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Phenolic compounds from cagaita (*Eugenia dysenterica* DC.) fruit prevent body weight and fat mass gain induced by a high-fat, high-sucrose diet

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ABSTRACT

Cagaita (*Eugenia dysenterica* DC.) is a Brazilian Cerrado biome tree, whose fruit and leaves are used as alternative medicine by local communities to treat diarrhea, diabetes, and jaundice. Phenolic compounds from cagaita fruit have demonstrated *in vitro* antioxidant potential and inhibitory actions toward the activity of enzymes involved in carbohydrate metabolism. Herein, we evaluated whether administration of phenolic-rich extracts from cagaita (CGT) affects obesity and its metabolic complications induced by the intake of a high-fat high-sucrose (HF/HS) diet during an eight-week period. Four groups of male C57BL/6J mice ($n = 9$–10 per group) fed either with a chow diet (Chow group) or an HF/HS diet were daily treated by gavage with water or CGT at doses of 7 and 14 mg gallic acid equivalent (GAE)/kg body weight (HF/HS, CGT7, and CGT14 groups). Treatment with both doses of CGT attenuated the increase in body weight gain and fat accumulation induced by the intake of an HF/HS diet without affecting food intake. Such protection from a diet-induced obesity by CGT was associated with the attenuation of fasting hyperglycemia, hypertriglyceridemia, and hypercholesterolemia, with no effect on glycemic control post-glucose challenge. Furthermore, CGT treatment also improved plasma antioxidant status. The fecal triglycerides excretion was increased by CGT phenolics at both studied doses. No significant alteration in liver glycogen and total cholesterol content was noted among the groups, but a significant decrease in liver triacylglycerol was found in the CGT7 group when compared to the HF/HS group. These results indicate that phenolics from cagaita may play an important role in the prevention of obesity and its associated abnormalities, and also point out their potential for the development of new applications and complementary strategies in therapeutic alternatives.

1. Introduction

The worldwide prevalence of overweight and obesity combined rose by 27.5% in adults and 47.1% in children in the last three decades (Ng, Fleming, Robinson, et al., 2014). This has resulted in a dramatic increase in the prevalence of obesity-associated chronic diseases including type 2 diabetes, cardiovascular complications, and certain types of cancer. Evidence-based studies have demonstrated that the consumption of regular amounts of fruits and vegetables reduces the risk of developing obesity and associated diseases; such benefits have been partially attributed to molecules with high antioxidant capacity, which includes phenolic compounds found in these foods (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005).

Brazil is the world's third largest fruit producer and the seventh largest producer of tropical fresh fruit (FAO, 2013). Due to its area, geographic location, climate, and soil conditions, Brazil has a natural abundance of native fruits; however, only a few of them are being exploited commercially, perhaps due to the limited amount of information available about their chemical composition, and biochemical and nutritional properties. The Brazilian Cerrado is the second largest biome in South America, occupying an area of more than 2 million hectares. In terms of biological diversity, the Brazilian Cerrado is recognized as the richest savanna in the world with 11,627 species of native plants (Ministério do Meio Ambiente, Brazil, 2015). Among this variety of species, some edible fruits are regularly consumed by the local population and commercially exploited such as pequi (*Caryocar brasiliense*), buriti (*Mauritia flexuosa*), cagaita or cagateira (*Eugenia dysenterica* DC.), and mangaba (*Hancornia speciosa*). The cagaita fruit is a yellow-orange roughly spherical berry with a diameter of approximately 3–4 cm and mass varying between 15 and 37 g that has a sweet–sour and slightly astringent taste (Cardoso, Martino, Moreira, Ribeiro, & Pinheiro-Sant'Ana, 2011; Clovegarden, 2015). The cagaita fruit is used in various regional preparations, such as jams, ice-cream, liqueurs, and juices; whereas both the fruit and the leaves are used as popular alternative medicine by local communities to treat diarrhea, diabetes, and jaundice (Lima et al., 2011). Cagaita is considered to be a source of vitamin C and phenolic compounds, including quercetin, ellagitannins, ellagic acid, and kaempferol (Cardoso et al., 2011; Gonçalves, Lajolo, & Genovese, 2010), which were previously shown to exert important

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biological functions preventing the development of obesity and type 2 diabetes (Del Rio, Borges, & Crozier, 2010; Scalbert et al., 2005).

Studies from our group have found that phenolic compounds from cagaita are potent inhibitors of enzymes involved in the carbohydrate metabolism such as α-amylase and α-glucosidase in *in vitro* enzymatic assays (Gonçalves et al., 2010). Here, we investigated whether the administration of CGT at two different doses protects mice from HF/HS diet-induced obesity and metabolic complications.

2. Materials and methods

2.1. Preparation and characterization of phenolic-rich extract from cagaita

The commercial frozen pulp of cagaita was obtained from Central do Cerrado, Urucuia® (Brasília DF, Brazil). Frozen samples were thawed at room temperature before extraction. A representative sample equivalent to 5.0 g of dry matter was homogenized and extracted in 100 mL of 80% aqueous methanol using a Turratec TE-102 (Tecnal) at 4000 rpm for 1 min, three times with intervals of 30 s, in an ice-bath, followed by rotation using a magnetic stirrer (200 rpm) at 5 °C for 30 min. The extract was separated by centrifugation at 5000 *g* for 30 min and filtered through a Whatman No. 3 filter paper. The precipitate was resuspended twice in the aqueous alcoholic solution for additional extraction steps and then centrifuged and filtered as described above. The pooled filtrates were concentrated at 39 °C to remove methanol on a rotary evaporator (Rotavapor R-210; Büchi, Sweden), and 40 mL of extract was made with distilled water.

The aqueous extract was added onto a preconditioned (100 mL of methanol, 150 mL of water) LC-18 SPE tube (10 g of LC-18 SPE, Supelclean™ LC-18, Supelco). After washing with water, the phenolic compounds were eluted with 200 mL of methanol. The eluate was evaporated to dryness and the residue resuspended in 70 mL of water, for CGT7 extract, or 35 mL of water, for CGT14 extract.

The extract was characterized regarding total phenolics (Singleton, Orthofer, & Lamuela-Raventos, 1999) and proanthocyanidin contents (Porter, Hrstich, & Chan, 1985), antioxidant capacity by the oxygen radical absorbance capacity (ORAC) assay (Dávalos, Gómez-Cordovés, & Bartolomé, 2004), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay (Brand-Williams, Cuvelier, & Berset, 1995), the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996), and the pancreatic lipase inhibitory activity (Nakai et al., 2005).

The identification and quantification of the main flavonoids and phenolic acids in cagaita were performed as described previously (Arabbi, Genovese, & Lajolo, 2004), adding the aqueous extract onto a preconditioned (20 mL of methanol, 60 mL of water) polyamide SC6 SPE tube (1 g, Macherey-Nagel GmbH and Co., Düren, Germany). After washing with water, phenolic compounds were eluted with 50 mL of methanol to elute neutral phenolics, and with 50 mL of methanol:ammonia (99.5:0.5) to elute acidic phenolics. These two fractions were pooled and evaporated to dryness under reduced pressure at 39 °C, re-dissolved in HPLC grade methanol (1 mL), and filtered through a 0.22 μm PTFE (polytetrafluoroethylene) filter (Millipore Ltd., Bedford, MA). Identification and quantification were performed using a Prodigy ODS3 reversed phase silica column (5 μm, 250 × 4.6 mm, Phenomenex Ltd., Torrance, CA), in an analytical reversed-phase HPLC (Hewlett-Packard 1100) system with an autosampler and a quaternary pump coupled to a diode array detector. Pure standards of quercetin, kaempferol, and ellagic acid dissolved in methanol (HPLC grade) were used to calibrate the standard curves and retention times. Calibration was performed by injecting the standards three times at five different concentrations. Peak identification was performed by comparing retention times and diode array spectral characteristics with the standards and the library spectra. Quantification of phenolic compounds was performed by comparing the peak area of the sample with that of the standards' peak area injected, and effected on the basis of external standard curves ($r \geq 0.998$) for peaks detected and identified at 270 nm with spectral characteristics similar to the their respective standard. In the case of quercetin and kaempferol derivatives, results were expressed as μg of aglycone. The results were expressed by mL of extract.

Total ellagic acid was determined after acid hydrolysis according to Pinto, Lajolo, and Genovese (2008). An aliquot of 0.5 mL of extract was dried under nitrogen and 2 mL of 2 N trifluoroacetic acid was added, and hydrolysis was performed at 120 °C for 90 min. The hydrolyzed samples were evaporated to dryness under nitrogen, redissolved in methanol and filtered for HPLC analysis.

2.2. Animals and experimental design

All animal procedures performed had been previously approved by the Ethical Committee for Animal Research of the Faculty of Pharmaceutical Science of University of São Paulo (CEUA/FCF/378). Forty 8-week old male C57BL/6J mice of approximately 23 g were used in this study. The mice were matched by weight and kept at 22 ± 1 °C under a 12-hour light/12-hour dark cycle. All mice were fed a low-fat chow diet (NUVILAB CR-1® Sogorb Inc., São Paulo — SP, Brazil) *ad libitum* for one week before beginning the experiment.

The animals were randomly divided into four groups of ten mice each, as follows: 1) a chow fed group that received a daily water administration by gavage (Chow); 2) an HF/HS fed group that received a daily water administration by gavage (HF/HS); 3) an HF/HS fed group that received a daily administration of phenolic-rich extract from cagaita by gavage (7 mg GAE/kg body weight, CGT7); and 4) an HF/HS fed group that received a daily administration of phenolic-rich extract from cagaita by gavage (14 mg GAE/kg body weight, CGT14).

According to the manufacturer, the chow diet provided 12.5 kJ/g of energy, of which, 63% was from carbohydrate, 25% from protein, and 12% from fat. The HF/HS diet provided 19.4 kJ/g, 41% of calories from sucrose, 20% from protein, and 39% from fat, as previously described (Lemieux, Picard, Labrie, Richard, & Deshaies, 2003). The chemical composition – excluding fiber and carbohydrates values – of the diets used in this study was as follows (in grams per 100 g of diet): protein, 23.3; lipids, 4.6; ash, 7.0; and moisture, 9.2 for chow diet; and 19.3; 19.6; 4.0; 4.6 for the HF/HS diet respectively. Diets and water were available *ad libitum* during the experimental period of 8 weeks. Body weight and food intake were recorded every two days, and fasting blood glucose (6 h) was measured from the caudal vein weekly throughout the study by using Accu-Chek Performa® (Roche, Mannheim, Germany).

After 8 weeks, the mice were anesthetized with isoflurane and immediately euthanized by decapitation. Blood was collected from the trunk and plasma separated by centrifugation at 3000 *g* for 20 min at 4 °C. Tissues, including heart, liver, gastrocnemius muscle, and adipose tissues (inguinal, retroperitoneal, epididymal and brown) were removed, separated, weighed, and immediately frozen under liquid nitrogen and stored at −80 °C.

2.3. Glucose tolerance test (GTT)

On the 7th week, the mice were fasted for 6 h and injected intraperitoneally with a glucose solution (1 g/kg body weight), and the glucose concentration was determined in tail blood collected at the baseline (prior to injection) and at 15, 30, 45, 60 and 90 min post-injection, using the Accu-Chek Performa® glucometer (Roche, Mannheim, Germany).

2.4. Plasma biochemical parameters

A LabMax 240® clinical chemistry analyzer and appropriated kits were used to determine plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol, urea and creatinine, following manufacturer's instructions (LABTEST, Lagoa Santa, MG, Brazil). Plasma antioxidant capacity was measured by ORAC (Dávalos et al., 2004) and ferric reducing ability of plasma (FRAP) assays (Benzie & Strain, 1996).