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Bacupari peel extracts (*Garcinia brasiliensis*) reduces the biometry, lipogenesis and hepatic steatosis in obese rats

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ABSTRACT

The aim was to evaluate the effect of the ethanol extract of bacupari peel (EEB) on biometric measurements, hepatic lipogenesis and progression of non-alcoholic fatty liver disease (NAFLD) in obese Wistar rats. 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). Animals treated with high fat diet plus EEB (BHFD) reduced body mass index (BMI), liver weight and hepatosomatic index in relation to the obese control. The food intake was similar between hyperlipid group (HFD) groups with or without EEB. However, the normal control group (AIN-93 M) presented higher food intake and lower final weight compared to the obese control (HFD). The PPAR-α, CPT-1a and the ADIPOR2 genes expressions, and the concentration of the PPAR-α and the adiponectin protein level increased in the BHFD group in relation to the obese control. The EEB promoted reduction of the SREBP-1c gene expression and the percentage of hepatic fat and the degree of steatosis in relation to HFD. It was concluded that EEB showed a protective effect on NAFLD, as it promoted a reduction in BMI, induced lipid oxidation, reduced lipogenesis and hepatic steatosis. Moreover, our results suggest an interaction that can lead to an agonist activity of the EEB to the PPAR-α receptor.

1. Introduction

Obesity results from an imbalance between food intake and energy expenditure, which leads to an excessive accumulation of adipose tissue (Jung & Choi, 2014). The obesity epidemic observed in the past decades has reached alarming proportions. Obesity is a complex, multifactorial, and largely preventable disease, affecting, along with overweight, over a third of the world's population today (Hruby & Hu, 2015). If secular trends continue, by 2030 an estimated 38% of the world's adult population will be overweight and another 20% will be obese (Kelly, Yang, Chen, Reynolds, & He, 2008). Evidences indicates that obesity is associated with an increased risk of metabolic diseases such as insulin resistance, type 2 diabetes, dyslipidemia and nonalcoholic fatty liver disease - NAFLD (Jung & Choi, 2014). NAFLD is considered a public health problem in Western countries and is directly associated with individuals' habits, such as increased consumption of hyperlipidic diets, sedentary lifestyle, and increased incidence of obesity and insulin resistance (Bedogni et al., 2005). The excess body weight can cause insulin resistance in adipose tissue, muscle, and liver. Increased glucose and insulin, caused by insulin resistance, increases lipolysis and consequently circulating lipid levels, thus inducing fat retention by the liver (Rolo, Teodoro, & Palmeira, 2012).

The development of hepatic steatosis is related to metabolic pathways, increasing the release of non-esterified fatty acids from white

Abbreviations: ACC, acetyl-CoA carboxylase; ADIPOR2, adiponectin 2 receptor; AIN-93 M, normal control; ALT, aspartate alanine aminotransferase; BHFD, high fat diet plus EEB; BMI, body mass index; CPT-1a, carnitine palmitoyl transferase; EEB, ethanol extract of bacupari peel; FAS, fatty acid synthase; HFD, obese control; NAFLD, non-alcoholic fatty liver disease; PPAR-α, peroxisome proliferator activated receptor alpha; RT-qPCR, quantitative reverse transcriptase polymerase chain reaction; SREBP-1c, palmitoyl transferase protein; TAG, triacylglycerol

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adipose tissue, increasing the de novo fatty acid synthesis and decreasing β-oxidation. Control and regulation of hepatic lipid homeostasis are performed by key transcriptional regulators such as sterol regulatory element binding protein 1 (SREBP-1c), the peroxisome proliferator activated receptor alpha (PPAR-α), receptor of adiponectin 2 (ADIPOR2) and carnitine palmitoyl transferase (CPT-1α) (Guerra et al., 2015).

PPAR-α regulates the metabolism of lipids, carbohydrates and amino acids and can be activated by ligands such as polyunsaturated fatty acids and drugs used to treat dyslipidemias (Contreras, Torres, & Tovar, 2013). SREBP-1c is a transcription factor that regulates fatty acid biosynthesis by enhancing the transcription of key enzymes, including fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). The expression of this transcription factor is elevated in insulin-resistant individuals, because it is increases lipogenesis, leading to the accumulation of triacylglycerol (TAG) in the liver and the development of hepatic steatosis (Shimomura, Bashmakov, & Horton, 1999).

Adiponectin is a cytokine that sensitizes insulin, stimulating the fatty acid oxidation in the liver and muscles (Guerra et al., 2015). According to Xu et al. (2003), increased levels of adiponectin reduced hepatomegaly, hepatic steatosis and aspartate aminotransferase, which are associated with NAFLD in rats. The improvement of the inflammatory state can stimulate the synthesis of ADIPOR2, a transcription factor, which regulates positively the activation of PPAR-α and CPT-1α, stimulating β-oxidation and reducing lipid biosynthesis (Yamauchi & Kadowaki, 2008).

The genus *Garcinia* (or *Rheedia*) belonging to the Clusiaceae family has been shown by phytochemical studies to possess a great diversity of bioactive compounds such as benzophenones, xanthones and flavonoids, such as 7-Epiclusianone and morelloflavone (Moreira et al., 2017). Many of these constituents are active principles with pharmacological properties against various diseases (Castro et al., 2015; Moreira et al., 2017). Santa-Cecília et al. (2012) reported that bacupari extract presentes anti-inflammatory and antioxidant activity and recent studies by our research group found that Bacupari peel extracts (EEB) has an antiobesogenic effect in obese *Wistar* rats treated with a hyperlipid diet (Moreira et al., 2017). The aim of this study was to evaluate the effect of EEB on the modulation of regulatory pathways related to lipolysis and hepatic lipogenesis in obese rats fed a hyperlipidic diet.

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 Botanical Garden (Horto Botânico) of the Federal University of Viçosa by Dr. João Augusto Alves Meira Neto. A voucher specimen (number VIC26240) 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. Lastly, the ethanolic extract of bacupari peel (EEB) was concentrated under reduced pressure.

2.2. Chromatagraphic profile and determination of flavonoid content of EEB

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 - Shimadzu, Kyoto, Japan) using an NST column (Nano Separation Technologies) C18-154 605 (5.0 µm in particle size; 150 × 4.6 mm). 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 being equilibrated at the initial conditions for 15 min. 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; Moreira et al., 2017).

The flavonoid content was measured using a previously developed colorimetric assay (Kalia, Sharma, Singh, & Singh, 2008). The flavonoid con tent was expressed as mg of quercetin equivalent (QE) per g of extract. Samples were analyzed in triplicate.

2.3. Biological assay and biometric measures

We used 24 male *Wistar* rats, 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 polyethylene boxes, consuming commercial food (Presence/In Vivo®) and distilled water ad libitum. The animals were housed under controlled temperature conditions (22 °C ± 3 °C) and a 12-h light/dark cycle with the light phase starting at 7 o'clock in the morning. On the 61th day, after reaching adulthood, the animals were kept in individual steel cages and randomly and evenly divided into three experimental groups (n = 8): the normal control group received AIN-93 M diet (Reeves, Nielsen, & Fahey JR., 1993) and the other two groups fed with high fat diet (HFD) in the formulation (Table 1) based of RESEARCH DIETS®, and distilled water ad libitum for seven weeks to induce obesity (Moreira et al., 2017).

Next, to start of the treatment with EEB (Table 1), the normal control (AIN-93 M) and one of the High Fat Diet (HFD) groups were maintained under the same food conditions, while the last group was introduced to bacupari peel extract (BHFD) in the high fat diet for 8 weeks. The extract was incorporated into the HFD at a dose of 300 mg/animal (equivalent to ± 674 mg/kg) that corresponds to 42 mg/animal (equivalent to ± 140 mg/kg) and 10.76 mg/animal (equivalent to ± 35.86 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; Moreira et al., 2017; Santa-Cecília et al., 2011). Weight body and food

Table 1
Formulation of the control and test diets.

	AIN-93 M (%)[d]	HFD (%)[e]	BHFD (%)[e]
Soybean oil	4.0	1.15	1.15
Lard	0.0	20.0	20.0
Cholesterol	–	0.15	0.15
Carbohydrate[a]	72.07	54.42	54.42
Protein[b]	14.0	19.5	19.5
Cellulose	5.0	5.0	5.0
Vitamin	1.43	1.43	1.43
Mineral	3.5	3.5	3.5
Caloric density	3.8	4.7	4.7
EEB (mg/kg)[c]	–	–	674

[a] Carbohydrate: maltodextrin, corn starch, saccharose.
[b] Protein: casein.
[c] EEB: ethanolic extract of bacupari peel; dose: mg/Kg. AIN-93 M: standard diet; HFD: High-fat diet; BHFD: HFD plus bacupari peel extract (incorporated in phase treatment of EEB).
[d] Reeves et al. (1993).
[e] Research Diet® (2006).

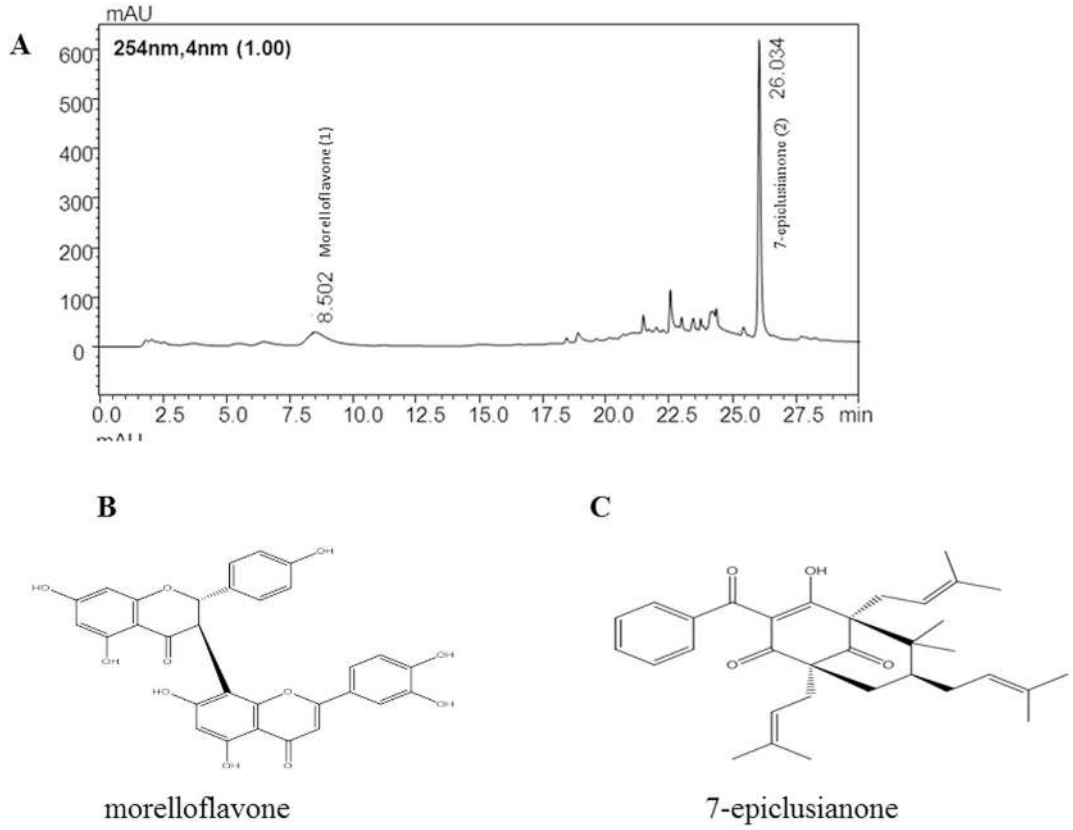

Fig. 1. Chromatograms obtained by high performance liquid chromatography (A) and the chemical structure of 7-epiclusianone (B) and morelloflavone (C).

intake were monitored weekly, during eight weeks.

At the end of the experiment, the animals were subjected to 12 h fasting 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 1009 xg.

for 10 min (Fanem-204, São Paulo, Brazil) to have plasma and serum, respectively. The major samples of the all tissues were immediately frozen in liquid nitrogen and stored at −80 °C before analysis. In addition, 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, Case N° 98/2015.

Liver, heart, cecum and 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.

The energy intake was calculated by multiplying the caloric density of the diet by the consumption in total (g). The feed efficiency ratio (FER) was obtained by the ratio between body weight gain (g) and total diet intake (g). The body mass index (BMI) was determined by the relation between body weight and the nasal–anal length quadratic. Rats with BMI > 0.68 kg/m^2 were considered obese (Novelli et al., 2007; Reynés, García-Ruiz, Díaz-Rúa, Palou, & Oliver, 2014).

2.4. Extraction of mRNA in liver tissue and cDNA synthesis

The tissues were macerated in liquid nitrogen under RNAse free conditions and the samples were aliquoted for total RNA extraction. Total RNA was extracted with a TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using the manufacturer's recommendations. The 2 µL of mRNA extracted was used to synthesize the cDNA using the M-MLV reverse transcription kit (Invitrogen Corp., Grand Island, NY) according to the manufacturer's protocol.

2.5. Determination of gene expression of proteins involved in hepatic lipogenesis and oxidation of fatty acids by quantitative reverse transcriptase polymerase chain reaction (RT-qPCR)

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. Sense and antisense primer sequences (Sigma- Aldrich®) were used to amplify protein sterol regulatory element-binding proteins 1c - SREBP-1c (5′ CGC TAC CGT TCC TCT ATC AAT GAC- 3′; and 5′- AGT TTC TGG TTG CTG TGC TGT AAG- 3′), carnitine palmitoyl transferase I – CPT-1a (5′- GTA AGG CCA CTG ATG AAG GAA GA- 3′; and 5′- ATT TGG GTC CGA GGT TGA CA- 3′); peroxisome proliferator-activated receptor alfa - PPAR-α- (5′ CCT GGC TTC CCT GTG AAC T-3′; and 5′- ATC TGC TTC AAG TGG GGA GA- 3′); and adiponectin receptor 2 - ADIPOR2 (5′- CAT GTT TGC CAC CCC TCA GTA- 3′; and 5′- ATG CAA GGT AGG GAT GAT TCC A- 3′). The relative expression levels of mRNA were normalized by the endogenous control glyceraldehyde 3-phosphate dehydrogenase - GAPDH (5′- AGG TTG TCT CCT GTC ACT TC- 3′; and 5′- CTG TTG CTG TAG CCA TAT TC- 3′). All steps were performed under open conditions with RNase.

2.6. Cytokine and transcription factor

Serum adiponectin concentrations were analyzed by immunoassay using a commercial ELISA specific kit (Adiponectin Cat. # EZRADP-62 K-Millipore®, Billerica, MA). Samples were added on microtiter plate and coated with antibody primary monoclonal Anti-adiponectin and was incubated for 2 h. Afterwards biotin conjugated antibody Horseradish Peroxidase (HRP) - Avidin was incubated for 1 h. The enzyme-substrate reaction finished with sulfuric acid solution addition

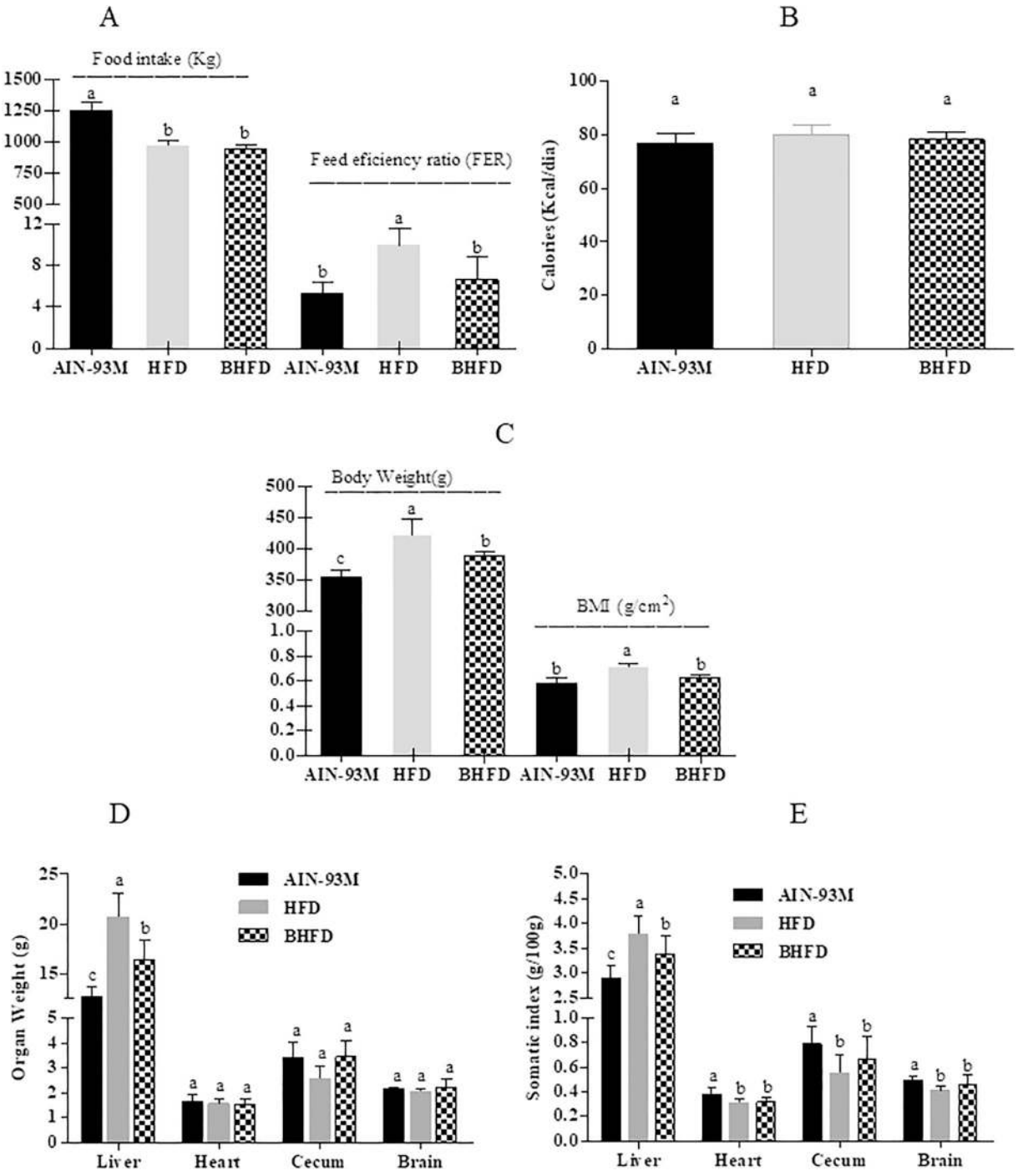

Fig. 2. Food intake and feed efficiency ratio - FER (A), caloric intake (B), body weight and Body Mass Index (C). The organ weigh the liver, heart, brain and cecum (D) somatic index (E) of rats treated with different diets. Each value is expressed as mean ± standard deviation. [a,b,c] Different letters indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman Keuls test. AIN-93 M: lean control group; HFD: obese control group; BHFD: high fat diet + bacupari peel extract.

and color change was determined spectrophotometrically (Awareness®, Stat Fax 2100) in a 450 nm wavelength. The results were determined by comparing absorbance samples with standard curve.

To determine peroxisome proliferator-activated receptor (PPAR-α) hepatic concentrations, hepatic tissue samples were homogenized using NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Scientific Fisher) to separate proteins from nuclear fraction and cytoplasmic, according to manufacturer's instructions. Nuclear fraction PPAR-α was assessed by immunoassay using Elisa PPAR-Rat Kit (Cat # E-EL-R0725-ra; Elabscience, USA), according to manufacturer's recommendations. The microplate provided in the ELISA kit was precoated with a PPAR-α antibody specific. Absorbance was measured spectrophotometrically in a 450 nm wavelength. The PPAR-α concentration on samples was calculated by comparing them with corresponding standard curve.

2.7. Histomorphometric analyses of the liver

The hepatic tissue samples were fixed in resin. Sections of 3 μm of thickness were cut,mounted on glass slides and stained with

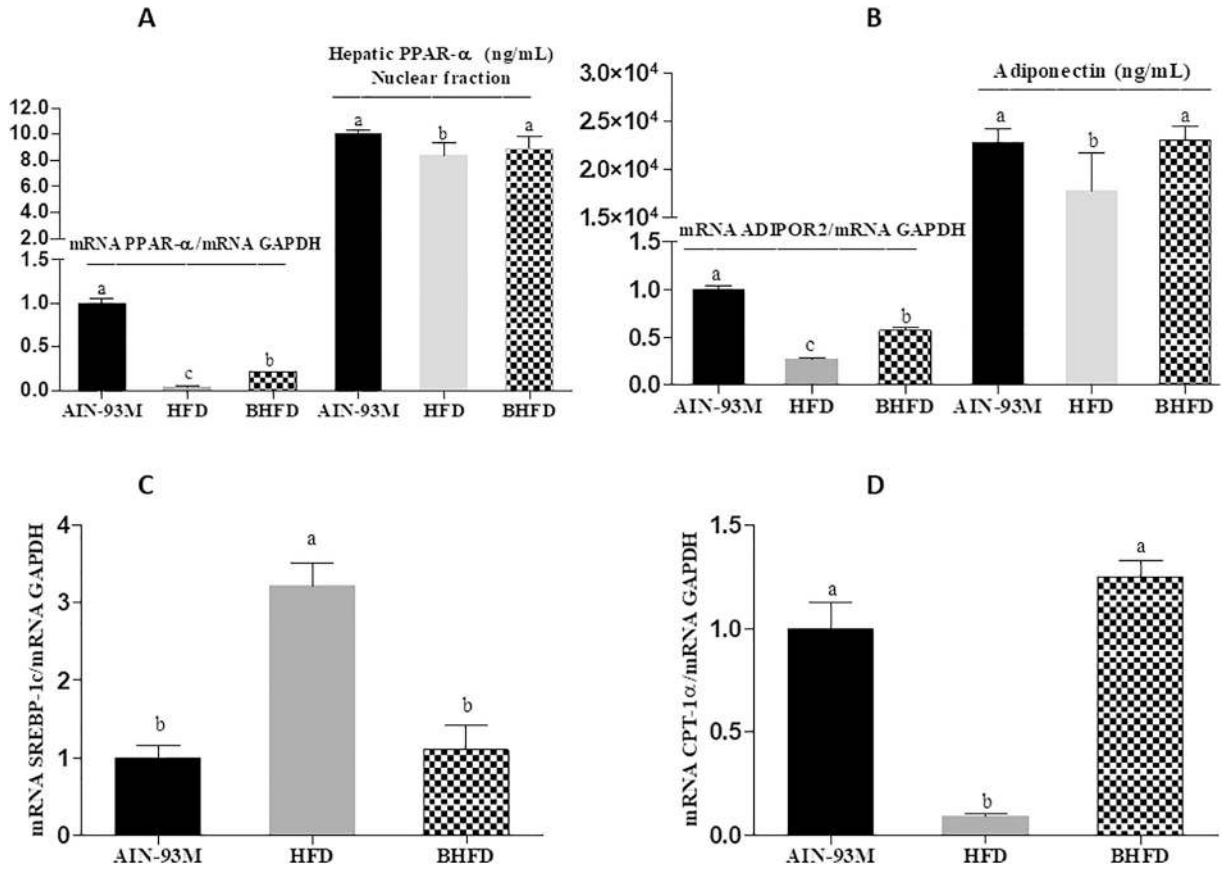

Fig. 3. PPAR-α gene expression and hepatic concentration increased (A), ADIPOR2 gene expression and adiponectin protein level (B), SREBP-1c and CPT-1a genes expression of obese Wistar rats fed with bacupari peel extract in a high fat diet for eight weeks. Each value is expressed as mean ± standard deviation. [a,b] Different letters indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman Keuls test. AIN-93 M: lean control group; HFD: obese control group; BHFD: high fat diet + bacupari peel extract;

hematoxylin and eosin. Glass slides analyzes were performed under a light microscope (Nikon Phase Contrast 0.90 Dry®, Japan) and images were captured using a DIGI-PRO 5.0 M digital camera via Micrometrics SE Premium Software (Accu-Scope®). Images of the histological sections were captured in a 40× objective. Fat vesicles, cytoplasm and nucleus were analyzed using Image J® version 1.5 software (Wayne Rasband). The steatosis degree was evaluated semi quantitatively according to 5 degrees scale: degree 0, if fat percentage was 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).

2.8. Molecular modeling

All computer applications were run on OpenSUS Tumbleweed. Structures of ligands 7-Epiclusianone, Morelloflavone and Cianidine (used as standard drug in this molecular docking studies) were constructed using Maestro 10.2.010 (Maestro, Version 10.2.010; Schrödinger, LLC, New York, NY, USA). The software LigPrep 3.4 (LigPrep, Version 3.4; Schrödinger, LLC) was used for the preparation of the ligands involved in these studies. The crystallographic structure of Peroxisome proliferator-activated receptor-alpha (PPAR-α) (Protein Data Bank [PDB] ID: 3G8I) (Bénardeau et al., 2009) was obtained from the database PDB and the software Protein Preparation Wizard (Schrödinger, LLC) was used for the preparation of this receptor. The OPLS3 force field in the MacroModel 9.9 (MacroModel, Version 9.9; Schrödinger, LLC) was used for optimization. Studies of molecular docking between PPAR-α and the ligands were performed using the Induced Fit Docking program (Induced Fit Docking, Version 9.9; Schrödinger, LLC). All computer programs belong to the Schrödinger suite.

2.9. Statistical analysis

The results were analyzed by ANOVA. For a significant "F-value", the post hoc Newman–Keuls test was used to compare the averages of all experimental groups. The main dispersion was expressed as the standard deviation. Statistical analyses were carried out using GraphPad Prism version 6.01 software. p-values < 0.05 were considered statistically significant.

3. Results

3.1. Chemical characterization

An analysis of the chromatogram and the chemical structure of morelloflavone (1B) and 7-epiclusianone (1C) are displayed in Fig. 1 A, B and C, (respectively). Chemical analysis of the bacupari peel extract identified 7-epiclusianone (2) as the major constituent (140.02 mg/g) (Fig. 1C) followed by morelloflavone (1) (35.86 mg/g) (Fig. 1B). The contents of total flavonoids were 22.52 mg as quercetin equivalents per g of the EEB (Fig. 1D).

3.2. Effect of bacupari extract on food intake and biometric measures of the experimental animals

The EEB added to HFD promoted the daily consumption of the morelloflavone (10.60 ± 0.55 mg/animal) and 7-epiclusianone

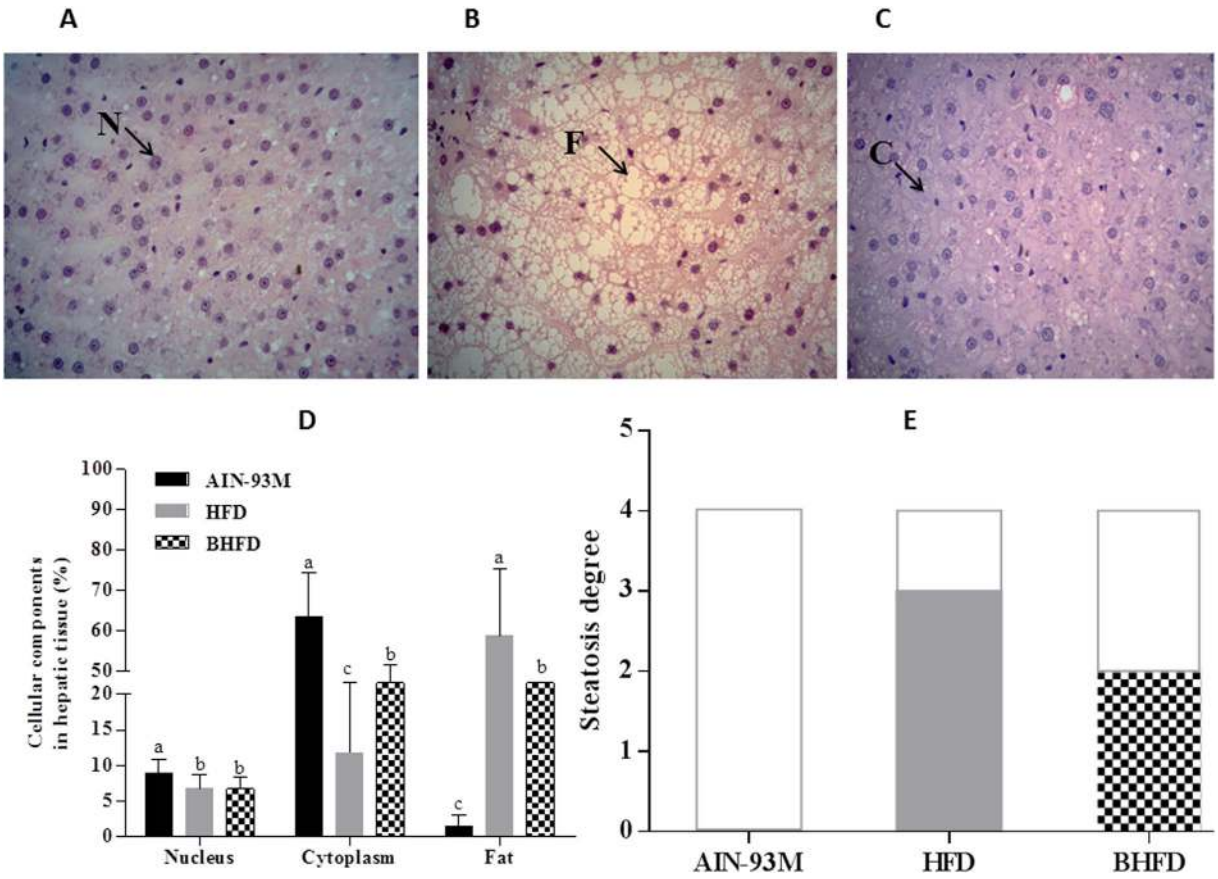

Fig. 4. Hepatic tissue phenotype (A, B, C), cellular components percentage (D), and steatosis degree (E) of obese Wistar rats treated with standard diet (AIN-93 M), high fat diet (HFD), high fat diet + bacupari peel extract (BHFD) for eight weeks. Each value is expressed as mean ± standard deviation. [a,b,c] Different letters indicate statistically significant difference ($p < 0.05$). ANOVA followed by Newman Keuls test. N: Nucleus; F: Fat vesicles; C: Cytoplasm. HE staining. Barr: 20 μm.

Table 2
Values of Glide Score (*GScore*), the number of interaction by Hydrogen bonds (*Hbond*) and by van der Waals (*good vdW*) between the ligands 7-Epiclusianone, Morelloflavone, the standard PPAR-α agonist Cianidine and PPAR-α receptor (Schrödinger Suite, Induced Fit Docking Program).

Ligand	GScore (kcal.mol^{-1})	Hbond	Good vdW
7-Epiclusianone	−11.505	0	649
Morelloflavone	−9.868	3	466
Cianidine	−9.561	3	238

(41.38 ± 2.14 mg/animal) (Moreira et al., 2017). The food intake was similar between HFD groups with or without EEB. However, the normal control group (AIN-93 M) presented higher food intake (Fig. 2A, $p < 0.05$) and lower final weight (Fig. 2C, $p < 0.05$) compared to the hyperlipid group (HFD). Moreover, the caloric intake was similar among experimental groups (Fig. 2B; $P > 0.05$). The feed efficiency ratio (FER) (Fig. 2A) of the obese control group was higher ($p < 0.05$) than the normal control and test groups.

Body mass index (Fig. 2C), liver weight (Fig. 2D) and hepatosomatic index (Fig. 2E) were the lowest ($p < 0.05$) in the BHFD group when compare to obese control. However, the cecum, the heart and the brain weight did not differ between the high fat diet groups (Fig. 2D; $p > 0.05$) but nevertheless, their somatic indices were lower ($p < 0.05$) in obese control and BHFD in comparison to the normal control group (Fig. 2E).

3.3. Cytokines and genes expression involved in lipogenesis and oxidation of fatty acid

The PPRA-α and its target gene, CPT-1a, are responsible for increasing insulin sensitivity, oxidation of free fatty acids and prevention of gluconeogenesis (Guerra et al., 2015). The PPAR-α gene expression and hepatic concentration increased ($p < 0.05$) in the BHFD group in relation to the obese control (Fig. 3A). Besides this, the EEB promoted reduction of SREBP-1c gene expression and increased CPT-1a gene expression in relation to obese control (Fig. 3C and D, respectively; $p < 0.05$).

The adiponectin receptor 2 (ADIPOR2) which positively regulates the activation of PPAR-α and CPT-1a, stimulates the β-oxidation and consequently lipid biosynthesis (Yamauchi & Kadowaki, 2008). This pathway may have been modulated by EEB since it promoted an increase of the ADIPOR2 gene expression and the adiponectin protein level compared to the obese control group (Fig. 3B; $p < 0.05$).

3.4. Effect of bacupari extract on hepatic tissue of the rats

It was by histological photomicrographs that fat deposition increased in the hepatocytes of the obese control group (Fig. 4B) in relation to the normal control (Fig. 4A) and BHFD groups (Fig. 4 C). The percentage of the nucleus did not differ between the BHFD and HFD groups. However, the cytoplasm percentage was higher in BHFD group compared to HFD ($p < 0.05$), while the percentage of the fat vesicles in the BHFD group was lower than the obese control group (Fig. 4D). The control group was classified as steatosis grade 0, the high-fat diet intake increased the steatosis into hepatic tissue to grade 3 and EEB was effective in decreasing steatosis to grade 2 (Fig. 4E).

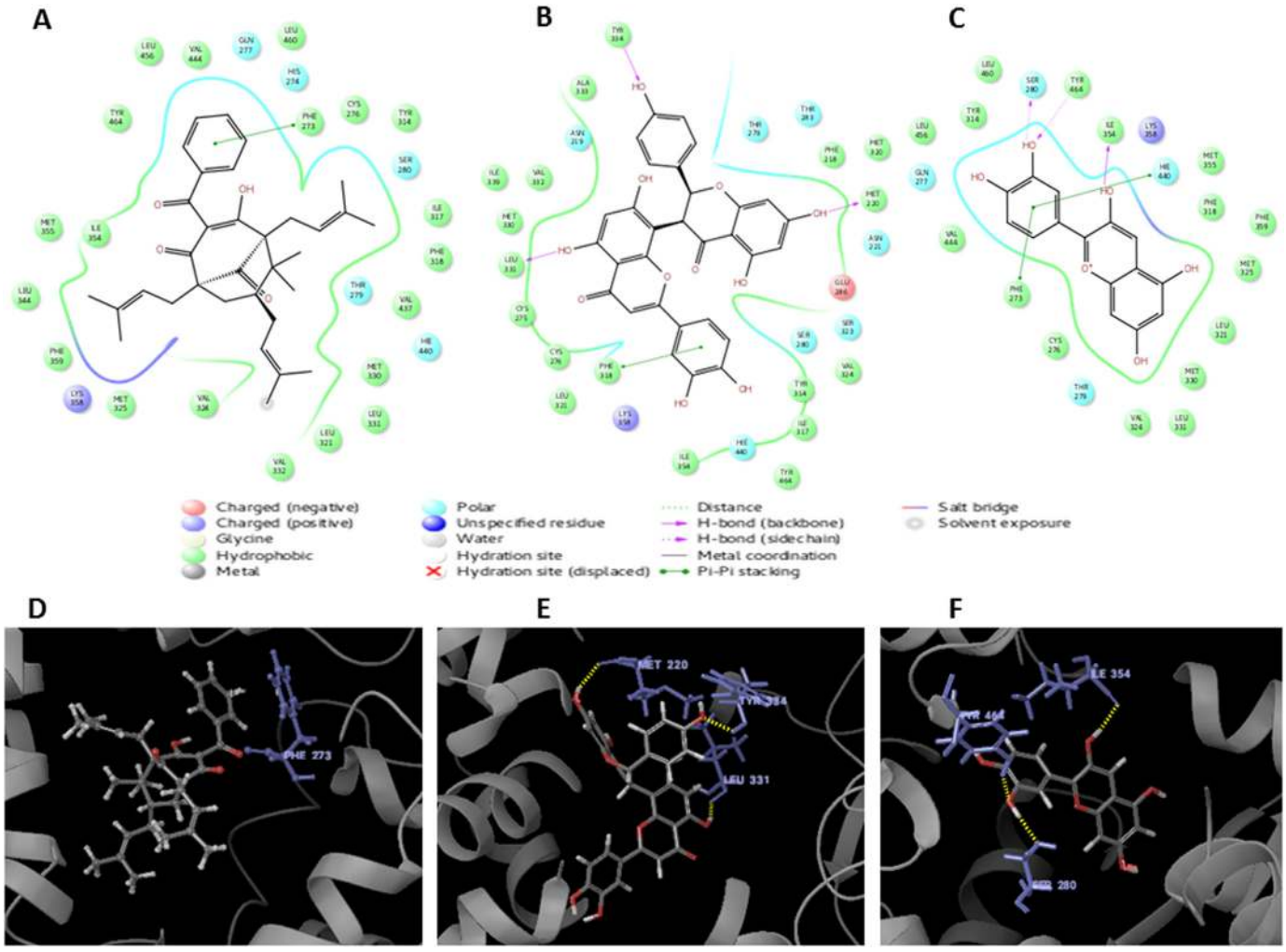

Fig. 5. Interactions between amino acids of PPAR-α receptor site and 7-Epiclusianone (A), Morelloflavone (B), the standard PPAR-α agonist Cianidine (C) and representation of molecular docking results between 7- Epiclusianone (D), Morelloflavone (E), Cianidine (F) and PPAR-α.

3.5. Molecular modeling

Molecular docking studies were accomplished in order to verify probable agonist profile of the compounds found in the bacupari peel, 7-Epiclusianone and morelloflavone with Peroxisome proliferator-activated receptor-alpha (PPAR-α). The PPAR-α agonist cianidin was used as a standard (Jia et al., 2013). The results presented good affinities values, corresponding to Gscore energy, for the three compounds analyzed (Table 2). 7-epiclusianone exhibited the best affinity value (GScore -11.505 kcal.mol^{-1}) of the three tested compounds and it was better than the known PPAR-α agonist Cianidin (GScore -9.561 kcal.mol^{-1}) used as standard in this studies. Morelloflavone also exhibits better but very similar affinity value (GScore -9.868 kcal.mol^{-1}) comparable to cianidin GScore. Fig. 5A, B and C represent the results of molecular docking in 2D format of the three compounds with amino acids of PPAR-α. Fig. 5D, E and F depicts the representation of the best poses of these molecular docking of three compounds evaluated.

4. Discussion

The ethanolic extract of bacupari peel (EEB) containing high concentration of 7-epiclusianone and morelloflavone showed an anti-obesogenic effect in *Wistar* rats (Moreira et al., 2017). In continuity with our study, the focus of this research was to investigate the potential benefits of the bioactive compounds (found in EEB) in reducing inflammation and alleviating the non-alcoholic fatty liver disease (NAFLD).

The use of hypercaloric or hyperlipidemic diets has been used as a model of obesity induction in animals, because of its similarity to the genesis and metabolic responses caused by obesity in humans (Rosini, Silva, & Moraes, 2012). This study thus proved that rats exposed to a high fat diet presented a higher food efficiency ratio (FER), confirming higher caloric density and the efficiency in promoting the weight gain in the animals, with lower food consumption. According to BMI measurements, the animals of the HFD group can be classified as obese (Novelli et al., 2007; Reynés et al., 2014). However, EEB reduced the weight gain of the animals that received the hyperlipid diet. Moreira et al. (2017) also observed that EEB added to the hyperlipid diet of obese rats promoted a lower percentage of adiposity and a lower glycemic index. Our study confirms the efficacy of EEB rich in bioactive compounds such as 7-epiclusianone and morelloflavone in the control of obesity, BMI and final weight.

The genus *Garcinia* is an important source of bioactive compounds reported in the literature (Gontijo et al., 2012; Martins et al., 2008; Santa-Cecília et al., 2012). Among such compounds are polyisoprenylated benzophenones, flavonoids, xanthones and proanthocyanidins (Castro et al., 2015). In our study (Moreira et al., 2017), was identified in the bacupari peel extracts tetraprenylated benzophenone 7-epiclusianone and the bioflavonoid morelloflavone which were associated with anti-obesogenic activity (Moreira et al., 2017). Moreover, these compounds showed that antioxidant and anti-inflammatory,

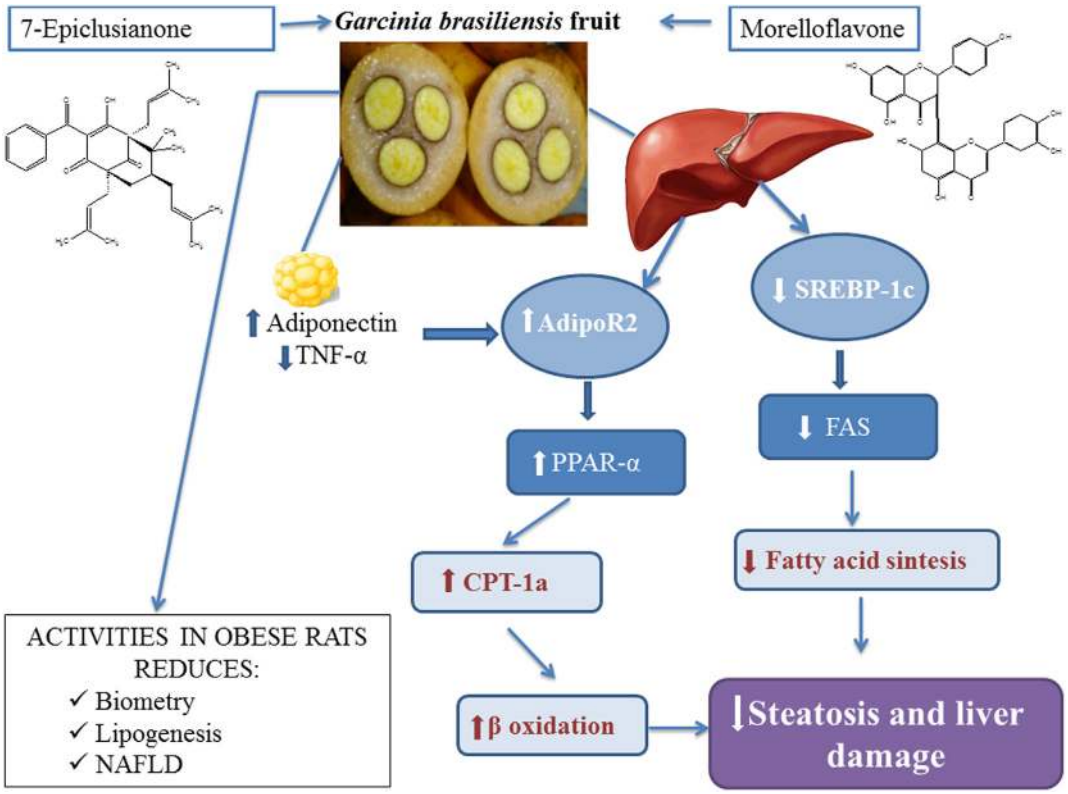

Fig. 6. Potential action mechanism of bioactive compounds present in bacupari peel extract in the lipogenic pathway and fatty acids lipolysis in the hepatic and adipose tissue of obese Wistar rats. Adipo R2: Adiponectin receptor 2; CPT-1a: Carnitine palmitoyltransferase 1; FAS: Fatty acid synthase; PPAR-α: Peroxisome proliferator-activated receptor α; SREBP1: Sterol regulatory element-binding proteins; TNF-α: tumor necrosis fator alpha; NAFLD: nonalcoholic fatty liver disease.

antimicrobial, leishmanicidal activities in studies in vivo and in vitro (Castro et al., 2015; Gontijo et al., 2012; Santa-Cecília et al., 2012).

Low levels of ADIPOR2 in NAFLD can play an important role in the pathogenesis of steatosis. Adiponectin has mechanisms by which it has demonstrated the reduction of hepatic lipid accumulation and the sensitization of ADIPOR2, which activates the nuclear receptor PPAR-α. The activation of the binding of this transcription factor is considered one of the main regulators of fatty acid oxidation (Guerra et al., 2015). The EEB in HFD obese rats increased ADIPOR2, PPAR-α, CPT-1a gene expression and adiponectin and PPAR-α protein levels. This effect can promote fatty acid oxidation and reduction of the inflammatory process through adiponectin induction in the PPAR-α pathway, resulting in the reduction of the percentage of hepatic and corporal fat (Yamauchi & Kadowaki, 2008). This pathway can be confirmed in our study by cellular changes in the liver observed in the HFD obese rat's intake EEB intake, which decreased liver weight, hepatosomatic index and percentage of liver fat, increasing the percentage of cytoplasm.

Adiponectin and TNF-α mutually inhibit production of one another in adipose tissue (Makki, Froguel, & Wolowczuk, 2013). Moreira et al. (2017) reported that animals treated with EEB had a lower concentration of TNF-α when compared to the obese control group. In this study we verified that the animals of the BHFD group showed greater quantification of adiponectin while the HFD group had lower values confirming as mentioned above that TNF-α can inhibit the expression of adiponectin. On the other hand, adiponectin stimulates the secretion of IL-10 (Moschen, Wieser, & Tilg, 2012). Previous studies by Moreira et al. (2017) reported that BHFD showed a high concentration of IL-10. This outcome may have been observed due to the fact that this group had greater quantification of adiponectin. In addition, Moreira et al. (2017) observed that animal treated with BHFD showed decreasing glycemic levels. This effect may be due to upregulation of adiponectin, which could increases the oxidation of carbohydrates and lipids and decreases appetite (Li, Shin, Ding, & Van Dam, 2009). Bioactive compounds of EEB, such as polyisoprenylated benzophenones, flavonoids, xanthones and proanthocyanidins, were efficient in improving morphology and liver function. A previous study conducted by Moreira et al. (2017) found that EEB reduced serum levels of aspartate alanine aminotransferase (ALT), suppressing the inflammatory process in the liver. Our study confirms the reduction of hepatic injury, since EEB reduced the degree of hepatic steatosis, improving the pathophysiological condition of the disease that is characterized by inflammation, fibrosis, cell death and insulin resistance (García-Ruiz, Baulies, Mari, García-Rovés, & Fernandez-Checa, 2013). The animals in the obese control group exhibited a high degree of steatosis, and the group that received the hyperlipid diet added with EEB presented a lower degree. This fact can be explained by the fact that the HFD group presented a lower concentration of adiponectin. Hypoadiponectinemia is often associated with higher grades of hepatic steatosis and has been considered a typical feature of NAFLD (Hui et al., 2004). Thus, substances that increase adiponectin levels may be considered therapeutic targets for NAFLD (Finelli & Tarantino, 2013). In this context, the bioactive compounds of EEB can be promising in the treatment of this disease.

The SREBP-1c gene expression was also reduced in the BHFD group, which indicate a preferential reduction lipogenic genes, among them acetyl CoA carboxylase (ACC), which converts acetyl CoA to malonyl CoA and fatty acid synthase (FAS), which converts malonyl CoA to palmitate (Strable & Ntambi, 2010). As previously observed by our research, decreases of FAS gene expression in this experimental model (Moreira et al., 2017). Thus, the potential benefit of EEB in the reduction of fatty acid synthesis and consequent in the accumulation of liver fat in animals, with a protective effect against NAFLD, preventing inflammatory processes and accumulation of fat, by increasing the serum concentrations of adiponectin, IL-10, PPAR-α and decreasing TNF-α (Fig. 6). Gene regulation and risk reduction of chronic non communicable diseases can be done by 7-epiclusianone and morelloflavone flavonoids, non-nutrient bioactive compounds found in EEB,

interacting with genes involved in intracellular signalling, interfering in lipid metabolism.

Molecular docking with these molecules and PPAR-α agonist showed affinity for the agonist PPAR-α, with higher docking score than the drug, cianidin. 7-epiclusianone is a molecule with more lipophilic structural characteristics than the others compounds evaluated and displays a high number of hydrophobic interactions of van der Waals (vdW Good: 649 interactions) and one π-π stacking interactions with the amino acid residue Phe273 leading to a higher affinity value. Morelloflavone presented three hydrogen bond interactions with the amino acids residues Met220, Phe318 and Tyr334 as well as the cianidine who also presented three hydrogen bond interactions with the amino acids residues Ser280, Ile354 and Tyr464. Despite the cianidine present more two π-π stacking interactions with the amino acid residues Phe273 and Hie440, it showed the lowest value of affinity among the three compounds analyzed. These results suggest that lipophilic compounds are better to interact with this site receptor of PPAR-α. These results suggest an interaction which can lead to an agonist activity of the 7-epiclusianone and morelloflavone in the EEB in relation to the PPAR-α receptor, which may have favored the hepatic lipolysis regulation.

The computational studies were performed with the aim to simulate and verify the affinity profile of EEB compounds with the enzymes studied. However, the limitation is that biotransformation could occur during digestion, absorption and transport, which could been not attributed the effect only to 7-epiclusianone and morelloflavone compounds of EEB. However 7-epiclusianone and morelloflavone in the EEB signalled this process.

5. Conclusion

Ethanolic extract of bacupari peel (EEB) (*G. brasiliensis*) has a protective effect against NAFLD and efficiently improves biometric parameters. This effect was the result of EEB increasing the ADIPOR2 gene expression and adiponectin protein, which decreased of the adipogenic genes transcription factors and activated β-oxidation, via PPAR-α, SREBP-1c and CPT-1a. The pharmacological activity presented by the EEB could be due to the agonist PPAR-α profile of studied compounds.

Author disclosure statement

No competing financial interests exist.

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