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

C.A. Lamas[a,*], S.A. Lenquiste[b], A.M. Baseggio[b], L. Cuquetto-Leite[a], L.A. Kido[a], A.C. Aguiar[c], M.N. Erbelin[c], C.B. Collares-Buzato[d], M.R. Maróstica Jr.[b], V.H.A. Cagnon[a]

[a] Department of Structural and Functional Biology, University of Campinas, Bertrand Russel Av, Campinas, São Paulo 13083-865, Brazil
[b] Department of Food and Nutrition, University of Campinas, 80 Monteiro Lobato St, Campinas, São Paulo 13083-852, Brazil
[c] ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas, Campinas, SP 13083-859, Brazil
[d] Department of Biochemistry and Tissue Biology, University of Campinas, 255 Monteiro Lobato St, Campinas, São Paulo 13083-970, Brazil

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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

* Corresponding author at: Department of Structural and Functional Biology, Biology Institute, State University of Campinas– UNICAMP, Bertrand Russel, Block A, Cidade Universitária Zeferino Vaz - Barão Geraldo, Zip code: 13083-865, Campinas, SP, Brazil.
E-mail address: celina.lamas@gmail.com (C.A. Lamas).

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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