted according to Benzie and Strain using sodium acetate buffer (0.3 mol/L, pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ, 10 mmol/L), and iron chloride (FeCl3, 20 nmol/L) for making the FRAP reagent. Ferrous sulfate (FeSO4·.7H2O, 3.9 mmol/L) was used as a standard curve. Reading was performed at 595 nm (Benzie & Strain, 1996).

SOD activity (U/g protein/minute) was determined using the technique described by Crouch et al. from the use of Tris buffer (50 mmol/L), EDTA (1 mmol/L, pH 8.5) and pyrogallol (2.6 mmol/L) to determine the ability of SOD present in the samples to inhibit pyrogallol autoxidation (Marklund, 1985).

CAT activity (pmol/g protein/minute) was determined in potassium phosphate buffer (5ommol/L, pH 7.0) and 10 % hydrogen peroxide to determine the ability of the enzyme present in the sample to consume hydrogen peroxide. Readings were performed at 240 nm (Aebi, 1984).

2.5. Statistical analysis

Data expressed as mean ± standard deviation or median (interquartile half-range). Comparison was determined by two-way ANOVA or Kruskal-Wallis followed by Tukey's post-hoc test considering an α of 5 %. The analyses were performed with SigmaStat 3.5 software and the graphs using GraphPad Prism software version 9.0 for Mac.

3. Results

The nutritional-metabolic profile at the end of the experimental protocol is shown at Table 1. The C+BLE group showed increased levels of insulin and insulin resistance compared to the C group. The groups that received HSF diet (HSF and HSF+BLE) had higher caloric intake, weight gain, final weight, presence of obesity, hypertriglyceridemia, insulin levels, and hypertension in relation to the control groups (C and C+BLE). In addition, animals in the HSF group had higher levels of fasting glucose and insulin resistance compared to the control group. In contrast, treatment with BLE promoted reduced levels of triglycerides, insulin and insulin resistance and increased levels of HDL in the HSF+BLE group compared to the HSF group.

The echocardiographic parameters in Table 2 shows that the HSF group showed cardiac remodeling, characterized by alterations in morphological variables (increase in PWT, IST, LVMI, LVRT and EFS), diastolic deterioration (higher EWDT and lower E/E'), and dysfunction systolic (reduction in LVPWSV and ejection fraction) compared to the control group. The HSF+BLE group showed reduced values of LVPWSV and increased E/E' compared to the Control+BLE group, which demonstrated reduced EFS and ejection fraction compared to the control group. Fig. 1 shows AST, ALT and AST/ALT ratio values at the end of 30 weeks which presents a significant difference was observed between the groups that consumed the control diet due to the reduction of AST in the C+BLE group in relation to the C group and an increase in the AST/ALT ratio of the groups that consumed the HSF diet (HSF and HSF+BLE) in relation to the controls (C and C+BLE). The addition of bergamot leaves extract treated cardiac remodeling and cardiac dysfunction in the HSF+BLE group when compared to the HSF group.

Fig. 1 shows AST, ALT and AST/ALT ratio values at the end of 30 weeks. A significant difference was observed between the groups that consumed the control diet due to the reduction of AST in the C+BLE group in relation to the C group and an increase in the AST/ALT ratio of the groups that consumed the HSF diet (HSF and HSF+BLE) in relation to the controls (C and C+BLE).

In relation to the kidneys, the HSF group developed renal disease, characterized by an increase in the protein/creatinine ratio and a

Table 1
Nutritional-Metabolic profile at the 30th week.

	Groups				Effects		
	C	C+BLE	HSF	HSF+BLE	Diet	Treatment	Interaction
Caloric intake (Kcal)	18949 ± 1082	18065 ± 2059	21462 ± 1757*	22003 ± 2416$^{▲}$	<0.001	0.776	0.242
Weight gain (g)	315 ± 53	281 ± 67	381 ± 66*	382 ± 83$^{▲}$	<0.001	0.458	0.424
Body weight (g)	482 ± 58	460 ± 74	578 ± 69*	575 ± 94$^{▲}$	<0.001	0.617	0.687
Adiposity index (%)	4.64 ± 1.17	4.27 ± 0.64	8.58 ± 1.69*	7.95 ± 2.49$^{▲}$	<0.001	0.344	0.802
Triglycerides (mg/dL)	31.1 ± 7.6	32.0 ± 8.1	100.1 ± 14.1*	65.4 ± 17.8$^{▲,◇}$	<0.001	<0.001	<0.001
HDL (mg/dL)	21.4 ± 2.0	20.2 ± 2.7	20.5 ± 3.3*	23.3 ± 3.2$^{▲,◇}$	0.233	0.384	0.034
Glucose (mg/dL)	87.1 ± 8.4	86.6 ± 5.3	96.4 ± 9.6*	91.1 ± 9.7	0.014	0.285	0.375
Insulin (μU/ml)	6.5 ± 2.3	15.6 ± 5.4*	26.5 ± 4.4*	20.6 ± 5.2$^{▲,◇}$	<0.001	0.275	<0.001
HOMA-IR	1.49 ± 0.38	3.63 ± 0.97*	6.69 ± 1.20*	4.39 ± 1.02$^{◇}$	<0.001	0.787	<0.001
SBP (mmHg)	115 ± 7	121 ± 9	142 ± 8*	144 ± 13$^{▲}$	<0.001	0.210	0.426

HDL – High Density Lipoprotein; HOMA-IR – Homeostatic model assessment – Insulin Resistance; SBP – Systolic Blood Pressure; C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet. Data expressed as mean ± standard deviation. Comparison by two-way ANOVA with Tukey's post hoc. *$p < 0.05$ vs. C; $^{◇}p < 0.05$ vs. HSF; $^{▲}p < 0.05$ vs. C+BLE.

Table 2
Doppler Echocardiogram at the end of 30 weeks.

	Groups				Effects		
	C	C+BLE	HSF	HSF+BLE	Diet	Treatment	Interaction
PWT	1.50 ± 0.06	1.48 ± 0.04	1.89 ± 0.20*	1.56 ± 0.25$^{◇}$	<0.001	<0.001	<0.001
IST[1]	1.52(1.53–1.50)	1.53(1.53–1.50)	1.94(2.14–1.79)*	1.56(1.63–1.23)$^{◇}$	<0.001	<0.001	<0.001
EFS	65.37 ± 2.60	60.53 ± 4.42*	55.34 ± 3.69*	58.27 ± 2.68$^{◇}$	<0.001	0.540	<0.001
LVMI	1.27 ± 0.10	1.33 ± 0.19	1.81 ± 0.17*	1.39 ± 0.29$^{◇}$	<0.001	<0.001	<0.001
LVRT	0.44 ± 0.02	0.45 ± 0.04	0.56 ± 0.03*	0.45 ± 0.06$^{◇}$	<0.001	<0.001	<0.001
LVPWSV	85.30 ± 3.80	81.00 ± 6.53	64.90 ± 9.64*	72.82 ± 5.28$^{◇,▲}$	<0.001	0.200	0.001
EWDT[1]	44.00(46.00–44.00)	46.00(46.00–45.00)	57.00(58.00–51.00)*	47.00(49.00–44.00)$^{◇}$	<0.001	0.011	<0.001
EF	0.96 ± 0.01	0.94 ± 0.02*	0.91 ± 0.02*	0.93 ± 0.01$^{◇}$	<0.001	0.969	<0.001
E/E'[1]	13.36(13.77–13.19)	12.49(11.55–13.83)	22.11(26.93–19.84)*	16.23(17.00–15.02)$^{◇,▲}$	<0.001	<0.001	0.001

PWT – Diastolic thickness of the left ventricle posterior wall (mm); IST – Diastolic thickness of the interventricular septum (mm); EFS – Endocardial fractional shortening (%); LVMI – Left ventricle mass; LVRT – Left ventricle relative thickness; LVPWSV – Left ventricle posterior wall shortening velocity (mm/s); EWDT – E wave deceleration time (ms); EF – Ejection Fraction; C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet. Data expressed as mean ± standard deviation or [1]median (interquartile range). Comparison by two-way ANOVA or [1]Kruskal-Wallis with Tukey's post hoc. *$p < 0.05$ vs. C; $^{◇}p < 0.05$ vs. HSF; $^{▲}p < 0.05$ vs. C++BLE.

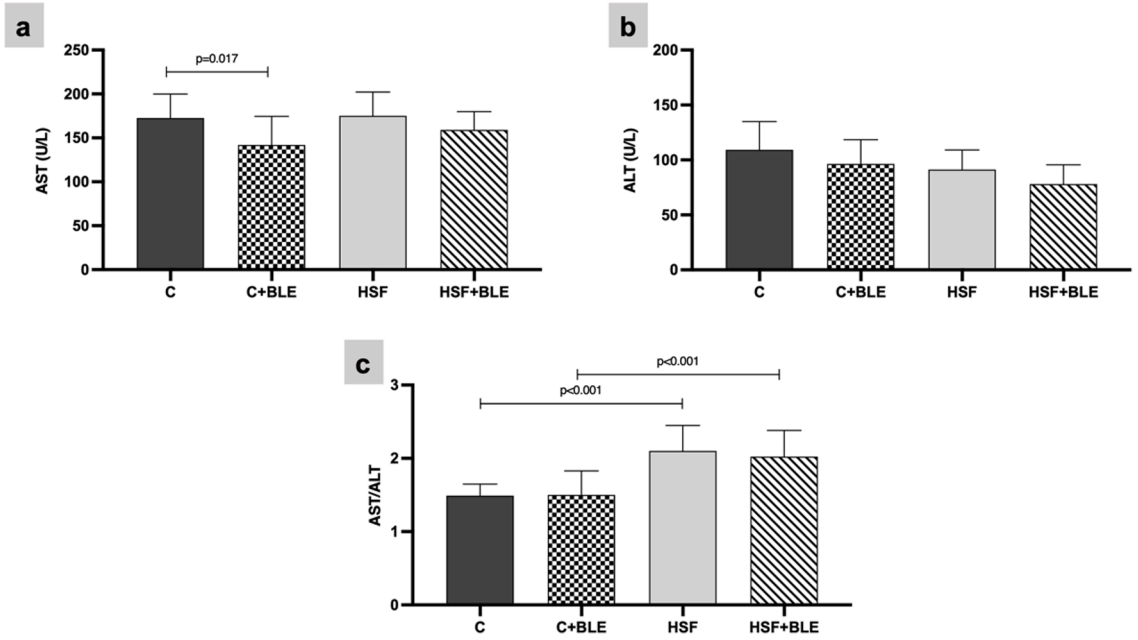

Fig. 1. Hepatic Function. (a) AST (U/L); (b) ALT (U/L); (c) AST/ALT. Data expressed as mean ± standard deviation. Comparison by two-way ANOVA with Tukey's post hoc. Significance of 5 %. C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet. AST – Aspartate aminotransferase; ALT – Alanine aminotransferase.

reduction in the GFR compared to the control group. The C+BLE group showed a reduction in the protein/creatinine ratio compared to the control group (Fig. 2). In contrast, the treatment with BLE promoted a reduction in proteinuria and improved renal function, as evidenced by the increase in GFR in the HSF+BLE group in comparison to the HSF group (Fig. 2).

Table 3 contains the Inflammatory state in the AT, heart, liver and kidneys at the end of 30 weeks. The animals from de HSF group presented increased levels of TNF-α and IL-6 in relation to the control group. The heart and liver from the obese animals had shown increased levels of MCP-1 and IL-10, which were decreased in liver and AT, respectively, when compared to the control group. However, treatment with BLE reduced the levels of TNF-α and IL-6 in the AT, heart, liver, and kidney; promoted lower levels of MCP-1 in the heart and liver; and lower IL-10 in the heart in comparison to the HSF group.

Table 4 contains the RedOx state in the AT, heart, liver and kidneys and the urinary levels of 8-OHdG at the end of the experimental protocol. The AT, heart and liver of the animals from de HSF group presented increased levels of MDA and decreased levels in the kidney in relation to the control group. The levels of 4-HNE were increased in AT, heart and kidneys. CBO levels presented higher levels in AT, heart and liver in comparison to the control group. AOPP levels were increased in the heart of the HSF group in comparison to the Control group. The antioxidant enzymes presented lower activity in the heart, liver and kidney of the HSF group in comparison to the control group and the FRAP assay presented lower levels in the AT, heart and liver. In addition, the animals in the groups that received the HSF diet (HSF and HSF+BLE) showed elevated levels of 8-OHdG compared to the groups that consumed the control diet (C and C+BLE). In contrast, in relation to the HSF group, the treatment with BLE promoted reduction in 8-OHdG levels; MDA levels on AT and liver; 4-HNE and AOPP levels on the AT, heart and kidneys; and CBO levels on the AT, heart, liver and kidney. In addition, the BLE treatment promoted increased activity of SOD enzyme (AT), CAT enzyme (AT, liver and kidneys) and FRAP (AT, heart, liver and kidneys).

4. Discussion

The aim was to evaluate the treatment effect of bergamot leaf extract (BLE) on the redox/inflammatory scenario in different organs of obese rats with metabolic syndrome. The diet provided to the HSF groups aims to mimic the Western diet, which consumed in excess, leads to obesity and metabolic syndrome. Due to its low molecular weight, the simple carbohydrates present in this diet are quickly absorbed, resulting in fat deposits, that play a key role in the pathophysiological processes that lead to the complications associated with obesity(Reyes-Farias, Fos-Domenech, Serra, Herrero, & Sánchez-Infantes, 2021; Rodríguez-Correa, González-Pérez, Clavel-Pérez, Contreras-Vargas, & Carvajal, 2020). Thus, the animals that consumed the HSF diet showed dyslipidemia, higher insulin levels and insulin resistance compared to the control group.

The literature has reported that the inadequate expansion of adipose tissue, especially due to adipocyte hypertrophy, is the main consequence of positive energy balance and results in several deleterious effects such as inflammation, alteration in adipokine secretion, hypoxia, fibrosis, and mitochondrial dysfunction with a consequent increase in ROS production (Bischoff et al., 2017; Da Silva & da Sobrinho, 2019; Francisqueti et al., 2017; Longo et al., 2019; SIES, 1985). As a result of adipose tissue dysfunction, impairments in metabolic signaling are common, culminating in insulin resistance and metabolic syndrome. In addition, adipose tissue dysfunction contributes to ectopic fat deposition in other organs, impairing their function (Longo et al., 2019). Confirming, the animals in the HSF group showed obesity, inflammation and oxidative stress in the adipose tissue, in addition to metabolic syndrome and cardiac, hepatic and renal functional impairments.

Chronic low-grade inflammation is one of the obesity characteristics and is associated with several negative outcomes, from favoring metabolic disorders to the manifestation of diseases such as heart and kidney failure and non-alcoholic fatty liver disease (Artemniak-Wojtowicz, Kucharska, & Pyrżak, 2020). In the heart, the literature has reported that inflammation is associated with hypertrophy, fibrosis, cardiac remodeling, and, ultimately, cardiac dysfunction (Gutiérrez-Cuevas et al., 2021; Wensley, Salaveria, Bulmer, Donner, & du Toit, 2013). In the liver, the inflammatory process resulting from obesity plays a crucial role in the development of non-alcoholic fatty liver disease and its progression to cirrhosis or liver cancer (Pilling, Karhadkar, & Gomer, 2021; Zhao et al., 2020). In the kidneys, inflammation resulting from obesity has been associated with renal fibrosis and irreversible deposition of extracellular matrix in the renal tissue, which leads to progressive loss of renal function (da Silva Junior, Bentes, Daher, & de Matos, 2017). Within this context, it is noted that the animals in the HSF group had inflammation in the heart, liver and kidneys, with consequent organ dysfunction, as demonstrated by the literature.

Oxidative stress is another obesity consequence, resulting from several mechanisms such as hyperleptinemia, adipose tissue dysfunction, impaired antioxidant defense, chronic inflammation and excessive production of ROS due to high caloric intake. Like inflammation, it has been associated with several disorders, such as diabetes and liver, heart and kidney dysfunction (Manna & Jain, 2015). In the heart, oxidative stress can damage cardiac tissue directly, altering its geometry and functionality, or indirectly through the carbonylation of proteins involved in the regulatory response of myocardial contractility, in addition to other mechanisms that contribute to myocardial failure due to a lack of adaptation (Dhalla, Elimban, Bartekova, & Adameova, 2022; Ferron et al., 2019; Tsutsui, Kinugawa, & Matsushima, 2011). In the liver, oxidative stress plays a fundamental role in activating mechanisms involved in the onset and worsening of liver diseases, in addition to modifying DNA, proteins and lipids that will impair the control of its biological functions (Li et al., 2015). In the kidneys, oxidative stress has been associated with podocyte apoptosis, leading to proteinuria in

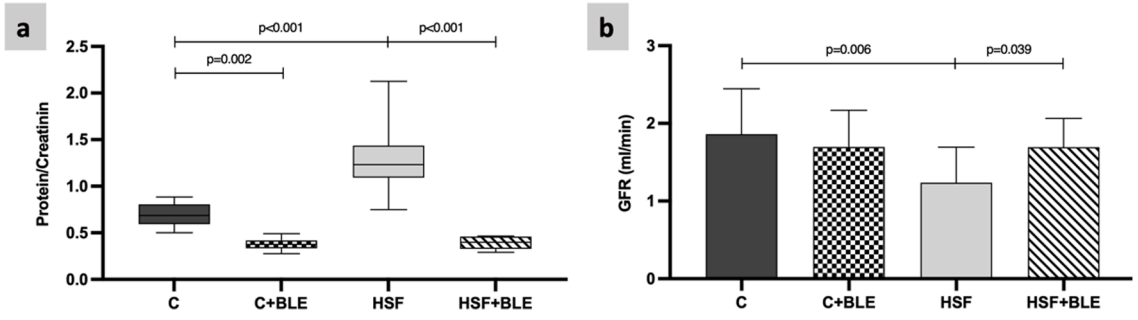

Fig. 2. Renal Function. (a) Protein/creatinine ratio; (b) Glomerular Filtration Rate – GFR (mL/min). Data expressed as mean ± standard deviation or median (interquartile range). Comparison by two-way ANOVA or Kruskal-Wallis with Tukey's post hoc. Significance of 5 %. C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet.

Table 3
Inflammatory State at the end of 30 weeks.

	Groups				Effects		
	C	C+BLE	HSF	HSF+BLE	Diet	Treatment	Interaction
TNF-α							
AT	3041 ± 1511	1830 ± 1368	4087 ± 1072*	2522 ± 14$^{\diamond}$	0.002	0.001	0.242
Heart	17625 ± 6111	21615 ± 5728	30159 ± 4463*	19847 ± 4927$^{\diamond}$	0.003	0.070	<0.001
Liver	3342 ± 744	2352 ± 616	3880 ± 947*	3458 ± 1315$^{\diamond}$	0.041	0.075	0.287
Kidney	158446 ± 95463	351976 ± 199187	647948 ± 414093*	345421 ± 128613$^{\diamond}$	0.003	0.483	0.003
IL-6							
AT	12296 ± 4721	11832 ± 4805	18160 ± 4176*	12617 ± 3187$^{\diamond}$	0.019	0.033	0.068
Heart[1]	25358(28926–22945)	27564(27565–23923)	124563(125385–56132)*	22754(26065–16801)$^{\diamond}$	<0.001	<0.001	<0.001
Liver	2496 ± 347	2281 ± 704	4705 ± 2072*	3887 ± 1524	0.007	0.191	0.520
Kidney	149328 ± 43172	190427 ± 64708	222268 ± 73990*	163210 ± 27789$^{\diamond}$	0.201	0.612	0.007
MCP-1							
AT	136 ± 40	137 ± 32	169 ± 136	158 ± 49	0.268	0.841	0.813
Heart	22.7 ± 4.8	24.9 ± 10.0	39.2 ± 10.5*	17.7 ± 5.4$^{\diamond}$	0.075	<0.001	<0.001
Liver	17.6 ± 4.7	17.4 ± 1.9	37.5 ± 5.6*	24.9 ± 5.9$^{\blacktriangle,\diamond}$	<0.001	<0.001	<0.001
Kidney	245 ± 63	246 ± 27	166 ± 48*	206 ± 53	<0.001	0.207	0.217
IL-10							
AT	14537 ± 2244	10723 ± 4046	8709 ± 2924*	10930 ± 3669	0.073	0.493	0.047
Heart	13607 ± 4960	19256 ± 8115	34078 ± 11,449*	17811 ± 11,029$^{\diamond}$	0.003	0.078	<0.001
Liver	3265 ± 856	4261 ± 1393	4902 ± 773*	5858 ± 1848$^{\blacktriangle}$	<0.001	0.041	0.690
Kidney	38601 ± 7989	44600 ± 10284	37018 ± 7,899	33743 ± 10,459	0.052	0.569	0.965

TNF-α – Tumor necrosis factor alpha (pg/g protein); IL-6 – Interleukin-6 (pg/g protein); MCP-1 – Monocyte chemoattractant protein-1 (pg/g protein); IL-10 – Interleukin-10; C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet. Data expressed as mean ± standard deviation or [1]median (interquartile range). Comparison by two-way ANOVA or [1]Kruskal-Wallis with Tukey's post hoc. *$p < 0.05$ vs. C; $^{\diamond}p < 0.05$ vs. HSF; $^{\blacktriangle}p < 0.05$ vs. C+BLE.

addition to fibrosis and mesangial expansion, progressively compromising their function (Tang, Cai, & Dong, 2016). In this study, it can be noted that the animals in the HSF group presented oxidative stress and functional impairment of the affected organs.

In contrast, animals that consumed the HSF diet and were treated with BLE showed improvement in metabolic parameters, inflammation and oxidative stress in different tissues. The search for new therapeutic strategies has become the subject of research as they demonstrate promising results in the face of metabolic diseases (Noce et al., 2021). In addition, analytical studies show that, for some plants, the leaf's phenolic composition is similar to that of the fruit or even richer or superior, indicating that they can be used as an alternative source in the development of nutraceuticals and functional foods (Carolo dos Santos, Santiloni Cury, & Ferraz, 2018; Ferlemi & Lamari, 2016). Additionally, Baron et al. (2016) quantified and compared in vitro the presence of polyphenolic compounds between the fruit and leaves of bergamot and demonstrated that the leaf shares some classes of polyphenols and, in some of them, has higher concentrations, such as neoeriocitrin, naringin, neohesperidin, melitidine and bruteridine, in relation to the fruit extract, proving to be promising in the face of comorbidities associated with the consumption of HSF diets(Baron et al., 2021).

Our obese animals treated with the BLE showed lower levels of triglycerides, insulin and insulin resistance and increased HDL cholesterol compared to the group that consumed only the HSF diet (Table 1). Mollace et al. (2011) and Miceli et al. (2007) demonstrated lower triglycerides levels, an increase in HDL cholesterol and a protective action on the hepatic parenchyma after treatment with the polyphenolic fraction from bergamot juice in hypercholesterolemic rats (Miceli et al., 2007; Mollace et al., 2011). The higher fecal excretion of sterols in experimental models receiving bergamot extract may be responsible for this outcome. In addition, the extract's flavonoids composition, particularly naringin and neohesperidin, acts by reducing the activity of enzymes involved in the formation of diacylglycerol in a hyperglycemic context, as phosphatidate phosphohydrolase, as well as by its effects on lipid availability for the assembly of lipoproteins through association with Apolipoprotein B, an integral part in the transport of lipids to tissues (Cha et al., 2001; Ighodaro, 2018; Mollace et al., 2011). Furthermore, some components present on bergamot, such as naringin, bruteridine and melitidine are similar to the structure of the substrate HMG-CoA reductase, inhibiting the activity of the enzyme (Di Donna et al., 2009; Kim et al., 2004). In addition, the BLE demonstrated, in vitro, anti-inflammatory action from the inhibition of nuclear transcription factor kappa-B (NF-kB) activation and antioxidant action through nuclear factor 2 related to erythroid-2 (Nrf2) activation, corroborating our results (Baron et al., 2021; Siqueira et al., 2022). Inhibition of pro-inflammatory cytokines release acts on glucose uptake, improving the sensitivity to insulin found in animals treated with the extract from the HSF+BLE group compared to the HSF group (Ferrari, Bock, Motta, & Helal, 2019). Corroborating, animals supplemented with BLE (HSF+BLE) showed a reduction in systemic genotoxicity compared to the HSF group.

The literature points to an improvement in cardiac function after administration of bergamot fruit extract, emphasizing the presence of flavonoids in oxidative stress prevention as the main protective mechanism (Carresi et al., 2018; Trombetta et al., 2010). Furthermore, the juice extract's hypoglycemic and hypolipidemic potential were associated with improvement in vascular reactivity in patients with hyperlipidemia and hyperglycemia, pointing to a potential protective role for the use of the fruit's polyphenolic fraction. These results can be extrapolated to the leaves extract action, since the comparative study between the fruit and leaves extracts showed higher levels of the polyphenolic fraction in the bergamot leaves extract (Baron et al., 2021). Corroborating, the addition of bergamot leaves extract treated cardiac remodeling, cardiac dysfunction (Table 4), inflammation (Table 2) and oxidative stress (Table 3) in the HSF+BLE group when compared to the HSF group.

De Leo et al. (2019) observed an improvement in microvesicular steatosis in only one animal fed a high-fat diet supplemented with bergamot juice, inferring a possible protective role played by the extract (De Leo et al., 2020). The authors assumed that the protective effects of the juice on hepatic steatosis could be achieved through the reduction of oxidative stress and inflammation since epidemiological and animal

Table 4
RedOx State at the end of 30 weeks.

	Groups				Effects		
	C	C+BLE	HSF	HSF+BLE	Diet	Treatment	Interaction
8-OHdG	93.8 ± 15.1	92.6 ± 27.7	148.3 ± 31.3*	118.6 ± 31.6$^{▲,◇}$	<0.001	0.081	0.107
MDA							
AT	294 ± 127	461 ± 138	931 ± 311*	471 ± 190$^{◇}$	<0.001	0.030	<0.001
Heart	343 ± 73	439 ± 66*	455 ± 181*	451 ± 78	0.088	0.200	0.023
Liver	159 ± 36	152 ± 34	197 ± 56*	182 ± 79$^{◇}$	0.123	0.050	0.040
Kidney	1620 ± 263	1550 ± 274	1239 ± 300*	1139 ± 281$^{▲}$	<0.001	0.310	0.818
4-HNE							
AT	0.30 ± 0.11	0.30 ± 0.12	0.53 ± 0.11*	0.38 ± 0.12$^{▲,◇}$	<0.001	0.036	0.047
Heart	3.62 ± 1.29	3.70 ± 2.13	6.36 ± 1.96*	3.75 ± 1.38$^{◇}$	0.036	0.055	0.043
Liver	0.779 ± 0.211	0.856 ± 0.196	0.556 ± 0.080	0.303 ± 0.112$^{▲}$	0.003	0.366	0.112
Kidney[1]	0.035(0.038–0.035)	0.024(0.024–0.017)	0.211(0.269–0.135)*	0.049(0.088–0.029)$^{◇}$	<0.001	0.001	0.007
CBO							
AT	48.5 ± 21.1	67.4 ± 16.9	135.0 ± 14.1*	65.4 ± 17.8$^{▲,◇}$	<0.001	0.426	0.007
Heart	134 ± 40	185 ± 41*	280 ± 74*	168 ± 22$^{◇}$	<0.001	0.050	<0.001
Liver	85.9 ± 10.1	88.8 ± 17.5	94.2 ± 16.5*	81.1 ± 13.5$^{◇}$	0.530	0.025	0.210
Kidney	26.1 ± 6.0	27.6 ± 5.4	22.7 ± 10.6	19.3 ± 4.3$^{▲,◇}$	0.168	0.155	0.015
AOPP							
AT[1]	55.9(67.7–49.4)	81.79(124.3–39.2)	83.7(87.3–81.2)	50.0(59.3–43.7)$^{▲,◇}$	0.854	0.721	0.010
Heart	80.5 ± 14.5	76.1 ± 31.2	118.7 ± 21.8*	78.1 ± 23.4$^{◇}$	0.029	0.016	0.048
Liver	70.4 ± 16.2	75.3 ± 7.1	60.7 ± 10.0	55.8 ± 8.6$^{▲}$	0.001	0.993	0.239
Kidney	111 ± 21	104 ± 22	107 ± 7	69 ± 27$^{▲,◇}$	0.013	0.006	0.053
SOD							
AT	365 ± 77	278 ± 79	440 ± 96	485 ± 133$^{▲}$	<0.001	0.496	0.042
Heart	143 ± 20	121 ± 13*	117 ± 8*	127 ± 19	0.049	0.252	0.003
Liver	6.14 ± 1.51	6.96 ± 1.55	4.04 ± 0.74*	5.39 ± 1.45$^{▲,◇}$	<0.001	0.002	0.533
Kidney	104 ± 10	83 ± 17*	90 ± 5*	101 ± 16$^{▲}$	0.601	0.221	<0.001
CAT							
AT	90.7 ± 17.4	69.2 ± 12.0*	88.5 ± 12.3	150.4 ± 35.5$^{▲,◇}$	<0.001	0.005	<0.001
Heart	55.9 ± 5.5	54.4 ± 7.2	44.4 ± 6.6*	47.8 ± 7.6$^{▲}$	<0.001	0.659	0.262
Liver	14.68 ± 2.24	17.26 ± 4.12	9.50 ± 1.72*	13.28 ± 3.28$^{▲,◇}$	<0.001	0.002	0.533
Kidney	145 ± 23	131 ± 18	114 ± 28*	159 ± 38$^{▲,◇}$	0.809	0.104	0.002
FRAP							
AT[1]	0.009(0.011–0.006)	0.007(0.008–0.005)	0.005(0.006–0.005)*	0.009(0.009–0.009)$^{◇}$	0.447	0.827	0.007
Heart	0.009 ± 0.002	0.006 ± 0.001*	0.007 ± 0.001*	0.009 ± 0.001$^{▲,◇}$	0.768	0.146	<0.001
Liver	0.009 ± 0.002	0.007 ± 0.001*	0.006 ± 0.001*	0.008 ± 0.002$^{◇}$	0.065	0.653	<0.001
Kidney	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.002	0,008 ± 0,002$^{▲,◇}$	0.003	0.015	0.364

MDA – malondialdehyde (nmol/mg protein); 4-HNE – 4-Hydroxynonenal (pg/g protein); CBO – Protein Carbonylation (nmol/mg protein); AOPP (Advanced oxidation protein products (umol/U cloramin/g protein); SOD – Superoxide Dismutase (U/g protein/minute); CAT – catalase (pmol/g protein/minute); FRAP – Ferric Reducing Antioxidant Power (uM FeSO4·H2O/ug protein); C – Control; BLE – Bergamot leaves Extract; HSF – High Sugar-Fat Diet. Data expressed as mean ± standard deviation or [1]median (interquartile range). Comparison by two-way ANOVA or [1]Kruskal-Wallis with Tukey's post hoc. *$p < 0.05$ vs. C; $^{◇}p < 0.05$ vs. HSF; $^{▲}p < 0.05$ vs. C+BLE.

studies have shown that increased production of ROS, lower levels of endogenous antioxidants, higher concentrations of pro-inflammatory cytokines, and dysfunctional lipid metabolism are present in fatty liver degeneration(Cheng et al., 2019; De Leo et al., 2020). Furthermore, after incubation with the polyphenolic fraction of the fruit, Mirachi et al. (2022) observed a reduction in intracellular lipid content in human hepatocytes, suggesting an increase in beta-oxidation as a mechanism (Mirarchi et al., 2022). Corroborating, HSF animals treated with BLE showed a reduction in inflammatory parameters (Table 2), oxidative stress and increased hepatic endogenous antioxidant activity compared to the HSF group (Table 3).

In the kidneys, BLE was able to improve kidney function, promoting a reduction in proteinuria and improved renal function, as evidenced by the increase in GFR in the HSF+BLE group in comparison to the HSF group (Fig. 2), in addition to reducing inflammatory cytokines (Table 2) and markers of oxidative stress levels and increasing catalase activity, an antioxidant enzyme (Table 3). Corroborating the finding, La Russa, Giordano, Marrone, Parafati, Janda, and Pellegrino (2019) observed redox imbalance attenuation after supplementation with the polyphenolic fraction of the bergamot fruit, associating obesity with kidney damage (La Russa et al., 2019). In addition, treatment with polyphenols and flavonoids acts by reducing inflammation and oxidative stress, factors associated with dysfunctional renal energy metabolism induced by a diet high in sugar and fat (Vargas et al., 2018).

Although there are no clinical trials evaluating the effect of BLE in humans, the extract shows promise in terms of development and progression of metabolic diseases. This study is pioneer in the application of BLE as an antioxidant and anti-inflammatory potential in different target tissues of obesity, opening space for future studies involving the search for ways of acting in the treatment of metabolic comorbidities in the target organs of obesity.

5. Conclusion

In summary, the HSF diet was able to induce obesity and metabolic syndrome, as well as cardiac, renal and hepatic inflammation, oxidative

stress and dysfunction. The treatment with bergamot leaves extract ameliorates the redox/inflammatory scenario in all target organs of obese rats with metabolic syndrome, showing a potential natural approach to treat the metabolic associated disorders in obesity.

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7. Ethics statement

The animal study protocol was approved by the Ethics Committee of Botucatu Medical School (1393/2021, approved on September 28, 2021).

CRediT authorship contribution statement

Juliana Silva Siqueira: Writing – original draft, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Erika Tiemi Nakandakare-Maia:** Methodology. **Taynara Aparecida Vieira:** Methodology. **Thiago Luiz Novaga Palacio:** Methodology. **Matheus Antônio Filiol Belin:** Methodology. **Giovanna Baron:** Methodology. **Silmeia Garcia Zanati Bazan:** Methodology. **Artur Junio Togneri Ferron:** Writing – original draft, Project administration, Data curation, Conceptualization. **Giancarlo Aldini:** Conceptualization. **Fabiane Valentini Francisqueti-Ferron:** Writing – original draft, Supervision, Project administration, Data curation. **Camila Renata Correa:** Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Aebi, H. (1984). [13] Catalase in Vitro. *Methods in Enzymology*, *105*(C), 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Anna, V. O., Mátyus, J., Sárkány, E., Horváth, A., & Fodor, B. (2010). New trends in the laboratory diagnostics of proteinuria and albuminuria. *Orvosi Hetilap, 151*(21), 864–869. https://doi.org/10.1556/OH.2010.28891

Artemniak-Wojtowicz, D., Kucharska, A., & Pyrżak, B. (2020). Obesity and chronic inflammation crosslinking. *Central European Journal of Immunology, 45*(4), 461–468. https://doi.org/10.5114/ceji.2020.103418

Baron, G., Altomare, A., Mol, M., Garcia, J. L., Correa, C., Raucci, A., … Aldini, G. (2021). Analytical Profile and Antioxidant and Anti-Inflammatory Activities of the Enriched Polyphenol Fractions Isolated from Bergamot Fruit and Leave. *Antioxidants, 10*(2), 141. https://doi.org/10.3390/antiox10020141

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Bischoff, S. C., Boirie, Y., Cederholm, T., Chourdakis, M., Cuerda, C., Delzenne, N. M., … Barazzoni, R. (2017). Towards a multidisciplinary approach to understand and manage obesity and related diseases. *Clinical Nutrition, 36*(4), 917–938. https://doi.org/10.1016/j.clnu.2016.11.007

Carolo dos Santos, K., Santiloni Cury, S., Costa Rodrigues Ferraz, A. P., Corrente, J. E., Mariani Gonçalves, B., De Araújo Machado, L. H., … Corrêa, C. R. (2018). Recovery of Cardiac Remodeling and Dysmetabolism by Pancreatic Islet Injury Improvement in Diabetic Rats after Yacon Leaf Extract Treatment. *Oxidative Medicine and Cellular Longevity*, *2018*(4), 1–9. Retrieved from %7C%7C.

Carresi, C., Musolino, V., Gliozzi, M., Maiuolo, J., Mollace, R., Nucera, S., … Mollace, V. (2018). Anti-oxidant effect of bergamot polyphenolic fraction counteracts doxorubicin-induced cardiomyopathy: Role of autophagy and c-kitposCD45negCD31neg cardiac stem cell activation. *Journal of Molecular and Cellular Cardiology*, *119*, 10–18. https://doi.org/10.1016/j.yjmcc.2018.04.007

Cha, J. Y., Cho, Y. S., Kim, I., Anno, T., Rahman, S. M., & Yanagita, T. (2001). Effect of hesperetin, a citrus flavonoid, on the liver triacylglycerol content and phosphatidate phosphohydrolase activity in orotic acid-fed rats. *Plant Foods for Human Nutrition (Dordrecht, Netherlands), 56*(4), 349–358. https://doi.org/10.1023/a:1011884200848

Cheng, K., Song, Z., Zhang, H., Li, S., Wang, C., Zhang, L., & Wang, T. (2019). The therapeutic effects of resveratrol on hepatic steatosis in high-fat diet-induced obese mice by improving oxidative stress, inflammation and lipid-related gene transcriptional expression. *Medical Molecular Morphology, 52*(4), 187–197. https://doi.org/10.1007/s00795-019-00216-7

Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I., & Kim, J. B. (2016). Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Frontiers in Endocrinology, 7*. https://doi.org/10.3389/fendo.2016.00030

Costa, M. R., Garcia, J. L., Silva, C. C. V. de A., Ferron, A. J. T., Francisqueti-Ferron, F. V., Hasimoto, F. K., … Moreto, F. (2019). Lycopene modulates pathophysiological processes of non-alcoholic fatty liver disease in obese rats. *Antioxidants*, *8*(8), 1–17. https://doi.org/10.3390/antiox8080276.

Da Silva, N. I., Sobrinho, H. M. da R., Blanch, G. T., Cruvinel, W. M., & Gomes, C. M. (2019). Adipocinas e sua relação com a obesidade. *Revista EVS - Revista de Ciências Ambientais e Saúde*, *46*(1). https://doi.org/10.18224/evs.v46i1.7179.

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society, 71*(2), 332–338. https://doi.org/10.1017/S0029665112000092

De Leo, M., Piragine, E., Pirone, A., Braca, A., Pistelli, L., Calderone, V., … Testai, L. (2020). Protective Effects of Bergamot (Citrus bergamia Risso & Poiteau) Juice in Rats Fed with High-Fat Diet. *Planta Medica, 86*(03), 180–189. https://doi.org/10.1055/a-1070-9325

Dhalla, N. S., Elimban, V., Bartekova, M., & Adameova, A. (2022). Involvement of Oxidative Stress in the Development of Subcellular Defects and Heart Disease. *Biomedicines, 10*(2), 393. https://doi.org/10.3390/biomedicines10020393

Di Donna, L., De Luca, G., Mazzotti, F., Napoli, A., Salerno, R., Taverna, D., & Sindona, G. (2009). Statin-like principles of bergamot fruit (Citrus bergamia): Isolation of 3-hydroxymethylglutaryl flavonoid glycosides. *Journal of Natural Products, 72*(7), 1352–1354. https://doi.org/10.1021/np900096w

Ferlemi, A. V., & Lamari, F. N. (2016). Berry leaves: An alternative source of bioactive natural products of nutritional and medicinal value. *Antioxidants, 5*(2). https://doi.org/10.3390/antiox5020017

Ferrari, F., Bock, P. M., Motta, M. T., & Helal, L. (2019). Biochemical and Molecular Mechanisms of Glucose Uptake Stimulated by Physical Exercise in Insulin Resistance State: Role of Inflammation. *Arquivos Brasileiros de Cardiologia*. https://doi.org/10.5935/abc.20190224

Ferron, A., Francisqueti, F., Minatel, I., Silva, C., Bazan, S., Kitawara, K., … Ferreira, A. (2018). Association between Cardiac Remodeling and Metabolic Alteration in an Experimental Model of Obesity Induced by Western Diet. *Nutrients, 10*(11), 1675. https://doi.org/10.3390/nu10111675

Ferron, A. J. T., Aldini, G., Francisqueti-Ferron, F. V., De Almeida Silva, C. C. V., Bazan, S. G. Z., Garcia, J. L., … Ferreira, A. L. A. (2019). Protective effect of tomato-oleoresin supplementation on oxidative injury recoveries cardiac function by improving β-adrenergic response in a diet-obesity induced model. *Antioxidants, 8*(9). https://doi.org/10.3390/antiox8090368

Formisano, C., Rigano, D., Lopatriello, A., Sirignano, C., Ramaschi, G., Arnoldi, L., … Taglialatela-Scafati, O. (2019). Detailed Phytochemical Characterization of Bergamot Polyphenolic Fraction (BPF) by UPLC-DAD-MS and LC-NMR. *Journal of Agricultural and Food Chemistry, 67*(11), 3159–3167. https://doi.org/10.1021/acs.jafc.8b06591

Francisqueti, F., Minatel, I., Ferron, A., Bazan, S., Silva, V., Garcia, J., … Corrêa, C. (2017). Effect of Gamma-Oryzanol as Therapeutic Agent to Prevent Cardiorenal Metabolic Syndrome in Animals Submitted to High Sugar-Fat Diet. *Nutrients, 9*(12), 1299. https://doi.org/10.3390/nu9121299

Francisqueti, F. V., Chiaverini, L. C. T., Dos Santos, K. C., Minatel, I. O., Ronchi, C. B., Ferron, A. J. T., … Corrêa, C. R. (2017). The role of oxidative stress on the pathophysiology of metabolic syndrome. *Revista Da Associacao Medica Brasileira, 63*(1), 85–91. https://doi.org/10.1590/1806-9282.63.01.85

Giannini, E. G. (2005). Liver enzyme alteration: A guide for clinicians. *Canadian Medical Association Journal, 172*(3), 367–379. https://doi.org/10.1503/cmaj.1040752

Gonc, D. F., Paola, B., Rafacho, M., Assis, B., Jaldin, G., Bruder, T., … Paiva, R. D. (2014). Vitamin D Induces Increased Systolic Arterial Pressure via Vascular Reactivity and Mechanical Properties. *PLoS One1, 9*(6), 1–9. https://doi.org/10.1371/journal.pone.0098895

Gutiérrez-Cuevas, J., Sandoval-Rodriguez, A., Meza-Rios, A., Monroy-Ramírez, H. C., Galicia-Moreno, M., García-Bañuelos, J., … Armendariz-Borunda, J. (2021). Molecular Mechanisms of Obesity-Linked Cardiac Dysfunction. *An Up-Date on Current Knowledge. Cells, 10*(3). https://doi.org/10.3390/cells10030629

Ighodaro, O. M. (2018). Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomedicine & Pharmacotherapy, 108*, 656–662. https://doi.org/10.1016/j.biopha.2018.09.058

Jayarathne, S., Koboziev, I., Park, O. H., Oldewage-Theron, W., Shen, C. L., & Moustaid-Moussa, N. (2017). Anti-Inflammatory and Anti-Obesity Properties of Food Bioactive Components: Effects on Adipose Tissue. *Preventive Nutrition and Food Science, 22*(4), 251–262. https://doi.org/10.3746/pnf.2017.22.4.251

Jung, U., & Choi, M.-S. (2014). Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences, 15*(4), 6184–6223. https://doi.org/10.3390/ijms15046184

Kalousová, M., Skrha, J., & Zima, T. (2002). Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. Retrieved from *Physiological Research, 51*(6), 597–604 http://www.ncbi.nlm.nih.gov/pubmed/12511184.

Kim, H.-J., Oh, G. T., Park, Y. B., Lee, M.-K., Seo, H.-J., & Choi, M.-S. (2004). Naringin alters the cholesterol biosynthesis and antioxidant enzyme activities in LDL receptor-knockout mice under cholesterol fed condition. *Life Sciences, 74*(13), 1621–1634. https://doi.org/10.1016/j.lfs.2003.08.026

La Russa, D., Giordano, F., Marrone, A., Parafati, M., Janda, E., & Pellegrino, D. (2019). Oxidative Imbalance and Kidney Damage in Cafeteria Diet-Induced Rat Model of Metabolic Syndrome: Effect of Bergamot Polyphenolic Fraction. *Antioxidants (Basel, Switzerland), 8*(3). https://doi.org/10.3390/antiox8030066.

Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., & Feng, Y. (2015). The Role of Oxidative Stress and Antioxidants in Liver Diseases. *International Journal of Molecular Sciences, 16*(11), 26087–26124. https://doi.org/10.3390/ijms161125942

Lo, A. T. C., Francisqueti, F. V., Hasimoto, F. K., Ferraz, A. P. C. R., Minatel, I. O., Garcia, J. L., … Corrêa, C. R. (2018). Brazilian Curcuma longa L. attenuates comorbidities by modulating adipose tissue dysfunction in obese rats. *Nutrire, 43*(1), 1–8. https://doi.org/10.1186/s41110-018-0085-y

Lobstein, T., Brinsden, H., Neveux, M., Cavalcanti, O. B., Barquera, S., Baur, L., … Wilding, J. (2022). *World Obesity Atlas 2022. World Obesity Federation 2022*. London: World Obesity Federation 2022.

Longo, M., Zatterale, F., Naderi, J., Parrillo, L., Formisano, P., Raciti, G. A., … Miele, C. (2019). Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *International Journal of Molecular Sciences, 20*(9), 2358. https://doi.org/10.3390/ijms20092358

Luvizotto, R. D. A. M., Nascimento, A. F., Imaizumi, E., Pierine, D. T., Conde, S. J., Correa, C. R., … Ferreira, A. L. a. (2013). Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. *The British Journal of Nutrition*, *110*(10), 1803–1809. https://doi.org/10.1017/S0007114513001256.

Manna, P., & Jain, S. K. (2015). Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metabolic Syndrome and Related Disorders, 13*(10), 423–444. https://doi.org/10.1089/met.2015.0095

Marklund, S. L. (1985). Product of extracellular-superoxide dismutase catalysis. *FEBS Letters, 184*(2), 237–239. https://doi.org/10.1016/0014-5793(85)80613-X

Martı, L., Felix-soriano, E., & Escote, X. (2018). Inflammation and Oxidative Stress in Adipose Tissue : Nutritional Regulation. In *Obesity* (pp. 64–92). https://doi.org/10.1016/B978-0-12-812504-5.00004-0.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, T. R., Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7), 412–419. https://doi.org/10.1007/BF00280883.

Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Analytical Biochemistry, 458*, 69–71. https://doi.org/10.1016/j.ab.2014.04.034

Miceli, N., Mondello, M. R., Monforte, M. T., Sdrafkakis, V., Dugo, P., Crupi, M. L., … Trovato, A. (2007). Hypolipidemic effects of Citrus bergamia Risso et Poiteau juice in rats fed a hypercholesterolemia diet. *Journal of Agricultural and Food Chemistry, 55*(26), 10671–10677. https://doi.org/10.1021/jf071772i

Mirarchi, A., Mare, R., Musolino, V., Nucera, S., Mollace, V., Pujia, A., … Maurotti, S. (2022). Bergamot Polyphenol Extract Reduces Hepatocyte Neutral Fat by Increasing Beta-Oxidation. *Nutrients, 14*(16), 3434. https://doi.org/10.3390/nu14163434

Mollace, V., Sacco, I., Janda, E., Malara, C., Ventrice, D., Colica, C., … Romeo, F. (2011). Hypolipemic and hypoglycaemic activity of bergamot polyphenols: From animal models to human studies. *Fitoterapia, 82*(3), 309–316. https://doi.org/10.1016/j.fitote.2010.10.014

Moufida, S., & Marzouk, B. (2003). Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange. *Phytochemistry, 62*(8), 1283–1289. https://doi.org/10.1016/S0031-9422(02)00631-3

Musolino, V., Gliozzi, M., Scarano, F., Bosco, F., Scicchitano, M., Nucera, S., … Sanyal, A. J. (2020). Bergamot Polyphenols Improve Dyslipidemia and Pathophysiological Features in a Mouse Model of Non-Alcoholic Fatty Liver Disease. *Scientific Reports, 10*(1), 1–14. https://doi.org/10.1038/s41598-020-59485-3

Nagueh, S. F., Appleton, C. P., Gillebert, T. C., Marino, P. N., Oh, J. K., Smiseth, O. A., … Evangelista, A. (2009). Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography. *Journal of the American Society of Echocardiography, 22*(2), 107–133. https://doi.org/10.1016/j.echo.2008.11.023

Nakandakare-Maia, E. T., Siqueira, J. S., Ferron, A. J. T., Vieira, T. A., Palacio, T. L. N., Grandini, N. A., … Corrêa, C. R. (2023). Treatment with bergamot (Citrus bergamia) leaves extract attenuates leptin resistance in obese rats. *Molecular and Cellular Endocrinology, 111908*. https://doi.org/10.1016/j.mce.2023.111908

Noce, A., Di Lauro, M., Di Daniele, F., Pietroboni Zaitseva, A., Marrone, G., Borboni, P., & Di Daniele, N. (2021). Natural Bioactive Compounds Useful in Clinical Management of Metabolic Syndrome. *Nutrients, 13*(2), 630. https://doi.org/10.3390/nu13020630

Organization, W. H. (Ed.). (2023). *WHO acceleration plan to stop obesity*.

Palacio, T. L. N., Siqueira, J. S., de Paula, B. H., Rego, R. M. P., Vieira, T. A., Baron, G., … Correa, C. R. (2022). Bergamot (Citrus bergamia) leaf extract improves metabolic, antioxidant and anti-inflammatory activity in skeletal muscles in a metabolic syndrome experimental model. *International Journal of Food Sciences and Nutrition, 1–8*. https://doi.org/10.1080/09637486.2022.2154328

Pilling, D., Karhadkar, T. R., & Gomer, R. H. (2021). High-Fat Diet-Induced Adipose Tissue and Liver Inflammation and Steatosis in Mice Are Reduced by Inhibiting Sialidases. *The American Journal of Pathology, 191*(1), 131–143. https://doi.org/10.1016/j.ajpath.2020.09.011

Popkin, B. M., Adair, Li. S., & Ng, S. W. (2012). NOW AND THEN: The Global Nutrition Transition: The Pandemic of Obesity in Developing Countries. *Nutrition Reviews*, *70*(1), 3–21. https://doi.org/10.1111/j.1753-4887.2011.00456.x.

Priest, C., & Tontonoz, P. (2019). Inter-organ cross-talk in metabolic syndrome. *Nature Metabolism, 1*(12), 1177–1188. https://doi.org/10.1038/s42255-019-0145-5

Reyes-Farias, M., Fos-Domenech, J., Serra, D., Herrero, L., & Sánchez-Infantes, D. (2021). White adipose tissue dysfunction in obesity and aging. *Biochemical Pharmacology, 192*, Article 114723. https://doi.org/10.1016/j.bcp.2021.114723

Risitano, R., Currò, M., Cirmi, S., Ferlazzo, N., Campiglia, P., Caccamo, D., … Navarra, M. (2014). Flavonoid fraction of bergamot juice reduces LPS-induced inflammatory response through SIRT1-mediated NF-κB inhibition in THP-1 monocytes. *PLoS One1, 9*(9), 1–9. https://doi.org/10.1371/journal.pone.0107431

Rodríguez-Correa, E., González-Pérez, I., Clavel-Pérez, P. I., Contreras-Vargas, Y., & Carvajal, K. (2020). Biochemical and nutritional overview of diet-induced metabolic syndrome models in rats: What is the best choice? *Nutrition and Diabetes, 10*(1). https://doi.org/10.1038/s41387-020-0127-4

SIES, H. (1985). Oxidative Stress: Introductory Remarks. In *Oxidative Stress* (pp. 1–8). Elsevier. https://doi.org/10.1016/B978-0-12-642760-8.50005-3.

da Silva Junior, G. B., Bentes, A. C. S. N., Daher, E. D. F., & de Matos, S. M. A. (2017). Obesity and kidney disease. *Jornal Brasileiro de. Nefrología, 39*(1). https://doi.org/10.5935/0101-2800.20170011

Siqueira, J. S., Vieira, T. A., Nakandakare-Maia, E. T., Palacio, T. L. N., Sarzi, F., Garcia, J. L., … Correa, C. R. (2022). Bergamot leaf extract treats cardiorenal metabolic syndrome and associated pathophysiological factors in rats fed with a high sugar fat diet. *Molecular and Cellular Endocrinology, 556*, Article 111721. https://doi.org/10.1016/j.mce.2022.111721

Tang, C., Cai, J., & Dong, Z. (2016). Mitochondrial dysfunction in obesity-related kidney disease: A novel therapeutic target. *Kidney International, 90*(5), 930–933. https://doi.org/10.1016/j.kint.2016.07.045

Trombetta, D., Cimino, F., Cristani, M., Mandalari, G., Saija, A., Ginestra, G., … Faulds, C. B. (2010). In Vitro Protective Effects of Two Extracts from Bergamot Peels on Human Endothelial Cells Exposed to Tumor Necrosis Factor-α (TNF-α). *Journal of Agricultural and Food Chemistry, 58*(14), 8430–8436. https://doi.org/10.1021/jf1008605

Tsutsui, H., Kinugawa, S., & Matsushima, S. (2011). Oxidative stress and heart failure. *American Journal of Physiology - Heart and Circulatory Physiology, 301*(6), 2181–2190. https://doi.org/10.1152/ajpheart.00554.2011

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry, 86*(1), 271–278. https://doi.org/10.1016/0003-2697(78)90342-1

Vargas, F., Romecín, P., García-Guillén, A. I., Wangesteen, R., Vargas-Tendero, P., Paredes, M. D., … García-Estañ, J. (2018). Flavonoids in Kidney Health and Disease. *Frontiers in Physiology, 9*. https://doi.org/10.3389/fphys.2018.00394

Wensley, I., Salaveria, K., Bulmer, A. C., Donner, D. G., & du Toit, E. F. (2013). Myocardial structure, function and ischaemic tolerance in a rodent model of obesity with insulin resistance. *Experimental Physiology, 98*(11), 1552–1564. https://doi.org/10.1113/expphysiol.2013.074948

Zhao, Q., Li, L., Zhu, Y., Hou, D., Li, Y., Guo, X., … Gong, K. (2020). Kukoamine B Ameliorate Insulin Resistance, Oxidative Stress, Inflammation and Other Metabolic Abnormalities in High-Fat/High-Fructose-Fed Rats. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 13*, 1843–1853. https://doi.org/10.2147/DMSO.S247844