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Euterpe oleracea Mart. (açaí) seed extract associated with exercise training reduces hepatic steatosis in type 2 diabetic male rats☆

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Abstract

Type 2 diabetes mellitus contributes to an increased risk of metabolic and morphological changes in key organs, such as the liver. We aimed to assess the effect of the açaí seed extract (ASE) associated with exercise training on hepatic steatosis induced by high-fat (HF) diet plus streptozotocin (STZ) in rats. Type 2 diabetes was induced by feeding rats with HF diet (55% fat) for 5 weeks, followed by a single low dose of STZ (35 mg/kg i.p.). Control and diabetic groups were subdivided into four groups that were fed with standard chow diet for 4 weeks. Control (C) group was subdivided into Sedentary C, Training C, ASE Sedentary C and ASE Training C. Diabetic (D) group was subdivided into Sedentary D, Training D, ASE Sedentary D and ASE Training D. ASE (200 mg/kg/day) was administered by intragastric gavage, and the exercise training was performed on a treadmill (30 min/day; 5 days/week). Treatment with ASE associated with exercise training reduced the blood glucose (70.2%), total cholesterol (81.2%), aspartate aminotransferase (51.7%) and hepatic triglyceride levels (66.8%) and steatosis (72%) in ASE Training D group compared with the Sedentary D group. ASE associated with exercise training reduced the hepatic lipogenic proteins' expression (77.3%) and increased the antioxidant defense (63.1%), pAMPK expression (70.2%), cholesterol transporters (71.1%) and the pLKB1/LKB1 ratio (57.1%) in type 2 diabetic rats. In conclusion, ASE treatment associated with exercise training protects against hepatic steatosis in diabetic rats by reducing hepatic lipogenesis and increasing antioxidant defense and cholesterol excretion.

Keywords: Type 2 diabetes; Hepatic steatosis; *Euterpe oleracea* Mart.; Exercise training; lipogenesis; oxidative stress

1. Introduction

Type 2 diabetes mellitus (T2DM) is a critical health problem in both developing and developed countries. T2DM contributes to an increased risk of developing metabolic changes and impairment of target organ, such as the liver [1].

Nonalcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of metabolic syndrome and is commonly associated with obesity and diabetes [2] The incidence of NAFLD is reported to be approximately in the 20%–30% range of the general population in various countries. However, it reaches nearly 70%–75% in individuals with T2DM and is almost certainly increasing [3]. T2DM has been associated with faster progression to steatosis with inflammation, necrosis and advanced fibrosis, supporting the concept that NAFLD should be considered as a complication of T2DM [4].

The *Euterpe oleracea* Mart. is a plant from Aracaceae family, popularly known as "açaí" and widely found in the Amazon region of Brazil. Popular reports indicate the beneficial effects of açaí to treat inflammation, anemia, fever, pain and flu [5].

Recent studies have demonstrated a significant beneficial effect of açaí extract on the metabolic changes associated with obesity, hepatic steatosis and DM [6–8]. Our group previously demonstrated that açaí seed extract (ASE), rich in catechin, epicatechin and polymeric proanthocyanidins [8,9], has a potent endothelium-dependent vasodilator effect [10]; antihypertensive, antioxidant [11–13] and anti-inflammatory properties [9]; as well as hypolipidemic action [8].

Exercise training has been shown to play an important role in the treatment and prevention of T2DM and is one of the first lifestyle interventions in the treatment of NAFLD [14]. Studies [15] have demonstrated that regular exercise, even without calorie restriction,

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reduces liver steatosis through an improvement in glycogen synthesis [16] and increase in free fat acid oxidation [17]. There is a large body of literature describing the beneficial metabolic consequences of exercise training on skeletal muscle metabolism, but the effects of exercise training on liver metabolism are still limited [14].

Until this moment, there is no report on the pharmacological activity of ASE associated with exercise training on hepatic steatosis in type 2 diabetic rats. Therefore, experiments were undertaken to determine the action of ASE associated with exercise training on hepatic steatosis with focus on hepatic lipid profile, and changes on lipogenesis, lipid excretion and oxidative status observed in adult type 2 diabetic rats.

2. Materials and methods

2.1. Induction of experimental diabetes in rats

The experiments were approved by the Ethics Committee for Experimental Animals Use and Care (CEA) of the Institute of Biology/Rio de Janeiro State University (protocol: CEA/058/2012). The animals were housed in a room with controlled temperature and dark–light cycles. Male Wistar rats weighing 180–200 g were randomly divided into two nutritional groups: a standard chow diet (Control; 10% energy from lipids, 76% from carbohydrate and 14% from protein; 3.8 kcal/g; n=40) and a high-fat diet (HF; 55% energy from lipids, 31% from carbohydrate and 14% from protein; 5.2 kcal/g; n=40). The diets were manufactured in accordance with the recommendations of the American Institute of Nutrition (AIN-93M) [18]. Three weeks after beginning the experimental diet, the HF group was fasted for 12 h (free access to water) and received streptozotocin (STZ) (35 mg/kg in citrate buffer i.p.; pH: 4.5), as previously described [19]. The control group received an intraperitoneal injection of the vehicle solution. Two weeks after STZ injection and 5 weeks after beginning the experimental diet, rats from HF group with blood glucose levels above 250 mg/dl were considered diabetic. At this point, the animals were submitted to exercise training and/or ASE treatment for 4 weeks. ASE (200 mg/kg/day) was administered by intragastric gavage, and the exercise training was performed in a treadmill (30 min/day; 5 days/week). During this period, all groups were fed with standard chow diet, and glycemia was determined by a glucometer (Accu-Chek Active, Roche, Manhein, Germany) once a week. Food intake and water consumption were estimated twice a week for 9 weeks. The food intake was not different between the groups, and the water consumption was increased in all diabetic groups compared with the controls (data not shown). Therefore, this study was performed with eight groups. The Control (C) group was subdivided into Sedentary C; Training C, ASE Sedentary C and ASE Training C. The Diabetic (D) group was subdivided into Sedentary D, Training D, ASE Sedentary D and ASE Training D.

2.2. Preparation of açaí (E. oleracea Mart.) seed extract

The hydroalcoholic extract was obtained from decoction of the açaí seeds as previously described [9]. Typically, 100 g of seed yielded approximately 5 g of lyophilized extract. The content of polyphenols in ASE, measured by analyzing for total phenol by Folin–Ciocalteu procedure, was around 265 mg/g of extract. Recently, an analysis of the composition of ASE by high-performance liquid chromatography showed the presence primarily of catechin and polymeric proanthocyanidins [8,9].

2.3. Exercise training

Exercise training was performed in a treadmill (Insight Equipments, Brazil), 5 days per week, 30 min per day for 4 weeks. The velocity was progressively increased to 50% to 60% of the maximal velocity obtained during a maximal treadmill stress test, 0% grade [20].

2.4. Serum assays

Serum total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride (TG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed by colorimetric assays (Bioclin, Belo Horizonte, Brazil).

2.5. Hepatic lipid profile

Liver lipid profile was evaluated according to the method described by Folch et al. (1957). Liver samples (50 mg) were homogenized in 1 ml isopropanol and centrifuged for 10 min at 4°C. TC and TG contents were measured by a colorimetric assay (Bioclin, Belo Horizonte, Brazil).

2.6. Hepatic glycogen content

Glucose produced through glycogen hydrolysis was measured using a commercial kit (Glucox, Doles, Goiânia, GO, Brazil). Liver samples were homogenized with 4 ml of trichloroacetic acid (10%) and then centrifuged (1000g, 10 min, 4°C). The supernatant (2 ml) was added to 5 ml of absolute ethanol and frozen. The mixture was centrifuged after 24 h (1000g, 10 min, 4°C), and supernatant was discarded. Glycogen was hydrolyzed through boiling the pellet for 30 min with 1 M HCl. After adding 1 ml of 1 M sodium hydroxide to neutralize the mixture, glucose was measured in 200 μl of the supernatant [21].

2.7. Western blotting

The expression of liver kinase B1 (LKB1), phosphorylated LKB1 (pLKB1), adenosine monophosphate activated protein kinase (AMPK), phosphorylated AMPK (pAMPK), sterol regulatory element-binding protein 1c (SREBP-1c), acetyl CoA carboxylase (ACC), phosphorylated ACC (pACC), MTP microsomal triglyceride transfer protein (MTP), 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMGCoA-R), ATP-binding cassette, subfamily G transporter (ABCG)5 and ABCG8 was evaluated in the eight groups. Liver samples were homogenized in cold lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCL, 0.1% SDS, 5 mM EDTA, 50 mM NaF and 1% Triton X-100) containing Complete Protease Inhibitor Cocktail Tablets (Roche, Basel, Switzerland) using an Ultra-Turrax homogenizer (IKA Werke GmbH & Co. KG, Staufen, Germany). The total protein content was determined by the BCA protein assay kit (Pierce, Rockford, IL, USA). Samples (20 μg total protein) were electrophoresed in 10% Tris-glycine sodium dodecyl sulfate polyacrylamide gels, except for pACC and LKB1 protein that used 8% gels. Proteins were transferred for polyvinylidene fluoride membranes (Hybond ECL; Amersham Pharmacia Biotech, London, UK). The blots were blocked 5% bovine albumin (Sigma-Aldrich Co., St. Louis, MO, USA) in T-TBS (0.02 M Tris/0.15 M NaCl, pH 7.5, containing 0.1% Tween 20) at room temperature for 1 h and incubated with primary antibodies (1:1000 concentration) overnight at 4°C. After washing with T-TBS, blots were incubated with corresponding secondary conjugated antibodies at 1:5000 and 1:10,000 concentration for 1 h. Antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). We also incubated all membranes with α-actin antibody to avoid possible inconsistency in protein loading and/or transfer. Blots were developed with enhanced chemiluminescence (ECL; Amersham Biosciences Inc., Piscataway, NJ, USA). The signals were visualized by ChemiDoc Resolutions System and determined by quantitative analysis of digital images of gels using Adobe Photoshop version 13.0 (Adobe System Incorporated).

2.8. Determination of oxidative damage: malondialdehyde and carbonyl protein assay

The lipid membrane damage was determined by formation of products of lipid peroxidation (malondialdehyde) concentration from plasma and liver homogenates using the thiobarbituric acid reactive substances method as previously described [22]. Protein carbonylation was determined in plasma and liver homogenates according to the method described by Levine et al. [23].

2.9. Immunohistochemistry

Liver tissue sections were initially dewaxed and rehydrated by serial passages through xylene and graded alcohols. To block endogenous peroxidase activity, they were placed in 3% H_2O_2, and to reduce nonspecific protein binding, they were incubated in phosphate-buffered saline supplemented with 5% bovine serum albumin for 20 min. Then, the slides were incubated with primary antibodies for 8-isoprostane at a dilution of 1:100 (polyclonal antibody; Oxford Biomedical Research, Oxford, MI, USA) at 4°C overnight in humidified chamber. After that, the samples were rinsed in phosphate-buffered saline and incubated with biotinylated linked antibody and peroxidase-labeled streptavidin, according to the product datasheet instructions (LSAB kit; Dako). The peroxidase activity was revealed by 3,30′-diaminobenzidine tetrachloride (K3466, DAB; DakoCytomation). Sections were counterstained with Mayer's hematoxylin, rinsed and then mounted. Digital images from the liver were obtained and studied by image analysis. A selection tool was used to identify the liver area with positive immunoreactions, and this selection was segmented in a black-and-white image, where white shows the immunostained area. The liver was delimited using an irregular AOI tool, and inside this delimited area, the tissue area occupied by white color was quantified using the image histogram tool. It was expressed as density stained per liver (%) using Image-Pro Plus version 7.0 (Media Cybernetics, Silver Spring, MD, USA).

2.10. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities

Antioxidant activity was determined in liver homogenates. SOD activity was assayed by measuring the inhibition of adrenaline auto-oxidation as absorbance at 480 nm [24]. CAT activity was measured in terms of the rate of decrease in hydrogen peroxide at 240 nm [25]. GPx activity was measured by monitoring the oxidation of NADPH at 340 nm in the presence of hydrogen peroxide [26].