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Bioactive compounds of the Ubá mango juices decrease inflammation and hepatic steatosis in obese *Wistar* rats

Dorina Isabel Gomes Natal [a], Kellen Cristina da Cruz Rodrigues [a], Maria Eliza de Castro Moreira [a], José Humberto de Queiróz [b], Laércio dos Anjos Benjamin [c], Marcelo Henrique dos Santos [d], Helena Maria Pinheiro Sant'Ana [a], Hércia Stampini Duarte Martino [a,*]

[a] Department of Nutrition and Health, Federal University of Viçosa, Viçosa, MG 36570-900, Brazil
[b] Department of Biochemistry, Federal University of Viçosa, Viçosa, MG 36570-900, Brazil
[c] Department of Veterinary, Federal University of Viçosa, MG 36570-900, Brazil
[d] Department of Chemistry, Federal University of Viçosa, MG 36570-900, Brazil

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Fructose (PubChem CID: 2723872)

ABSTRACT

Obesity is a serious epidemic pathology whose dangerous visceral fat accumulation initiates nonalcoholic fatty liver disease. This study evaluated the effect of the bioactive compounds of the control and peel extract enriched Ubá mango juices on hepatic steatosis associated with inflammation in obese *Wistar* rats. Juices were good sources of the β-carotene, presented high concentration of mangiferin and contributed to decrease the liver weight in animals. Total antioxidant capacity was higher in a group fed with control Ubá mango juice and resistin concentration reduced in both test groups intake Ubá mango juices became similar to normal control. In addition, the percentages of fat vesicles and inflammatory infiltrate in the liver was higher to the animals that intake HFD, and both juices reduced these parameters. Therefore, Ubá mango has potential as a functional food and effect to reduce metabolic risk of the hepatic steatosis associated with inflammation.

1. Introduction

Obesity is a serious epidemic disease, closely associated with inflammation, whose prevalence and incidence are increasing around the word, in adults, children and adolescents. This pathology is characterized by accumulation of fat into the body tissues; it is extremely dangerous to change their normal function and to cause chronic diseases like type 2 diabetes, cerebral and cardiovascular diseases, dyslipidemia and cancer (Guerra et al., 2015; Emamat et al., 2016; WHO, 2016).

Nonalcoholic fatty liver disease (NAFLD) is the most common disease of the liver and ranges from simple steatosis to chronic cirrhosis. Its pathogenesis include reduction in adiponectin levels and insulin sensitivity, increase of resistin levels, inflammatory infiltrate, changes to redox reactions and lipid metabolism due firstly to visceral fat accumulation (Guerra et al., 2015; Emamat et al., 2016; Kobyliak et al., 2016). Adiponectin is an anti-inflammatory cytokine synthesized by adipose tissue that allows macrophage growing inhibition, fat acids oxidation and anti-atherogenic effect. Otherwise, resistin is a pro-inflammatory cytokine, which increases triacylglycerol oxidation and lipids metabolism in a condition of high adiposity (Pereira & Alvarez-Leite, 2014). Thus, the metabolic disturbance identified in this clinic condition makes it important to develop a new treatment, especially in order to modulate fat accumulation and inflammatory response.

The Ubá mango is a Brazilian tropical fruit that presents vitamins and phenolic compounds with anti-inflammatory and antioxidant actions in its pulp and peel; the latter contains a concentration of those phenolic compounds 32 times higher than

* Corresponding author at: Department of Nutrition and Health, Federal University of Viçosa, P.H. Rolfs Avenue, University Campus S/N, Viçosa, MG 36570-900, Brazil.
E-mail addresses: dorinanatal@gmail.com (D.I.G. Natal), kellen.rodrigues.nut@gmail.com (Kellen Cristina da Cruz Rodrigues), elizamoreira@yahoo.com.br (Maria Eliza de Castro Moreira), jqueiroz@ufv.br (J.H. de Queiróz), laercio@ufv.br (L.A. Benjamin), marcelo_hs@yahoo.com.br (M.H. dos Santos), helenapinheirosantana@gmail.com (H.M.P. Sant'Ana), hercia72@gmail.com, hercia@ufv.br (H.S.D. Martino).

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the pulp (Ribeiro, Barbosa, Queiroz, Knödler, & Schieber, 2008; Ribeiro, Rocha, de Queiroz, Campos, & Sant'Ana, 2007). Recently, a research in our laboratory investigated the capacity of the Ubá mango juices on inflammatory markers in adipose tissue in obese *Wistar* rats, showing reduction in biometry, insulin resistance, pro-inflammatory cytokine, TNF-α, and fat accumulation in the adipose epididymal tissue (Natal et al., 2016).

There was no research about the potential of the Ubá mango juices bioactive compounds to increase the total antioxidant capacity and reduce biometry, inflammation and fat accumulation in the liver. By taking into account our previous positive results in the adipose tissue, the purpose of this study was to evaluate the effect of the bioactive compounds of the Ubá mango juices on hepatic steatosis associated with inflammation in obese *Wistar* rats fed with high-fat diet (HFD).

2. Materials and methods

2.1. Plant material collection and preparation

The Ubá mango was collected in the city of Ubá, Minas Gerais, in February 2013. Fruits were selected based on maturation level (Ribeiro, Santos, & Neto, 2013). Then, they were washed in current water, sanitized with 200 mg kg^{-1} chlorine solution and submitted to heat treatment at 90 °C for 15 min (to inactivate polyphenol oxidase enzymes and preserve the phenolic compound) and cooled at ice bath to stop the reaction (Mercali, Jaeschke, Tessaro, & Marczak, 2013). Pulp and peel were stored in polyethylene bags at −22 °C.

2.2. Elaboration of mango peel extract and mango juices

The peel extract was prepared by boiling a mix of mango peel and filtrate water (1:5 w/w) at 100 °C for 5 min. This extract was added to mango pulp (1:2 w/w) to prepare the enriched juice and the control juice was prepared with mango pulp plus filtrate water (1:2 w/w). Both control and enriched mango juice were purified with a 6 mm steel mesh filter to standardize the content of insoluble particles.

2.3. Physicochemical profile

Before preparing the juices with quality standard of the Brazilian legislation, the physicochemical parameters, namely soluble solids (SS), hydrogen potential (pH) and titratable acidity (TA), were analyzed in the pulp.

Total soluble solids were measured by using a digital refractometer (Instrutherm®, RTD-45, Brazil) at 25 ± 1 °C and hydrogen potential (pH) was determined by potentiometric method with directly measure in the pH meter (Tecnal®, Tec-3MP, Brazil) at the same temperature. To evaluated the titratable acidity, the samples diluted in distillated water (1:6 w/v) were titratable with standard solution NaOH 0.1 N until achieving a permanent light pink color for 30 s, using pH indicator phenolphthalein. The acidity percentage was calculated by relation between the volume titratable and the sample weight (Santhirasegaram, Razali, & Somasundram, 2013).

These parameters were lastly determined in the control and enriched Ubá mango juices in three repetitions.

2.4. Proximate composition

The content of water was determined in lyophilize (Terroni®, LC 1500, Brazil) trough vacuum sublimation. Lipids was evaluated by Soxhlet, proteins by Kjeldahl, ashes by incineration and the dietary fibers by enzymatic gravimetric method, as reported by AOAC (2012). The digestible carbohydrates concentration was obtained as the difference among the total of the sample and the other components describing. Finally, the caloric value was calculated by summing the calories supplied by proteins, carbohydrates and lipids, using the conversion factors 4 kcal g^{-1}, 4 kcal g^{-1} and 9 kcal g^{-1}, respectively (Brasil, 2001).

2.5. Beta-carotene concentration

Ubá mango juice's β-carotene was determined by high performance liquid chromatography (HPLC), with three repetitions for each juice.

Approximately 5 g of the each sample were diluted in 60 mL of acetone, homogenized in micro grinder (Tecnal®, TE 102, Brazil) and filtrated in a Büchner funnel under vacuum, to extraction and purification. This procedure was repeated until the residue became colorless and pigments were transferred to petroleum ether, each fraction being washed with distilled water for complete acetone removal.

Each extract rate 8 mL were dried under nitrogen gas flow, and the residue redissolved in 2 mL of the acetone. After being filtrated in the 0.45 μm membrane (Milipore®, Billerica, MA), 50 μL of the extract were injected in the chromatographic column to analyze. This analyses was performed in HPLC system (Shimadzu®, SCL 10AT VP, Japan) equipped with diode array detector (DAD) (Shimadzu®, SPD-M10A, Japan), high pressure pump (Shimadzu®, LC-10AT VP, Japan), autosampler with loop of 500 μL (Shimadzu®, SIL-10AF, Japan), and helium degassing system. The following chromatographic conditions were used: Kinetix C-18 column (150 × 4.6 mm id, 5 μm) equipped with a C-18 guard column (4 mm × 3 mm) (Phenomenex®, Torrance, CA, USA) according to Sant'Ana, Stringheta, Brandão, and de Azeredo (1998). The mobile phase was methanol:acetonitrile:ethyl acetate (80:10:10) with a flow rate of 2 mL min^{-1} and the chromatograms were obtained at 449 nm.

2.6. Ascorbic acid content

The content of ascorbic acid (Vitamin C) was determined by high performance liquid chromatography (HPLC), in three repetitions for each juice.

The samples were extracted with 15 mL of the extraction solution (3% metaphosphoric acid, 8% acetic acid, H_2SO_4 (0.3 N) and 1 mM EDTA) and finally centrifuged and filtrated in a Büchner funnel under vacuum to purification. After filtrated in the 0.45 μm membrane (Milipore®, Billerica, MA, USA), 50 μL of the extract were injected in the chromatographic column to analyze. This analyses was performed in HPLC system (Shimadzu®, SCL 10AT VP, Japan) equipped with diode array detector (DAD) (Shimadzu®, SPD-M10A, Japan), high pressure pump (Shimadzu®, LC-10AT VP, Japan), autosampler with loop of 500 μL (Shimadzu®, SIL-10AF, Japan), and helium degassing system. The following chromatographic conditions were used: Kinetix C-18 column (150 × 4.6 mm id, 5 μm) equipped with a C-18 guard column (4 mm × 3 mm) (Phenomenex®, Torrance, CA) according to Sant'Ana et al. (1998). The mobile phase was 1 mM NaH2-PO4:1 mM EDTA, pH 3.00, with a flow rate of 1 mL min^{-1} and the chromatograms were obtained at 450 nm (Della Lucia, Campos, Oliveira, & Pinheiro-Sant'ana, 2008).

2.7. Mangiferin analyses

The extraction and purification of mangiferin was carried out as described previously (Ribeiro et al., 2008). Separation of phenolic compounds was performed using a HPLC system (Shimadzu®, LC-10AD, Japan) and samples were extracted with metanol:water

solution (60:40 v/v) and purified by centrifugation. The column used was a 150 × 3.0 mm i.d., 4 μm C18 Hydro-Synergy (Phenomenex®, Torrance, CA, USA) with a 4.0 × 2.0 mm i.d. C18 ODS guard column, maintained at 25 °C and the mobile phase consisted of metanol: trifluoroacetic acid 1% (60:40 v/v). Nitrogen was used as the dry gas at a flow of 1.0 mL/min and spectra were recorded from 258 nm.

2.8. Biological assay

We used 32 male rats, *Wistar*, recently weaned, 21 days of age and weighing 69 g, from the Animal Center of Biological and Health Sciences, Federal University of Viçosa (UFV). From the 21th to 60th day the animals were maintained in groups of four, in polyethylene boxes, consuming commercial food (Presence/In Vivo®) and distilled water *ad libitum*. Animals was housed under controlled temperature conditions (22 °C ± 3 °C) and a 12-h light/dark cycle with light phase starting at 7 o'clock in the morning.

On the 61th day, after attained the adult phase, the animals were kept in individual steel cages and randomly and evenly divided into four groups (n = 8), in such a way that the difference between mean weights of the groups did not exceed 3.0 g (AOAC, 2012). The normal control received diet AIN-93M (Reeves, Nielsen, & Fahey, 1993) and the others were fed with high fat diet (HFD) and distilled water *ad libitum* for seven weeks to induce obesity (Table 1).

Next, to start of the treatment with Ubá mango juices, the normal control (AIN-93M) was maintained and the animals fed with the HFD were re-grouped to have the similar weight body (Natal et al., 2016), as the following: obese control (HFD) and two news groups: one with HFD and Ubá mango control juice (MHFD); and other with HFD plus Ubá mango enriched juice (HMHFD) (Barbalho et al., 2012). The juices were given to rats in drinking fountain at 35 mL/day according to the lower juice intake checked in pre-testing during 7 days. The beverages intake were controlled two times per day and there was not juice intake lower than 35 mL/day. If a rat had ingested all the juice given to him before the next day, this animal received distilled water *ad libitum*. Weight gain and food intake were monitored weekly, during eight weeks.

At the end of experiment, the animals were fasted 12 h and submitted to euthanasia by cardiac puncture after anesthesia with isofluran (Isoforine, Cristália®). Blood was centrifuged in test-tube with or without anticoagulant under 4 °C at 1006*g* for 10 min (Fanem-204, São Paulo, Brazil) to have plasma and serum, respectively. The liver, heart, cecum and brain was retired and weighed by calculated the indexes. The major samples of the all tissues were immediately frozen in liquid nitrogen and stored at −80 °C before analysis and a little samples of the liver were fixed into 10% buffered formalin for histomorphological analysis.

This research was approved by the Animal Experimentation Ethics Committee of the Federal University of Viçosa, Viçosa, MG, in 2013 April, Case N° 34/2013.

Table 1
Composition of the experimental diets (g·100 g^{-1}).

Ingredients	AIN 93M	Calories (kcal)	HFD	Calories (kcal)
Casein	14	56	19.5	78
Maltodextrin	15.5	62	10	40
Corn starch	46.57	186.28	5.32	21.28
Saccharose	10	40	34.1	136.4
Soybean oil (mL)	4	36	1	9
Animal fat	0	–	20	180
Cellulose	5	–	5	–
Mineral mix	3.5	–	3.5	–
Vitamin mix	1	–	1	–
Bitartrate choline	0.25	–	0.25	–
L-cystine	0.18	–	0.18	–
Cholesterol	0	–	0.15	–
BHT	0.0008	–	0.004	–
Total	100	380.3	100	464.7
CD (kcal g^{-1})	3.8	–	4.7	–

BHT: butylated hydroxytoluene; CD: caloric density.

2.9. Biometric measures

Body weight and diet intake were monitored weekly. Liver, heart, cecum e brain weight were determined in the euthanasia and the tissues indexes were calculated by the relation among tissues weight and body weight, multiply for 100.

2.10. Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) was determined in the plasma for enzymatic immunoassay with a specific kit (Sigma®) according to Santos et al. (2008). The reaction mixture contained ABTS (2.2′-azino-bis 3-etilbenzotiazolina-6-ácido sulfônico) radical cation, hidrogen peroxide (H_2O_2) and a peroxidase (metmyoglobin). This assay is based in the oxidation of the ABTS and resulted in the production of the blue-green solution. So, the antioxidants present in test plasma resulted in suppression of this reaction and the color production decrease proportionally to their concentration. The absorbance was measured in an spectrophotometer (Thermo Scientific®, model Multiskan GO) at 405 nm and the amount of antioxidants was standardized using Trolox, a water-soluble vitamin E analogue. From the calibration curve, the results obtained were expressed as Trolox equivalent [Mm].

2.11. Cytokines

The cytokines pro and anti-inflammatory were analyzed in the plasma by immunoassay using specific kit to resistin and adiponectin (Millipore®, Billerica, MA) according to Noratto, Martino, Simbo, Byrne, and Mertens-Talcott (2015). Samples were added to appropriate microtiter plate well with biotin-conjugated antibody specific and enzyme Adivin conjugated to Horseradish Peroxidase (HRP) in a reaction that exhibit change in color. After addition of sulphuric acid this reaction is finished and the absorbances are measured in spectrophotometer (Awareness®, Stat Fax 2100) at a wavelength of 450 nm. The results were determined by comparing samples to standard curve using the program MultCalc®.

2.12. Histomorphometric analyses of the liver

The fixed hepatic tissue was embedded in resin and the blocks were sliced with semi-serial sections of 3 μm thickness for Hematoxilin/Eosin (HE) staining. The blades analysis were carried out on an light microscopy (Nikon Phase Contrast 0.90 Dry®, Japan) and images were captured with a ful-DIGI-PRO 5.0 M digital camera through Software Micrometrics SE Premium (Accu-Scope®) at 40× magnification. To quantify areas of steatosis and hepatic inflammation were recorded the percentage of fat vesicles and inflammatory infiltrate by manual counting points on tissue, using the mean of ten fields for each animal. Following, steatosis was assessed semiquantitatively according to a 5 grade scale: grade 0, if the fat percentage absent or <5%; grade 1, if ≥5% and <25%; grade 2, if ≥25% and <50%; grade 3, if ≥50% and <75%; and grade 4, if ≥75% (Turlin et al., 2001).

2.13. Statistical analyses

The experiments were arranged in completely randomized design and data submitted to one-Way ANOVA followed by *post hoc* test Tukey for physicochemical or Duncan for biological, both at 5% probability. Results are showed as mean ± standard deviation an all the analyses were performed in software SPSS Statistics, version 20.0, 2011 and graphics made with system Sigma Plot, version 11.0, 2008.

3. Results

3.1. Physicochemical profile and proximate composition

Soluble solids (SS) must be investigated in function of the sample concentration and dilution (Brasil, 2013). As expected, the pulp presented higher content of SS than both control and enriched Ubá mango juices showed the lowest concentration ($p < 0.05$). The hydrogen potential (pH) range vary from acid to basic (1.0–14.0) and set the probability of the microbial growth and enzymatic activity. Otherwise, titratable acidity (TA) is the physicochemical measure, which determine the organic acid concentrations. So, both are related with food quality or deterioration levels in a sample, since each microorganism or enzyme has a wide range of pH and a specific food matrix for activity or proliferation (Santhirasegaram et al., 2013; Wang, Selvam, Chan, & Wong, 2013). There was no change in pH among pulp, control and enriched juices ($p > 0.05$). However, acidity was higher in enriched juice with peel extract ($p < 0.05$) when compared to control juice, and was highest in mango pulp ($p < 0.05$, Fig. 1A).

The Ubá mango pulp showed lower water content than the mango juices, which become its proximate compounds the highest ($p < 0.05$, Fig. 1B and C). The addition of the peel extract in the mango juice increased significantly the amount of fat, the soluble dietary fiber (SDF), digestible carbohydrates and calories (kcal) compare to control mango juice ($p < 0.05$). Mango peel extract also added 13.8% of the total dietary fiber (TDF) to juice and showed a tendency to increase this nutrient ($p = 0.094$) (Fig. 1D and E). In addition, mango peel extract increased 7% of the total dietary fiber concentration, 5% of the digestible carbohydrate and 7% of the calories (Fig. 1F), based in the Dietary Reference Intake (DRI) in a 200 mL serving size (Brasil, 2003a).

3.2. Bioactive compounds concentration: β-carotene, ascorbic acid and mangiferin

The bioactive compounds ascorbic acid (vitamin C), mangiferin and β-carotene were analyzed in the control and enriched Ubá mango juices (Fig. 2A and B). The ascorbic acid and mangiferin concentrations were higher in the enriched juice compare to control juice ($p < 0.05$), highlighting that mango peel extract added 7% to enriched juice in relation to control. The β-carotene did not differ among the juices, although they have a high content of the β-carotene based in the DRI ($p > 0.05$; Fig. 2C and D).

3.3. Food intake and biometric measures of the experimental animals

The food intake in the normal control group (AIN-93M) was the highest and the body weight was the lowest ($p < 0.05$) in all experimental weeks (Fig. 3A and B). Intake of both control and enriched juices did not change the body weight in HFD groups (groups fed with the high-fat diet) until the seventh week ($p > 0.05$), period in which it showed a tendency to decrease ($p \leq 0.122$; Fig. 3B). Liver weight was the highest in the obese control (HFD, $p < 0.05$), decreased ($p < 0.05$) in the control (MHFD) and enriched (HMHFD) Ubá mango groups, and was the lowest in the normal control (AIN-93M, $p < 0.05$). The HMHFD group decreased heart weight compare to obese control group ($p < 0.05$) and MHFD present the same heart weight than the normal control group. Cecum weight was the lowest in the HFD group ($p < 0.05$) and increased ($p > 0.05$) in the MHFD and HMHFD, becoming similar to AIN-93M. Brain weight was also observed and the normal control showed higher values when compared to the obese control ($p < 0.05$). The Ubá mango

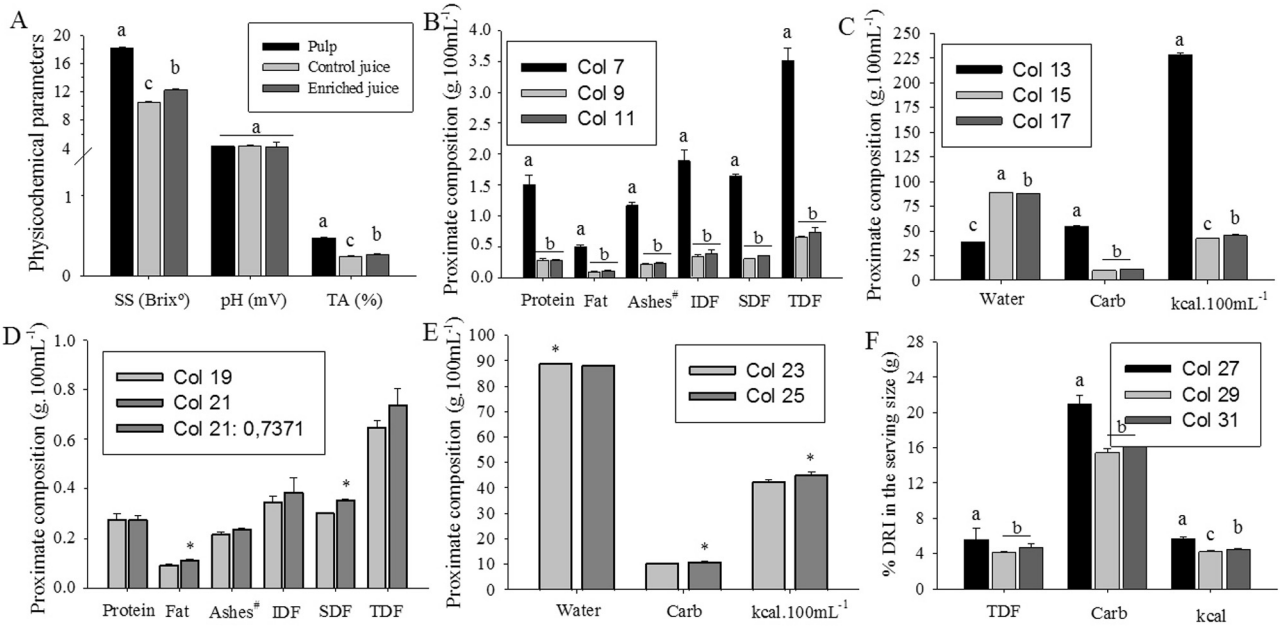

Fig. 1. Physicochemical (A), proximate composition (B–E) and percentages (F) of the Dietary Reference Intake (DRI) of the Ubá mango pulp and both juices control and enriched. *The means followed by the lower letter in the same graphic differed by Tukey test's at 5% of probability. *The means followed by the star differed between juices by ANOVA at 5% of probability. SS: soluble solids; pH: hydrogen potential; TA: titratable acidity; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF: total dietary fiber; Carb: digestible carbohydrates; kcal: calories. #Ashes was represent in mg·100 g^{-1}.

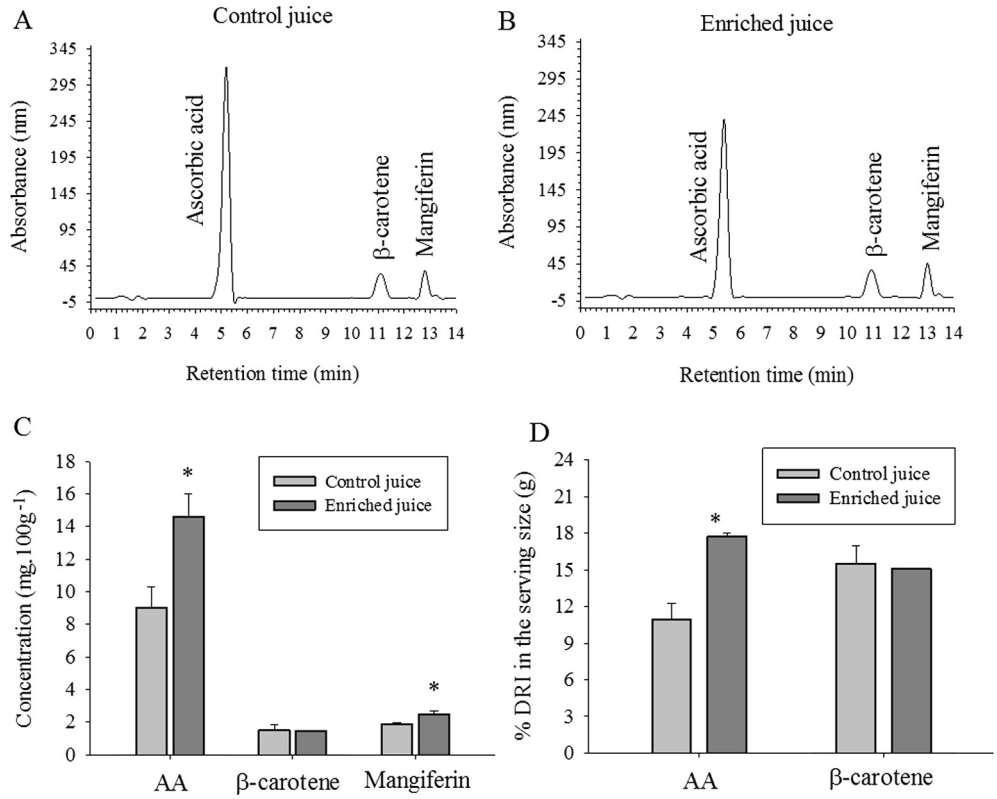

Fig. 2. Chromatograms (A and B), concentration (C) and percentages (D) of the Dietary Reference Intake (DRI) to ascorbic acid, β-carotene and mangiferin of the control and enriched Ubá mango juices. *The means followed by the star differed between juices by ANOVA at 5% of probability. AA: ascorbic acid.

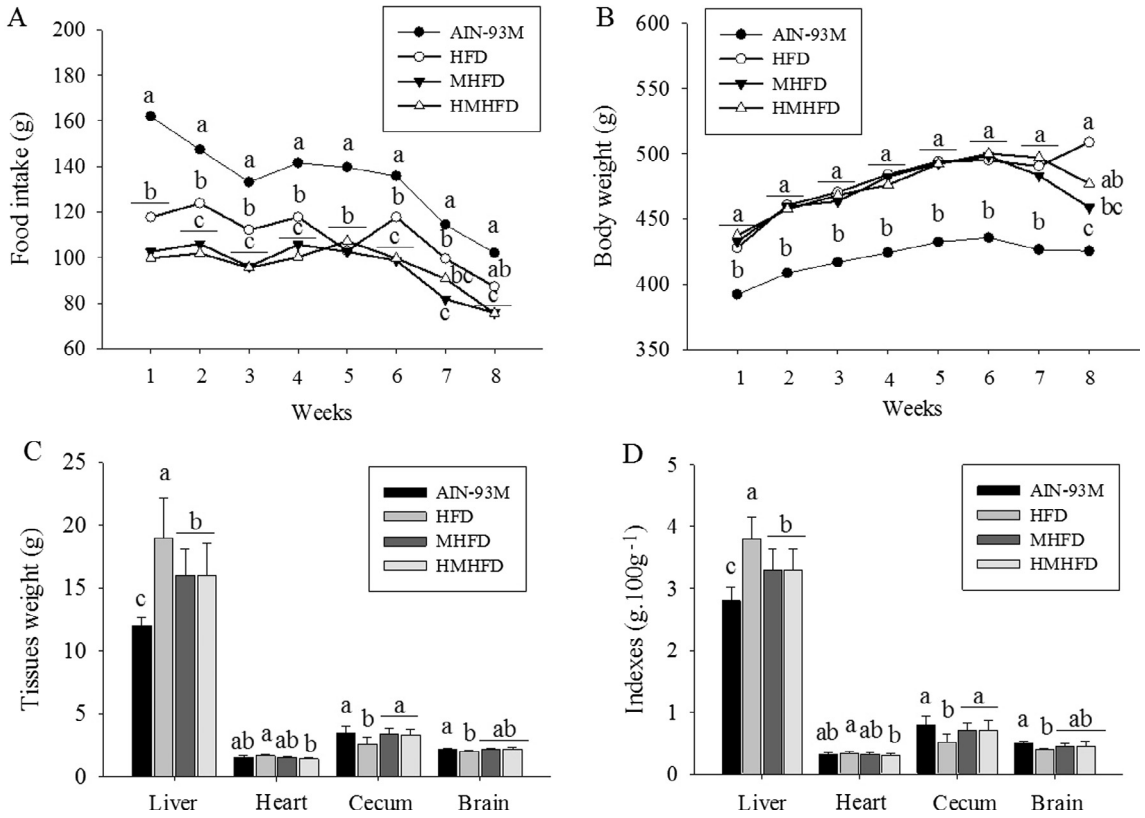

Fig. 3. Food intake (A) and biometric measure (B–D) of the experimental animals before and after treatments with Ubá mango juices. *Means followed by the same letter in the same graphic did not differed by Duncan test at 5% of probability. AIN-93M: normal control group; HFD: obese control group; MHFD: test group with mango control juice; HMHFD: test group with mango enriched juice with mango peel extract.

juices intake showed a tendency of return to normal standard physiological in the MHFD and HMHFD ($p = 0.057$; Fig. 3C and D).

3.4. Effect of the Ubá mango juices intake on plasma total antioxidant capacity

According to bioactive compounds concentration in the two mango juices, its intake by the animals was calculated. The total antioxidant capacity (TAC) in plasma was higher ($p < 0.05$) in the MHFD group when compared to HMHFD (Fig. 4A and B), showing so negative correlation between the TAC and bioactive compounds intake (sum of the ascorbic acid, β-carotene and mangiferin) ($p < 0.001$; Fig. 4C).

3.5. Cytokines concentration after the treatment with Ubá mango juices

The anti-inflammatory cytokine levels (adiponectin) did not differ among the experimental groups ($p > 0.05$, Fig. 5A); however, a positive correlation was observed between bioactive compounds and adiponectin ($p < 0.001$, Fig. 5C). The Ubá mango juices reduced the pro-inflammatory cytokine concentration (resistin) in MHFD and HMHFD groups became similar to normal control group. In addition, negative correlation was observed between resistin and TAC ($p < 0.001$, Fig. 5B and D).

3.6. Hepatic tissue of the rats after the treatment with Ubá mango juices

Changes were observed in the liver tissue of the obese control group (HFD) (Fig. 6A, B, D, E). The percentage of the fat vesicles in HFD group was higher than normal control group (AIN-93M) and both Ubá mango juices were able to decrease the hepatic steatosis ($p < 0.05$, Fig. 6C). Similarly, the content of the inflammatory infiltrate also increased in the obese control and decreased after juices treatment ($p < 0.05$), became similar to AIN-93M (Fig. 6F). The correlations between the pairs fat vesicles/liver weight and inflammatory infiltrate/liver weight indicated a positive correlation coefficients and tend to increase together ($p < 0.05$, Fig. 6G and H). The control group was classified as steatosis grade 0, the high-fat diet intake increased the steatosis into hepatic tissue to grade 2 and both Ubá mango juices were effective to decrease steatosis to grade 1 (Fig. 6I).

4. Discussion

The present research focus was the potential benefits of the bioactive compounds present in control and enriched Ubá mango juices to increase the total antioxidant capacity and to reduce inflammation, alleviating the hepatic steatosis.

We know that the fruit composition change in function of the variety researched, conditions of the cultivation, maturation, harvest and processing method. These characteristic are essential to a good quality and acceptability of the food and so required standardization. Both pulp and juices presented the physicochemical parameters in the standard range specified by Brazilian legislation to soluble solids (SS: up to 10.0 Brix°), hydrogen potential (pH: 3.3–4.5) and titratable acidity (% TA: up to 0.20) (Brasil, 2000, 2003b). The mango peel extract added soluble solids (SS) and increased the titratable acidity percentage (% TA) to enriched mango juice. Only one publication at this moment evaluated the

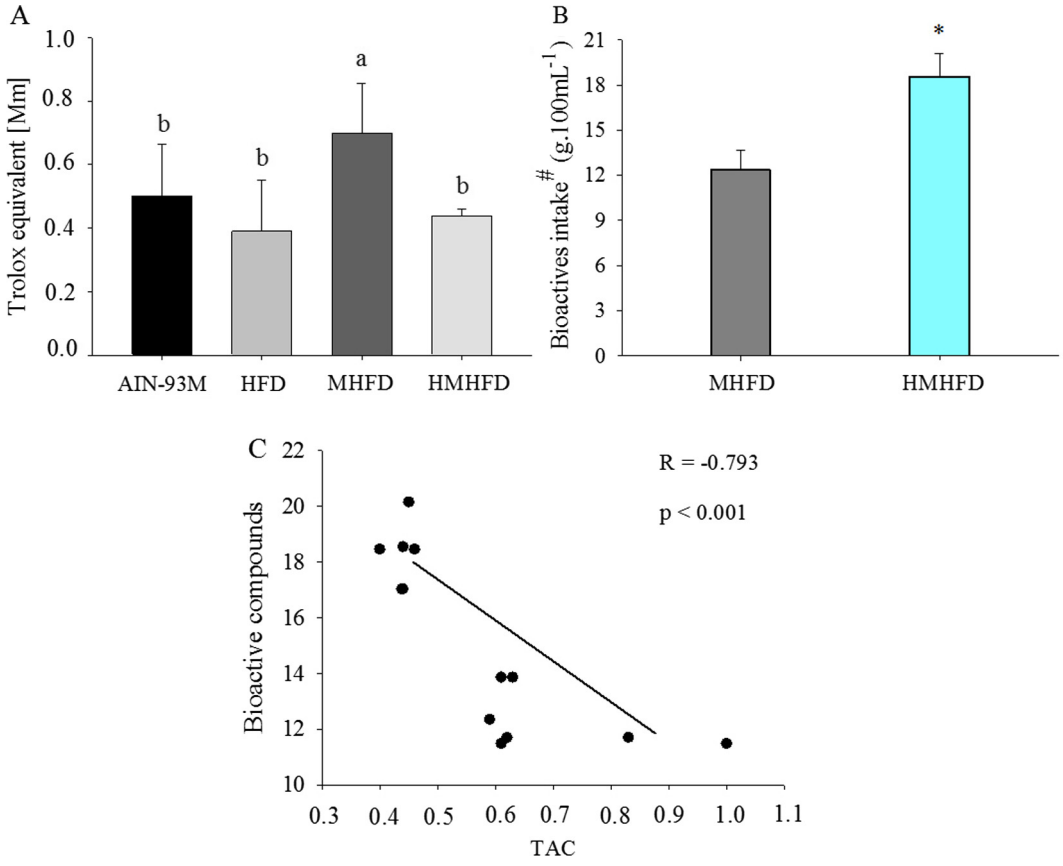

Fig. 4. Total antioxidant capacity (TAC) (A), bioactive compounds intake (B) and correlation between these variables (C). *Means followed by the same letter or star in the same graphic did not differed by Duncan test at 5% of probability. #Calculated by sum of ascorbic acid, β-carotene and mangiferin. AIN-93M: normal control group; HFD: obese control group; MHFD: test group with mango control juice; HMHFD: test group with mango enriched juice with mango peel extract; TAC: total antioxidant capacity.

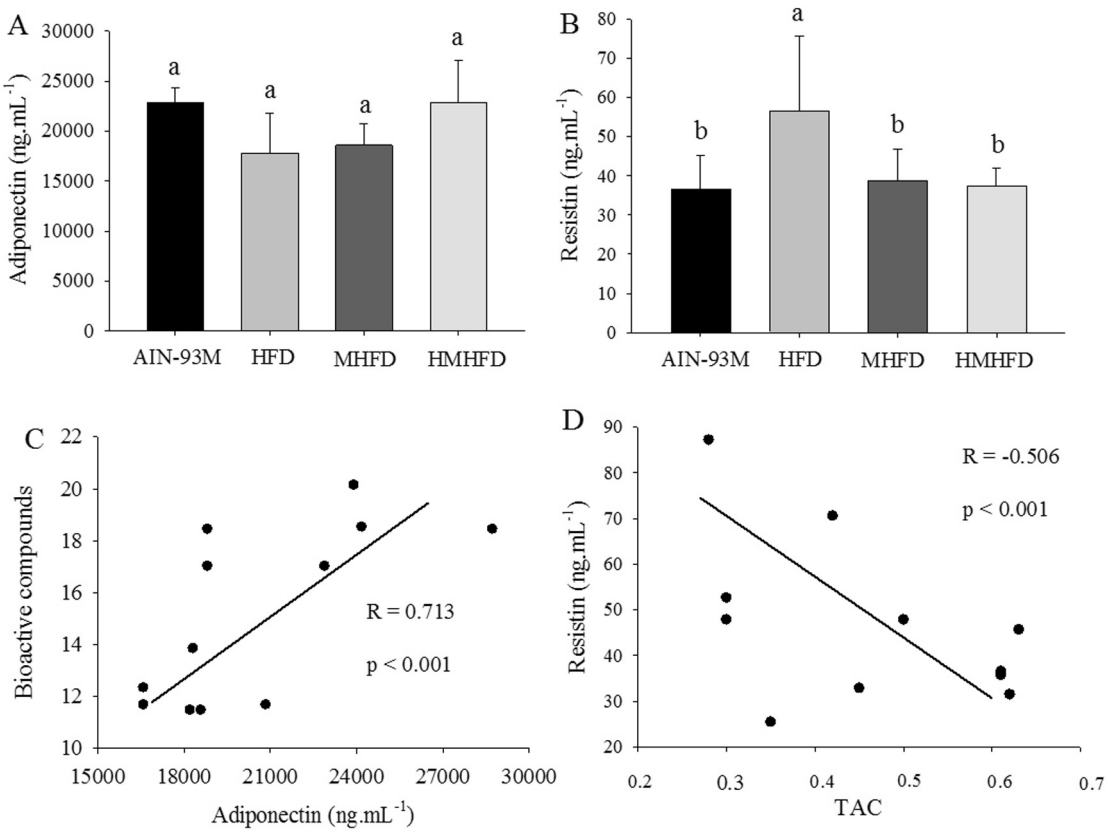

Fig. 5. Ubá mango juices effect on anti-inflammatory (A) and pro-inflammatory (B) hormones levels in the plasm of the *Wistar* rats and correlation between bioactive compounds/adiponectin (C) and resistin/total antioxidant capacity (D). *Means followed by the same letter in the same graphic did not differed by Duncan test at 5% of probability. AIN-93M: normal control group; HFD: obese control group; MHFD: test group with control mango juice; HMHFD: test group with enriched mango juice with mango peel extract; TAC: total antioxidant capacity.

morphological characteristics in different Brazilian mango fruits, including the Ubá mango variety (Ribeiro et al., 2013). However, this paper evaluated just the soluble solids that are in accordance with this present work and shows high values to this parameter. Additionally, Santhirasegaram et al. (2013) found similar results to physicochemical parameters in Chokanan mango, although it is a different variety of the fruit.

The Ubá mango pulp showed two times lower water and proportionally higher concentration of all other nutrients available in comparing with the table of food composition of the United States Department of Agriculture to other varieties of this fruit (USDA, 2016). Pulp, control and enriched mango juices showed respectively 52%, 59%, 70% dietary fiber concentration standard classified as sources by Brazilian legislation (Brasil, 2012). The beverages development in this present work added digestible carbohydrates naturally present in the mango fruit (like fructose), did not contain sugar addition and thus had 20% lower kcal in the 200 mL serving size (Brasil, 2003a) than industrial mango juices. In addition, Ubá mango juices also did not present sodium and chemical preservatives, which contribute to healthy characteristics.

Mango is a tropical fruit that contains high amounts of vitamins and phytochemicals with anti-inflammatory and antioxidant action, hence its potential to prevent or alleviate chronic diseases and fat accumulation (Manthey & Perkins-Veazie, 2009; Natal et al., 2016). Ascorbic acid is an important co-factor to enzymes in the metabolism of minerals (iron and cooper), metalloenzymes and showed further antioxidant activity. Otherwise, β-carotene is precursor of the vitamin A, which is required for vision normality, gene expression, reproduction and immune function (IOM, 2006). According to the average DRI (IOM, 2006) for adult women and men aged 19–50 years old and the 200 mL serving size, the control mango juice contributed with 11% of the recommended intake and was classified as good source of ascorbic acid. It's important highlight that the mango peel extract contributed to become enriched mango juice as source of the Vitamin C. Moreover, both control and enriched Ubá mango juices were classified as β-carotene sources, contributing with 16% and 15% of the DRI, respectively.

There was no DRI to mangiferin, since there were not studies focused in the tolerable upper intake levels to non-nutrient bioactive compounds, but the phytochemical intake is related to inflammation reduction, antioxidant defense and tumor growth suppression. Ubá mango peel extract added 25% of this compound to mango juice and so have protective effect against common chronic diseases, such as cancer, obesity, cardiovascular and nonalcoholic fatty liver disease (Banerjee, Kim, Krenek, Talcott, & Mertens-Talcott, 2015; Guerra et al., 2015; Leiherer, Mündlein, & Drexel, 2013). In this work, we evaluated vitamins and mangiferin of both control and enriched Ubá mango juices, verifying that the peel contains more antioxidant compounds than the pulp. However, the plasma total antioxidant capacity (TAC) of the HMHFD reduced, instead increasing as the MHFD group though the highest bioactive compounds intake. We expected an increase, due to its antioxidant properties. The excessive concentrations of this compounds may have been poorly absorbed, reducing the oxygen available, and increasing the pH or rapidly metabolized into other molecules. So, did not showed the expected biological effect to TAC. Otherwise, a high exogenous antioxidant compounds intake decreased the mobilization or synthesis of endogenous enzymes, like catalase, superoxide dismutase and glutathione, beyond other

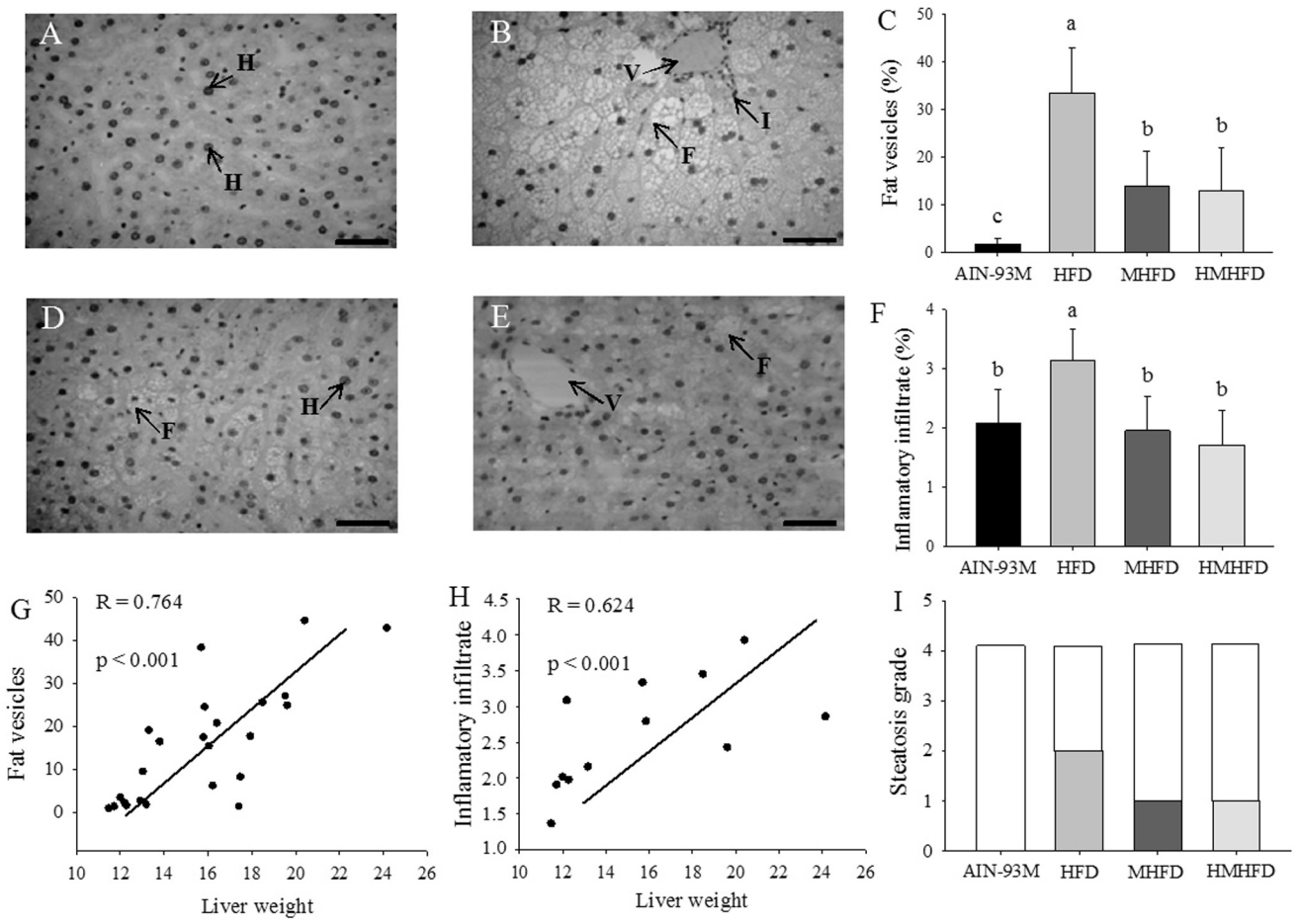

Fig. 6. Effect of control and enriched Ubá mango juice on the hepatic tissue phenotype (A, B, D, E) and on percentages of the fat vesicles (C), inflammatory infiltrate (F), correlations between fat vesicles/liver weight (G) and inflammation/liver weight (H) and steatosis grade (I) of the *Wistar* rats hepatic tissue's. (A) AIN-93M: normal control group; (B) HFD: obese control group; (D) MHFD: test group with control mango juice; (E) HMHFD: test group with enriched mango juice with mango peel extract H: hepatocytes; V: blood vessels; F: fat vesicles; I: inflammatory infiltrate. HE staining. Barr: 50 μm. *Means followed by the same letter in the same graphic did not differed by Duncan test at 5% of probability.

antioxidants for plasma. Also, the fruit that was used is more adequate than pure compounds and ensures consistent results, showing that there may be interference in the action of bioactive compounds with the food matrix. Therefore, we did not take only the TAC of plasma that must be interpreted in association with other parameters to answer about biological effect of the phytochemicals (Pompella et al., 2014; Vihakas, Pälijärvi, Karonen, Roininen, & Salminen, 2014).

Both Ubá mango juices introduced fiber and calories to obese rats, resulting in satiety and lower dietary consumption. Moreover, the lower food intake of the obese control group occurred as a function of the regulation of food intake due to its higher caloric density (Sampey et al., 2011) and the reduction in food intake verified in all groups indicate the lower energy needs to adult animals. The weight is the variable less sensitive whose changes are observed later than biomolecular or cellular markers. In addition to that, old age is an important factor to take into account in studies of body weight loss, since old animals have a tendency to stay overweight. So, the expected reduction in body weight after bioactive compounds intake present in Ubá mango juices was observed only in the last experimental week and showed limitation or inhibition in the action of the phytochemicals to improve body measures (Guerra et al., 2015; Reynés, García-Ruiz, Díaz-Rúa, Palou, & Oliver, 2014; Yang et al., 2011). The high weight of the liver and the heart in HFD control obese group indicated fat accumulation after high-fat diet intake but the bioactive compounds of the Ubá mango juices resulted in lower weight and low fat in the both groups MHFD and HMHFD (Leiherer et al., 2013). Cecum weight decreased in the HFD group due to the lower intake of nutrients, such as dietary fibers and bioactive compounds, which was reversed in the Ubá mango juices, MHFD and HMHFD groups, in which the cecum weight was similar to the normal control group (Natal et al., 2016). Finally, the phytochemicals present in both Ubá mango juices were able to modulate the neurotrophic signaling pathway and increase the beneficial cells differentiation, which improved the tissue function and increased brain weight (Moosavi, Hosseini, Saso, & Firuzi, 2016).

The resistin levels decreased in the test groups, indicating that the bioactive compounds present in the pulp and peel of the Ubá mango could be effective to modulate both inflammation and oxidation. In our previous work, we also verified decrease in cytokine pro-inflammatory TNF-α levels after the intake of Ubá mango juices, in obese *Wistar* rats (Natal et al., 2016). The increase in adiponectin was related to a decrease in adiposity, but no significant difference was observed regarding this anti-inflammatory cytokine in the groups with control and enriched Ubá mango juices, when compared to HFD. However, studies showing the effect of antioxidant compounds in other foods reducing pro-inflammatory molecules and increase anti-inflammatory markers, such as IL-10 and adiponectin. The short experimental time in the present study was probably not enough to verify these effects (Ibrahim, El-Denshary, & Abdallah, 2015; Noratto et al., 2015; Yang et al., 2011).

The present study also showed the ability of bioactive compounds present in pulp and peel of the Ubá mango juices to control obesity though reducing lipid storage and immune cells infiltrates in the hepatic tissue of obese *Wistar* rats with obesity induced by high-fat diet. As well as in previous studies, we verified reduction in steatosis, to grade 1, in the MHFD and HMHFD groups due to the concentrations of ascorbic acid, β-carotene and mangiferin present in both Ubá mango juices (Lim et al., 2014; Pan, Lai, Tsai, & Ho, 2014). Additionally, Emamat et al. (2015) found NAFLD reduction after the treatment with onion accompanying a healthy diet, since that the onion have also high content of flavonoids, antioxidants and anti-inflammatory agent.

We know that biological experiment with rats cannot be extended to human individuals, since there are significant metabolic differences. But, considering the Ubá mango juices intake/animals weight, their fast metabolism and the duration of the experiment, an average 70 kg human need an intake of at least one juice's serving size/day to have the same improvements in biometry, cytokines concentration and histology observed in this research.

5. Conclusion

In conclusion, the control and enriched Ubá mango juices presented high content of vitamins and mangiferin, with anti-inflammatory and antioxidant effects, which improved biometric measures, cytokines concentration and was beneficial for obese *Wistar* rats with adiposity and inflammation in the liver. Therefore, Ubá mango has potential as a functional food, indicating potential to prevent and combat metabolic risk of obesity, hepatic steatosis and other comorbidities.

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