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Mango leaf tea promotes hepatoprotective effects in obese rats

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ABSTRACT

As mango leaf tea contains mangiferin and other bioactive compounds, this study investigated its anti-inflammatory, antioxidant and hepatoprotective effects on rats with high-fat induced obesity. Three groups were established: a control group (AIN93M diet), an obese group (high-fat diet HFD) and a treatment group (HFD with mango leaf tea for 8 weeks). Mango leaf tea increased antioxidant enzymes, total antioxidant capacity, AdipoR2 and PPAR-α mRNA and proteins expressions and, it also inhibited the NF-κB p65 and SREBP1c genes expressions in the liver. This beverage also leads to Cpt1 overexpression and a significant decrease in the accumulation of fat droplets, improving the hepatic steatosis. Molecular docking suggested a positive interaction between mangiferin, the main bioactive compound of mango leaf tea, and PPAR-α. Mango leaf tea exhibited a hepatoprotective effect through activating PPARα and decreasing the NF-κB p65 expressions, reducing oxidative stress and steatosis, and improving the lipid metabolism.

1. Introduction

Obesity is one of the most prevalent disorders worldwide and the main risk factor for the development of inflammatory process and oxidative stress to name a few. In 2016, an estimated of 41 million children (under the age of 5 years), 340 million children and adolescents (aged 5–19), and 650 million adults (aged 18 years and over) were overweight or obese. In accordance to the World Health Organization these global estimates are increasing (Fernández-Sánchez et al., 2011; World Health Organization, 2016). Fats excessive consumption results in a lipid metabolism disorder which can increase lipid delivery to the liver and reduce fatty acid oxidation. This can manifest in an accumulation of fatty acids (as triacylglycerols) in hepatocytes, causing hepatic steatosis (Fabbrini, Sullivan, & Klein, 2010; Koo, 2013). The body has a particular sensitivity to high-fat consumption, so it is more exposed to an imbalance of the redox homeostasis (between reactive oxygen species and antioxidants) and an increasing of proinflammatory mediators (Fernández-Sánchez et al., 2011; Vincent, Innes, & Vincent, 2007). Therefore, obesity by high fat diet intake is strongly related to oxidative stress and hepatic steatosis (Koo, 2013). During obesity, chronic inflammation occurs due to inflammatory molecules production (cytokines) and cells activation of the immune system (neutrophils and macrophages), as a result of the excessive fat cells accumulation, leading to the activation of different signalling pathways. The nuclear factor kappa B (NF-κB) and peroxisome proliferator-activated receptor (PPAR) pathways has been demonstrated to be involved in obesity inflammation process (Asghar & Sheikh, 2017; Lee, 2013; Pawlak, Lefebvre, & Staels, 2015; Tailleux, Wouters, & Staels, 2012).

Due to the serious effects of obesity on the human metabolism, many new treatments are being developed, including the use of natural and phytotherapeutic products. Teas and extracts containing multiple bioactive compounds have been widely studied and used. Teas are natural, inexpensive, and contain several bioactive compounds with functional properties, representing a great alternative for the treatment and prevention of obesity and its alterations (Chakrabarti, 2009; Jobu et al., 2013; Lee et al., 2011; Moreira et al., 2017).

Mango (*Mangifera indica*) is a tropical fruit rich in bioactive compounds with high therapeutic potential. The mango leaf is less used and it is considered a kind of crop waste. However, it is an important source of mangiferin, phenolic, flavonoids, benzophenones and antioxidants

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with free radical scavenging activity. These bioactive compounds has been linked to biological activities including anti-inflammatory, antioxidant, antidiabetic and others (Medina Ramírez et al., 2016; Pan et al., 2018; Ribeiro & Schieber, 2010; Ribeiro, Barbosa, Queiroz, Knödler, & Schieber, 2008; Zhang et al., 2011). Since tea is important to human life and mango leaves contain many bioactive compounds, we developed and studied a tea using processed mango leaves of Ubá variety. We have previously showed that the mango leaf tea contain 0.72 ± 0.08 mg ml^{-1} of mangiferin, 1.59 ± 0.11 mg GAE mL^{-1} of total phenolics and 80.33 ± 0.18% of radical scavenging activity (Medina Ramírez et al., 2016). Also we demonstrated that this beverage decreases the visceral fat accumulation, regulates glucose metabolism, stimulates the anti-inflammatory markers and improves adipocytes hypertrophy, confirming its anti-obesity effects (Medina Ramírez et al., 2017). In contrast, currently, there are not studies that report its effects on the liver of obese rats after treatment with mango leaf tea. Consequently, we hypothesized that mango leaf tea can modulate markers related to hepatic lipid accumulation, enhance antioxidation, and improve liver alterations caused by the high fat-diet intake. Thus, the current research aims to evaluate the hepatoprotective effects of mango leaf tea on liver damage in obese rats.

2. Materials and methods

2.1. Tea preparation

Young leaves from *M. indica*, Ubá variety, were collected in October (spring, 2015) from the Zona da Mata area (20°60′ S, 43°06′ W, 183 m), Minas Gerais State (Brazil). The plants were identified, and voucher specimens were deposited at the herbarium of the Federal University of Viçosa under the number No. VIC37611. The leaves were washed, sanitized, dried and crushed as described previously (Medina Ramírez et al., 2016). The fine powder obtained (50 g) was blended with one litre of water (5% of final concentration), boiled for 5 min and then filtered (Melitta filter paper N°4). The mango leaf tea was previously characterized by Medina Ramírez et al. (2016), where mangiferin was analysed via high-performance liquid chromatography (HPLC), total phenolic was estimated colorimetrically using the Folin-Ciocalteu reagent and the antioxidant activity was analysed by using the 2,2-diphenyl-1-picrilhidrazil-DPPH assay.

2.2. Assay biologic

A total of twenty-four, sixty-day old, male Wistar rats (200 ± 50 g) were placed under controlled conditions: 12/12-h light–dark cycle (AM 07:00-PM 07:00), room temperature 22 ± 3 °C and constant humidity (80%). The animals were supplied by the Animal Laboratory of the Biological Science and Health Centre, Federal University of Viçosa. All the experimental procedures were performed in accordance with the Ethic Committee for Animal Research of the Federal University of Viçosa, Brazil (approval registered under the number 29/2016). The experimental design and dietary intervention were previously described (Medina Ramírez et al., 2017). According to the method, the control non-obese group (CG) received AIN-93M diet and water, the obese group (OB) was fed a high-fat diet-HFD and water, and the treated group (TF) received a HFD and 50 mL/day of mango leaf tea, during eight weeks. OB and TF groups were both fed for 7 weeks with HFD before the intervention started. Water was administered to rats *ad libitum*. The tea was prepared daily and administered via oral using sipper bottles which were washed and changed daily, simultaneously with the tea. The experimental diets are presented in Table 1 (RESEARCH-DIETS®, 2006). The animals were anesthetized with Isoforine® 100% (Cristália, SP, Brazil) and euthanized by cardiac puncture. Blood and liver tissue were collected and stored at −80 °C, and a liver fragment was separated in order to perform histological analysis.

Table 1
Diets composition and caloric density.

Ingredients (g 100 g^{-1})	AIN-93M	HFD
Casein	14.00	19.50
Maltodextrin	15.50	10.00
Saccharose	10.00	34.10
Corn starch	46.57	5.32
Soybean oil (mL)	4.00	1.00
Lard	0.00	20.00
Cellulose	5.00	5.00
Mineral mix	3.50	3.50
Vitamin mix	1.00	1.00
Bitartarate choline	0.25	0.25
L-cystine	0.18	0.18
Cholesterol	0.00	0.15
Butylated hydroxytoluene	0.0008	0.004
Total	100.00	100.00
Calories (Kcal)		
Casein	56.00	78.00
Maltodextrin	62.00	40.00
Saccharose	40.00	136.40
Corn starch	186.28	21.28
Soybean oil (mL)	36.00	9.00
Lard	–	180.00
CD	3.80	4.70

CD: caloric density (Kcal g^{-1})

2.3. Food consumption, body weight and serum parameters

Tea and food consumption were measured daily and once per week respectively. For the tea intake control, drink spills and the surplus beverage in drinking bottles were collected and measured. The animals weight was monitored once per week at the same time. Food efficiency was calculated as follows: FE = [body-weight gain (g)/energy intake (kcal)] * 1000. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triacylglycerols (TG), were determined previously (Medina Ramírez et al., 2017), using commercial kits (Bioclin Quibasa, SP, Brazil).

2.4. Analyses of liver oxidative stress

All chemicals were of analytical grade and were purchased from Sigma-Aldrich (Saint Louis, USA). Liver homogenate was prepared homogenizing 200 mg of the liver tissue (one per animal of each group) in Tris-HCL buffer solution (10 mM pH 7.4). Afterwards it was centrifuged for 10 min at 4 °C and 12,000*g*. The supernatant was carefully collected and stored at −80 °C. Values obtained were normalized by the amount of total protein.

2.4.1. Bradford protein assay

Total protein was quantified by Bradford protein assay (Bradford, 1976). For the reaction, 5 µl of homogenate, 395 µl of distilled water and 100 µl of Bradford reagent (0.01% Coomassie Brilliant Blue [G250], 4.7% ethanol [95%], and 8.5% phosphoric acid [85%]), were combined. Samples were vortex for 30 s and allowed to stand for 15 min, without light illumination at room temperature (20 °C). The absorbance was read (spectrophotometer Thermo Scientific MultiSkan™ GO) at 595 nm. Values were expressed as milligrams of protein per millilitre (mg Ptn mL^{-1}), using a standard curve of Bovine serum albumin protein (2 mg mL^{-1}) with concentrations varying from 2 to 44 µg mL^{-1}.

2.4.2. Superoxide dismutase (SOD)

SOD activity was determined using the method based on the enzyme capacity to inhibit 50% of pyrogallol oxidation (Marklund & Marklund, 1974). For the reaction 20 µl of liver homogenate, 6 µl of MTT (bromide (3- [4,5-dimethylthiazol-2 M] -2,5-diphenyltetrazolium), 1.25 mM), 15 µl of pyrogallol (1 mM, HCL 10 mM) and 259 µl of Tris-EDTA buffer