

Goji Berry (*Lycium barbarum*) Extract Improves Biometric, Plasmatic and Hepatic Parameters of Rats Fed a High-Carbohydrate Diet

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Abstract: GB (goji berry) has bioactive components capable of reversing the metabolic syndrome. This work investigated systemic, biometric and metabolic parameters of male rats fed with standard diet (group CD) or high-carbohydrate diet (group HC). At 90 days of age the HC group was subdivided: one was given vehicle solution (HCD) and the other was given GB extract (HCDGB), for 60 days. The vehicle was also given to the CD group. At 150 days of age, glucose tolerance test, tissue collection, plasmatic determinations, lipid content, *in situ* perfusion and oxidative stress of the liver were carried out. The GB supplementation improved the parameters of the metabolic syndrome caused by the HC diet, including decreased body weight gain, adiposity index, dyslipidemia, hyperinsulinemia, NAFLD, liver oxidative stress and gluconeogenesis. Together with the diminished insulin resistance, these results indicate the GB extract is an important adjuvant in the treatment of the metabolic syndrome.

Key words: Metabolic syndrome, insulin resistance, NAFLD, liver metabolism, goji berry.

1. Introduction

Lycium barbarum, popularly known as goji berry, is a millenary plant from the Solanaceae family, used in traditional Chinese medicine and widely indicated as functional food. It is rich in polysaccharides, carotenoids, vitamins, amino acids, minerals and phenolic compounds [1, 2]. Among these, the polysaccharides are considered active primary compounds with a wide range of pharmacological properties [3]. The bioactive compounds of the fruits have a variety of beneficial effects, such as reduction of glycemic and lipidemic levels, improvement of insulin

sensitivity, anti-inflammatory, anti-aging, immunomodulating, cytoprotective and especially anti-oxidant actions [3, 4].

Recently, Zanchet et al. [5] demonstrated that the daily supplementation with GB was effective in preventing cardiovascular diseases in patients with metabolic syndrome (MS). This is characterized by a number of metabolic abnormalities, such as abdominal obesity, insulin resistance (IR), hyperglycemia, dyslipidemia and arterial hypertension that predispose to type 2 diabetes mellitus and cardiovascular diseases [6]. Abdominal obesity, in turn, is strongly correlated with the increased prevalence of non-alcoholic fatty liver disease (NAFLD) [7] and IR [8]. The hepatoprotective effect of GB in suppressing steatosis

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and oxidative stress has been observed in NAFLD patients [5] and in an animal model of obesity [9, 10].

The capacity of the polysaccharides of GB in reverting the parameters of MS has been studied in models of fat-rich diet [11, 12], which are effective in developing obesity [13, 14]. However, studies were not found using sugar-rich diets, which as well resemble human MS. Therefore, this investigation assessed whether the daily treatment with GB extract, commercially used by humans, improved the MS in rats fed with sugar-rich diet.

2. Material and Methods

2.1 Preparation of the GB Extract

The powdered extract of the GB fruits of Chinese origin had 40.81% polysaccharides. The analyses and approval of the product were carried out by Quimer® (Brazil) using physico-chemical methods.

A sample of the GB was analyzed in UHPLC-QTOF-MS in positive and negative ionization modes for identification of the compounds with the aid of Data Analysis 2.3. As a source for compound identification, three online databases were used (Human Metabolome Database, Respect and Mass Back). These provide the fragmentation profiles of several compounds in both ionization modes at several voltages. After identification, the confirmation of 19 compounds was possible, such as amino acids (glycyl-L-proline, L-asparagine), polysaccharides (mannose-6-phosphate, glucose), organic acids (quinic acid, 4-hydroxycinnamic acid, phenyllactic acid), and phenolic compounds (naringenin, saponarin, apigenin, myricitrin, isoxanthohumol).

The extract of GB was prepared by manipulation pharmacy (Brazil) at a concentration of 250 mg/mL [15]. It was dissolved in a vehicle containing 3% propylene glycol, 40% liquid sorbitol, 0.15% methylparaben and 0.5% xanthan gum to allow the intragastric administration.

2.2 Animals, Diets and Treatment

This study was approved by the Ethics Committee for the Use of Animals in Experiments of the State University of Maringa (Maringa, Brazil), statement number 4373180216.

Fifty-four male Wistar rats aging 21 days (weaned, 45-50 g) were kept under controlled temperature (23 °C) and photoperiod (12 h light/12 h dark, lights on at 6 am) in polypropylene cages. Eighteen animals were fed with standard rodent diet (control group, CD) and 36 rats were fed with high-carbohydrate diet (HC). At 90 days of age the HC group was subdivided in two groups of 18 animals each; one group (HCD) was given vehicle solution and the other (HCDGB) was given GB extract, daily, for 60 days. The vehicle was also given to the CD group during this period. Food and water were supplied *ad libitum*.

The GB extract or vehicle solution was given intragastrically through gavage; each animal was given a volume of 0.1 mL/100 g body weight (BW) of the vehicle or GB extract (at the dose of 250 mg/kg BW).

The standard diet was Nuvilab CR1 (Nuvital®, Brazil). The HC diet was prepared according to Lima et al. [16] and was composed as follows: 33% standard chow, 33% condensed milk (Nestlé®, Brazil), 7% crystalized sugar (União®, Brazil) and 8.6% water. The nutritional values of the standard and HC diet were, respectively: 402 and 428 kcal/100 g; 57.5 and 68% carbohydrate; 30 and 16% protein; 12.5 and 16% fat.

2.3 Biometric Parameters

Water and food ingestion were monitored every two days in order to obtain an average daily intake over the entire experimental period. The weight gain was calculated as the difference between the final weight and the initial weight (150 and 21 days, respectively). At the end of the experimental period the final weight and naso-anal length were used to calculate the Lee index [17].

2.4 Intravenous Glucose Tolerance Test (ivGTT)

At the end of the experimental period polyethylene cannula was inserted in the right jugular vein [18] under intraperitoneal anesthesia (thionembatal 40 mg/kg BW plus lidocaine 5 mg/kg BW) one day before the test. The cannula was used to administrate glucose (100 mg glucose/100 g BW) and to collect the blood samples at the times 0, 5, 15, 30, 45 and 60 min. The plasma glucose was measured using commercial kits (Gold Analisa[®], Brazil) and insulin by radioimmunoassay (Wizard2 Automatic Gamma Counter[®], TM-2470, PerkinElmer, Shelton-CT, EUA). The fasting insulinemia and glycemia were used to calculate the insulin resistance (IR), expressed in terms of the homeostasis model assessment of insulin resistance-HOMA index [19]. Plasma glucose was expressed as mg/dL and insulinemia as ng/mL.

2.5 Removal of Tissues and Collection of Blood

After the tolerance test, the animals were anesthetized with a lethal intravenous dose of thionembatal (100 mg/kg BW). Blood samples were collected by cardiac puncture to obtain serum and plasma that were used to measure fructosamine, total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides (TG). In addition, the liver, gastrocnemius muscle and the fat deposits (epididymal, mesenteric, retroperitoneal and subcutaneous) were removed and weighed. The livers were also used to measure total lipid content and oxidative stress. The adiposity index was calculated and was defined as the sum of the weights of the fat deposits/100 g BW [20].

2.6 Serum Biochemical Analyses

Total cholesterol (TC), high-density lipoprotein (HDL) and triacylglycerol (TG) were analyzed by standard methods using commercial kits (Gold Analisa[®], Brazil), VLDL levels were calculated using the equation by Friedewald et al. [21] and LDL levels were determined by subtracting HDL and VLDL from TC. These values were expressed as mg/dL. The

atherogenic index was calculated as the relation between TC and HDL.

Fructosamine was used as a marker of glycemic control and formation of glycation end-products. The levels of fructosamine and liver damage markers (aspartate aminotransferase, AST and alanine aminotransferase, ALT) were evaluated in blood samples using commercial kits (Gold Analisa[®], Brazil). Fructosamine was expressed as mmol/L while AST and ALT were expressed as U/L.

2.7 Hepatic Lipid Content

The liver total lipid content was determined using the gravimetric method by Folch et al. [22], which is based on the extraction of lipids from homogenized liver samples (approximately 1.0 g) in a chloroform-methanol mixture (2:1). The TC and TG contents were determined after fat suspension in 2% Triton, followed by agitation and heating to 55 °C. They were measured using commercial kits (Gold Analisa[®]). The results were expressed as g of total fat/100 g of liver wet weight.

2.8 Determination of the Liver Oxidative Stress

To analyze the redox state of the liver, generation of mitochondrial reactive oxygen species (ROS), content of mitochondrial and cytosolic reduced glutathione (GSH) and level of lipid peroxidation were determined. A sample of the liver was used to obtain the mitochondrial fraction and another to obtain the homogenate. The mitochondrial fraction was obtained by differential centrifugation in mannitol-sucrose medium pH 7.4 [23]. The intact mitochondria were used to measure the generation of ROS and those disrupted by freezing-thawing were used to measure GSH content. The homogenate was used to measure the content of GSH and the level of lipid peroxidation.

The generation of ROS was determined by the oxidation of 2',7'-dichlorofluoresceine diacetate as described by Berson et al. [24] and was expressed as pmol of DCF produced/min per mg protein. The GSH

contents were measured using o-phthalaldehyde according to the method described by Hissin, Hilf [25] and were expressed as μg GSH/mg protein. Malondialdehyde (MDA) was used to measure the levels of lipid peroxidation by means of direct spectrophotometry [26] and the results were expressed as nmol/mg protein (ϵ , $1.56 \times 10^5 \text{ M}^{-1} \times \text{cm}^{-1}$).

2.9 In Situ Liver Perfusion

The liver perfusion was carried out as previously described [27, 28]. After a 12-h fasting, the rats were anesthetized (thionembutal 40 mg/kg plus lidocaine 5 mg/kg, i.p.) and the liver was perfused. Krebs/Henseleit-bicarbonate (KH) buffer was perfused for 10 min and then a test substance was added. Glucagon (1 nM) as glycogenolytic agent and L-alanine (5 mM) as gluconeogenic substrate were perfused sequentially for 30 min each. The effluent fluid (perfusate) was collected each five min to determine glucose, L-lactate and pyruvate.

Glucose concentration in the perfusate was determined with commercial kit (Gold Analisa[®],

Brazil), while L-lactate and pyruvate, used to calculate the rate of glycolysis [(L-lactate+pyruvate)/2] were determined according to Bergmeyer [29]. The perfusate concentrations were expressed as $\mu\text{mol/g}$ liver.

2.10 Statistical Analysis

The results were expressed as mean \pm standard error (SE). GraphPad Prism 6.0 was used to calculate the area under the curve (AUC) and perform the statistical analyses (One-way ANOVA followed by the Tukey's test). The level of significance was set at 5%.

3. Results

3.1 Determination of Biometric and Plasmatic Parameters

Table 1 presents several biometric and plasmatic parameters of the three groups. At the age of 150 days, rats fed with the HC diet since weaning had a gain of 28.59% in body weight and of 5.26% in Lee index, and decreased water ingestion by 29.3% compared with

Table 1 Biometric and plasmatic parameters of rats fed standard diet (CD); high-carbohydrate diet (HCD) and HCD treated with GB extract (HCDGB).

Parameters	CD	HCD	HCDGB
Body weight gain (g)	386.95 \pm 6.36 ^a	497.60 \pm 14.70 ^b	449.60 \pm 8.20 ^c
Lee Index	285.42 \pm 1.84 ^a	300.42 \pm 2.89 ^b	306.91 \pm 2.19 ^b
Water consumption (mL/dia)	43.00 \pm 0.40 ^a	30.40 \pm 0.60 ^b	27.30 \pm 2.80 ^b
Food consumption (g/dia)	26.00 \pm 0.05 ^a	28.30 \pm 0.50 ^a	26.60 \pm 2.60 ^a
Liver weight (g/100g BW)	3.02 \pm 0.06 ^a	3.19 \pm 0.06 ^a	3.04 \pm 0.09 ^a
Gastrocnemius weight (g/100g BW)	1.13 \pm 0.022 ^a	1.01 \pm 0.035 ^b	0.97 \pm 0.0332 ^b
Glycemia (mg/dL)	81.15 \pm 2.38 ^a	80.46 \pm 4.15 ^a	86.85 \pm 3.64 ^a
Insulinemia (ng/mL)	1.89 \pm 0.15 ^a	3.03 \pm 0.03 ^b	1.94 \pm 0.15 ^a
Homa-IR index	9.51 \pm 0.87 ^a	14.71 \pm 0.72 ^b	10.29 \pm 0.71 ^a
Triglycerides (mg/gL)	62.19 \pm 7.00 ^a	155.94 \pm 9.64 ^b	109.44 \pm 6.36 ^c
Total cholesterol (mg/dL)	96.43 \pm 4.72 ^a	112.53 \pm 3.68 ^b	95.19 \pm 3.36 ^a
HDL-cholesterol (mg/dL)	33.00 \pm 1.61 ^a	29.50 \pm 2.10 ^a	32.33 \pm 1.90 ^a
LDL-cholesterol (mg/dL)	56.43 \pm 2.65 ^a	46.21 \pm 2.80 ^a	45.36 \pm 4.62 ^a
VLDL-cholesterol (mg/dL)	11.61 \pm 1.49 ^a	34.61 \pm 2.75 ^b	21.89 \pm 1.27 ^c
Atherogenic Index	3.01 \pm 0.18 ^a	3.97 \pm 0.33 ^b	3.05 \pm 0.18 ^a
AST (U/L)	124.50 \pm 13.24 ^a	141.50 \pm 13.02 ^a	144.33 \pm 9.47 ^a
ALT (U/L)	64.00 \pm 7.23 ^a	62.50 \pm 5.26 ^a	69.50 \pm 5.06 ^a
Fructosamine (mmol/L)	0.65 \pm 0.05 ^a	1.13 \pm 0.05 ^b	0.59 \pm 0.03 ^a

Results represent mean \pm SE of 8 to 9 animals. Different letters indicate statistical difference between groups ($p < 0.05$).

group CD. Daily chow ingestion did not differ between the groups. The supplementation with GB extract (250 mg/kg) for 60 days was capable of partially reducing the body weight gain without changing daily chow and water ingestion compared with the HCD animals. Liver weight was not different between the groups, although there was a reduction of gastrocnemius muscle weight in the animals of the groups given the HC diet. The supplementation did not interfere with these changes.

The plasmatic evaluations showed no difference in fasting blood glucose between the groups, however insulinemia was higher in group HCD; this was reversed by GB supplementation. The HOMA index, a measure of IR, displayed a similar pattern, that is, it was increased in the HCD animals and was reversed to control values in group HCDGB.

As for the lipid profile, HCD animals had high levels of TG, TC, VLDL and atherogenic index (the relation of TG to HDL-cholesterol that indicates the risk of cardiovascular disease). The supplementation with GB was effective in significantly improving the lipid profile, decreasing TG and VLDL and reversing TC and atherogenic index to control values. The HDL and LDL-cholesterol fractions did not differ between the groups.

The markers of liver injury, AST and ALT, were not changed. However, plasmatic protein glycation, determined by fructosamine, was higher in the HCD

animals and was normalized by GB supplementation.

3.2 Intravenous Glucose Tolerance Test (ivGTT)

The glucose tolerance test was carried out through the intravenous infusion of glucose (1 g/kg BW). The values of glycemia and insulinemia obtained during the test are shown in Figs. 1A and 1B, respectively. The histograms are AUC of both measures. Group HCD had higher glycemic and insulinemic indices than group CD, thus confirming a lower glucose tolerance. The supplementation with GB was capable of reversing these indices, thus improving insulin sensitivity, as shown by the AUC values.

3.3 Determination of the Adiposity Index

The adiposity index presented in Fig. 2 was calculated by the sum of the epididymal, retroperitoneal, subcutanea and mesenteric white adipose tissues. The animals of group HCD had a significant increase of all these fat deposits. The supplementation with GB promoted a significant decrease of the epididymal, retroperitoneal and subcutanea fats, but did not change the mesenteric adipose tissue. The adiposity index displayed a similar profile, that is, increased in group HCD and decreased in group HCDGB, because of the decreased weight of the epididymal, retroperitoneal and subcutanea fats.

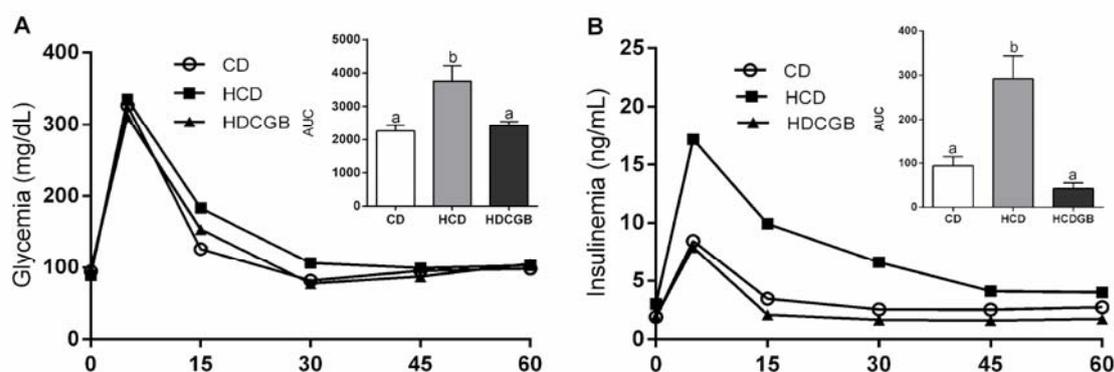


Fig. 1 Glucose tolerance test.

Glycemic (A) and insulinemic (B) curve of rats submitted to intravenous glucose infusion (1 g/kg BW). Histograms represent the AUC values of the respective curves. Rats fed standard diet (CD); high-carbohydrate diet (HCD) and HCD treated with GB extract (HDCGB). The results were expressed as mean \pm SE from 7 to 9 animals. Different letters represent statistical difference ($p < 0.05$).

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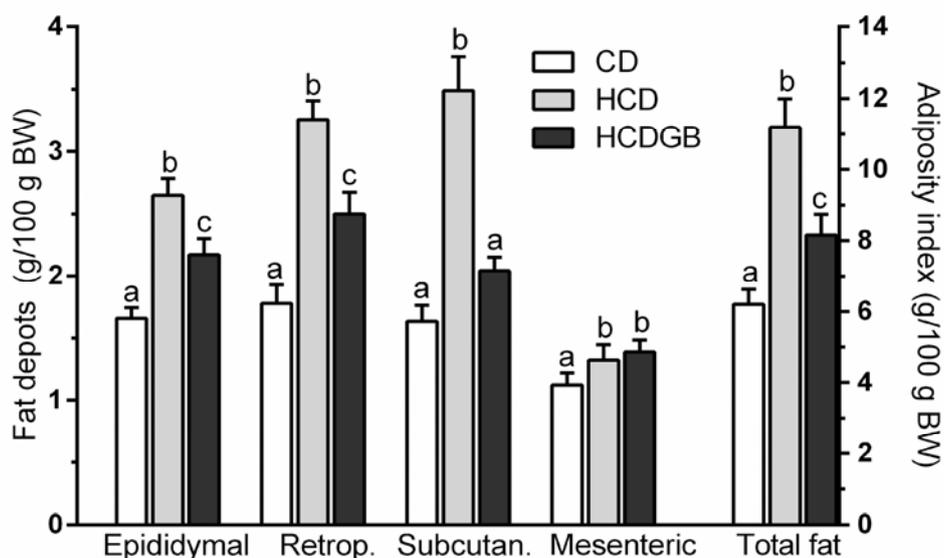


Fig. 2 Deposits of white adipose tissue.

Weight of epididymal, retroperitoneal, subcutanea and mesenteric fat deposits and adiposity index (total fat). Rats fed standard diet (CD); high-carbohydrate diet (HCD) and HCD treated with GB extract (HCDGB). Results were expressed as mean \pm SE of 9 animals. Different letters represent statistical difference ($p < 0.05$).

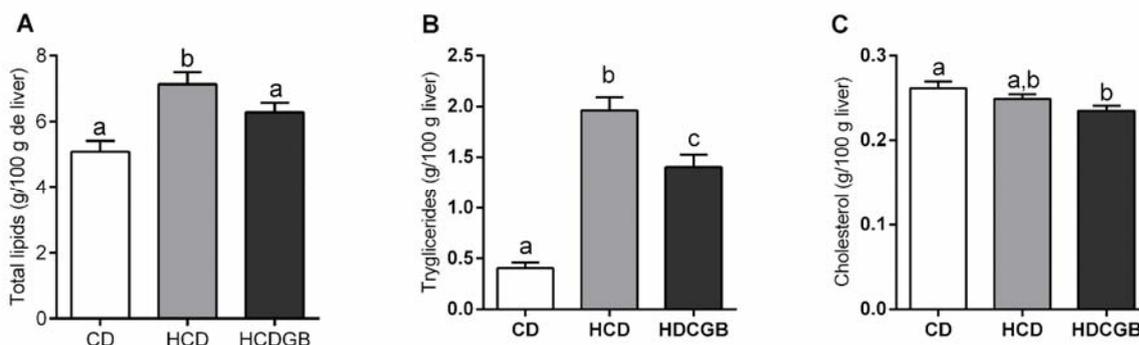


Fig. 3 Liver lipid profile.

Hepatic contents of total lipids, triglycerides and total cholesterol of rats fed standard diet (CD), high-carbohydrate diet (HCD) and HCD treated with GB extract (HCDGB). Values were expressed in g per 100 g of liver weight as mean \pm SE of 8 to 9 animals. Different letters represent statistical difference ($p < 0.05$).

3.4 Determination of Liver Fat Content

The determination of the liver total lipid content revealed that the HCD rats had total lipids significantly higher than group CD, a feature of steatosis which was completely prevented by the supplementation with GB (Fig. 3A). The levels of TG (Fig. 3B) and total cholesterol (Fig. 3C) were also determined and showed a marked increase of TG in the liver of the HCD rats and partial reversal by the supplementation. The levels

of total cholesterol were not changed by the diet, but they were decreased by the supplementation with GB compared with group CD.

3.5 Determination of Liver Oxidative Stress

Fig. 4 shows the analysis of the liver redox state, where the generation of ROS and the mitochondrial and cytosolic contents of GSH were assessed. As seen in Fig. 4A, ROS production was 170.88% higher in group HCD than in group CD and the supplementation

with GB was capable of reversing this parameter. The mitochondrial (Fig. 4B) and cytosolic (Fig. 4C) GSH contents were reduced in group HCD and were not restored by the supplementation with GB, although there is a tendency of increased cytosolic GSH content.

As for lipid peroxidation through MDA quantification, although there was no difference between groups HCD and CD, there was a discrete, non-significant increase in group HCD, which was significantly decreased by the supplementation with GB (Fig. 4D).

3.6 Assessment of Liver Metabolism

Liver metabolism was investigated through *in situ* perfusion, as shown by the graphic in Fig. 5A. The

glucose, either released or produced by the liver, and the rate of glycolysis, were expressed as AUC. The liver of rats given the HC diet had higher basal (Fig. 5B) and glucagon-stimulated (Fig. 5C) glucose release compared with the control group, while the rate of glucagon-stimulated glycolysis was not different (Fig. 5D). The supplementation with GB was not effective in restoring these parameters. However, when perfused with L-alanine, the liver of the HCD rats showed high gluconeogenic capacity, observed by the larger glucose production (Fig. 5E), while glycolysis was not altered when compared with the control (Fig. 5F). These two parameters were clearly influenced by the supplementation with GB, resulting in inhibition of gluconeogenesis (lower glucose production) and reduced glycolysis, as shown in Figs. 5E and 5F.

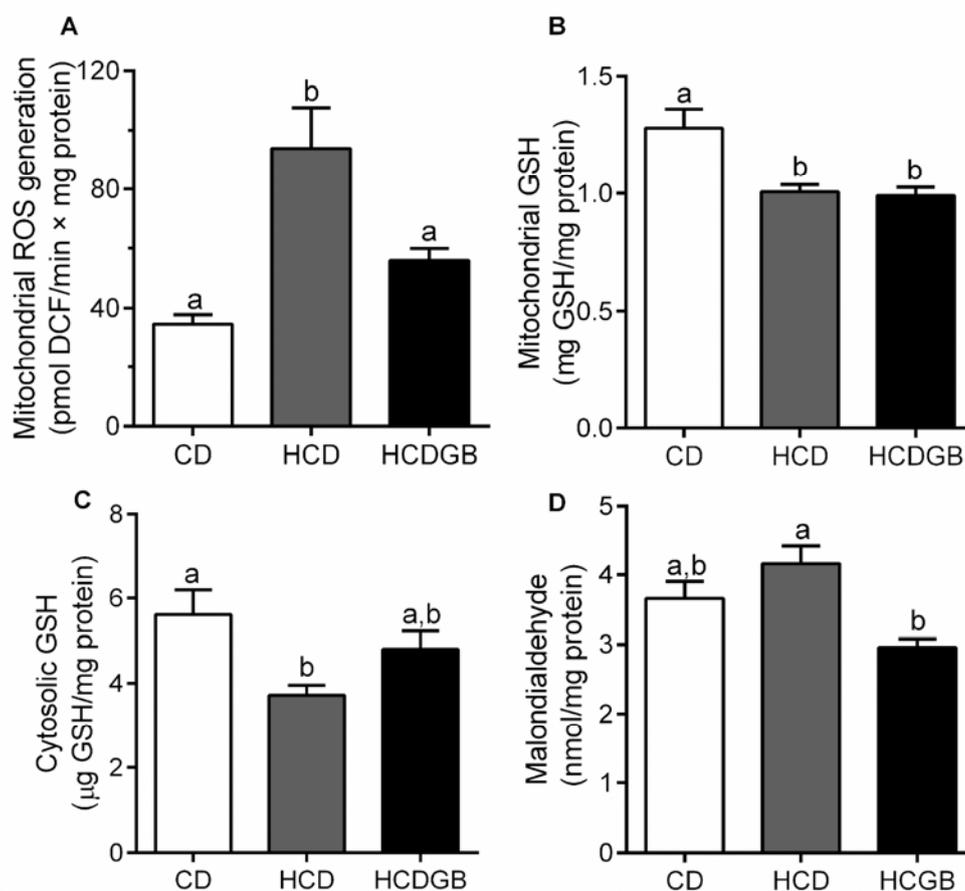


Fig. 4 Liver oxidative stress.

Generation of Mitochondrial ROS (A) and mitochondrial GSH (B), cytosolic GSH (C) and Malondialdehyde (D) contents of rats fed standard diet (CD), high-carbohydrate diet (HCD) and HCD treated with GB extract (HCDGB). Values were expressed as mean ± SE of 5 to 6 animals. Different letters represent statistical difference ($p < 0.05$).

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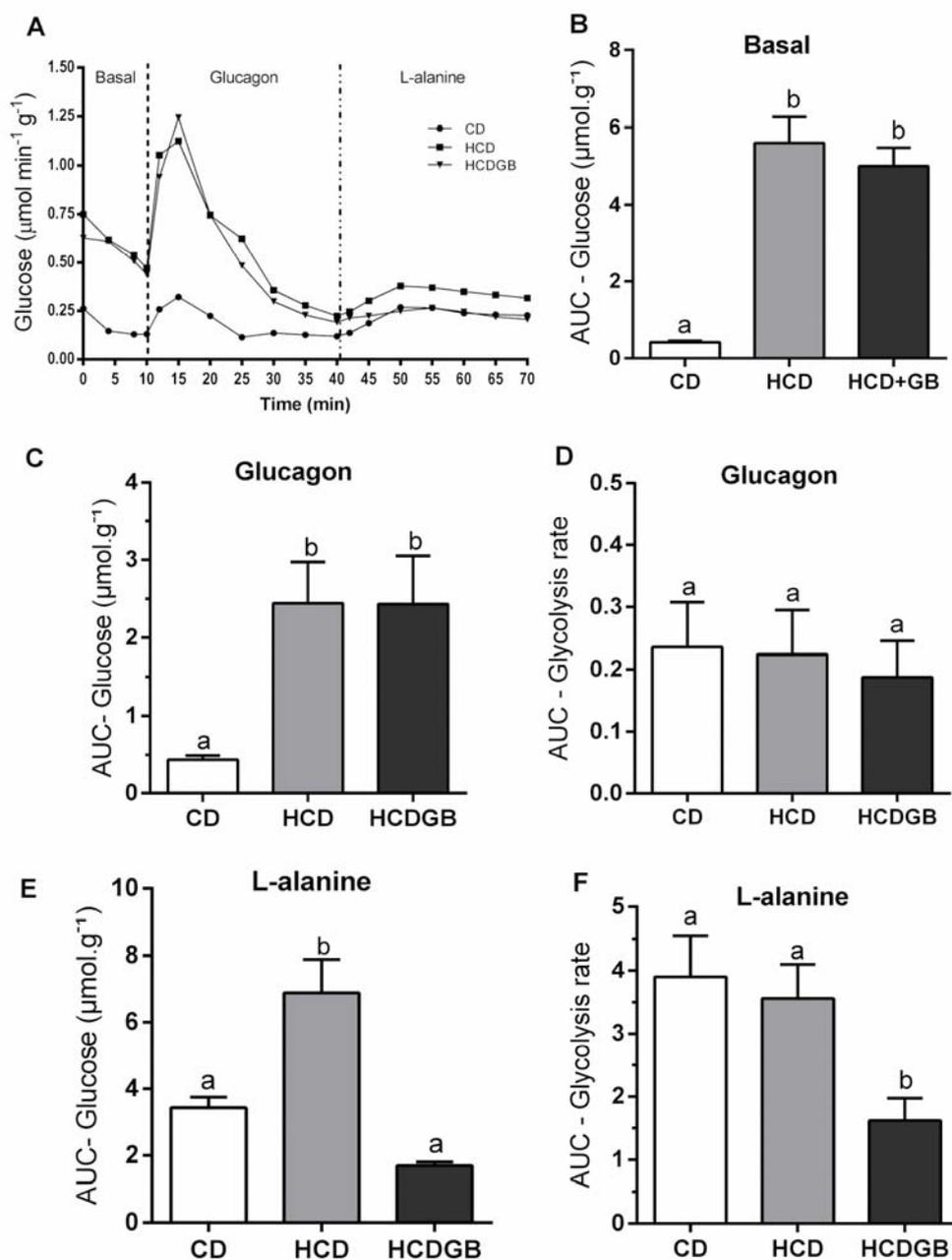


Fig. 5 Liver perfusion.

Experiment demonstrates the release of glucose (A), AUC of basal glucose release (B), glucose release (C) and glycolysis rate (D) during Glucagon infusion (1nM) and glucose release (E) and glycolysis rate (F) during infusion of L-alanine (5mM). Rats fed standard diet (CD); high- carbohydrate diet (HCD) and HCD treated with GB extract (HCDGB). The results were expressed as mean \pm SE from 7 to 9 animals. Different letters represent statistical difference ($p < 0.05$).

4. Discussion and Conclusion

Polysaccharides are considered the most important functional constituents of GB, representing 5 to 8% of the dry fruit [30]. The soluble powder of the fruit extract

of GB used in this study is composed by 40.81% polysaccharides, which allowed assessing whether this commercially used extract could reverse the parameters of MS triggered by the HC diet.

The animals fed with the HC diet developed

metabolic impairments similar to those observed by Lima et al. [31] and in animals fed with high-fat (HF) diet [11, 32], including increased body weight and adiposity, dyslipidemia, NAFLD and liver oxidative stress, typical of human MS [6].

Although fasting glycemia did not differ between the groups, the high insulinemia, HOMA index and insulin and glucose peaks after glucose load showed the decreased insulin sensitivity in this experimental model. These alterations were accompanied by high levels of plasmatic fructosamine, which reflects the glycation of serum proteins and allows the detection of rapid fluctuations in the plasma glucose levels [33].

Body weight gain promoted by the HC diet can be ascribed to the higher adiposity index of the animals, as they had reduced muscle mass indicated by the weight of the gastrocnemius and that can be the result of the lower protein content of the diet [34]. The observation of decreased body weight gain with the daily supplementation with GB, without change in daily chow ingestion was similar to those in mice [35] and rats [36] fed with HF diet and treated with polysaccharides isolated from GB. The lower water ingestion can be attributed to the HC diet, which has a greater water and fat content and lower protein level; together, these factors result in greater availability of water, higher production of metabolic water, and lower renal clearance due to the lower excretion of nitrogenous wastes.

The adiposity index, an indicator of obesity that accurately determines the percent of body fat, was high in the rats fed the HC diet, characterizing central/visceral obesity. In addition, the lipid content of the liver was increased in this group. Measurements of insulin resistance are significantly correlated with the degree of abdominal adiposity in humans [37]. Studies show that the high intra-abdominal adiposity is associated with increased IR and glucose intolerance [14], which is in accordance with the results of this investigation. Insulin resistance increases adipose tissue lipolysis and the circulating levels of free fatty

acids (FFA) that overload the liver if not oxidized [38]. The greater influx of FFA to the liver increases the synthesis of TG and the production/exportation of VLDL from the perivenous hepatocytes, which finally leads to hypertriglyceridemia [39, 40]. This was also noticed in this study and by Lima et al. [16]. In addition, carbohydrate-rich diets stimulate liver *de novo* lipogenesis through increased glycolysis, pyruvate formation, mitochondrial generation of citrate and conversion to fatty acids in the cytosol [41]. This favors *in situ* lipid deposition and massive steatosis [42].

The supplementation with the extract of GB not only decreased the adiposity index (which can partially explain the decreased body weight gain), but also reversed dyslipidemia and hepatic steatosis. The GB extract improved the IR together with a reversal of steatosis, in addition to reversing the risk of cardiovascular disease as indicated by the atherogenic index.

Studies show that the polysaccharides of GB decrease the expression of lipogenic genes and the accumulation of hepatic TG in mice fed an HF diet [15, 35]. Therefore, these compounds have biological activity towards regulation of lipid metabolism and the development of steatosis.

Another important feature of the HC diet is that the overload of TG in the hepatocytes leads to oxidative cellular damage [43]. Studies have shown that liver steatosis is linked to oxidative stress, including elevated production of mitochondrial ROS, decreased content of mitochondrial and cytosolic GSH, increased lipid peroxidation and decreased activity of antioxidant enzymes [20, 44]. The liver of the HCD rats of this study displayed a similar profile, with increased mitochondrial ROS, reduced mitochondrial and cytosolic GSH and slightly higher lipid peroxidation. The supplementation with GB was capable of decreasing the levels of ROS and the lipid peroxidation and tended to increase cytosolic GSH, although it did not alter the mitochondrial GSH.

The antioxidant activity of GB can be explained by

the presence of phenolic compounds, organic acids and vitamins that neutralize free radicals detected by chromatography [45, 46]. Additionally, in the liver of mice fed HF diet and treated with polysaccharides isolated from GB for 24 weeks, these were capable of activating PI3K/Akt/Nrf2, repress the activation of JNK, increase the expression of catalase, superoxide dismutase and GSH, and simultaneously decrease the levels of ROS and improve IR [10].

In the mitochondrion, the redox cycle of the GSH is the major endogenous antioxidant system protecting against ROS [47]. Although the generation of mitochondrial ROS has been decreased by the treatment, the levels of mitochondrial GSH were not reestablished, suggesting that the enzymes of the GSH redox cycle could be altered. Additional investigations are needed to test this hypothesis.

Another important effect of the extract of GB was to prevent cellular damages induced by the generation of ROS, as observed by the reduced levels of MDA in liver of rats given the HC diet. The MDA, considered a marker of the degree of lipid peroxidation, is a highly reactive compound formed by the action of ROS on the cell membrane lipids, especially poly-unsaturated fatty acids [48]. Decreased levels of MDA were already observed in studies testing GB in animals [49, 50], as well as the antioxidant potential of GB *per se* [9, 49, 51-53].

Glucose homeostasis depends on a balance between liver glucose release and glucose uptake by insulin-dependent peripheral tissues (skeletal muscle and adipose tissue). Our results showed that HCD animals had higher liver glycogen content without change in the glucagon-stimulated glycolysis rate. In IR, glycogen synthesis is enhanced [54] due to the reduced glucose use and down-regulation of liver glycolytic enzymes [55]. The liver glycogen content and the glycolysis rate did not differ in animals treated with the extract of GB. These results are not compatible with studies showing that the polysaccharides of GB were effective in increasing the activity and mRNA

expression of glucokinase and pyruvate kinase, key glycolytic enzymes, in animals fed with HF diet [10, 12], suggesting that this difference could be related to the composition of the diet.

The supplementation with the extract of GB was effective in inhibiting the glucose production from L-alanine, in accordance with studies demonstrating the effect of GB in suppressing gluconeogenesis by decreasing the mRNA expression of key enzymes of this pathway, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [10]. The decreased glycolysis during the infusion of L-alanine in the HCDGB group could be due to the exhausted glycogen store after the action of glucagon and/or the reduced gluconeogenesis.

The enhanced glucose releases when the livers were perfused with L-alanine confirm the high liver glucose production typical of IR [39, 56] because of the suppression of the inhibitory effect of insulin on gluconeogenesis [57, 58]. Additionally, the elevated flux and oxidation of TG in the liver speeds gluconeogenesis by providing a continuous energy source [59].

In conclusion, the treatment with GB extract was capable of improving the parameters of the metabolic syndrome caused by the HC diet, including decreased body weight gain, adiposity index, dyslipidemia, hyperinsulinemia, NAFLD, liver oxidative stress and gluconeogenesis. Together with the decreased insulin resistance, these effects make the extract of GB an important adjuvant in the treatment of MS.

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