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Jaboticaba extract prevents prediabetes and liver steatosis in high-fat-fed aging mice

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ABSTRACT

We developed a patented jaboticaba peel extract (PJE) aiming to investigate PJE dose-dependent effect in liver and metabolism of high-fat-fed aging mice. Male FVB mice were distributed as follows: YG(young; 3-months-old), AG(aged; 11-months-old), HfAG(aged + high-fat diet), JAGI(aged + 2.9gPJE/Kg), JAGII(aged + 5.8gPJE/Kg), HfJAGI(aged + high-fat diet + 2.9gPJE/Kg) and HfJAGII(aged + high-fat diet + 5.8gPJE/Kg). PJE showed a potent antioxidant activity and high bioactive compounds content. Both PJE doses prevented weight gain, dyslipidemia, hyperglycemia, reduced COX-2 level and improved HDL-cholesterol, pIRS-1, and PPARγ levels in high-fat-fed aging mice. Only HfJAGII showed lower TNFα level, insulin resistance, and glucose intolerance relative to HfAG. Taking aging into consideration, PJE prevented dyslipidemia and hyperglycemia, reduced TNFα besides increasing pIRS-1 and PPARγ levels, and restoring the hepatic structure in aged mice. Thus, PJE anti-inflammatory, hypoglycemic and lipid modulation capacity prevented the pre-diabetes and NAFLD in this model, being indicated as potential therapy to prevent hepatic and metabolic disorders associated to obesity, diabetes, and aging.

1. Introduction

Aging is directly associated with a reduction in metabolic capacity, and hepatic, glucose and lipid metabolism alterations (Chiang, Huang, Paul, Lee, & Lin, 2016; Daskalova et al., 2015). The impairment of blood glucose level control, the increase of liver triglyceride deposition, and hormonal imbalance during aging are alterations that are associated to dyslipidemia, hyperinsulinemia, diabetes, and cardiovascular complications, which are very common disorders in elderly people (Matsudo, Matsudo, & Barros Neto, 2000; Oh et al,. 2016). Considering that, most of the health problems related to aging are chronic diseases, the engagement in a healthier behavior, such as physical activities and good nutrition, can prevent or delay these age-related complications (WHO, 2015). Regarding the increase in life expectancy of the human population, there has been a great interest in studying possible alternatives to mitigate the aging process (WHO, 2015).

It is known that overweight and obesity during adult life contribute to an increase in morbidity and mortality (Matsudo et al., 2000). Nowadays, obesity is considered a public health problem, being associated with several diseases/disorders that include hypercholesterolemia, insulin resistance, metabolic syndrome, diabetes and hepatic complications such as the nonalcoholic fatty liver disease (NAFLD) (Song, Lai, Tang, & Cheng, 2016; Wang et al., 2016). The high intake of lipid and carbohydrate-enriched food together with sedentary habits are considered to be crucial risk factors for the development of obesity (Wang et al., 2016). Lenquiste et al. (2012) observed that high-fat diet ingestion by rodents promotes body weight gain associated with increased hepatic fat besides higher epididymal and retroperitoneal

Abbreviations: AG, aged experimental group; CAT, catechin; COX-2, ciclooxygenase-2; Cyn-3-glu, cyanidin-3-glycoside; GAE, gallic acid equivalent; ESI, electrospray ionization; FRAP, ferric reducing antioxidant power; HfAG, high-fat diet and aged experimental group; HfJAGI, high-fat diet and aged ingesting PJE dose I experimental group; HfJAGII, high-fat diet and aged ingesting PJE dose II experimental group; pIRS-1, phosphorylated insulin receptor substrate 1; JAGI, aged ingesting PJE dose I experimental group; JAGII, aged ingesting PJE dose II experimental group; NAS, nonalcoholic fatty liver disease activity score; NAFLD, nonalcoholic fatty liver disease; ORAC, oxygen radical absorbance capacity; PJE, patented jaboticaba extract; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxigen species; T2DM, type 2 diabetes mellitos; TE, trolox equivalent; TNFα, tumor necrosis factor alpha; YG, young experimental group

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adipose tissue mass. In addition, Araújo, Esteves, Dessimoni-Pinto, and Batista (2014) confirmed that even the consumption of a moderately high-fat diet can stimulate dyslipidemia and high glucose levels in rats. The increased adiposity generates a pro-inflammatory and pro-oxidative microenvironment, which plays a central role in obesity pathogenesis (Song et al., 2016; Stinkens, Goossens, Jocken, & Blaak, 2015).

Many natural products have been studied for their therapeutic uses such as the prevention or mitigation of metabolic damages. Fruit can be an important source of several bioactive compounds concentrated mostly in the peel (Dragano et al., 2013; Lenquiste et al., 2012). Among the fruits, the jaboticaba peel has been indicated as a promising natural product in the obesity treatment (Baptista et al., 2014; Dragano et al., 2013; Lenquiste et al., 2015).

Jaboticaba (Myrciaria jaboticaba) is a Brazilian berry, with a purple peel, found throughout the country (Baptista et al., 2014). The jaboticaba peel has a great number of phenolic compounds, including anthocyanin, quercetin, ellagic acid and gallic acid (Abe et al., 2007). Recent studies have shown that the replacement of 4% of the total daily food intake by freeze-dried jaboticaba reduced weight gain, and improved HDL-cholesterol level (Lenquiste et al., 2012; Lenquiste et al., 2015). According to these findings, Araújo et al. (2014) verified that the replacement of 10% of the total daily food intake by jaboticaba peel flour prevents dyslipidemia and reduces glucose serum levels in rats fed with a moderately high-fat diet. The antioxidant and anti-inflammatory properties, the regulation of intestinal absorption and lipid oxidation, as well as the capacity to modulate hormones and enzymes involved in the satiation process, are the main ways in which the phenolic compounds exert their anti-obesity effects (Dragano et al., 2013; Lenquiste et al., 2012, 2015; Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012).

Despite having studies that point to the beneficial effects of the jaboticaba peel on the metabolic response in the organism; these studies had only been applied to the jaboticaba peel added to animal feed. However, it was necessary to provide a large intake volume of jaboticaba peel intake to reach the desired beneficial effect. Therefore, the aim of the present study was to evaluate a patent jaboticaba peel extract (PJE) for its dose-dependent effect on dyslipidemia and insulin resistance, besides evaluating for the first time its beneficial action against prediabetes and NAFLD in obese/aged mice.

2. Methods

2.1. Patented jaboticaba peel extract (PJE)

An efficient extraction should maximize the compounds bioactivity as well as have reduced toxicity. The method used to prepare the PJE (130 mg jaboticaba peel/mL PJE) was patented (Maróstica et al., 2017), and consisted in the solubilization of freeze-dried jaboticaba peel (*Myrciaria cauliflora* (Vell.) Berg), in ethanol with subsequently solvent removal. The PJE compounds and their *in vitro* antioxidant activity were analyzed as described below.

2.2. Patented jaboticaba peel extract (PJE) bioactive compounds and in vitro antioxidant activity

2.2.1. Total phenolic compounds

The quantification method was based on *Folin-Ciocalteu* reduction in the presence of phenolic compounds (Roesler et al., 2007). The PJE used was prepared in triplicate and diluted (1:200) in distilled water. It was added 50 µL of the diluted sample/blank (water)/curve, 800 µL of distilled water and 50 µL of *Folin-Ciocalteu* reagent in microtubes, which were incubated in the dark for 3 min. Then, 100 µL of saturated sodium carbonate solution was added to the mixture, which was incubated in the dark for 2 h and the absorbance was read at 725 nm in microplate reader (SynergyHT Biotek, Wionooski, USA). The curve (gallic acid) was diluted in water and ranged from 100 to 16 µg/mL of gallic acid equivalents (GAE). The results were expressed as mg of GAE per g of a dry sample.

2.2.2. Monomeric anthocyanin

This analysis was performed according to that described by Wrolstad (1976) and adapted by Abe, Mota, Lajolo, and Genovese (2007), using the pH differential method. The PJE used was prepared in triplicate, diluted (1:100) in potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). The samples/blank (water) were read at 510 nm and 700 nm in microplate reader (SynergyHT Biotek, Wionooski, USA). The diluted sample absorbance was calculated using the following formula: A = (Ab 510 − Ab 700) pH 1.0 − (Ab 510 − Ab 700) pH 4.5. The monomeric anthocyanin concentration in the original sample was calculated using the following formula: Monomeric Anthocyanin (mg/100 g) = (A × MW × DF × 100)/(e × 0.71*). Considering: Ab = Absorbance; MW = molecular weight of the sample predominant anthocyanin; DF = dilution factor e = molar absorptivity of the predominant anthocyanin in the sample. The results were expressed as mg of cyanidin-3-glucoside equivalent per 100 g of dry sample.

2.2.3. Total flavonoids

The protocol used was based on a colorimetric method (Herald, Gadgil, & Tilley, 2012). The PJE used was prepared in triplicate and diluted (1:50) in distilled water. It was added 100 µL of distilled water, 50 µL of sodium nitrite (5%) and 25 µL of the diluted samples/blank (acetone solution)/curve in a microplate, which was incubated for 5 min. Subsequently, 15 µL of aluminum chloride (10%) was added and incubated for 6 min. It was mixed to 50 µL of sodium hydroxide (1 M) and 50 µL of distilled water. The microplate was read at 510 nm in microplate reader (SynergyHT Biotek, Wionooski, USA). The curve (catechin) was diluted in acetone and ranged from 5 to 250 µg/mL of catechin equivalent (CAT). The results were expressed as mg of CAT per g of dry sample.

2.2.4. UPLC-ESI-QTOF-MS/MS analyses

To analyze the PJE composition through UPLC-ESI-QTOF-MS/MS, each extract was solubilized in methanol (HPLC grade, Merck S.A.; Rio de Janeiro, Brazil) to a concentration of 3 mg/mL. Then, the analyte solution was separated by HPLC (Agilent 1290 Series Liquid Chromatography equipment, Agilent Technologies, USA) using a Zorbax Eclipse Plus C18 1.8 µm, 2.1 mm i.d., 100 mm column (Agilent Technologies, USA) and a mobile phase consisting of methanol (phase B) and water (phase A) (Milli-Q, Millipore, Billerica, MA). The gradient method was as follows: 0–48 min, 3–97% B; 48–50 min, 97–3% B; 50–53 min, 3% B. The flow rate was 0.5 mL/min at 40 °C, and the injected volume was 2 µL. Mass spectra was collected using a QTOF instrument (Q-TOF 6550) and ESI ionization (Dual AJS-ESI) using the following conditions: drying gas at 290 °C, drying gas flow 11 L/min, nebulizer at 20 psi; sheath gas at 350 °C; sheath gas flow 12 L/min, VCap 3000; fragment 110 V, OCT 1 RF Vpp 750 V, different collision energy using N2. Mass spectrometer parameters acquisition ranged from *m/z* 50 to *m/z* 1600. Automatic MS/MS experiments were carried out using collision energies (20 eV, 30 eV and 40 eV). Integration and data elaboration were performed using MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA).

2.2.5. UPLC-MS/MS bioactive compounds quantification

The quantification of free bioactive compounds at the PJE was performed using a UHPLC–MS/MS 8040 (Shimadzu, Kyoto, Japan) instrument consisting of a liquid chromatography system coupled to a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source based on the procedure proposed by Bataglion, da Silva, Eberlin, and Koolen (2015). The extracts were solubilized in methanol (HPLC grade, Merck S.A.; Rio de Janeiro, Brazil) at a 3 mg/mL concentration. The chromatographic separation was performed on Zorbax Eclipse Plus C18 1.8 µm, 2.1 mm i.d., 100 mm column (Agilent

Technologies, USA) maintained in a thermostated oven at 40 °C. The autosampler temperature was 10 °C and the injection volume was 2 µL. Mobile phases consisted of 0.1% formic acid (formic acid grade HPLC; Sigma-Aldrich, St. Louis, USA) in water (eluent A) and methanol (eluent B) at a flow rate of 0.40 mL/min. The elution conditions were: 0–1 min, 5% B; 1–4 min, 5–60% B; 4–7 min, 60–70% B; 7–10 min, 70–100% B; 10–10.50 min, 100% B; 10.50–11 min, 100–5% B; 11–15 min, 5% B. ESI source was operated in the negative ion mode, according to the following parameters: capillary voltage, 3.5 kV; heat block temperature, 300 °C; desolvation line temperature, 250 °C; drying gas flow (N2), 20 L/min; nebulizing gas flow (N2), 3 L/min; collision induced dissociation gas pressure (Ar), 224 kPa. Bioactive compounds (ascorbic acid, rutin, gallic acid, ellagic acid) were identified by comparison of their UHPLC-ESI(-)-MS/MS dissociation patterns and retention time with those of authentic standards (purity ≥ 96%; Sigma-Aldrich; St. Louis, USA). The quantification of the identified bioactive compounds was performed by comparison to the calibration curve of each standard. Stock solutions of each standard compound (1 mg/mL) were used to prepare the calibration curves (9 points ranging from 30 to 1000 ng/mL). The calibration curves were prepared in a synthetic matrix (sugars mixture: glucose, fructose, arabinose, xylose and sucrose: 20 ng/mL of each sugar), since the food matrix affects the ionization of analytes. Data were acquired and processed by Labsolution software (version 5.53 SP2, Shimadzu). Results were expressed as mg per 100 g of dry sample (mg/100 g dry sample).

2.2.6. Antioxidant assay in vitro

The PJE *in vitro* antioxidant capacity was evaluated by oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) and ABTS assays, using Trolox as standard.

The ORAC hydrophilic assay evaluated the antioxidant molecules capacity to inhibit the oxidant agents' action, protecting against free radicals *in vitro* (Davalos, Gomez-Cordoves, & Bartolome, 2004). For this assay, the fluorescein was used as a probe, and the results were expressed as µmol Trolox per g dry sample.

In the ABTS assay, the absorbance decrease was monitored (30 min, considering 1-minute interval) and the differences between sample and control were calculated. The results were expressed as µmol Trolox per g dry sample (Rufino et al., 2010).

The FRAP assay evaluated the capacity of antioxidant compounds to reduce the 2,4,6-tripiridil-s-triazine ferric complex (Fe3 + - TPTZ). The results were expressed as µmol Trolox per g dry sample (Benzie & Strain, 1996).

2.3. Animals and experimental design

The study was carried out using a total of 70 male FVB mice, obtained from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science of the University of Campinas (UNICAMP), Brazil. All the experimental procedures were submitted to the Ethics Committee on Animal Use of UNICAMP/Protocol: 3421-1.

The animals were randomly distributed in the following experimental groups (n = 10 animals/group): Young group (YG): 3-month-old mice, receiving the PJE vehicle daily by gavage, water and a standard diet (Nuvital CR1, Colombo, Paraná, Brazil) (composition: 22 g% protein; 53 g% carbohydrate; 4.5 g% lipid and 2.9 kcal/g); Aged group (AG): 11-month-old mice, receiving the PJE vehicle daily by gavage, water and a standard diet; High-fat diet group (HfAG): 11-month-old mice, receiving the PJE vehicle daily by gavage, water and a high-fat diet (20 g% protein; 50 g% carbohydrate; 21 g% lipid and 4.5 kcal/g); Aged/PJE I group (JAG I): 11-month-old mice, receiving PJE (2.9 g PJE/Kg body weight) daily by gavage, water and standard diet; Aged/PJE II group (JAG II): 11-month-old mice, receiving PJE (5.8 g PJE/Kg body weight) daily by gavage, water and standard diet; High-fat diet/PJE I group (HfJAG I): 11-month-old mice, receiving PJE (2.9 g PJE/Kg body weight) daily by gavage, water and high-fat diet; High-fat diet/PJE II group (HfJAG II): 11-month-old mice, receiving PJE (5.8 g PJE/Kg body weight) daily by gavage, water and high-fat diet.

All treatments lasted 60 days. The animals were housed one per cage, with a 12 h light–dark cycle; water and food were provided *ad libitum*. After the treatment period, the mice were weighted on a semi-analytical scale (Marte AS 5500 – São Paulo, Brazil), anesthetized with xylazine hydrochloride (5 mg/kg i.m.; König, Sao Paulo, Brazil) and ketamine hydrochloride (60 mg/kg i.m.; Fort Dodge, Iowa, EUA). The animals were submitted to euthanasia by increasing the anesthetic level. Then, blood samples were collected by left ventricle punction in tubes containing EDTA. Liver sections were collected and frozen at − 80 °C for Western-Blotting analyses or processed for light microscopy evaluation.

2.4. Relative body weight gain (%), food and energy intake

The relative body weight gain was calculated using the following formula: Relative Body Weight Gain (%) = (WG × 100)/IW, considering: WG (final weight – initial weight) and IW (initial weight). Moreover, the food intake was determined considering the amount of food (in grams) given to the animals at the beginning of a week, subtracted from the food amount measured at the end of a week. The energy intake was calculated multiplying the food intake by the energetic amount of each diet (Standard diet: 2.9 kcal/g and High-fat diet: 4.5 kcal/g).

2.5. Fasting glucose, glucose tolerance test and insulin tolerance test

The fasting glucose level (after 12 h fasting period) was evaluated in the plasma using a commercial kit (Glicemia enzimática AA; Wiener lab®). The glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed as previously described using a glucometer (BreezeTM – Bayer®) (Oliveira, Maschio, Carvalho, & Collares-Buzato, 2015).

2.6. Lipid profile

Plasma samples were collected from 12 h-fasted mice to evaluate the total cholesterol, HDL-cholesterol and triglyceride levels using the following commercial kits: Colestat enzimático AA Wiener lab®, HDL-Colesterol Reactivo Precipitante Wiener lab® e TG Color GPO/PAP AA Wiener lab®. The LDL-cholesterol level was estimated considering the following formula: CLDL = (Total Cholesterolplasma − HDL-cholesterol − (Triglycerides × 0.16)) (Duivenvoorde et al., 2011).

2.7. Light microscopy

Liver fragments collected during euthanasia for light microscopy analysis were fixed in Bouin solution for 24 h. After that, the samples were rinsed in ethanol (70%), dehydrated in increasing ethanol series and embedded in plastic polymers (Paraplast Plus, ST. Louis, MO, EUA). The liver samples were 5 µm sectioned using a micrometer (Hyrax M60, Zeiss, Germany) and stained with hematoxylin and eosin (Luz & Zancheta Neto, 2002).

2.8. Liver histopathological analysis

The liver histopathological analysis was done using the softwares NIS-Elements/Image and Image Pro-Plus in digital microscopic images obtained with the Nikon Eclipse E-400 microscope (Nikon, Tokyo, Japan). A grid containing 200 intersections was superimposed over 10 randomly obtained images (400× magnification) per animal. A total of 2000 points were sampled per animal and the following parameters were quantified: mononucleate hepatocyte, binucleate hepatocyte, total hepatocyte cytoplasm, lipid hepatic content, blood vessel and inflammatory infiltrate.

In addition, a histological score to determine the NAFLD degree was applied, considering the following parameters: steatosis (score 0–3), inflammation (score 0–3) and hepatocellular ballooning (score 0–2). According to Takahashi and Fukusato (2014), the ballonized hepatocytes are described as swollen hepatocytes with little cytoplasm, containing fat droplets and nuclei displaced to the cell periphery, reflecting hepatocellular injury. The sum of these scores gave the final NAFLD activity score (NAS), that ranged from 0 to 8 (Kleiner et al., 2005; Roth et al., 2012).

2.9. Liver protein extraction and Western-Blotting analyses

The liver fragments frozen at −80 °C were homogenized in RIPA buffer (Radio-Immunoprecipitation Assay), containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), using a Polytron (Kinematica). The protein concentration of each sample was determined using the Bradford reagent (Bio-Rad Laboratories, Hercules, CA. USA). An aliquot of each sample containing 50 µg of protein was applied in SDS-polyacrylamide gel under reducing conditions and transferred to nitrocellulose membranes (Amersham). Subsequently, the membranes were blocked with bovine serum albumin (BSA: 1–5%), diluted in TTBS buffer for 1 h and overnight incubated with the following primary antibodies diluted in 1% BSA solution: β-actin (mouse monoclonal; sc-81178, Santa Cruz Biotechnology), COX-2 (mouse monoclonal; sc-376861, Santa Cruz Biotechnology), TNFα (mouse monoclonal; ab8348, Abcam), pIRS-1 (goat polyclonal; sc17196, Santa Cruz Biotechnology) and PPARγ (mouse monoclonal; sc-7273, Santa Cruz Biotechnology). After that, the membranes were washed in TTBS buffer and incubated for 2 h with the following secondary antibodies diluted in 1% BSA solution (dilution range of 1:5000–1:6000): anti-mouse (W4021 - Promega) or anti-goat (sc-3851, Santa Cruz Biotechnology). The membranes were incubated with a chemiluminescence solution (Pierce Biotechnology Western blotting) for 5 min and the blots were captured using the G-Box Chemi associated with the GeneSnap (Syngene, Cambridge, UK) image acquisition software. The β-actin was used as an endogenous control. The labeling intensity was identified by densitometry using the Image J software (USA).

2.10. Statistical analysis

The statistical analyses of the results were performed using t-student test or analysis of variance (One-Way ANOVA) followed by Tukey's multiple range post-test. The significance limit was set at $p < 0.05$ and all data were expressed as mean ± standard deviation (Zar, 1999).

3. Results

3.1. PJE bioactive compounds and in vitro antioxidant activity

The PJE was analyzed by UPLC-ESI-MS/MS and 19 compounds could be identified (Table 1). Peak identification was performed based on the exact mass of the compounds and fragmentation spectra together with the information previously reported in the literature. Table 2 shows the results of the total polyphenols, total flavonoids, monomeric anthocyanins and some specific bioactive compounds quantified in the PJE. In addition, PJE *in vitro* antioxidant capacity of PJE was verified using the FRAP, ORAC and ABTS assays (Table 2).

3.2. Relative body weight gain (%), food and energy intake

The mice from the AG group did not display body weight gain during the experimental period; in fact, the final total weight in this group was lower than the initial weight (Fig. 1). In contrast, YG mice showed a 15% increase in body weight gain during the same period. When aged mice were fed a high-fat diet, a significant increase in body weight gain was observed in these animals (HfAG) compared to the AG

Table 1
UPLC retention times and MS data of PJE bioactive compounds identified.

RT (min)	Compound	Molecular formula	$[M-H]^-$ (m/z)	Main fragments (m/z)	Error (ppm)	Reference
1.5	Gallic acid[a]	$C_7H_6O_5$	169.0140	125.0246	1.77	Erşan, Üstündağ, Carle, and Schweiggert (2016)
1.8	HHDP-galloylglucose	$C_{27}H_{22}O_{18}$	633.0751	481.0640; 300.9990; 275.0209; 169.0142	3.6	Plaza et al. (2016)
3.0	Bis-HHDP-glucose (Casuariin)	$C_{34}H_{24}O_{22}$	783.0681	481.0626; 300.9984; 275.0202; 249.0426	−1.14	Plaza et al. (2016)
4.9	Bis-HHDP-glucose isomer (Pedunculagin)	$C_{34}H_{24}O_{22}$	783.0668	481.0611; 300.9982; 275.0190	−2.81	Plaza et al. (2016)
4.9	HHDP-galloylglucose isomer	$C_{27}H_{22}O_{18}$	633.0751	481.0684; 300.9982; 275.0196	3.64	Plaza et al. (2016)
6.8	(-)-Epicatechin[a]	$C_{15}H_{14}O_6$	289.071	245.0821; 221.0816; 203.0719; 125.0246; 109.0298	1.03	Hofmann, Nebehaj, and Albert (2016)
7.2	Galloyl-bis-HHDP-glucose (Casuarinin)	$C_{41}H_{28}O_{26}$	935.0791	783.0485; 633.0682; 447.0928; 300.9982; 275.0168	3.95	Plaza et al. (2016)
8.2	Galloyl-bis-HHDP-glucose (Casuarictin)	$C_{41}H_{28}O_{26}$	935.0740	783.0672; 633.0822; 300.9999; 275.0190	−1.5	Plaza et al. (2016)
8.3	HHDP-digalloylglucose (Tellemagrandin I)	$C_{34}H_{26}O_{22}$	785.0857	633.0741; 483.0799; 300.9982; 275.0156; 1249.0415	2.54	Plaza et al. (2016)
8.4	Kaempferol hexoside	$C_{21}H_{20}O_{11}$	447.0941	285.406	3.13	Erşan et al. (2016)
9.3	Chlorogenic acid[a]	$C_{16}H_{18}O_9$	353.0874	191.0565	0.28	Ibrahim et al. (2015)
11.3	HHDP-trigalloylglucose (Tellemagrandin II)	$C_{41}H_{29}O_{26}$	937.0953	785.0885; 767.0757; 465.0714; 300.9997; 275.0188; 169.0134	0.64	Plaza et al. (2016)
15.2	Pentagalloyl hexose	$C_{41}H_{31}O_{26}$	939.1070	787.0988; 769.0866; 617.0778; 602.0824; 465.0688; 447.0559; 431.0604; 295.0449	3.51	Plaza et al. (2016)
15.3	Myricetin-rhamnoside	$C_{21}H_{20}O_{12}$	463.0858	316.0225	−4.1	Negri and Tabach (2013)
17.1	Ellagic acid pentoside	$C_{19}H_{14}O_{12}$	433.0403	300.9971; 229.0140	0.93	Plaza et al. (2016)
17.6	Ellagic acid[a]	$C_{14}H_6O_8$	300.9984	229.0148	−0.99	Plaza et al. (2016)
18.3	Quercetin-3-rhamnoside (Quercitrin)	$C_{21}H_{20}O_{11}$	447.0936	301.0351	2.0	Plaza et al. (2016)
20.8	Quercetin[a]	$C_{15}H_{10}O_7$	301.0348	151.0036; 178.9994	-0.1	Ibrahim et al. (2015)
20.9	Naringenin[a]	$C_{15}H_{12}O_5$	271.0610	151.0037; 119.0509; 107.0141	1.1	Santos, Pinto, Silvestre, and Neto (2010)

[a] Identification based on the fragmentation pattern of the analytical standard.

Table 2
PJE bioactive compounds and *in vitro* antioxidant capacity.

Parameters		
Total Phenolic Compounds	mg GAE g^{-1}[a]	121.0 ± 3.51
Monomeric Anthocyanins	mg cyd 3-glu 100 g^{-1}[b]	1381.0 ± 64.09
Total Flavonoids	mg CAT g^{-1}[c]	24.5 ± 0.26
Specific bioactive compounds		
Ellagic acid	mg 100^{-1} g	19.6 ± 0.02
Rutin	mg 100^{-1} g	2.02 ± 0.02
Gallic acid	mg 100^{-1} g	1.73 ± 0.07
Ascorbic acid	mg 100^{-1} g	1.21 ± 0.06
In vitro antioxidant capacity		
ORAC	μM TE^{-1}[d]	4252.0 ± 613.3
ABTS°	μM TE^{-1}[d]	6834.5 ± 77.9
FRAP	μM TE^{-1}[d]	316.2 ± 211.03

[a] Values expressed in Gallic Acid Equivalents.
[b] Cyanidin-3-glucoside.
[c] Catechin.
[d] Values expressed in Trolox Equivalents. All results are expressed in jaboticaba peel dry weight.

group. Both PJE doses, in the HfJAGI and HfJAGII groups, presented an anti-obesity effect showing reduced body weight gain compared to HfAG group. There were no significant differences among AG, JAGI and JAGII groups (Fig. 1).

The food intake was lower in HfAG group compared to AG group. Nevertheless, a higher energy intake was observed in aged animals that ingested high-fat diet in comparison to aged animals receiving a standard diet. PJE treatments, at both doses, induced a significant reduction in food and energy intake in HfJAGI and HfJAGII groups compared to HfAG group. There were no significant differences in these parameters among AG, JAGI and JAGII groups (Fig. 1).

3.3. Lipid profile

Aged animals showed a significant increase in plasma levels of total and LDL-cholesterol compared to young animals (YG). Both PJE treatment (JAGI and JAGII groups) significantly reduced plasma levels of total and LDL-cholesterol in AG mice, reaching similar levels to that displayed by the YG group (Fig. 1D and E). As expected, high-fat diet consumption worsened dyslipidemia in aged animals (HfAG group), showing a significantly higher level of total, LDL and HDL-cholesterol compared to the AG group. Both doses of PJE ameliorated the high-fat-induced dyslipidemia in HfJAGI and HfJAGII groups, showing lower levels of total and LDL-cholesterol as well as higher levels of HDL-cholesterol (Fig. 1E and F), compared to HfAG group. No significant differences in triglyceride levels were observed among the experimental groups (Fig. 1G).

3.4. Fasting glucose, glucose tolerance test and insulin tolerance test

The PJE treatment promoted a dose-dependent response, reducing the fasting glucose in aged mice (JAG I and II groups) compared to AG group (Fig. 2A). In addition, aged mice that received the highest tested dose of PJE (JSEII), showed an even lower glycemia than that displayed by AG and JAGI mice. The high-fat diet ingestion by aging mice significantly increased the fasting glucose compared to aged animals that received the standard diet. The PJE treatment ameliorated the high-fat-diet-induced hyperglycemia in a dose-dependent manner (Fig. 2A).

Moreover, the high-fat diet intake by aging mice promoted glucose intolerance (Fig. 2B and C) and insulin resistance (Fig. 2D and E) compared to aged mice that received a standard diet. Both PJE doses significantly inhibited the insulin intolerance state induced by the ingestion of the high-fat diet by aged animals, compared to the HfAG group (Fig. 2D and E). Meanwhile, the glucose intolerance was only significantly reduced by the highest tested dose of PJE, in the HfJAGII group, compared to the HfAG group (Fig. 2B and C).

3.5. Liver histopathology

The YG group showed a typical liver morphology and, therefore, was scored as 0 regarding the NAS index (Table 3; Fig. 4A). As shown in Fig. 4, the hepatocytes displayed a polygonal format with 1–2 central nuclei (mononuclear or binuclear); these cells were arranged in anastomosing plates, with borders that faced either sinusoid capillaries or adjacent hepatocytes. Some inflammatory infiltrates were also observed in few areas of the hepatic parenchyma.

The liver morphology of aged mice showed an increased frequency of ballooning hepatocytes (score 1) as well as in the lipid accumulation within hepatocyte cytoplasm, indicating an initial liver steatosis (score 2). Moreover, a higher frequency of inflammatory infiltrates was verified in AG group in relation to the YG group. The NAFLD activity score determined for the AG group was 3 (Table 3; Figs. 3 and 4B).

The high-fat diet ingestion intensified the hepatic alterations seen in aged mice, pointing to a severe steatosis. It was confirmed semi-quantitatively by the NAS index, in which HfAG group was scored as 8 (Table 3). In this group, an increased frequency of hepatocyte cytoplasm (Fig. 3D) and ballooning cells (Fig. 4C) (score 2) as well as a higher hepatocyte lipid accumulation (Fig. 3C and 4C) (score 3) were observed. These alterations were associated with a reduction in blood vessel frequency (Fig. 3F) (score 3) compared to the AG group.

Both PJE doses administration for aged animals led to a reduction in lipid accumulation within hepatocyte cytoplasm compared to the AG group, decreasing the steatosis level (score 1). Only mice from the JAGII group showed a lower total hepatocyte cytoplasm volume in relation to aged animals. Nevertheless, the administration of both PJE doses induced a decrease in the frequency of ballooning hepatocytes (score 0) and inflammatory infiltrates (score 0) compared to the AG group. Moreover, a higher blood vessels frequency was verified in the liver of aged mice receiving PJE (JAGI and JAGII groups) in relation to the AG group, in a dose-dependent manner. Thus, the JAGI and JAGII groups scored 1 regarding the NAS index (Table 3; Figs. 3 and 4E and F).

PJE treatment also reduced the steatosis induced by the ingestion of high-fat diet by aged mice. Thus, the HfJAGI and HfJAGII groups presented the scores 2 and 1, respectively, considering the NAFLD activity (Table 3; Figs. 3 and 4G and H). Mice from these groups showed a decrease in hepatocyte lipid accumulation (score 1) (Figs. 3C, 4G and H), which was associated with the lower hepatocyte cytoplasm volume observed. Moreover, HfJAGI and HfJAGII groups displayed a reduced frequency of ballooning cells (score 1 for HfJAGI and score 0 for HfJAGII) and inflammatory infiltrates (score 0) besides a higher frequency of blood vessels in comparison with the HfAG group.

3.6. Western-Blotting evaluation in liver

3.6.1. COX-2

The AG group showed increased hepatic levels of COX-2 compared to the YG group. After the high-fat diet ingestion, it was observed an even higher level of this molecule in the HfAG group in relation to the AG group. The PJE treatment led to a reduction in hepatic COX-2 levels, in the HfJAGI and HfAGII groups, compared to the HfAG group. Also, the PJE dose-dependent effect was observed in this analysis due to the fact that the HfAGII group showed lower COX-2 level than that verified in the HfAGI group. (Fig. 5A)

3.6.2. TNFα

The TNFα hepatic content increased in aged animals compared to young animals. The HfAG group showed an even higher level of this molecule in relation to aged animals. Both PJE treatments reduced the TNFα hepatic level in aged mice. However, only the highest PJE dose

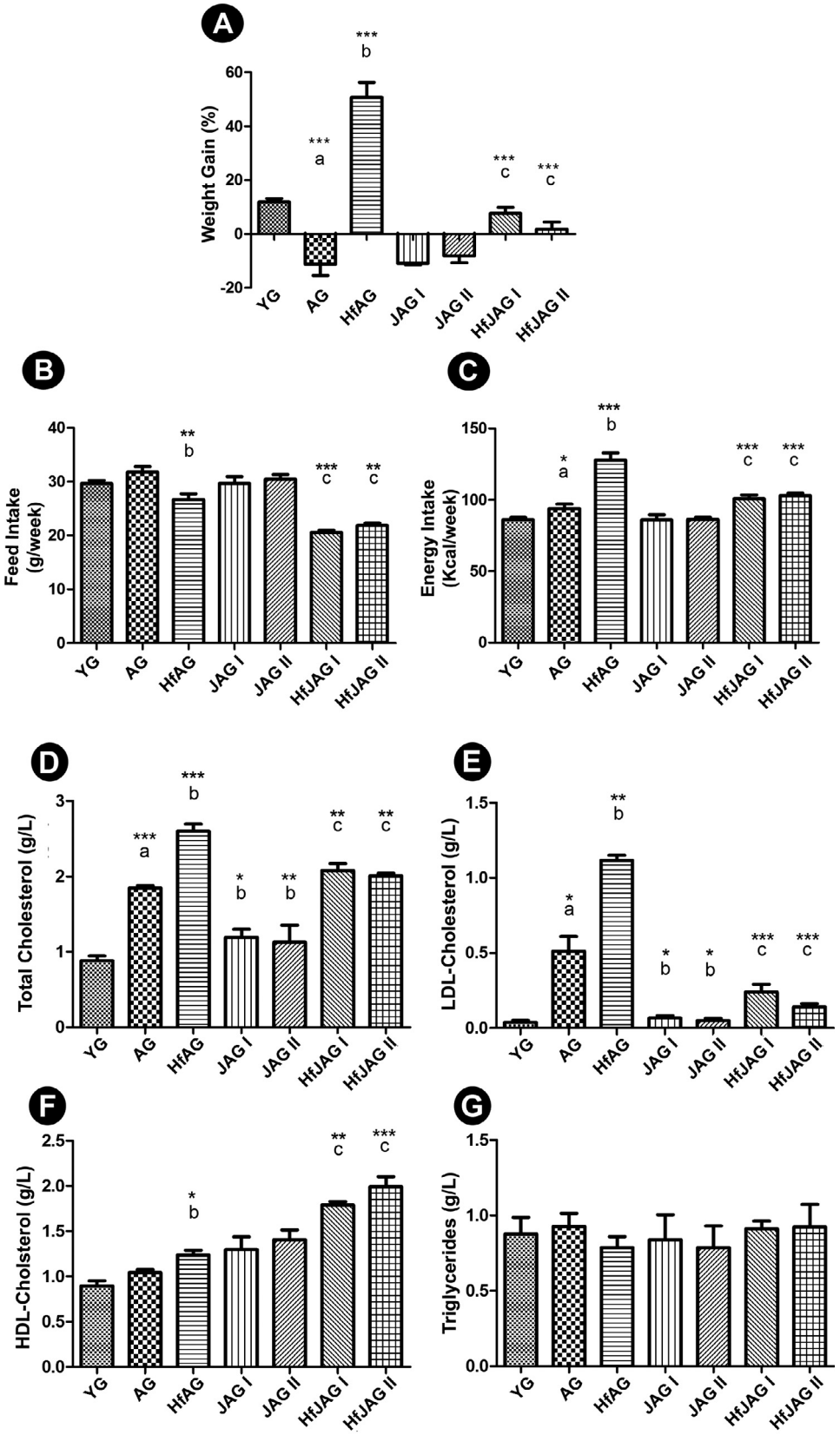

Fig. 1. (A) Relative Body Weight Gain. (B) Weekly Feed Intake (g). C) Weekly Energy Intake (Kcal). (D) Total Cholesterol. (E) LDL-cholesterol. (F) HDL-cholesterol. (G) Triglycerides. Significant differences were observed for: [a] relative to YG group; [b] relative to AG group. [c] relative to HfAG group. Considering: $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$.

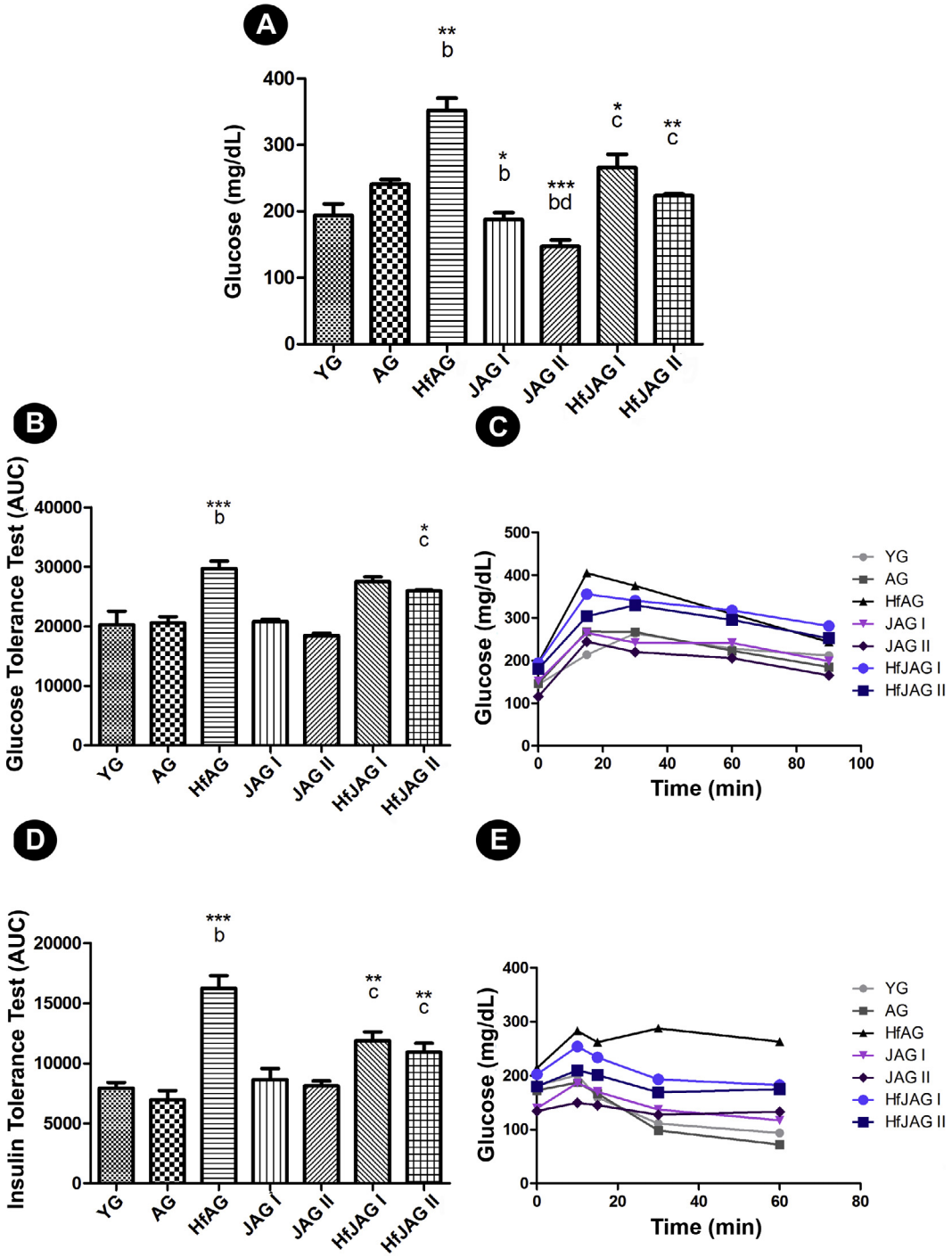

Fig. 2. (A) Fasting Glucose Dosage. (B) Glucose Tolerance Test (AUC). (C) Mean blood glucose levels at 0, 15, 30, 60 e 90 min after glucose administration. (D) Insulin Tolerance Test (AUC). (E) Mean blood glucose levels at 0, 10, 15, 30 e 60 min after insulin administration. Significant differences were observed for: [b] relative to AG group. [c] relative to HfAG group. [d] relative to JAGI group. Considering: $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$.

Table 3
Histopathological Score for NAFLD.

Experimental Groups	Steatosis	Balloning	Inflammation	NAS
YG	0	0	0	0
AG	2	1	0	3
HfAG	3	2	3	8
JAGI	1	0	0	1
JAGII	1	0	0	1
HfJAGI	1	1	0	2
HfJAGII	1	0	0	1

reduced TNFα tissue content in high-fat-fed aged animals, being having observed lower levels of this molecule in the HfAGII group in relation to the HfAG group (Fig. 5B).

3.6.3. pIRS-1

The AG group showed 50% lower hepatic amount of pIRS-1 in relation to young animals. The high-fat diet ingestion by aged animals reduced the pIRS-1 level even more compared to AG group. The highest PJE dose increased the pIRS-1 level in the JAGII group compared to the AG and JAGI groups, characterizing the PJE effect as dose-dependent. Moreover, the PJE treatment increased the pIRS-1 level even more than

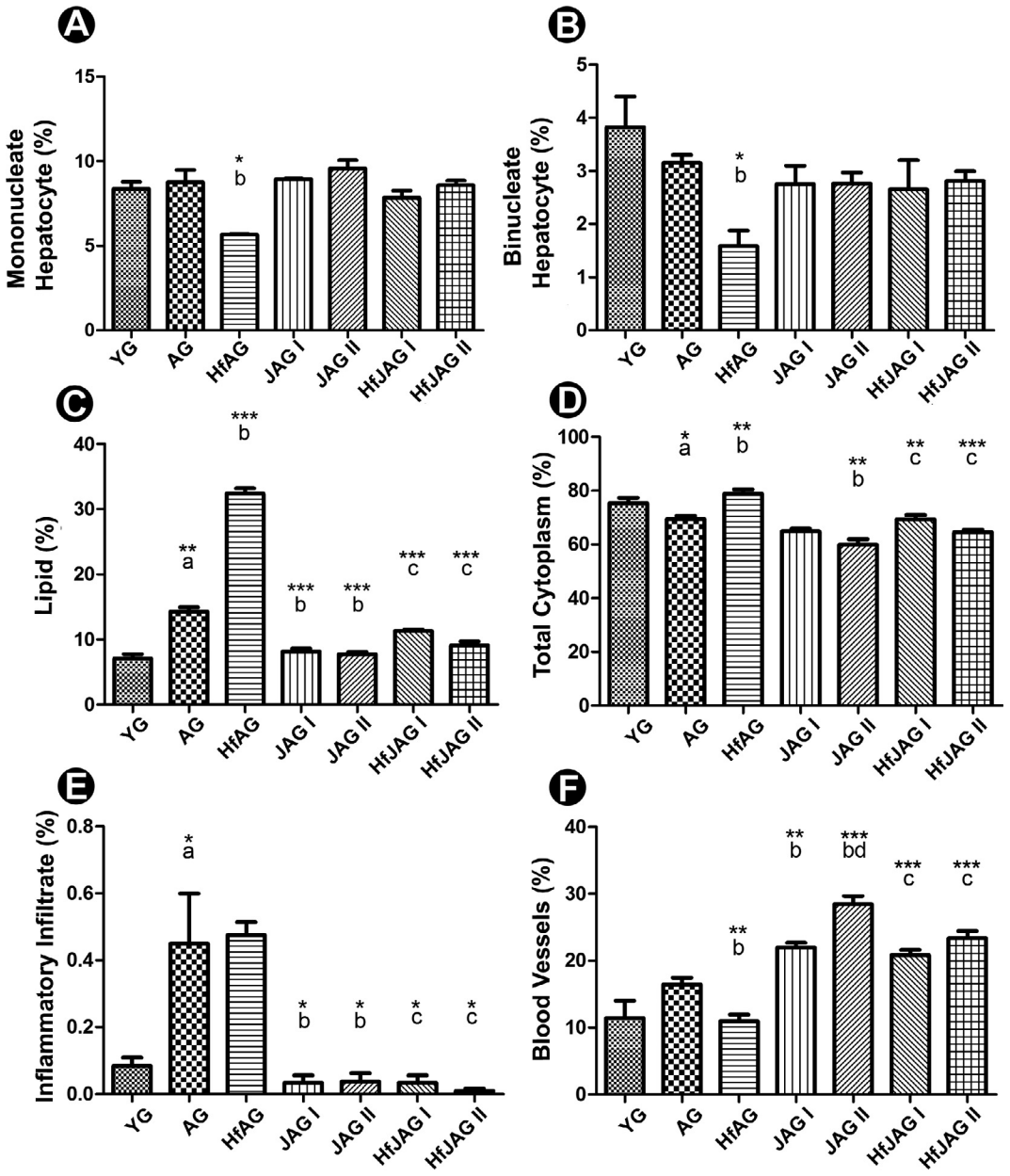

Fig. 3. Histopathological analysis. (A) Mononucleate Hepatocyte (%). (B) Binucleate Hepatocyte (%). (C) Lipeid (%). (D) Total Cytoplasm (%). (E) Inflammatory Infiltrate (%). (F) Blood Vessels (%). Significant differences: [a] relative to YG group; [b] relative to AG group. [c] relative to HfAG group. [d] relative to JAGI group. Considering: $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$.

50% in the HfJAGI and HfJAGII groups compared to the HfAG group (Fig. 5C).

3.6.4. PPARγ

The aging process itself increased the PPARγ hepatic level in AG group, compared to young animals. The high-fat diet ingestion decreased the level of this transcription factor compared to the AG group. Regarding the aged animals, the PPARγ hepatic level increased only in the JAGII group in relation to the AG group. Both PJE doses increased the PPARγ level in HfJAGI and HfJAGII groups compared to HfAG group (Fig. 5D).

4. Discussion

For the first time in scientific literature, our results showed the patented jaboticaba peel extract (PJE) dose-dependent effect in the prevention of obesity, prediabetes and the development of NAFLD in high-fat-fed aged mice. The PJE effects also included the recovery of aging-related hepatic and metabolic alterations. Moreover, the PJE exerted a better anti-obesogenic effect than previously observed after the jaboticaba peel addition to the animals' feed (Lenquiste et al., 2012; Dragano et al., 2013; Baptista et al., 2014). The advantages related to the PJE treatment are mainly related to the precise control of the dose intake and the high concentration of bioactive compounds, which allow the administration of a low dose of extract, becoming a more viable therapy. Also, this study was innovative because for the first time in literature a study focused on aging metabolism that shows specific modifications aggravated by obesity.

The PJE used in this study presented a phenolic content and an *in vitro* antioxidant capacity higher than that described in literature considering the jaboticaba peel. The extract used herein showed at least 100% higher phenolic content compared to that described by Lenquiste et al. (2015) after a methanolic and aqueous jaboticaba peel extraction, using the same technique for total phenolic detection. Moreover, other

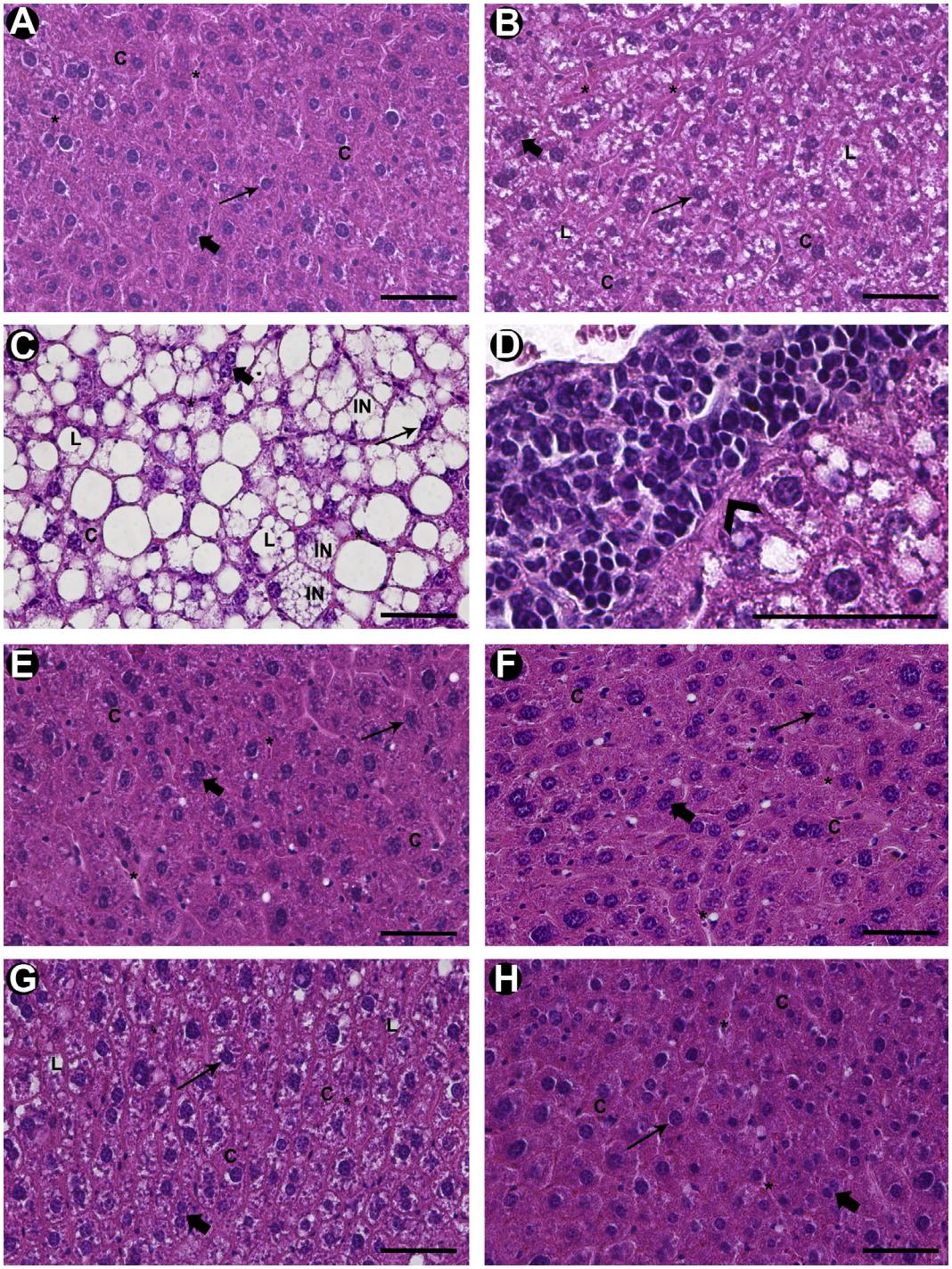

Fig. 4. Photomicrographs of liver stained with hematoxylin and eosin. (A) YG group. (B) AG group. (C–D) HfAG group. (E) JAGI group. (F) JAGII group. (G) HfJAGI group. (H) HfJAGII group. Thin arrow (mononucleate hepatocyte); thick arrow (binucleate hepatocyte); arrow head (inflammatory infiltrate); C (cytoplasm); L (lipid); IN (ballooning cell) e * (blood vessels). Bar = 50 μm.

fruits ethanolic extraction such as the grape peel and mulberry ethanolic extraction provided a 42% and 71% lower phenolic content, respectively, compared to our PJE (Wang, Xiang, Wang, Tang, & He, 2013; Mezni, Aoua, Khazri, Limam, & Aouani, 2017). The anthocyanins are described as one of the main bioactive compounds present in this fruit peel, nevertheless, literature data remains controversial. When compared to the present study, Baptista et al. (2014) reported higher values after a methanolic jaboticaba peel extraction. However, our jaboticaba peel extraction provided more than 100% monomeric anthocyanin in relation to a grape peel aqueous or ethanolic extraction, considering the performance of the same assay with another anthocyanin as standard (Ky and Teissedre, 2015).

In relation to the flavonoid content, our result showed a 200% higher level than that described in the literature by means of a methanolic and aqueous jaboticaba peel extraction (Lenquiste et al., 2015). Also, the PJE flavonoid content was 21%, 42% and more than 100% higher than the mulberry ethanolic extract, the grape peel ethanolic extract and the raspberry aqueous extract, respectively (Gulçin et al., 2011; Wang et al., 2013; Mezni et al., 2017).

Among compounds identified in PJE by UPLC-ESI-MS/MS, eight are

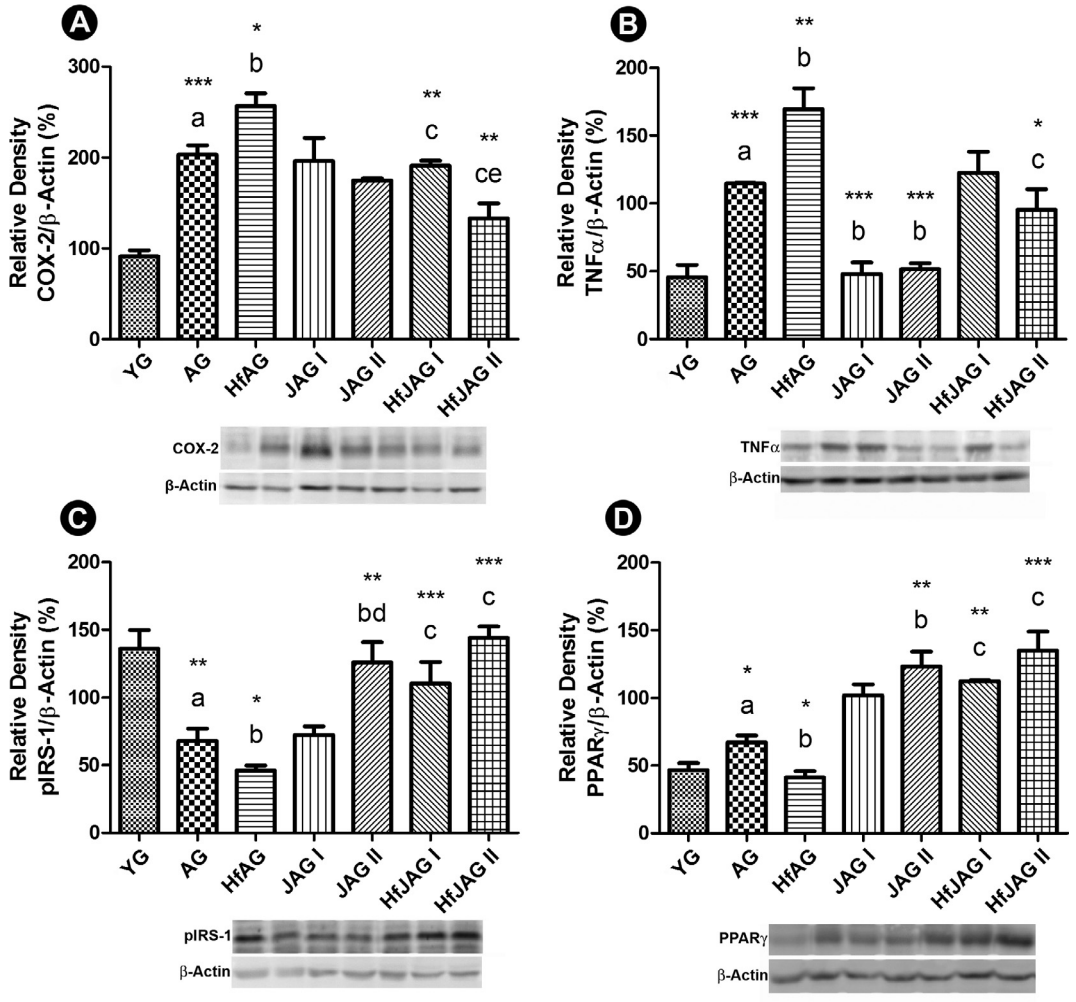

Fig. 5. Western blotting analysis of COX-2, TNFα, pIRS-1 and PPARγ levels from liver of the experimental groups. (A) COX-2. (B) TNFα. (C) pIRS-1. (D) PPARγ. Significant differences : [a] relative to YG group; [b] relative to AG group. [c] relative to HfAG group. [d] relative to JAGI group. [e] relative to HfJAGI group. Considering: $^{*}p < 0.05$; $^{**}p < 0.01$ and $^{***}p < 0.001$.

ellagitannins (casuarinin, casuarictin, pedunculagin, casuariin, HHDP-galloylglucose, HHDP-galloylglucose isomer, tellimagrandin I and tellimagrandin II), one is a gallotannin (pentagalloyl hexose), six are flavonoids (quercetin-3-rhamnoside, (-)-epicatechin, kaempferol hexoside, myricetin-rhamnoside, quercetin and naringenin), three phenolic acids (gallic acid, chlorogenic acid and ellagic acids) and one ellagic acid derivative (ellagic acid pentoside). The phenolic profile is in agreement with data reported in the literature for jaboticaba peel (Wu, Dastmalchi, Long, & Kennelly, 2012; Inada et al., 2015; Plaza et al., 2016).

The PJE antioxidant capacity evaluated by FRAP and ORAC were at least 100% higher than the results described in literature after a methanolic jaboticaba peel extraction (Baptista et al., 2014). Regarding the ABTS technique, the PJE antioxidant capacity was 200% higher than that obtained by Lenquiste et al. (2015) after a methanolic and aqueous jaboticaba peel extraction. The PJE *in vitro* antioxidant potential was at least 100% higher compared to a grape peel ethanolic extraction, considering ORAC and ABTS techniques (Ky and Teissedre, 2015). In addition, results, which were at least 100% higher, were also observed comparing our PJE to a raspberry aqueous extract, by means of ABTS and FRAP assays.

The apparent discrepancy among the studies regarding PJE analyses may be related to the different techniques used for the detection of bioactive compounds; to the different solvents used for the extractions and to the different concentrations of solvent used in the sample preparation. Therefore, the ethanolic extraction was more effective in the present study than the aqueous or the methanolic jaboticaba peel extraction and, also, when compared to the ethanolic extraction in other fruits (grape or mulberry). This fact was confirmed by results that showed the maintenance of the phenolic content and its antioxidant capacity.

It is known that climatic, geographic and sunlight exposure factors influence the biosynthesis of flavonoids, resulting in phenolic compounds variability in the jaboticaba (Jaakola & Hohtola, 2010; Lenquiste et al., 2015). Moreover, the sensitivity of these compounds to the factors described above may explain the variation of results found regarding the antioxidant potential and phenolic composition of the PJE. Nevertheless, the extraction carried out in the present study preserved the bioactive compounds and their *in vitro* antioxidant activity in a higher level than the ones described in the literature for jaboticaba peel and other dark fruits extracts, as discussed above (Wang et al., 2013; Lenquiste et al., 2015; Ky & Teissedre, 2015; Mezni et al., 2017).

In the present study, the PJE treatment significantly ameliorated the metabolic alterations, which indicate the type 2 prediabetes state (Tripathy & Chavez, 2010; Oliveira et al., 2014, 2015), induced by high-fat diet intake in aging mice. These alterations included moderate hyperglycemia, remarkable insulin resistance, glucose intolerance associated with dyslipidemia (hypercholesterolemia and high LDL-cholesterol level) and increased body weight gain. It is well known that a high-fat diet consumption and weight gain is correlated with increased insulin resistance, total cholesterol and its LDL fraction (Emanuela

et al., 2012; Song et al., 2016, Wang et al., 2016). These metabolic alterations are mainly consequences of adipocyte hypertrophy, which promotes a pro-inflammatory microenvironment due to fatty acid release into the circulation (Emanuela et al., 2012). In addition, there is evidence that reactive oxygen species (ROS), also produced in obesity, contribute to β-cell dysfunction and lipid peroxidation, further aggravating insulin resistance, glucose accumulation, dyslipidemia, and consequently the prediabetes state (Brand & Nicholls, 2011; Pepin et al., 2016). Taking into consideration that the aging organism is predisposed to inflammation and oxidative stress, the excessive weight during this period promoted a more severe metabolic imbalance.

Thus, considering the present results, we suggest that the high-fat diet stimulated weight gain because of its higher energy intake, despite weight loss due to aging. The excessive weight gain caused by the chronic high-fat diet consumption reduced the PPARγ levels and possibly promoted a lipid-processing imbalance related to the large intake of saturated fatty acids (Sahin et al., 2017). Furthermore, the weight gain and the altered PPARγ levels possibly stimulated the inflammatory processes, interfering in the COX-2 and TNFα pathways. It probably led to a glycemic impairment by reducing the level of active insulin receptor substrate, and, as a consequence, triggering the initial phase of type 2 diabetes mellitus (T2DM). The weight gain, the total-cholesterol and LDL-cholesterol reduction after the PJE treatment in high-fat diet-fed aged mice are in agreement with the PJE beneficial effects on the glucose metabolism. These data resulted in a remarkable decrease of hyperglycemia and insulin resistance, and improved glucose sensitivity in our animal model of T2DM.

The PJE treatment reduced the food intake and, consequently, the energy intake and weight gain in high-fat-diet-fed aged mice. Corroborating these results, Lenquiste et al. (2015) have also reported a reduction in weight gain in obese adult rats, after the water substitution by a jaboticaba peel aqueous extract. It is known that phenolic compounds have the ability to modulate enzymes involved in the digestion process, such as α-amylase, α-glucosidase and pancreatic lipase, contributing to lower intestinal absorption (Sergent et al., 2012). Wu et al. (2013) demonstrated that juices from fruits rich in anthocyanins, stimulated fatty acid oxidation and increased CPT 1 expression, which is a gene related to β-oxidation, in rodents consuming a high-fat diet. Despite controversial results in the scientific literature, Sahin et al. (2017) related the weight loss to a high PPARγ levels in the liver of high-fat-fed rats after a carotenoid treatment. The bioactive compounds have the ability to modulate hormones and adipokines, due to the fact that products of fruits rich in anthocyanin increased the adiponectin and reduced the leptin levels in overweight animals (Wu et al., 2013; Song et al., 2016). Therefore, the interference of PJE in food and energy intake, especially associated to high-fat diet, could be a result of bioactive compounds effects on hormones, adipokines, enzymes and transcription factors. In addition, it could be suggested that the decrease in food/energy intake and consequently in lipid ingestion are the main factors implicated in the beneficial effects of PJE on the glucose homeostasis verified in our high-fat diet-fed mice.

Moreover, recent studies have shown that anti-inflammatory and antioxidant properties of polyphenols contribute to diabetes improvement, reducing total and LDL-cholesterol, besides increasing HDL-cholesterol levels (Qatanani & Lazar, 2007; Seymour et al., 2009; Lenquiste et al., 2012; Dragano et al., 2013). Dragano et al. (2013) demonstrated that the replacement of 4% of the total daily food intake by jaboticaba peel reduced inflammatory hallmarks in liver, contributing to the increase in insulin peripheral response. In addition, the modulation of enzymes, involved in ROS production and in the lipid peroxidation, was verified after the jaboticaba peel aqueous extract administration to overweight rats (Lenquiste et al., 2015). These effects could be related to the maintenance of β-cell functional status, insulin resistance reduction (Qatanani & Lazar, 2007) and LDL-cholesterol oxidation increase (Rodrigues et al., 2003; Lenquiste et al., 2012). Thus, the partial recovery of glucose and lipid metabolism dysfunctions after PJE treatment, in overweight mice, could be associated to the ability of PJE bioactive compounds to interfere in COX-2, TNFα and oxidative stress pathways. Moreover, high pIRS-1 level after PJE treatment suggested that the extract increased the insulin receptor substrate and consequently the insulin peripheral sensitivity and response, improving the T2DM state. The PJE consumption was also able to enhance the HDL-cholesterol level, which can contribute to the prevention of obesity-related dysfunctions. Furthermore, it should be mentioned that the PJE exerted its positive effect in our animal model at a much lower dose than those described in the literature (Lenquiste et al., 2012; Dragano et al., 2013; Baptista et al., 2014).

Several obesity-related factors, such as oxidative stress, lipid peroxidation and inflammation, contribute to fat accumulation, especially in the liver (Stinkens et al., 2015; Song et al., 2016). The hepatic steatosis due to high-fat diet consumption is related to alterations in the expression of genes responsible for fatty acid oxidation, cholesterol biosynthesis and liver glucose uptake (Song et al., 2016). It is known that free insulin can stimulate genes, as SCREP-1c, which reduces lipolysis, and stimulates fatty acid synthesis in the liver (Carvalheira, Zecchin, & Saad, 2002). Moreover, lower levels of IRS-1 and PPARγ in the liver; have been associated with lipid accumulation in this organ (Sahin et al., 2017). Therefore, we conclude that high-fat diet consumption during aging intensified the inflammatory process and decreased the pIRS-1 and PPARγ levels, worsening the lipid and glucose metabolism. These aspects possibly contributed to aggravating NAFLD development in elderly mice, which already display a basal inflammation state and metabolism dysregulation due to aging.

In the present study, PJE also prevented NAFLD in aged animals that consumed a high-fat diet. It is believed that anthocyanins may decrease hepatic steatosis, mainly due to their anti-inflammatory action, lipolysis promotion, lipogenesis and oxidative stress reduction (Song et al., 2016; Wang et al., 2016). Therefore, we can suggest that the ability of PJE to prevent prediabetes and the onset of dyslipidemia, previously discussed, also contributed to NAFLD prevention in high-fat diet-fed aged animals. The PJE capacity to reduce the pIRS-1 level suggests a regulation of insulin signaling, possibly contributing to stimulate lipolysis and reduce the liver steatoses (Carvalheira et al., 2002). Sahin et al. (2017) related the increase in IRS-1 and PPARγ levels in the liver with the fatty reduction in this organ. Thus, the PJE anti-inflammatory and antioxidant effect observed in the present study, in addition to their capacity to impair insulin and lipid metabolism, may also be related to their hepatic-protective effects.

Interestingly, PJE also affected, in a dose-dependent manner, the lipid and glucose metabolism and liver morphology of aged animals that received a standard diet, indicating a protective action of this compound on the aging-related dysfunctions. According to literature, the mechanisms that correlate aging, glucose and lipid homeostasis have not been fully explained (Yue et al., 2016). Despite this, dyslipidemia and higher glucose levels in aging continue to be considered an important risk factor of cardiovascular disease and diabetes (Gobal & Mehta, 2010; Daskalova et al., 2015, Liu & Li, 2015, Yue et al., 2016). Liver dysfunction and hormonal changes are some of the mechanisms described to explain alterations in the lipid profile during senility (Le Couteur et al., 2007; Gobal & Mehta, 2010).

PJE significantly reduced glycemia, total and LDL-cholesterol plasma levels in aged mice without affecting food intake, which indicates a direct action of PJE on the metabolism of these animals. Also, the PJE hepatic-protective effect was observed considering only senescence, as revealed by the significant reduction in lipid accumulation, inflammatory infiltrate and in the inflammatory markers (COX-2 and TNFα) evaluated in the liver of these animals. Daskalova et al. (2015) have demonstrated a reduction of total and LDL-cholesterol after the consumption of black plum juice by aged animals. The jaboticaba bioactive compounds have already been described as potentially able to modulate intermediary metabolites of the insulin pathway and enzymes involved in cholesterol synthesis (Valcheva-Kuzmanova et al., 2007;

Dragano et al., 2013; Daskalova et al., 2015; Song et al., 2016). These effects are in agreement with our results, considering that the PJE treatment increased the pIRS-1 and PPARγ levels in aged mice. The PJE antioxidant and anti-inflammatory properties as well as bioactive compound ability to interfere in fatty acid oxidation and biosynthesis (Wu et al., 2013; Song et al., 2016; Wang et al., 2016), are probably related to the effects observed after PJE consumption by aged animals. Therefore, we could suggest that PJE interfered in typical aging processes such as oxidative stress and inflammation, by means of glucose and lipid metabolism regulation. This fact may have led to the hypercholesterolemia and glycemia reduction associated with hepatic morphological reestablishment in aged animals.

By and large, the PJE exerted a dose-dependent beneficial effect regarding the glucose metabolism, inflammatory process and liver morphology. Although the PJE dose-dependency was statistically significant mainly in the parameters described above, both PJE doses were effective and did not show apparent side effects or toxicity signs. A recent research in humans demonstrated an improvement in both the serum antioxidant capacity and glucose and insulin response after jaboticaba peel ingestion, in agreement with our results (Plaza et al., 2016). Therefore, considering the PJE benefits verified in the present research we encourage the development of human being studies since life expectancies and obesity have increased in the world, thus contributing to the increase of metabolic syndrome incidence.

5. Conclusion

The PJE chemical characterization demonstrated an antioxidant activity and a phenolic content, which contributed to its protective metabolic effect observed in the present study. The PJE administration associated with high-fat diet intake resulted in a dose-dependent reduction of food consumption and, consequently, a decrease in weight gain and dyslipidemia. These effects could be associated to the HDL-cholesterol level increase and the prevention of metabolic and hepatic alterations, which are indicative of T2DM and NAFLD. Moreover, considering only the aging process, the PJE also had positive systemic actions mainly in preventing hypercholesterolemia, high LDL-cholesterol plasma levels and hepatic structural changes. Thus, the PJE regulated typical aging processes, reducing the organism overload. These PJE effects are probably related to its ability to interfere with the inflammatory process, insulin action and lipid metabolism. The high-fat diet consumption worsened the metabolic alterations observed during aging. The higher energy intake increased body adiposity, inflammatory state and alterations in lipid and glucose homeostasis, triggering severe metabolic conditions such as prediabetes and NAFLD. Therefore, our results pointed to beneficial effects of PJE on hepatic and metabolic dysfunctions related to obesity, T2DM and aging. Further studies with this PJE in human beings could lead to new therapeutic alternatives to treat and/or the above-mentioned these metabolic disorders.

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Conflict of interest

The authors declared that there is no conflict of interest.

Ethical statement

(1) This material has not been published in whole or in part elsewhere;
(2) The manuscript is not currently being considered for publication in another journal;
(3) All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.
(4) The experiments were carried out according to the Guide for Care and Use of Laboratory Animals and were approved by the Committee of Ethics in Animal Experimentation of UNICAMP.
(5) The authors ensure that the manuscript corresponds to an entirely original work, and that other works were appropriately cited.
(6) The authors acknowledge the National Council for Scientific and Technological Development (CNPq – 141766/2015-8 and 301108/2016-1) and the São Paulo Research Foundation (FAPESP – 2015/25714-1) for financial support.
(7) All the authors declared no conflict of interest.
(8) The authors undertake to notify the journal editor or publisher if any significant error or inaccuracy in the manuscript is identified in the future.

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