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Phenolic compounds from cambuci (*Campomanesia phaea* O. Berg) fruit attenuate glucose intolerance and adipose tissue inflammation induced by a high-fat, high-sucrose diet

Carlos M. Donado-Pestana [a], Thiago Belchior [b], William T. Festuccia [b], Maria Inés Genovese [a,*]

[a] Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580, Bloco 14, São Paulo, SP, CEP 05508-900, Brasil
[b] Departamento de Fisiologia e Biofísica, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1524, São Paulo, SP CEP 05508-000, Brasil

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ABSTRACT

Elevated intake of certain dietary components, such as phenolic compounds of fruits and vegetables, reduces the risk of developing obesity and other metabolic diseases characterized by a chronic, low-grade inflammation. Cambuci (*Campomanesia phaea* O. Berg) is a Brazilian Atlantic Coastal Forest fruit rich in phenolic compounds including ellagitannins and proanthocyanidins that were previously shown to display anti-inflammatory actions. Herein we investigated whether the administration of phenolic-rich extract from cambuci (CBC) at two different doses protects mice from diet induced obesity, insulin resistance, and chronic peripheral inflammation. In order to verify this, C57BL/6J mice fed either with a chow or a high-fat, high-sucrose (HFHS) diet were daily treated by gavage with water or CBC (18 and 32 mg gallic acid equivalent, GAE/kg body weight) for 8 weeks and evaluated for body mass, adiposity, glucose tolerance, and adipose tissue inflammation. Despite the absence of changes in body weight gain and adiposity, administration of CBC at two different doses protected mice from the disarrangements in glucose homeostasis induced by HFHS feeding as evidenced by the reduced fasting glycemia and insulinemia and improved glucose tolerance featured by CBC treated mice. Along with glucose homeostasis, CBC administration also protected mice from adipose tissue inflammation induced by HFHS feeding as evidenced by the lower expression of proinflammatory cytokines tumor necrosis factor-alpha (*TNF-α*) and interleukin-6 (*IL-6*) and macrophage markers *CD11b*, *F4/80*, and *CD86* in retroperitoneal adipose tissue. Furthermore, the higher dose of CBC increased plasma HDL-cholesterol while reducing LDL-cholesterol levels. Altogether, our findings indicate that phenolic compounds from cambuci have potentially beneficial actions protecting mice from HFHS diet-induced glucose intolerance and adipose tissue inflammation.

1. Introduction

Obesity, defined as an abnormal or excessive accumulation of body fat, is a serious health issue threatening modern society. Obesity, the prevalence of which has markedly increased in the last decades, is associated with the development of serious chronic diseases such as cancer, diabetes and cardiomyopathies, among others, being a leading risk factor for deaths globally and an economic burden to society. Recent estimation indicates that overweight and obesity (body-mass index of 25 kg/m^2 or greater) affects approximately 37% of the world population, or 2.1 billion individuals worldwide (Ng, Fleming, Robinson, et al., 2014). This prevalence has also increased substantially in children and adolescents in developed and developing countries. In addition, overweight and obesity were estimated to cause 3.4 million deaths, in most cases, associated with cardiovascular diseases (Lim et al., 2012).

Several predisposing factors have been associated with obesity such as gender, ethnicity, socioeconomic condition, education level, and diet and regular physical activity with interactions among these factors (Paeratakul, Lovejoy, Ryan, & Bray, 2002). Of special interest to this study is the finding that intake of diets rich in fruits and vegetables decreases the prevalence of obesity and associated diseases namely type 2 diabetes, cardiovascular diseases and cancer (Carter, Gray, Troughton, Khunti, & Davies, 2010). Although the plausible mechanisms by which fruits and vegetables exert those protective actions are unknown, they may be related to natural antioxidant and anti-inflammatory properties featured by the chemical components of these crops (Carter et al., 2010; Cherniack, 2011). These components include a wide range of molecules of different structural patterns, such as phenolic compounds, carotenoids, vitamins and minerals (Wang, Melnyk, Tsao, & Marcone, 2011).

Brazil is the world's third largest fruit producer and seventh producer of tropical fresh fruit (FAO, 2013), among which are included a large number of native and exotic fruit species of potential interest to the food industry that remain underexplored. Among these are the *Myrtaceae*, a

* Corresponding author. Tel.: +55 11 3091 3656; fax: +55 11 3815 4410.
E-mail address: genovese@usp.br (M.I. Genovese).

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large family which is comprised of about 4000 species, including some Brazilian native fruits, such as jaboticaba (*Myrciaria cauliflora* Berg), camu-camu (*Myrciaria dubia* McVaugh), and cambuci (*Campomanesia phaea* O. Berg).

Cambuci grows in Brazil's Atlantic Coastal Forest, which is the world's richest forest in terms of biodiversity. Cambuci is unique in the genus, featuring an ovoid-rhomboidal structure with a horizontal ridge along with a strong sweet scent, but tasting extremely sour like lemons. These fruits are used in various typical preparations, mainly jams, jellies, ice-cream, and liqueurs (Kawasaki & Landrum, 1997). Despite its consumption, limited information is available on the composition and functionality of bioactive compounds of cambuci and their potential health benefits. In a previous study evaluating ethanolic extracts of seven fruits from the Brazilian Atlantic Coastal Forest, cambuci featured the highest concentration of phenolic compounds (Haminiuk et al., 2011), such findings that were also observed when comparing commercial frozen pulps (Genovese, Pinto, Gonçalves, & Lajolo, 2008).

Phenolic compounds from cambuci were also found to be potent inhibitors of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase) in *in vitro* enzymatic assays (Gonçalves, Lajolo, & Genovese, 2010). The suppression of glucose absorption through the inhibition of these enzymes may be one therapeutic approach to prevent the postprandial hyperglycemia. Thus, in the present study, we investigated whether the administration of phenolic-rich extract from cambuci (CBC) at two different doses protects mice from diet-induced obesity, hyperglycemia, insulin resistance and chronic peripheral inflammation. For this, C57BL/6J mice fed either with a chow or a high-fat, high-sucrose (HFHS) diet were daily treated by gavage with water or CBC (18 and 32 mg gallic acid equivalent, GAE/kg body weight) for 8 weeks and evaluated for body mass, adiposity, glucose tolerance, and adipose tissue inflammation.

2. Materials and methods

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

The commercial frozen pulp of cambuci was obtained from Sítio do Bello (Paraibuna — São Paulo, Brazil). Frozen samples were thawed at room temperature before extraction was performed. 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 centrifuging for 30 min at 5000 *g* 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 CBC18 extract, or 35 mL of water, for CBC32 extract.

The extract was characterized in relation to 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), and the ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996), and pancreatic lipase inhibitory activity (Nakai et al., 2005).

The identification and quantification of the main flavonoids and phenolic acids in cambuci 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 evaporated to dryness under reduced pressure at 39 °C, redissolved in HPLC grade methanol (1 mL) and filtered through 0.22 μm PTFE (polytetrafluoroethylene) filters (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. Total ellagic acid was determined after acid hydrolysis according to Pinto, Lajolo, and Genovese (2008). An aliquot of 2 mL of extract was dried under nitrogen and 2 mL of 2 N trifluoroacetic acid were 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 were previously approved by the Ethical Committee for Animal Research of the Faculty of Pharmaceutical Science of University of São Paulo (No. CEUA/FCF/378).

Forty 8-week old male C57BL/6J mice, weighting approximately 24 g, were used in this study. The mice were matched by weight and kept at 22 ± 1 °C and 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) The Chow group received the low-fat chow diet and water by gavage; 2) the HFHS group received the high-fat, high-sucrose diet and water by gavage; 3) the CBC18 group received the high-fat, high-sucrose diet and the phenolic-rich extract from cambuci by gavage (18 mg GAE/kg body weight); and 4) the CBC32 group received the high-fat, high-sucrose diet and the phenolic-rich extract from cambuci by gavage (32 mg GAE/kg body weight). The water and extracts were administered every day for 8 weeks.

According to the manufacturer, the chow diet provided 12.5 kJ/g as energy, of which 63% was from carbohydrate, 25% from protein, and 12% from fat. The HFHS 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). 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, mice were anesthetized with isoflurane, immediately euthanized by decapitation and their trunk blood was collected and the plasma separated by centrifugation at 3000 *g* for 20 min at 4 °C. The tissues, including heart, liver, gastrocnemius muscle, and adipose tissues (inguinal, retroperitoneal, epididymal and brown) were removed, weighed, and immediately frozen under liquid nitrogen and stored at −80 °C.

2.3. Glucose tolerance test (GTT)

GTT was performed on the mice at week 7 of the experiment. The animals 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 baseline (prior to injection) and at 15, 30, 45, 60 and 90 min post-injection, using the Accu-Chek Performa® glucometer (Roche, Mannheim, Germany). Blood samples