Original article

*Vaccinium virgatum* fruit extract as an important adjuvant in biochemical and behavioral alterations observed in animal model of metabolic syndrome

Pathise Souto Oliveira\(^a\), Marta Gazal\(^a\), Natália Porto Flores\(^a\), Aline Rigon Zimmer\(^b\), Vitor Clasen Chaves\(^c\), Flávio Henrique Reginatto\(^c\), Manuella Pinto Kaster\(^d\), Rejane Giacomelli Tavares\(^a\), Roselia Maria Spanevello\(^e\), Claiton Leoneti Lencina\(^a,\)**, Francieli Moro Stefanello\(^a,\)**

\(^a\)*Laboratório de Biomarcadores, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário s/n, Pelotas, RS, Brazil
\(^b\)Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
\(^c\)Programa de Pós-Graduação em Farmácia, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
\(^d\)Departamento de Bioquímica, Universidade Federal de Santa Catarina, Campus Universitário, Córrego Grande, 88040900, Florianópolis, SC, Brazil
\(^e\)*Laboratório de Neuroquímica, Inflamação e Câncer, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Campus Universitário s/n, Pelotas, RS, Brazil

**ARTICLE INFO**

Article history:
Received 5 December 2016
Accepted revised form 13 January 2017
Accepted 20 January 2017

Keywords:
Metabolic syndrome
Blueberry
Phenolic compounds
Metabolic parameters
Neurochemical parameters

**ABSTRACT**

The aim of this study was to investigate the effect of blueberry (*Vaccinium virgatum*) fruit extract on metabolic, behavioral and oxidative stress parameters in the hippocampus and cerebral cortex of mice submitted to an experimental model of metabolic syndrome induced by a highly palatable diet (HPD). Mice C57BL/6 were divided into 4 experimental groups: (1) received standard chow and saline orally, (2) received standard chow and blueberry hydroalcoholic extract, (3) received HPD and saline orally, (4) received HPD and blueberry hydroalcoholic extract. The animals were treated for 150 days. Our results showed that the animals fed with HPD presented insulin resistance, increased body weight, visceral fat, glucose, triglycerides, and total cholesterol when compared to the control group. The blueberry extract prevented the increase of these metabolic parameters. Also, the extract was able to reduce the levels of thiobarbituric acid reactive substances in the cerebral cortex and hippocampus of animals submitted to HPD. In contrast, no differences were observed in the total thiol content, activity of the antioxidant enzymes catalase and superoxide dismutase. In addition, the HPD fed animals showed a significant increase in immobility time in the forced swimming test and blueberry prevented this alteration, although no changes were observed in the ambulatory behavior, as well as in the anxiolytic profile of these animals. Overall, our findings suggest that chronic consumption of blueberry extract exhibits hypoglycemic, hypolipidemic, antidepressant-like and antiperoxidative effects in an animal model of metabolic syndrome.

© 2017 Published by Elsevier Masson SAS.

1. Introduction

Obesity is a chronic pathological condition characterized by the accumulation of adipose tissue associated with an increased risk of multiple morbidities and mortality [1–3]. Further, obesity is causally linked to metabolic syndrome (MetS), a cluster of factors including insulin resistance, increased abdominal fat, dyslipidemia and hypertension [4]. MetS is also characterized by low high-density lipoprotein (HDL) cholesterol in association with elevated triglyceride levels that commonly precedes the development of...
type 2 diabetes and heart disease [4,5]. Furthermore, the increase in plasma free fatty acids (FFA) concentrations in normal subjects to levels comparable to those observed in obese individuals also induces oxidative stress, inflammation and subnormal vascular reactivity [6].

Abnormally high levels of free radicals and the loss of antioxidant defense mechanisms lead to damage to the cellular organelles and enzymes, increased lipid peroxidation, DNA damage, and protein derivatives, the development of insulin resistance, depression and neurobehavioral and cognitive deficit [2,7–10]. Epidemiological studies reveal that bioactive compounds produced by the secondary metabolism of plant foods such as anthocyanins, flavonoids and other phenolic compounds show multiple biological functions including antioxidant, anti-inflammatory, anti-hypertensive, anti-hyperlipidemic and hypoglycemic activities [11–14]. Blueberries (Vaccinium sp) are known to contain high levels of anthocyanins, flavonoids and phenolic compounds [15–17]. Extracts of roots, stems, leaves and blueberry fruits contain several active ingredients that may reduce complications of diabetes and obesity [18–20]. In addition, these extracts have protected against damage from oxidative stress in different pathologic conditions, such as cardiovascular disorders, advancing age-induced oxidative stress inflammatory responses, degenerative diseases, and triggered genetic signaling to promote human health and prevent disease [21–24].

Some authors demonstrated that animals supplemented with blueberry fruit have decreased weight gain and food intake, improved cognitive and motor performance and protection against short-term memory decline [25–27]. Moreover, blueberry supplementation prevented impairment in neurochemistry, synaptic transmission and behavior in rodent models of brain aging [28,29].

In animals and humans, symptomatic features of MetS are linked to the consumption of high-carbohydrate diets, inducing inflammation, oxidative stress and cognitive disorders. In this respect, we assessed the effects of blueberry extracts on the lipid and glycemic profile, oxidative stress and behavioral parameters in mice fed with a highly palatable diet.

2. Materials and methods

2.1. Chemicals and procedures

All reagents were purchased from Sigma Aldrich® Co. (St. Louis, MO, USA). All solvents were purchased from Vetec® AG (Rio de Janeiro, Brazil). Acetonitrile (HPLC grade) was provided by Tedia® (Brazil). Water was purified on a MilliQ system (Millipore®, Bedford, USA).

2.2. Extraction

Vaccinium virgatum fruits were harvested in the orchard at the Federal University of Pelotas [31 ’48°12.48”S and 52°30’34.08”W]. The extracts were prepared according to Bordignon et al. [30], with modifications. Briefly, unprocessed freeze-dried rabbiteye blueberry fruits (30 g) were sonicated for 30 min at 25 °C in 90 mL 70:30 v/v ethanol–water. The pH solution was adjusted to 1.0. The crude extracts were filtered; the ethanol evaporated under reduced pressure and the remaining aqueous solution was lyophilized yielding the test samples. These procedures were performed in triplicate and sheltered from light.

2.3. Total flavonoid content

The total flavonoid content in samples was determined according to Millauskas et al. [31] with minor modifications. The calibration curve was prepared by mixing aliquots of 1 mL of an ethanolic solution of rutin at concentrations of 100, 150, 200, 300, 450 and 500 μg/mL with 1 mL of an ethanolic solution of 20 g/L aluminum chloride and diluted to 25 mL of ethanol. The absorptions were measured at 415 nm after 40 min. These readings were used to draw the calibration curve. The absorption of each blueberry sample was measured under the same conditions. Data are mean ± SD values expressed as milligrams of rutin per 1 g of dried extract. All analyses were performed in triplicate.

2.4. Total phenolic content

The total phenolic content was determined according to Singleton et al. [32] with slight modifications. The calibration curve was first prepared by mixing 125 mL aliquots of an ethanolic solution of gallic acid at concentrations of 100, 150, 200, 300, 450 and 500 μg/mL, 500 μL of distilled water and 125 μL of Folin–Ciocalteau reagent. After 3 min 1.25 mL of a 7% solution of sodium carbonate was added and 1 mL of distilled water making a final volume of 3 mL. The readings were performed after 90 min on a wavelength of 760 nm. Data are mean ± SD values expressed as milligrams of gallic acid per 1 g of dried extract.

2.5. Total anthocyanin content

Anthocyanins were quantified by the pH differential method [33,34]. Calculation of the anthocyanin concentration was based on cyanidin-3-glucoside in a molar extinction coefficient of 26,900 and molecular weight of 449.2 g/mol. Data are mean ± SD values expressed as milligrams of cyanidin-3-glucoside per 1 g of dried extract. All analyses were performed in triplicate.

2.6. LC/MS

Anthocyanin profile analysis was performed by liquid chromatography (UPLC, Waters Acquity mode) coupled to high resolution mass spectrometry (Xevo G2 QToF model), equipped with electrospray ionization source and controlled by MassLynx v.4.1 software. Separation was performed on a C18 column (100 × 2.1 mm – 1.7 μm – Kinetix(Phenomenex) at 40 °C. The mobile phase consisted of a linear gradient of 0.1% formic acid (A) and acetonitrile (B) using the range of 5% to 58% B over 8 min at a flow rate of 0.5 mL/min. A mass spectrum was obtained in positive mode, with a mass range of m/z of 100–1000. The capillary voltage used was 2.0 kV and the cone voltage 50 V. Nebulization and desorption were performed with nitrogen at 300 and flow 10 L/h⁻¹. Desorption and source temperature were respectively 300 °C and 120 °C.

2.7. Animals and drug treatments

Male C57Bl/6 mice, aged 21 days old, were maintained at 21–25 °C with free access to water and food, under a standard dark–light cycle (lights on between 7:00 a.m. and 7:00 p.m.). Animal care followed the official governmental guidelines in compliance with the Federation of Brazilian Societies for Experimental Biology and was approved by the Committee of Ethics and Animal Experimentation of the Federal University of Pelotas, Brazil (CEEA n° 10265).

Mice were divided into four groups: (1) normal diet group (ND) + vehicle, which received standard laboratory rat chow (50% carbohydrate; from starch, 22% protein and 4% fat) and saline orally; (2) ND + blueberry, which received standard laboratory rat chow (50% carbohydrate; from starch, 22% protein and 4% fat) and 200 mg/kg/day of blueberry orally; (3) highly palatable diet group (HPD)+vehicle, which received an enriched sucrose diet (65%
carbohydrates being 34% from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% of fat) and saline orally (4) HPD + blueberry, which received an enriched sucrose diet (65% carbohydrates being 34% from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% of fat) and 200 mg/kg/day of blueberry orally. All animals had free access to food and water. The HPD protocol was performed according to the method described by Souza et al. [35]. The dose of blueberry extract used in this study was chosen according to Leibowitz et al. [36].

2.8. Body weight gain and food intake

Changes in body weight and food intake patterns of mice were measured throughout the experimental period. The weight of each mouse was recorded on day 0 and at weekly intervals throughout the course of the study. The quantity of food consumed by each group was recorded weekly, and the food consumption per mouse was calculated for all groups.

2.9. Sample collection and biochemical assay

After 150 days of food and extract co-administration and 24 h after the last behavioral test, the animals of all groups were sacrificed by decapitation after 6 h of fasting. At sacrifice, visceral fat was weighed and the blood collected. Serum was obtained by centrifugation at 4000 rpm (4 °C) for 15 min. Cerebral cortex and hippocampus were collected and stored at −80 °C for subsequent biochemical analyses.

2.10. Glucose tolerance test

Mice were injected intraperitoneally with a 50% glucose solution load of 2 mg/g of body weight. The glucose levels for all the groups were estimated by the glucometer (AccuChek Active, Roche Diagnostics®, USA) at 30, 60, and 120 min after the injection by a small tail puncture.

2.11. Biochemical parameters

Measurements of serum glucose, total cholesterol and triglyceride levels were determined using commercially available diagnostic kits supplied by Labtest® (Labtest, MG, Brazil).

2.12. Behavioral analysis

All behavioral experiments were conducted, after 150 days of treatment, between 9 a.m. and 6 p.m under low-intensity light. All apparatuses were cleaned with an ethanol solution and then dried paper after each trial.

2.12.1. Forced swim test (FST)

The depressive-like behavior was evaluated by the total duration of immobility in the forced swimming test, as previously described Kaster et al. [37]. Briefly, in the FST, mice were individually forced to swim in an open cylindrical container (diameter, 10 cm; height, 25 cm), with water at 25 ± 1 °C and the total duration of immobility during a 6 min period was scored: mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their head above water.

2.12.2. Open-field test

The ambulatory behavior was evaluated in an open-field test as previously described by Kaster et al. [38]. The apparatus consisted of a box measuring 40 × 60 × 50 cm with the floor of the arena divided into 12 equal squares and placed in a sound-free room. Animals were placed in the rear left square and left to freely explore it for 8 min during which time the number of squares crossed with all paws (crossing) was counted.

2.12.3. Elevated plus-maze

The anxiety-related behavior was evaluated using the elevated-plus-maze [39]. The maze consists of a cross, made of two closed arms and two open arms (each 31 cm long, 5 cm wide and 15 cm tall), set up 40 cm above the floor. Mice were placed at the center of the cross and left to explore the apparatus during a total of 5 min. One entry was considered when the mice placed the four paws inside an arm. We recorded the time spent in the open and closed arms, as a measure of anxiety.

2.13. Oxidative stress parameters

2.13.1. Tissue preparation

Cerebral cortex and hippocampus were homogenized in sodium phosphate buffer pH 7.4 containing KCl (1:10, w/v). The homogenates were centrifuged at 3500 rpm for 10 min at 4 °C. The supernatant was immediately separated and used for biochemical determinations.

2.13.2. Thiobarbituric acid-reactive substances (TBARS)

TBARS, a measure of lipid peroxidation, was determined according to the method described by Okhawa et al. [40]. Briefly, tissue supernatant was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBARS were determined by the absorbance at 535 nm and reported as nmol TBARS/mg protein.

2.13.3. Total thiol content assay

Total thiol content was determined using the DTNB (5,5'-dithiobis-2-nitrobenzoic acid) method as described by Aksenov and Markesbery [41] with some modifications. Briefly, 50 mL of the sample were mixed with 980 mL of PBS, pH 7.5, containing 1 mM EDTA. The reaction was started by the addition of 30 mL of 10 mM DTNB. The amount of TNB formed was determined at 412 nm. The results were reported as nmol of TNB/mg protein.

2.13.4. Catalase (CAT) assay

CAT activity was assayed according to Aebi [42] based on the decomposition of H2O2 monitored at 240 nm. The specific activity is reported as units/mg protein.

2.13.5. Superoxide dismutase (SOD) assay

SOD activity was measured by the method described by Misra and Fridovich [43]. This method is based on the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer adjusted at 480 nm. The specific activity of SOD was reported as units/mg protein.

2.13.6. Protein determination

Protein was determined by the method of Lowry [44] using bovine serum albumin as standard.

2.14. Statistical analysis

The values are expressed as mean ± S.E.M. Glucose tolerance was analyzed by Repeated Measures analyses of variance (ANOVA) and Bonferroni’s post-hoc test. Parametric variables were tested by Two-way ANOVA and Bonferroni’s post-hoc test. A value of P < 0.05 was considered to be significant. Analyses were performed using the GraphPad PRISM 5® software.
3. Results

3.1. Phytochemical characterization

Total anthocyanin content in blueberry was 13.03 ± 0.44 mg/g of dried extract. As to the total flavonoid content, 156.52 ± 4.86 mg/g of dried extract was detected. In addition, the total phenolic content was 302.70 ± 8.13 mg/g of dried extract [45].

3.2. Anthocyanin identification

Anthocyanin identification was based on fragmentation pattern and exact mass (Table 1). Five aglycone fragments, cyanidin (m/z 287.0552), peonidin (m/z 301.0711), delphinidin (m/z 303.0506), petunidin (m/z 317.0668) and malvidin (m/z 331.0814) were identified. Aglycone fragmentation pattern and exact mass allowed the identification of nine monoglucosilated anthocyanins [45].

3.3. Metabolic parameters

Table 2 shows that there was no significant difference in the initial weight of the animals of all groups (blueberry treatment: [F(1,35) = 3.52, P = 0.07], diet: [F(1,35) = 1.32, P = 0.26], interaction: [F(1,35) = 4.20, P < 0.05]). However, treatment with blueberry (200 mg/kg, p.o.) reduced the weight gain of the animals fed with the ND, as well as HPD (blueberry treatment: [F(1,35) = 49.14, P < 0.001], diet: [F(1,35) = 31.90, P < 0.001], interaction: [F(1,35) = 4.86, P < 0.05]) and reduced visceral fat (blueberry treatment: [F(1,28) = 34.28, P < 0.001], diet: [F(1,28) = 316.01, P < 0.001], interaction: [F(1,28) = 9.56, P < 0.05]).

Additionally, blueberry extract treatment prevented the increase of serum glucose levels (blueberry treatment: [F(1,36) = 53.61, P < 0.001], HPD: [F(1,36) = 52.02, P < 0.001], interaction: [F(1,36) = 36.57, P < 0.001]), triglycerides (blueberry treatment: [F(1,11) = 7.0, P < 0.05], HPD: [F(1,11) = 6.89, P < 0.05], interaction: [F(1,11) = 38.87, P < 0.001]) and total cholesterol (blueberry treatment: [F(1,14) = 1.09, P = 0.31], HPD [F(1,14) = 56.46, P < 0.001], interaction: [F(1,14) = 8.69, P < 0.05]) caused by HPD.

To access the insulin resistance profile, we performed the glucose tolerance test. As can be seen in Fig. 1, the blueberry treatment prevented the impairment of glucose tolerance induced by HPD (P < 0.05).

3.4. The depressant-like effect of the highly palatable diet in the FST is prevented by chronic consumption of blueberry extract

As shown in Fig. 2A, mice submitted to HPD exhibited a significant increase in immobility time in the FST as compared to control animals, indicative of a depressive-like state. Chronic treatment with blueberry extract prevented the increase in immobility time (blueberry treatment: [F(1,35) = 5.19, P < 0.05], HPD: [F(1,35) = 7.04, P < 0.05], interaction: [F(1,35) = 5.05, P < 0.05]).

In order to rule out nonspecific motor effects that could influence activity in the FST, mice were also submitted to the open-field test (Fig. 2B). No significant alteration in the ambulatory behavior was observed, suggesting that the effect of blueberry consumption on the FST is not related to changes in the locomotor activity (blueberry treatment: [F(1,35) = 2.99, P = 0.09], HPD: [F(1,35) = 0.22, P = 0.64], interaction: [F(1,35) = 1.05, P = 0.31]).

3.5. Anxiogenic profile of mice submitted to highly palatable diet and chronic blueberry consumption in the elevated plus-maze

No differences were found in the anxiogenic profile (time spent in the open arm of the elevated plus maze); see Fig. 3A (blueberry treatment: [F(1,34) = 0.65, P = 0.43], HPD: [F(1,34) = 0.03, P = 0.87], interaction: [F(1,34) = 0.06, P = 0.81]), or number of open arm entries; (Fig. 3B) of mice submitted to the HPD and/or blueberry consumption (blueberry treatment: [F(1,34) = 0.80, P = 0.38], HPD: [F(1,34) = 0.17, P = 0.68], interaction: [F(1,34) = 0.48, P = 0.49]). The absence of ambulatory behavior alterations was further confirmed in the plus-maze apparatus since no change was found in the total number of entries between groups (Fig. 3C) (blueberry treatment: [F(1,34) = 0.30, P = 0.58], HPD: [F(1,34) = 1.07, P = 0.30], interaction: [F(1,34) = 0.34, P = 0.56]).

Table 1

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>[M]+ (Molecular formula, ppm)</th>
<th>Fragment ion [M – X]+ (Molecular formula, ppm)</th>
<th>Tentative identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.84</td>
<td>449.1074 (C23H20O11, -2.20)</td>
<td>287.0552 [M – hexosyl group]+ (C12H30O6, -1.0)</td>
<td>Cyanidin-3-O-hexoside</td>
</tr>
<tr>
<td>1.86</td>
<td>449.1075 (C23H20O11, -2.00)</td>
<td>287.0552 [M – hexosyl group]+ (C12H30O6, -1.0)</td>
<td>Cyanidin-3-O-hexoside</td>
</tr>
<tr>
<td>2.03</td>
<td>419.0969 (C20H14O9, -2.10)</td>
<td>287.0552 [M – pentosyl group]+ (C12H30O6, -1.0)</td>
<td>Cyanidin-3-O-pentoside</td>
</tr>
<tr>
<td>2.13</td>
<td>463.1230 (C22H20O11, -2.20)</td>
<td>301.0711 [M – hexosyl group]+ (C12H30O6, -1.3)</td>
<td>Peonidin-3-O-hexoside</td>
</tr>
<tr>
<td>2.40</td>
<td>463.1231 (C22H20O11, -1.90)</td>
<td>301.0711 [M – hexosyl group]+ (C12H30O6, -1.3)</td>
<td>Peonidin-3-O-hexoside</td>
</tr>
<tr>
<td>1.62</td>
<td>465.1028 (C23H20O12, -1.10)</td>
<td>303.0505 [M – hexosyl group]+ (C12H30O6, 0.0)</td>
<td>Delphinidin-3-O-hexoside</td>
</tr>
<tr>
<td>1.83</td>
<td>435.0922 (C22H20O11, -1.10)</td>
<td>303.0505 [M – pentosyl group]+ (C12H30O6, 0.0)</td>
<td>Delphinidin-3-O-pentoside</td>
</tr>
<tr>
<td>1.96</td>
<td>479.1180 (C22H20O12, -2.10)</td>
<td>317.0668 [M – hexosyl group]+ (C12H30O6, 0.3)</td>
<td>Petunidin-3-O-hexoside</td>
</tr>
<tr>
<td>2.18</td>
<td>493.1338 (C22H20O12, -1.00)</td>
<td>331.0814 [M – hexosyl group]+ (C12H30O6, -1.5)</td>
<td>Malvidin-3-O-hexoside</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>ND/Vehicle</th>
<th>ND/Blueberry</th>
<th>HPD/Vehicle</th>
<th>HPD/Blueberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>16.00 ± 1.13</td>
<td>20.80 ± 0.50</td>
<td>17.30 ± 1.43</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>29.80 ± 0.97</td>
<td>27.40 ± 0.50*</td>
<td>36.22 ± 0.62***</td>
</tr>
<tr>
<td>Viseral fat mass (g)</td>
<td>0.54 ± 0.16</td>
<td>0.40 ± 0.01</td>
<td>1.92 ± 0.21***</td>
</tr>
<tr>
<td>Glycmia (mg/dL)</td>
<td>82.82 ± 3.98</td>
<td>76.62 ± 3.55</td>
<td>147.3 ± 6.20***</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>68.01 ± 7.48</td>
<td>79.63 ± 4.91</td>
<td>132.0 ± 4.14*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>86.18 ± 1.74</td>
<td>97.08 ± 3.32</td>
<td>113.2 ± 3.46***</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n = 5-10). (*** Denotes P < 0.001 as compared to the ND/Vehicle control group. (*) Denotes P < 0.05 as compared to the ND/Vehicle group. (†) Denotes P < 0.01 as compared to H/D/Vehicle group.)
3.6. Measurement of oxidative stress parameters in the cerebral cortex and hippocampus

Fig. 4A shows that blueberry treatment was able to prevent the increase in TBARS levels induced by HPD in the cerebral cortex (blueberry treatment: \( F(1,35) = 19.63, P < 0.001 \), HPD: \( F(1,35) = 1.39, P = 0.24 \), interaction: \( F(1,35) = 12.15, P < 0.01 \)). The sulfhydryl content (Fig. 4B) and the activity of the antioxidant enzymes CAT (Fig. 4C) and SOD (Fig. 4D) were not different in the HPD or in the group treated with blueberry extract.

We also evaluated the effects of blueberry on oxidative stress parameters in the hippocampus. Likewise, blueberry treatment (200 mg/kg, p.o.) was able to prevent the increase in TBARS levels induced by HPD (Fig. 5A) (HPD: \( F(1,15) = 1.80, P < 0.05 \), blueberry treatment: \( F(1,15) = 5.40, P < 0.05 \), interaction: \( F(1,15) = 8.60, P < 0.01 \)), without interfering in the sulfhydryl content (Fig. 5B) (blueberry treatment: \( F(1,15) = 0.34, P = 0.57 \), HPD: \( F(1,15) = 4.43, P < 0.05 \), interaction: \( F(1,15) = 1.76, P = 0.20 \)), CAT activity (Fig. 5C) (blueberry treatment: \( F(1,14) = 2.22, P = 0.16 \), HPD: \( F(1,14) = 0.22, P = 0.64 \), interaction: \( F(1,14) = 8.60, P = 0.08 \)) or SOD activity (Fig. 5D) (blueberry treatment: \( F(1,11) = 7.20, P = 0.21 \), HPD: \( F(1,11) = 0.73, P = 0.41 \), interaction: \( F(1,11) = 2.98, P = 0.11 \)).

Fig. 2. Effect of chronic blueberry consumption on the highly palatable diet (HPD) in immobility time in the FST (A) and ambulatory behavior in the open-field test (B). The results are expressed as mean ± S.E.M. (n = 9–10 for group). *P < 0.05 when compared to control group (vehicle/ND). #P < 0.05 when compared to vehicle/HPD group. Two-way ANOVA followed by Bonferroni post-hoc test.
4. Discussion

The present study investigated the ability of blueberry extract to improve some biochemical and behavioral parameters related to the pathogenesis of the MetS, in particular, blood lipid and glycemic profile, oxidative stress and depressive-like behavior.

HPD-induced changes in biochemical parameters (insulin resistance, glucose, cholesterol and triglycerides) and lipid peroxidation (TBARS) were significantly prevented by the treatment with blueberry extract. However, no significant effect was observed on the total thiol content, SOD or CAT activity after treatment with blueberry extract.

It must be noted that recent studies have not confirmed this biochemical effect on humans. Basu et al. [46] demonstrated that the consumption of freeze-dried blueberry beverage daily for 8 weeks in a randomized controlled trial decreased the systolic and diastolic blood pressures, without affecting serum levels of glucose and lipid profiles.

The metabolic effects observed in this study on blueberries may be associated with the great concentration of anthocyanins. Li et al. [47] demonstrate that anthocyanin supplementation exerts beneficial metabolic effects on subjects with type 2 diabetes by improving dyslipidemia, enhancing antioxidant capacity, and preventing insulin resistance, corroborating our results. Another
interventional study with hyperlipidemic adults receiving anthocyanins (fruit extract) or placebo capsules twice a day for 4 weeks demonstrated that the fruit extract significantly reduced total cholesterol [48]. Anthocyanins, such as cyanidin-3-O-glucoside, in vitro regulate the expression and activity of key enzymes involved in lipid metabolism including LPL, fatty acid synthase and ABCA1. These findings might explain the observed effects of blueberry consumption on the lipid profile [49]. Phenolic acids and flavonoids are also important phytochemical classes in MetS treatment. The antiobesity, hypolipidemic properties of phenolic acids such as gallic acid, and their ability to reduce oxidative stress in animal models have been shown [50,51]. Moreover, coumaric acid can reduce weight as well as serum cholesterol, triacylglycerol, insulin and leptin [51,52]. In turn, flavonoids, such as quercetin, have also potential antiobesity effects through inhibition of preadipocyte differentiation and induction of apoptosis of mature adipocytes [52,53]. There are also reports that rutin can reduce the blood levels of insulin and leptin, as well as inhibit glycerol-3-phosphate dehydrogenase, an enzyme linked to glycerol and triacylglycerol conversion into adipose tissue and liver [52].

Many dietary polyphenols are antioxidants that can protect against oxidative damage by directly neutralizing reactive oxidants [50]. Reactive oxygen species (ROS) are unavoidable products during normal intracellular metabolism. They play essential roles in cell differentiation, proliferation, and host defense response. However, ROS can cause oxidation of DNA, proteins and lipids [54]. As a result, the action of reactive species can lead to the destruction of cell membrane permeability and cellular dysfunction, related to neurodegenerative diseases and MetS [55–57].

In this study, we evaluated the activity of the enzymes SOD and CAT in the cerebral cortex and hippocampus of animals subjected to HPD and treated with blueberry extract, since the enzymatic antioxidant defenses help to control reactive species levels protecting the cells against oxidative damage [54]. However, no significant difference in the activity of these enzymes was
observed in any of the groups tested. Similarly, no significant difference was found in the tissues studied regarding total thiol content, which is a biomarker of oxidative damage to proteins. In contrast, a study performed by Sinha and collaborators [58] with obese rats presenting all components of the MetS found a significant decrease in SOD and CAT activity in the cerebral cortex, indicating that the components present in the MetS may be involved in the decrease in antioxidant defenses. Furthermore, studies suggest that increase in energy intake can induce oxidative stress and higher ROS production in adipocytes [59–62]. Thus, our study indicated that TBARS levels, a lipid peroxidation biomarker, had a significant increase in cerebral cortex and hippocampus of animals subjected to HPD. These results suggest that higher energetic intake can be involved in this oxidative damage.

Alternatively, chronic ingestion of blueberry in animals subjected to HPD prevented the increase in TBARS levels in the studied tissues. Indeed, blueberry fruit extract is protective against oxidative stress damages in different pathological conditions indicating that it could be a potent inhibitor of lipid peroxidation when compared to other classic antioxidants [21–24]. These positive effects have been attributed to anthocyanins, flavonoids and other phenolic presence [15–17]. We further reinforce that the present study evaluated the effects of the whole berry and at this point we are not able to determine the specific contribution of the isolated polyphenols.

Our study shows that MetS induces lipid peroxidation in the cerebral cortex and hippocampus of mice. Indeed, some data show that oxidative stress in several brain regions may play a role in the pathogenesis of depression [63–65]. In addition, an association has been demonstrated between depression and MetS [66,67]. To confirm this hypothesis, we investigated whether depressive behavior is elicited by HPD consumption and the influence of blueberry treatment. Our findings demonstrate that mice exposed to a MetS model presented a depressive-like behavior in FST and administration of blueberry extract prevented this effect. Furthermore, no significant changes in the ambulatory behavior were observed in animals submitted to the open field test, which suggests that the effect of blueberry intake on the FST is specific and not related to alterations in locomotor activity. The Vaccinium genus demonstrated antidepressant properties in stressed mice, possibly by modulation of the nitric oxide signaling pathway [24].

Herein we also demonstrate that despite the absence of effects on the anxiolytic profile, animals subjected to HPD had a significant increase in immobility time on FST. In addition to oxidative damage, the depressive-like behavior observed in our data may be explained due to the fact that individuals with MetS show elevated levels of proinflammatory cytokines [68]. Further, high concentrations of insulin and C-reactive protein in depressed individuals seem to be important indicators that support the hypothesis of interaction between depression and MetS [69].

In summary, our results suggest that in this animal model treatment with blueberry extract produces antidepressant-like and antiperoxidative effects, besides altering body composition in association with improvement in glucose and lipid profiles. Therefore, these data may contribute to the development of a new pharmacological intervention in patients with MetS.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported in part by grants from “Fundação de Amparo à Pesquisa do Rio Grande do Sul” (FAPERGS), “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES). The authors also thank Hedy L. Hofmann for the corrections of the English version.

References


In thiobarbituric acid-reducing syndrome, of Kaster, O.H., M.Y., C.L., neuronal damage centrally enhances oxidative stress and cellular toxicity. Evidence includes increased oxidative stress markers such as 4-HNE formation, elevated lipid peroxidation, and decreased antioxidant capacity.


A. Avignon, M. Hokayem, C. Bisbal, K. Lambert, Dietary antioxidants: do they have a role to play in the ongoing fight against abnormal glucose metabolism, Nutrition 28 (2012) 715–721.


