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Bacupari peel extracts (*Garcinia brasiliensis*) reduce high-fat diet-induced obesity in rats

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ABSTRACT

The aim of this study was to determine the effect of ethanol extract of bacupari peel (EEB) on the adiposity and inflammation modulation in obese Wistar rats. The group treated with high fat diet plus EEB (BHFD) presented weight gain, visceral fat, and lee, and an adiposity index similar to the negative control group (AIN-93M). Also, the BHFD group showed antioxidant and anti-inflammatory effect, increase of peroxisome proliferator activated receptor-γ (PPAR-γ) and Interleukin-10 (IL-10) expression, and decreasing expression of lipoprotein lipase (LPL) and fatty acid synthase (FAS), and reduced the tumor necrosis factor-α (TNF-α), blood levels of glucose, alanine aminotransferase, and adipocyte hypertrophy. The molecular docking showed that morelloflavone and 7-epiclusianone compounds from bacupari extract interacted with PPAR-γ receptor, hydrophobic interaction, indicating an agonist activity of these compounds. Thus, we demonstrated that extract of bacupari presented anti-obesity activities.

1. Introduction

The growing prevalence of obesity worldwide has become an immediate public health concern. According to the World Health Organization, obesity is a global epidemic due to the parallel rise of related morbidity and risk factors. Currently, >30% of the world population is estimated to be overweight or obese (Lankford, Hardman, Dankmeyer, & Schmid, 2013). Obesity is characterized by an excess of fat resulting from the interaction between genetic and environmental factors, mainly caused by positive energy balance, which leads to expansion of adipocyte mass. It is well known that a high fat diet leads to an accumulation of adipose tissue and the development of metabolic changes associated with weight gain, particularly in genetically predisposed individuals (Dewulf et al., 2011), besides the environment and lifestyle. Obesity is associated with a low-grade chronic inflammation because it can lead to increased levels of inflammatory cytokines (Rodríguez-Hernández, Simental-Mendía, Rodríguez-Ramírez, & Reyes-Romero, 2013).

This inflammatory process may be controlled by peroxisome proliferator activated receptor (PPAR) since its activation inhibits the transcription of pro-inflammatory genes (Monsalve, Pyarasani, Delgado-Lopez, & Moore-Carrasco, 2013). A variety of natural products, including crude extracts and isolated compounds from plants, have been widely used to treat obesity. Several studies indicate that numerous bioactive components from nature are potentially useful in obesity management or control (Wang et al., 2014), for example: strawberries, blueberries, pomegranates,

Abbreviations: EEB, ethanol extract of bacupari peel; PPAR-γ, peroxisome proliferator activated receptor-γ; LPL, lipoprotein lipase; FAS, fatty acid synthase; IL-10, Interleukin-10; TNF- α, tumor necrosis factor-α; AIN-93M, diet negative control; HFD, high fat diet; BHFD, HFD plus EEB; AST, aspartate aminotransferase; ALT, alanine aminotransferase; OGTT, the oral glucose tolerance test; TAOC, plasma total antioxidant capacity; ABTS, Azinodiethyl-benzthiazoline sulphate; CBA, Cytometric Beads Array Kit; Log P, octanol-water partition coefficient; SAR, structure-activity relationship.

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oranges, and compounds such as anthocyanins, catechins, quercetin and rutin (Brown, Poudyal, & Panchal, 2015; Joseph, Edirisinghe, & Burton-Freeman, 2016). A significant research effort has recently been undertaken to explore the PPARγ-activating potential of a wide range of natural products originating from traditionally used dietary sources (Wang et al., 2014).

The genus Garcinia is an important source of bioactive secondary metabolites such as polyprenylated benzophenones, flavonoids, and proanthocyanins that have proven effective against diseases such as peptic ulcer, urinary tract infections, and tumors (Martins et al., 2008). For example, the fruit peel of *G. cambogia* has been shown to have an anti-obesity effect and anti-lipidemic effect (Chuah, Ho, Beh, & Yeap, 2013), inhibiting the cytoplasmic lipid accumulation, as well as adipogenic differentiation of preadipocytes (Kim, Kim, Kwon, & Park, 2004). Moreover, water-soluble calcium hydroxycitrate, as *G. atroviridis*, has been used for weight control of obese women (Roongpisuthipong, Kantawan, & Roongpisuthipong, 2007), while *G. indica* (hydroxycitric acid) has been used as a weight loss supplement for obese patients (Onakpoya, Hung, Perry, Wider, & Ernst, 2011). The species *Garcinia brasiliensis* (Mart.), also known as *Rheedia brasiliensis* Planch and Triana, is native to the Amazon region and is cultivated throughout Brazil. In Brazil it is popularly known as bacupari, bacuri, porocó and bacuripari (Martins et al., 2008). The extract of bacupari peel was studied as anti-inflammatory and antioxidant (Martins et al., 2008; Santa-Cecília et al., 2011), antimicrobial (Naldoni et al., 2009), and antinociceptive (Santa-Cecília et al., 2011) therapies. However, no evidence is available to prove the potential of bacupari peel to assist in adipogenesis regulation. In the present study, we investigated the potential of ethanol extract of bacupari peel to reduce obesity complications in Wistar rats with high-fat diet induced obesity.

2. Material and methods

2.1. Plant material, preparation of the extract and characterization

2.1.1. Plant material

The fruits of *G. brasiliensis* (Mart.) were collected on the *campus* of the Federal University of Viçosa-MG, Brazil in February (summer) of 2011. Botanical identification was performed in the Horto Botânico of the Federal University of Viçosa by Dr. João Augusto Alves Meira Neto. A voucher specimen (number VIC2604) was deposited at the Herbarium of Federal University of Viçosa.

2.1.2. Sample preparation and extraction

The extract was obtained according to Castro et al. (2015). Briefly, peel of *G. brasiliensis* was first dried in an oven with a circulating air temperature of 40 °C for eight days and then pulverized (1 kg) and subjected to extraction by re-maceration in absolute ethanol. The ethanolic extract of bacupari peel (EEB) was lastly concentrated under reduced pressure.

2.1.3. Chromatographic analysis and quantification

The chromatographic analysis of the extract and the quantification of their isolated molecules present in the extract were performed on a Liquid Chromatography device (Shimadzu HFLC 20) using an NST column (Nano Separation Technologies) C18-154 605 (150 × 4,6 mm; 5.0 mm in particle size) 24 (24). The mobile phase consisted of a mixture of acetic acid (5 mM) (eluent A) and methanol/acetic acid (0.1% v/v) (eluent B). The injection volume was 20.0 μL at a flow rate of 1.0 mL/min. During the initial 10 min, an analysis was performed using 50.0% of eluent B followed by an increase in the concentration of eluent B to 100.0% over 20 min. The analysis continued for 30 min at a concentration of 100.0% eluent B. After the analysis with eluent B was concluded, the column was prepared for the next analysis. Chromatograms were obtained at 254 nm and the peaks were compared with the peaks of the compounds that had been previously isolated in the laboratory (Castro et al., 2015).

2.1.4. Determination of total flavonoids

In a 10 mL test tube, 0.5 mL of 20% (w/v) BEE, 1.5 mL of ethanol, 0.1 mL of 10% (w/v) $AlCl_3{\cdot}6H_2O$ and 0.1 mL of 1 M potassium acetate were combined and mixed. The volume was then brought up to a final volume of 5.0 mL with H_2O. After 30 min, the mixture was measured at 425 nm. The standard curve for total flavonoids was generated with a quercetin standard solution (25–120 g/mL), using the same procedure as above. The total flavonoids were determined as quercetin equivalents (mg quercetin/g extract), and the values are presented as the means of triplicate analyses (Castro et al., 2015).

2.2. Dietary intervention, biometric measurements, and plasma and tissue collection

Adult male Wistar rats (180–220 g) obtained from the Central Animal Facility of the Federal University of Viçosa were housed under controlled light (12:12 h light-dark cycle; lights on at 06:00 am) and temperature conditions (22 ± 1 °C) with access to water *ad libitum*. The animals were kept in a specific pathogen-free facility and acclimated to their housing environment for one week prior to the experiment. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals.

In Phase I, the animals were divided into 3 experimental groups (n = 8): a negative control group which was fed AIN-93M diet, while the other two groups received a high fat diet (HFD) in the formulation (Table 1) of RESEARCH DIETS®. (2006) and distilled water *ad libitum* for 7 weeks to induce obesity. Weight gain and food consumption were monitored weekly. At the end of this phase, the rats were fasted for 12 h to collect blood samples by caudal puncture. Analysis of glucose and triglycerides levels were performed to calculate the biometric indicators and to confirm the effect of HFD.

At the beginning of Phase II, the two groups fed with the HFD were relocated to have similar biometric and biochemical indicators. The negative control (AIN-93M) and one of the High Fat Diet (HFD) groups were maintained under the same food conditions as in phase I, while the last group was introduced to bacupari peel extract (BHFD) in the high fat diet (positive control - HFD) for 8 weeks. The weight and food consumption were monitored weekly. The extract was incorporated into the HFD at a dose of 300 mg/animal that corresponding to 42 mg/kg and 10.76 mg/kg of 7-epiclusianone and morelloflavone, respectively, based on the previously demonstrated anti-oxidant and anti-inflammatory activity of these compounds (Castro et al., 2015; Santa-Cecília et al., 2011).

At the end of the experiment, the animals were anesthetized (isoflurane using 100% Isoforine, Cristália®) and euthanized by cardiac puncture. Blood samples were collected for biochemical analyses. The abdominal and epididymal adipose tissues were removed and weighed for later calculations of biometric measurements, frozen in liquid nitrogen, and stored at a temperature of -80 °C until further analysis. Lee index was calculated by the relationship between the cube root of the body weight and the nose-to-anus length. For the calculation of adiposity index, the abdominal and epididymal adipose tissue weights were added and the result was divided by total body weight and multiplied by 100. Food efficiency was calculated by dividing body-weight gain by energy intake (in kcal), and multiplying the result by 1000.

Table 1
Composition of the experimental diets (g/100 g).

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
Lard	0	–	20	180
Cellulose	5	–	5	–
Mineral mix	3.5	–	3.5	–
Vitamin mix	1	–	1	–
Bitartarate 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	–
EEB**			300	

*BHT: butylated hydroxytoluene; CD: caloric density; EEB: ethanolic extract of bacupari peel **dose: mg/kg animal (incorporated in phase 2).

2.3. Biochemical analyses

Creatinine, uric acid, fasting glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL) was assessed (Cobas Roche Diagnostic, Basel, Switzerland) in the Laboratory of Clinical Analysis of the Health Division of UFV, Viçosa, MG, Brazil .

The oral glucose tolerance test (OGTT) was performed one week prior to euthanasia. After 12 h of fasting, glucose solution at a concentration of 2 g·kg^{-1} body weight was administered by gavage. Blood samples were collected by puncturing tail vein of each conscious rat sequentially before and after OGTT. Blood glucose was measured in an Accutrend® GCT device at 0, 30, 60, 90 and 120 min.

2.4. Plasma total antioxidant capacity – TAOC

Plasma TAOC was measured using the Azinodiethylbenzthiazoline sulphate (ABTS; Sigma-Aldrich) method (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993). In this assessment, incubation of ABTS with H_2O_2 and a peroxidase (metmyoglobin) resulted in the production of the blue–green radical cation ABTS, which was measured at 405 nm. Antioxidants in test plasma resulted in the suppression of this color production which was proportional to their concentration. The system was standardized using Trolox, a water-soluble vitamin E analogue. The results were expressed as mM Trolox.

2.5. Quantification of cytokines by flow cytometry

The anti and pro-inflammatory cytokine, Interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α), respectively, were quantified in the plasma of the animals using the Cytometric Beads Array Kit (CBA) from BD® brand (CA, USA). Beads population with distinct fluorescence intensities were combined with a capture antibody specific for each cytokine and were mixed to form the CBA. In the CBA, the cytokine capture beads were mixed with the detection antibody conjugated to the fluorochrome PE and then incubated with the test samples to form the "sandwich". Test tubes used in the analysis were prepared with 50 μL of sample, 50 μL of beads and mixing with 50 μL of the detection reagent. A standard curve was obtained by performing the same procedure. The tubes were homogenized and incubated for two hours at 22 °C in the dark. One mL of wash buffer was added and centrifuged. The supernatant was discarded. Mix in the vortex briefly to resuspend the bead, and read on FACSVerse flow cytometer (BD®). The results were obtained with the FACSuite software (BD®) and analysed by FCAP Array V3 software (BD®).

2.6. Gene expression levels in adipose tissue

The total RNA extraction was performed using Trizol Reagent (Invitrogen, CA, USA) and a Mirvana™ miRNA Isolation Kit (Ambion® by Life Technologies™) used according to the manufacturer protocols. The RNA concentration and purity were evaluated by μDrop plate spectrophotometer Multiskan™ GO (Thermo Scientific, DE, USA), and the integrity was confirmed by electrophoresis agarose gel. A M-MLV Reverse Transcriptase Kit (Invitrogen) was used for cDNA synthesis. Relative quantification of gene expression was performed by RT-qPCR using the equipment AB StepOne Real Time PCR System and the reagent Fast SYBR Green Master Mix (Applied Biosystems, CA, USA). The initial parameters used in the run were 20 s at 95 °C (203°F) and then 40 cycles at 95 °C (3 s), 60 °C (30 s) followed by melting curve analysis. The primers used for amplification are as follows: FAS (fatty acid synthase): AGCCCCTCAAGTGCACAGTG (forward) and TGCCAATGTGTTTT CCCTGA (reverse); LPL (lipoprotein lipase): CAGCTGGGCCTA ACTTTGAG (forward) and CCTCTCTGCAATCACACGAA (reverse); PPAR-γ (peroxisome proliferator-activated receptor gamma) CATTTCTGCTCCACACTATGAA (forward) and CGGGAAGGACTT-TATGTATGAG (reverse); and housekeeping gene GAPDH (Glyceraldehyde-3-phosphate dehydrogenase): AGGTTGTCTC CTGTCACTTC (forward) and CTGTTGCTGTAGCCATATTC (reverse); The indicators were designed with the use of Primer3 plus program (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi), which meets requirements for real time PCR.

2.7. Molecular modeling

All computer applications were run on Unis CentOS 5.0. Structures of ligands 7-epiclusianone, morelloflavone and rosiglitazone (standard drug) were constructed using Maestro 9.2 (Maestro, Version 9.2; Schrödinger, LLC, New York, NY, USA). The software LigPrep 2.5 (LigPrep, Version 2.5; Schrödinger, LLC) was used for the construction and preparation of the ligands involved in this studies. The crystallographic structure of Peroxisome proliferator-activated receptor-gamma (PPAR-γ) (Protein Data Bank [PDB] ID: 4Y29) was obtained from the database PDB and the software Prime 3.0 (Prime, Version 3.0; Schrödinger, LLC) was used for the preparation of this receptor. The OPLS 2005 force field in the MacroModel 9.9 (MacroModel, Version 9.9; Schrödinger, LLC) was used for

optimization. Studies of molecular docking between PPAR-γ and the ligands were performed using the program Induced Fit Docking (Induced Fit Docking, Version 9.9; Schrödinger, LLC]. All computer programs belong to the Schrödinger suite.

2.8. Histological study of adipose samples

Visceral fat collected after euthanasia was separated and weighed. The tissues were fixed with 10% formaldehyde. Visceral fat was immersed in paraffin. Sections were cut at a thickness of 5 μm, mounted on glass slides, and stained with hematoxylin and eosin. The slides were mounted with Entellan (Merck®) and analysed under a light microscope (Nikon Phase Contrast 0.90 Dry, Japan). The images of histological sections were captured with a 20X objective. Diameter, circumference, and area of adipocytes were analysed with a software program (Image-Pro Plus 4.5).

2.9. Statistical analysis

Data were analysed using the GraphPad software program Version 5.0 (GraphPad Software, San Diego, CA) and expressed as the mean ± S.D. Statistically significant differences between the groups were calculated using analysis of variance (ANOVA) followed by the Newman-Keuls or Dunnett test. The blood glucose area under the curve (AUC) was calculated using the trapezoidal rule. P–values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Chemical characterization

3.1.1. Chromatographic analysis, limit of detection and quantification of the G. brasiliensis EEB and standards

An analysis of the chromatogram is displayed in Fig. 1A, B and C. The retention times and areas and the quantification of the isolated compounds in the extract are shown in Fig. 1D. Chemical analysis of the bacupari peel extract identified 7-epiclusianone as the major constituent (140.02 mg/g) followed by morelloflavone (35.86 mg/g) (Fig. 1A and D). These peaks may be compared with the standards of these compounds (Fig. 1B and C).

3.1.2. Flavonoid level

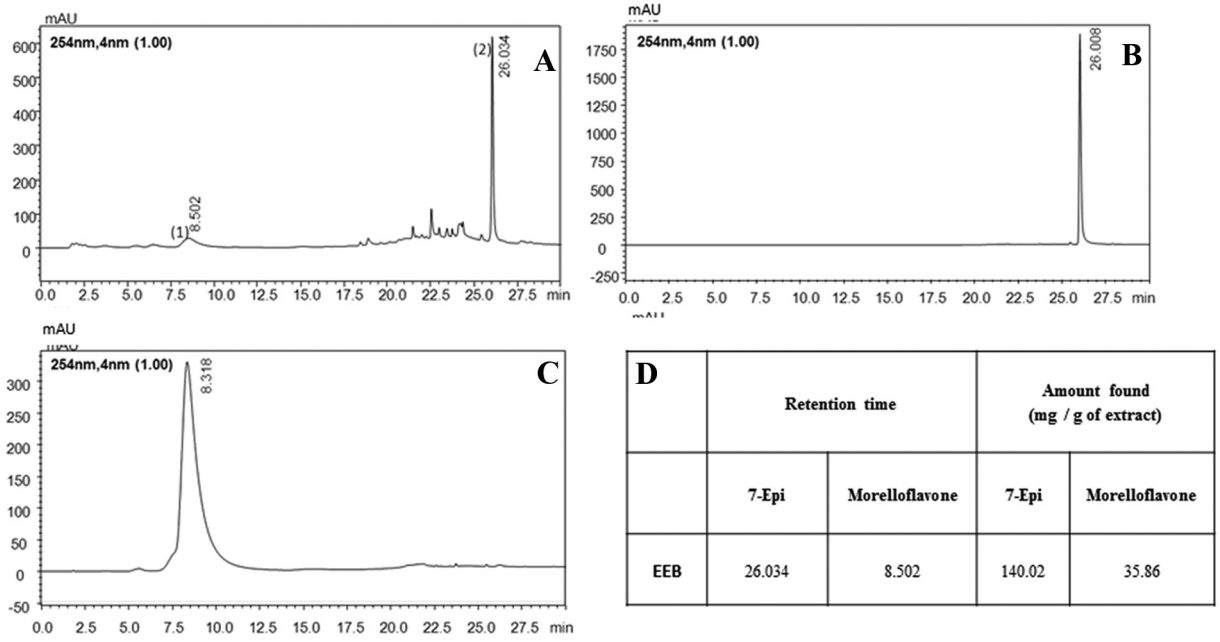
The total flavonoids of the bacupari peel extract of *G. brasiliensis* was determined from the calibration curve regression equation and expressed in terms of quercetin/gram (querc/g) of extract. The total amount of flavonoid found in the ethanolic peel extract of *G. brasiliensis* was 22.52 mg in querc equivalents/g of extract.

3.2. Effect of diets on body weight, food intake, biometric indicators, biochemical variables, and TAOC

At the end of eight weeks, the HFD group increase weight gain ($p < 0.05$) when compared to the AIN-93M and BHFD groups, while the weight gain of treated animals with AIN-93M and BHFD were similar (Table 2). However, the weekly weight did not show difference between BHFD and HFD groups (Fig. 2A). With regard to consumption, the HFD and BHFD groups consumed a lower amount of the diet than the AIN-93M group ($p < 0.05$) since the energy density of the AIN-93M diet was smaller than the high fat diet. However, the intake of calories was similar among groups ($p > 0.05$) (Table 2). After treatment with bacupari peel extract, the food efficiency of the BHFD group was similar to that of the AIN-93M. However, it was lower than the HFD ($p < 0.05$) group. The adipose tissue, adiposity, and Lee indexes decreased at BHFD group compared to HFD group.

Means and standard deviations of biochemical variables of all groups are expressed in Table 2. The group treated with AIN-93M and BHFD showed a difference in fast serum glucose, uric acid, and ALT when compared with the positive control (HFD). However the cholesterol, HDL cholesterol, creatinine, and AST did not differ.

Fig. 2B and C shows the results of the OGTTs performed after 8 weeks of high-fat feeding. The blood glucose increased in the first



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	Retention time		Amount found (mg / g of extract)	
	7-Epi	Morelloflavone	7-Epi	Morelloflavone
EEB	26.034	8.502	140.02	35.86

Fig. 1. Chromatograms obtained by high performance liquid chromatography: (A) The chromatogram of the *G. brasiliensis* peel extract, demonstrating the separation of morelloflavone (1) (8.502 min) and 7-epiclusianone (7-epi) (2) (26.034 min). (B) The standard 7-epi chromatogram showing the retention time (26.008 min). (C) The standard morelloflavone chromatogram of A, demonstrating the retention time (8.318 min). (D) The retention times and areas and the quantification of the 7-epiclusianone and morelloflavone in the extract.

Table 2
Mean and standard deviation of total food intake, biometric index and biochemical parameters in rats during experiment.

	AIN-93	HFD	BHFD	Reference values[1]
Food intake (kcal/phase II)	4728.34 ± 292.21[a]	4485.49 ± 216.63[a]	4388.00 ± 155.60[a]	
Food intake (g/day)	22.21 ± 1.37[a]	17.41 ± 0.90[b]	17.03 ± 0.92[b]	
Food efficiency (g/kcal)	13.85 ± 3.40[b]	22.56 ± 4.25[a]	15.73 ± 3.73[b]	
Body-weight gained (g)	65.00 ± 13.51[b]	100.00 ± 15.27[a]	68.14 ± 16.17[b]	
Adipose tissue (g)	21.03 ± 9.17[c]	45.15 ± 13.66[a]	34.17 ± 4.58[b]	
Adiposity (%)	4.27 ± 1.44[b]	8.97 ± 2.30[a]	6.94 ± 0.81[b]	
Lee index (g/cm3)	0.28 ± 0.01[b]	0.30 ± 0.01[a]	0.28 ± 0.01[b]	
Fasting serum glucose (mg/dL)	114.5 ± 8.20*	135.80 ± 8.18	120.30 ± 7.80*	79–144
Triacylglycerol (mg/dL)	64.40 ± 9.84	74.00 ± 22.28	81.20 ± 15.12	42–160
Cholesterol (mg/dL)	68.17 ± 8.42	65.50 ± 11.33	56.57 ± 6.16	55–79
HDL cholesterol (mg/dL)	23.20 ± 1.30	21.83 ± 4.62	21.60 ± 2.07	16–54[#]
Creatinine (mg/dL)	0.58 ± 0.10	0.61 ± 0.08	0.63 ± 0.10	0.44–0.64
Uric acid (mg/dL)	1.52 ± 0.40*	2.45 ± 0.23	1.82 ± 0.09*	0.9–2.0
AST (mg/dL)	150.40 ± 47.40	158.40 ± 51.75	120.70 ± 27.56	81–180
ALT (mg/dL)	47.83 ± 7.39*	224.75 ± 78.77	56.71 ± 23.22*	36–58

[1]Melo, Doria, Serafini, & Araujo, 2012; [#]Spinelli, Cruz, Godoy, Motta, and Damy (2014). Each value is expressed as mean ± standard deviation (n = 7). Food efficiency = body-weight gain by energy intake x 1000; AST: aspartate aminotransferase; ALT: alanine aminotransferase. [a,b,c]Different lower case letters in in the rows indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman-Keuls test; *biochemical parameters: Dunnett's test compared HFD ($p < 0.05$).

60 min after intake of glucose (Fig. 2B), differing among the groups. The mean values of blood glucose in the AIN-93M and BHFD groups were lower than in the HFD group in times 60 and 90 min ($p < 0.05$). However, after 120 min of intake, the values were similar ($p > 0.05$). The oral glucose tolerance tests of the HFD group showed significantly greater area under the curve (AUC; Fig. 2C) compared to the AIN-93M and BHFD groups ($p < 0.05$), and the AUC of BHFD group was similar to the AIN-93M group ($p > 0.05$; Newman-Keuls). Fig. 2C shows the plasma antioxidant capacity of the animals that received a high fat diet. The AIN-93M and BHDF groups demonstrated higher antioxidant capacity when compared to the control group - HFD (129 and 92%, respectively). In addition, there were not significant differences in the antioxidant capacity between the AIN-93M and BHFD groups. Fig. 2E shows the daily consumption of the morelloflavone (10.60 ± 0.55 mg/kg weight/day) and 7-epiclusianone (41.38 ± 2.14 mg/kg weight/day) by BHFD.

3.3. Cytokines and gene expression levels in adiposity tissue

The TNF-α act by inhibiting lipogenesis (via inhibition of the expression of LPL, GLUT4, and acetyl CoA synthetase), and increasing lipolysis which leads to a change of the number or volume of adipocytes (Warne, 2003). The IL-10 is an anti-inflammatory cytokine and is produced by M2 macrophages in the adipose tissue. Their main function appears to be regulation of the immune system, significantly inhibiting the expression/synthesis of cytokines or adipokines pro-inflammatory by negative counter regulation (Galic, Oakhill, & Steinberg, 2010). The TNF-α levels were significantly decreased in animals treated with BHFD and AIN-93. Moreover, the group treated with BHFD and AIN-93M showed increased levels of IL-10 when compared to the HFD group (Newman-Keuls test; $p < 0.05$) (Fig. 3A).

Expressions of lipogenesis-related gene (FAS), fatty acid uptake-related gene (LPL and PPAR-γ) in epididymal adipose tissue were measured by RT-qPCR (Fig. 3B, C and D). The increased PPAR-γ (Fig. 3D) and LPL (Fig. 3C) gene expression in treated group with BHFD was 1.24 and 1.38-fold higher than in negative control (respectively), while the group treated with HFD was reduced 0.14 and 0.27-fold (PPAR-γ and LPL, respectively). On the other hand, the group treated with HFD increased of gene expression of the FAS (2.68-fold) when compared to the AIN-93M group (Fig. 3B; $p < 0.05$), however the BHFD group was similar to the negative control group.

3.4. Molecular modeling

Molecular Docking of the prenylated benzophenone 7-epiclusianone and biflavonoid morelloflavone and the standard drug rosiglitazone with PPAR-γ receptor presents docking score values of −12.847, −14.514 and −9.931 kcal·mol^{-1} (Table 3), and the main interactions are shown in Fig. 4(A, B, C, D, E and F), demonstrating that the interaction can lead to the agonist receptor activity. Fig. 4A, B and C represent the results of molecular docking in 2D format of 7-epiclusianone, morelloflavone and rosiglitazone, respectively, with amino acids of PPAR-γ. Fig. 4D, E and F present the representation of the best poses of these molecular docking of three compounds evaluated. Fig. 4G and H show the mains groups of the 7-epiclusianone (G) and morelloflavone (H) related with the biological activity.

3.5. Histological study of adipose samples

Fig. 5 shows the measure of the area (Panel A), diameter (Panel B), and perimeter (Panel C) of epididymal adipose tissue cells and epididymal adipose tissue photomicrographs. The measures of the adipocytes were lower in the groups treated with AIN-93M and BHFD compared to HFD group. In photomicrographs (Panel D) hypertrophy of adipose cells of the HFD group was observed compared to groups treated with bacupari peel extract and negative control group.

4. Discussion

Research concerning new natural compounds addressing the management of obesity is increasing due to their low side effects compared with conventional pharmacological agents. In this study we evaluated the properties of bacupari peel extract to modulate fat and inflammation in rats fed with a high-fat diet.

High fat diet-induced obesity has been considered the most popular model among researchers due to its high similarity of mimicking the usual route of obesity episodes in humans and also because it is considered as a reliable tool for studying obesity since the animals will readily gain weight when fed high-fat diets (Guerra et al., 2015). In the present study, the administration of ethanolic extract from bacupari peel in the diet showed effect to control obesity in obese rats during intake of a HFD. The group treated with BHFD presented a higher total food intake (g/day) compared to the AIN-93M group and similar to the HFD

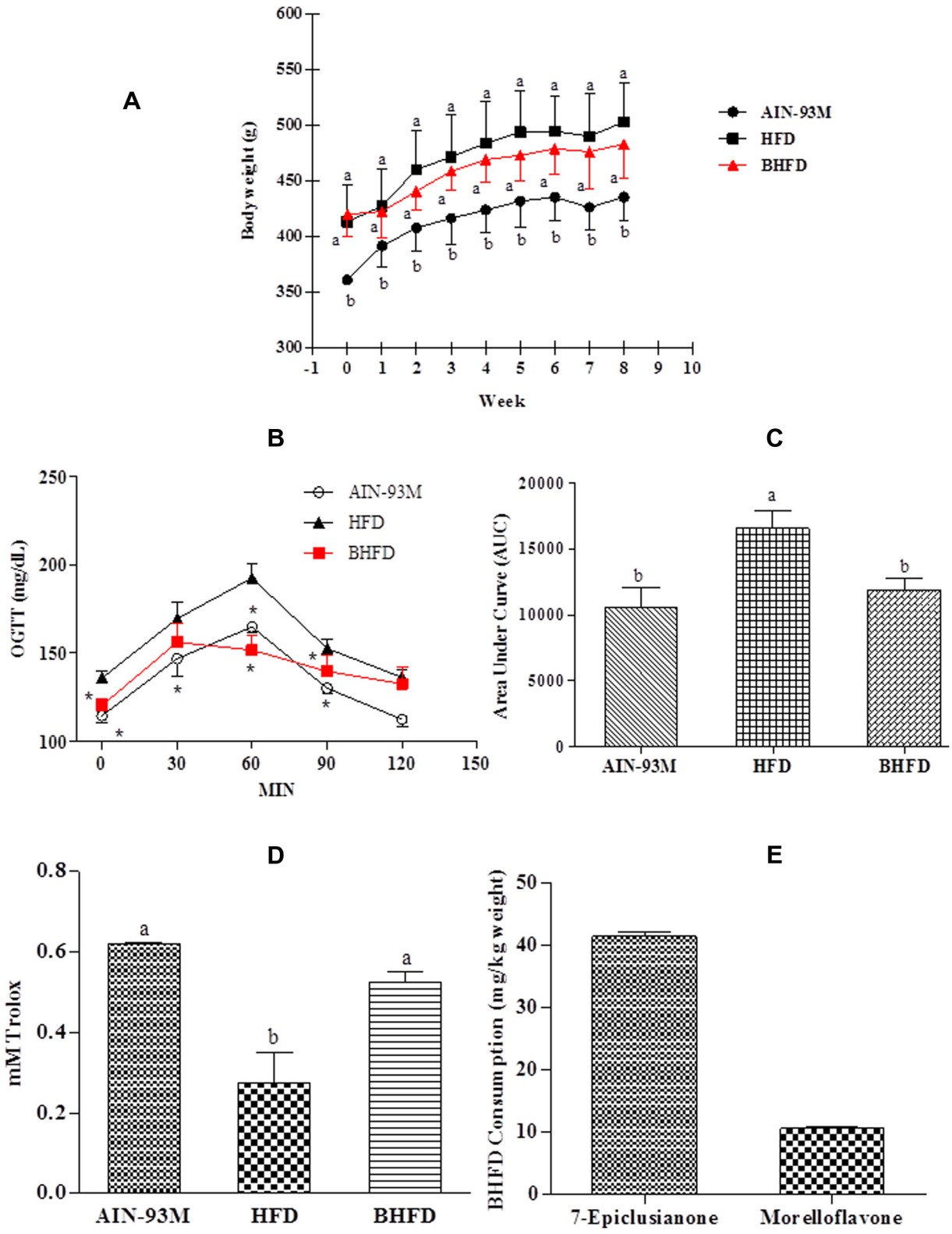

Fig. 2. The weekly body weight for the 8 weeks (Panel A). The oral glucose tolerance test (OGTT) measured at times 0, 30, 60, 90 and 120 min after the start of the test (Panel B), and values of area under the curve AUC obtained in the OGTT blood glucose values measured at times 0, 30, 60, 90 and 120 min after the start of the test (Panel C). The activity of plasma total antioxidant capacity – TAOC of rats treated with standard diet (AIN-93M), high fat diet (HFD), high fat diet + bacupari peel extract (BHFD) (Panel D) and consumption of the compounds with antioxidant activity present in bacupari peel extract (Panel E). Each value is expressed as mean ± standard deviation (n = 5). *Dunnett's test compared to the HFD ($p < 0.05$). a,b Different letters indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman-Keuls test.

group. However, the BHFD group showed body-weight gain and food efficiency similar to AIN-93M group and lower than the HFD group. This observation suggests that an increase in body weight is independent of the amount of food consumed by the animals, in agreement with Guerra et al. (2015). In addition, the group treated with BHFD remarkably decreased the weight of adipose tissue, adiposity, and lee index of rats fed on a high-fat diet.

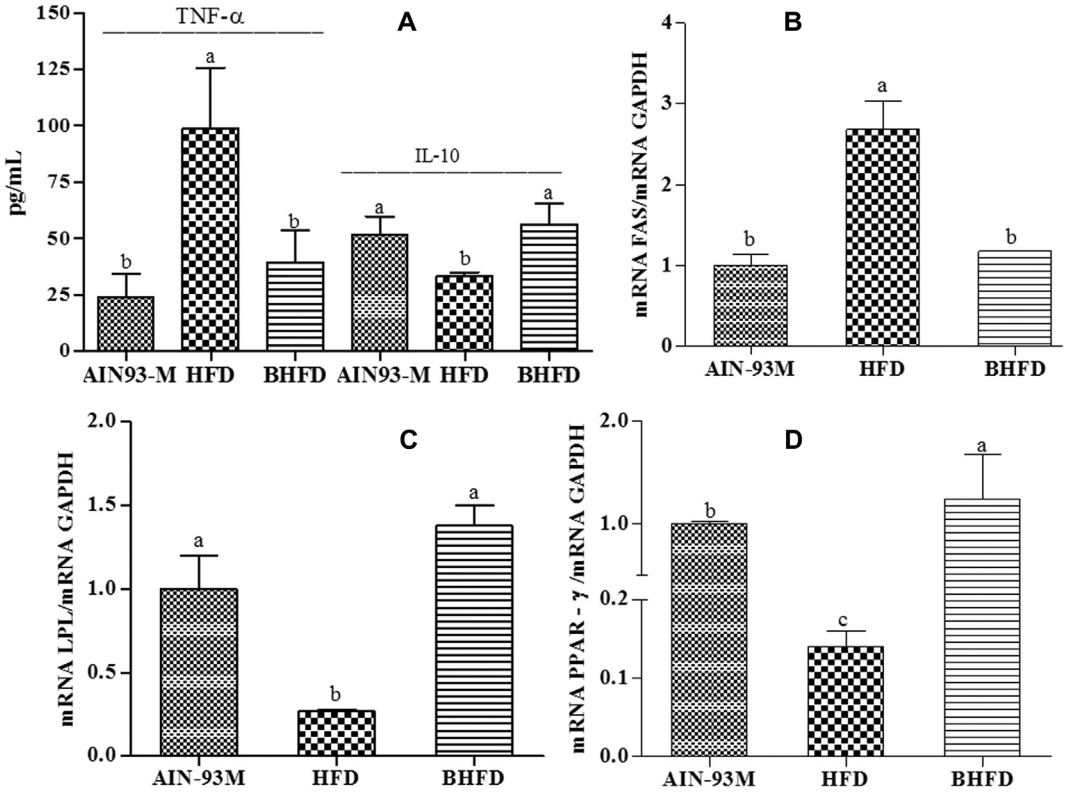

Fig. 3. Concentrations of cytokines (Panel A) and gene expression of FAS (Panel B) and LPL (Panel C) and PPAR-γ (Panel D), normalized with GAPDH. Each value is expressed as mean ± standard deviation (n = 4). a,b,c Different letters indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman-Keuls test.

Table 3
Values of Glide Score (*GScore*), the number of interaction by hydrogen Bonds (*Hbond*) and by van der Waals (*good vdW*) between the ligands and PPAR-γ receptor (Schrödinger Suite, Induced Fit Docking Program).

Ligand	GScore (kcal·mol^{-1})	Hbond	Good vdW
7-Epiclusianone	−12.847	1	569
Morelloflavone	−14.514	2	419
Rosiglitazone	−9.931	2	277

High fat diet consumption showed contribute to diseases such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis (Guerra et al., 2015). The present study demonstrated the increase in fast serum glucose levels in the group treated with HFD and reduction in the group BHFD. The results obtained for the oral glucose tolerance test indicated an increase in blood glucose levels at 30, 60, and 90 min in the HFD group. After 2 h the values were similar among groups. The values of area under the curve confirmed these data where the BHFD group showed a smaller AUC then the HFD group (39%). Evidence in the literature indicates the same that was observed in our study, which high-fat diets result in disturbance in glucose metabolism and impair glucose tolerance (Guerra et al., 2015), by impairing insulin sensitivity possibly due to the reduction of PPAR-γ expression and their target genes (Monsalve et al., 2013). However, bacupari extract favoured glucose metabolism in animals fed high-fat diets.

In addition, the hyperglycemia decreases the functional activity of beta cells, and this effect is associated with an intense oxidative stress (Miyazaki et al., 2007). Furthermore, hyperlipemic diet can modify the metabolism of oxygen because the molecules of fatty acids with double bonds are vulnerable to oxidative reactions that may lead to lipid peroxidation and, consequently, oxidative stress (França et al., 2013). In the present study the plasma total antioxidant capacity increased in the group treated with BHFD and decreased in the group of animals treated with HFD. Intake of foods containing antioxidants can increase the total antioxidant capacity of blood and biological fluids, reducing oxidative stress due to a decrease of cerebral oxidative reactions by the direct removal of free radicals (Wang et al., 2012). The increased plasma total antioxidant activity of animals treated with BHFD may be due to the compounds presented in the *Garcinia brasiliensis* extract, tetraprenylated benzophenone 7-epiclusianone and the bioflavonoid morelloflavone, which the rats showed higher consumption. These compounds showed that antioxidant and anti-inflammatory, antimicrobial, leishmanicidal activities in studies *in vivo* and *in vitro* (Castro et al., 2015; Gontijo et al., 2012; Santa-Cecília et al., 2011), which effects were confirmed in our study.

The group BHFD decreased the concentrations of ALT compared to the HFD group, suppressing the inflammatory process in the liver, which is associated with high fat diet intake. The inhibition of ALT may be due to the suppression of TNF-α, up-regulation of PPAR-γ, and/or via increasing adiponectin (Elmazar, El-Abhar, Schaalan, & Farag, 2013). Moreover, AST did not differ among groups, which can be attributed to half-life of only 12 h and rapidly normalized the blood. Furthermore, AST located in the cytosol and mitochondria, while the ALT located only in the cytosol, which can hinder the detection of AST (Ramaiah, 2007). On the renal function of the rats, the bacupari peel extract produced a significant effect on the uric acid measured in comparison with the control group treated HFD. However, creatinine levels were similar between groups. It is known that uric acid, besides being a biomarker metabolic disorder has a direct effect on oxidative metabolism and endothelial cells (Kramer et al., 2010). Since the bacupari extract decreased uric acid and creatinine remained at normal levels (Melo, Doria, Serafini, & Araujo, 2012), it may suggest the extract

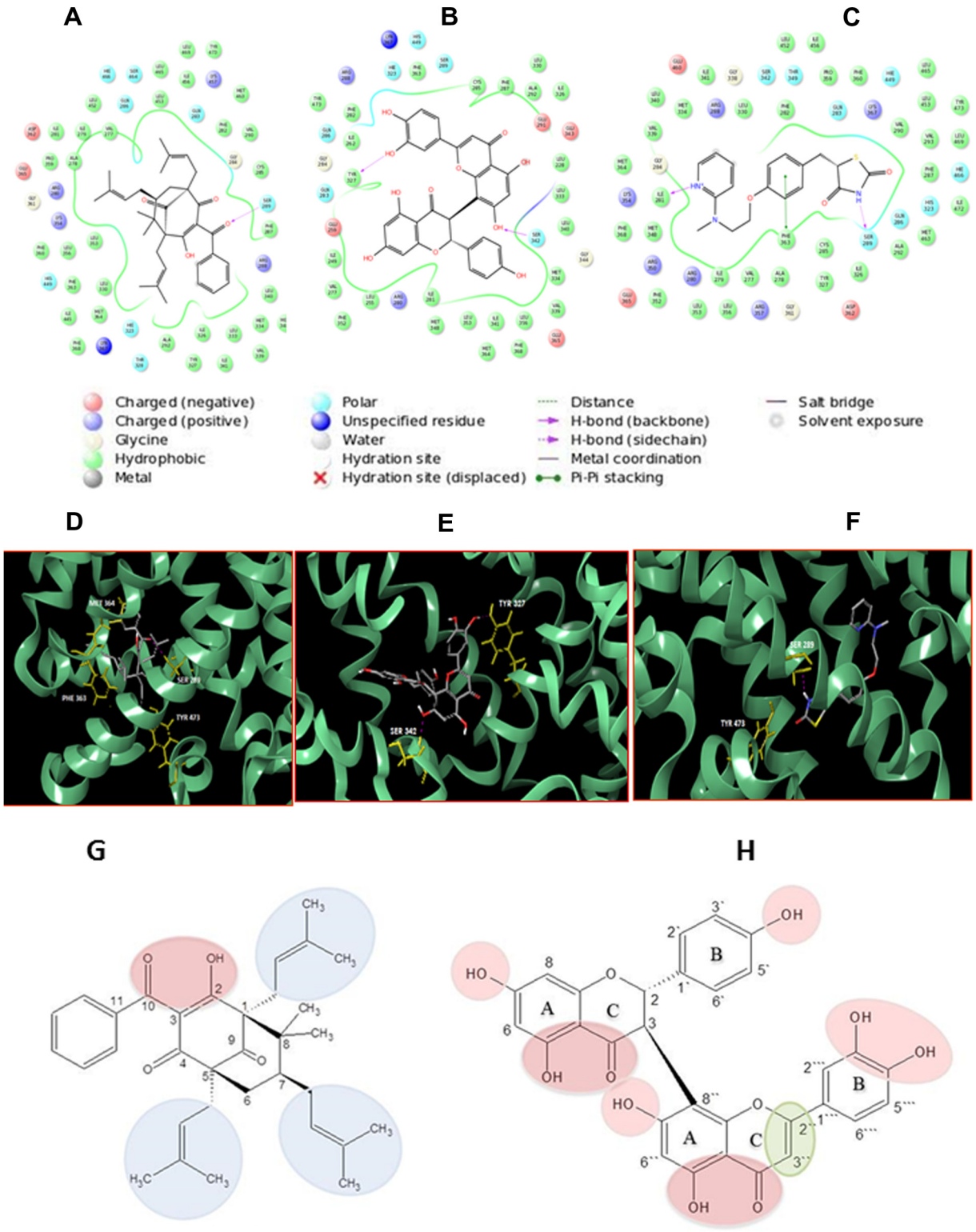

Fig. 4. Interaction between amino acids of PPAR-γ receptor site and 7-Epiclusianone (A), Morelloflavone (B), standard drug - Rosiglitazone (C) and representation of better interaction between 7-Epiclusianone (D), morelloflavone (E), rosiglitazone (F) with PPAR-γ receptor site and main groups of the 7-Epiclusianone (G) and morelloflavone (H) relationship with the biological activity.

is devoid of any effect whether therapeutic or adverse on the renal functions of rats.

In the present study, the hypertrophy of adipose tissue and increase of TNF-α protein in the group HFD was observed, however the group BHFD decreased circulating TNF-α. The excess consumption of saturated fatty acids in our study can explain enhances of white adipose tissue expansion and adipocyte hypertrophy (Kennedy, Martinez, Chuang, LaPoint, & McIntosh, 2009), which induced macrophage infiltration and increased inflammation with increased production of proinflammatory adipokines. In addition,

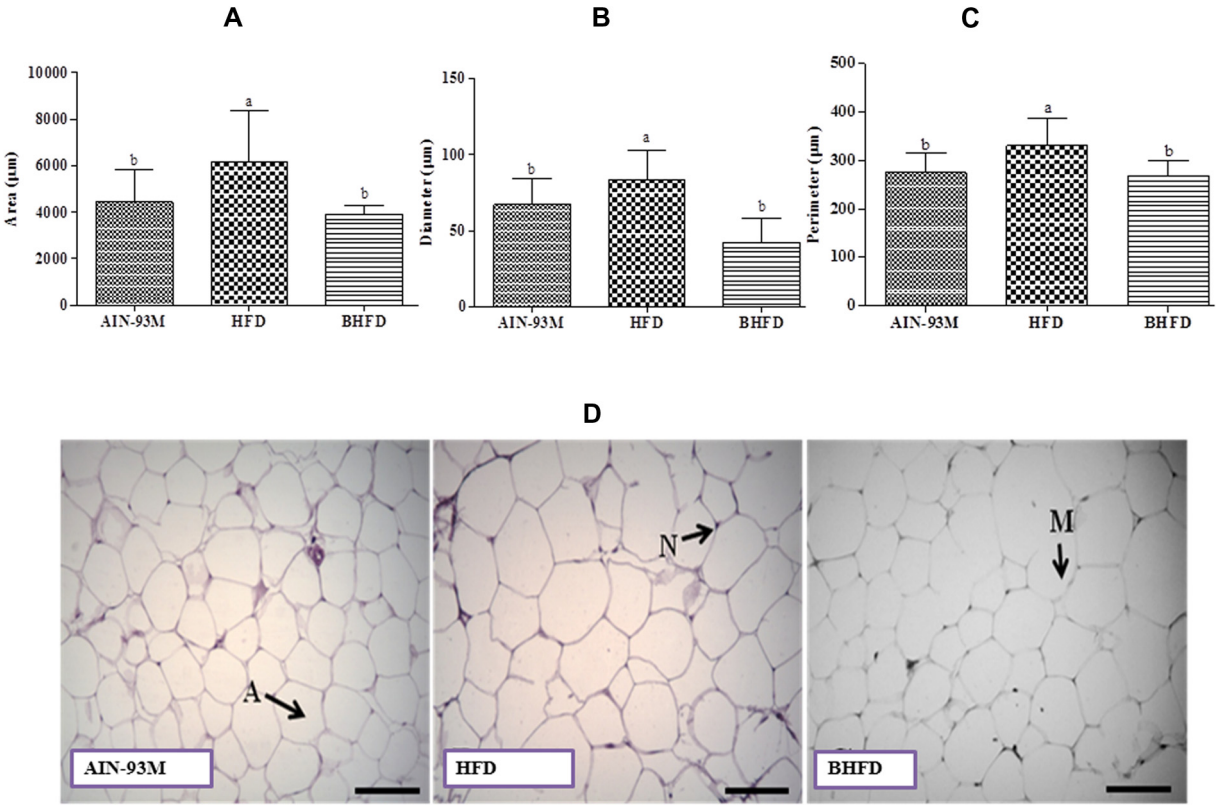

Fig. 5. The measures of area (Panel A), diameter (Panel B) and perimeter (Panel C) of epididymal adipose tissue cells (µm) and epididymal adipose tissue photomicrographs (Panel D) of the differents experimental groups. Coloração HE. Barra de 100 µm. A: adipocyte; N: Nucleo; M: membrane. Means followed by the same letter did not differ by Newman-Keuls test at 5% of probability.

saturated fatty acids have a lower ability to bind transcription factors (PPAR) which explains in part their proinflammatory characteristics such as TNF- α (Arslan, Erdur, & Aydin, 2010; Kennedy et al., 2009). The group BHFD decreased measures diameter, area and perimeter of adipocytes, and IL-10 protein confirming the protective effect of this extract in reverting these effects. The volume of adipose tissue can be reduced by inhibition of adipogenesis, which may be regulated via transcriptional key factors, such as the PPAR-γ (Kong, Kim, Eom, & Kim, 2010), however the PPAR-γ increased in the present study. Corroborating our data, Jang et al. (2015), reported that the expression secretion TNF-α protein increased in obese humans and animals, positively correlated with increased volume of adipocytes. In contrast, the obese rats decreased the levels of anti-inflammatory adipokines, such as IL-10 (Arslan et al., 2010).

In this study, the PPRA-γ mRNA in the adipose tissue was significantly overexpressed in the BHFD group, suggesting receptor activation by bacupari extract without increase adipogenesis. In accordance with Brown and Plutzky (2007), PPAR activation can reduce the inflammatory process involved in the metabolic disorders of obesity. On the other hand, the expression was downregulated by the HFD group. Moreover, TNF-α has been shown to reduce lipoprotein lipase (LPL) activity in adipose tissue. Regulation of LPL by TNF-α occurs at the level of LPL gene transcription and post transcriptionally (Morin, Schlaepfer, & Eckel, 1995). In the present study BHFD showed decreased TNF-α and increased gene expression of LPL. On the other hand, FAS expression decreased in the BHFD group when compared to the HFD group.

Chemical study demonstrated that the genus *Garcinia* is an important source of natural products with a wide variety of biologically active metabolites, including polyisoprenylated benzophenones, flavonoids, xanthones and proanthocyanidins, which have proven to be effective against diseases (Castro et al., 2015). In our study, was identified in the bacupari peel extracts tetraprenylated benzophenone 7-epiclusianone and the bioflavonoid morelloflavone. Molecular docking with these molecules and PPAR-γ receptor showed affinity for the receptor PPAR-γ, with higher docking score than the drug, rosiglitazone. Rosiglitazone is an antidiabetic drug in the thiazolidinedione class of drugs. The mechanism of action of thiazolidinediones involves their binding to the nuclear PPARγ receptors in fat cells and making the cells more responsive to insulin (Chiarelli & Di Marzio, 2008). The morelloflavone and 7-epiclusianone showed highest hydrophobic (van der Waals, eletrostatic and π-stacking interactions) interaction with PPAR-γ, although have been observed a hydrogen bond between the oxygen of phenolic carbonyl of 7-epiclusianone with the serine 289, the same amino acid with which the rosiglitazone also presented an interaction for hydrogen bond. Furthermore, interaction between morelloflavone and PPAR-γ was observed two hydrogen bonds with tyrosine 326 and serine 342. Agonists of the nuclear receptor PPAR-γ are therapeutically used to combat hyperglycaemia associated with the metabolic syndrome and type 2 diabetes. Several PPAR-γ agonists were identified in plants used as culinary spices, beverages or food sources, opening the possibility to consider modulation of the activity of this nuclear receptor through dietary interventions (Wang et al., 2014).

Our results allow us to establish correlations between the biological activity observed with structural characteristics of other compounds described in the literature. The structure-activity relationship (SAR) associated with PPAR-γ and diabetes activity, lipophilicity and structural character of morelloflavone and 7-epiclusianone with prenyl and number/type of hydroxyl groups in the structure have high activity. Thus, the morelloflavone despite has a lipophilic character (Log P 5.22) has seven OH groups

in the appropriate pattern, which enable effective interaction with receptor center. Moreover, the analysis of SAR showed that the flavonoids containing two hydroxyl groups in B ring and 5,7-hydroxyl groups in A ring with C-2, 3 double bond inhibited FAS competitively with acetyl CoA and most likely react mainly on acyl transferase domain (Tian, 2006). The 7-epiclusianone demonstrates more lipophilic character (Log P 10.43) due to the presence of three prenyl groups, one phenyl ring and only one hydroxyl group on C-4. These groups increase the pharmacological activities of some classes of aromatic secondary metabolites, including the anti-inflammatory effect (Alhassan, Abdullahi, Uba, & Umar, 2014). In addition, the presence of hydroxyl chelatogenic may potentiate the hydrophilic activity of the 7-epiclusianone. Then, the molecular interactions promoted by morelloflavone and 7-epiclusianone showed an agonist activity of the bacupari extract in relation to the PPAR-γ receptor, decreasing the inflammation and adiposity in obese rats. In others studies the tetraprenylated benzophenone 7-epiclusianone isolated from the fruits of *G. brasiliensis* (Santos, Nagem, Oliveira, & Braz-Filho, 1999) showed schistosomicidal activity (Castro et al., 2015), and analgesic and anti-inflammatory activities (Santa-Cecília et al., 2011), which its activity have been assigned by benzophenone group. In addition, the morelloflavone obtained from *Garcinia* spp, demonstrated leishmanicidal, antiproteolytic and antioxidant activities (Gontijo et al., 2012).

In conclusion, it was demonstrated that ethanolic extract of bacupari peel (*G. brasiliensis*) presented anti-obesity activities. The anti-obesity effect of EEB was related to the down regulation of lipogenic gene FAS, and TNF-α protein and up regulation IL10 protein, decreasing adipogenese, adipocyte size, body weight, and the bacupari extract have strong agonist activity to the PPAR-γ receptor. Finally, we suggest that ethanolic extract of bacupari peel may be used as a potential therapeutic for obesity management.

Author disclosure statement

No competing financial interests exist.

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