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Original Article

Effects of hydroalcoholic extract of *Celtis iguanaea* on markers of cardiovascular diseases and glucose metabolism in cholesterol-fed rats

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ABSTRACT

Celtis iguanaea (Jacq.) Sarg., Cannabaceae, is popularly used in the treatment of diabetes mellitus. However, chemical and pharmacological investigations are lacking. In this study, we investigated the effects of the hydroalcoholic extract from *C. iguanaea* on markers of cardiovascular diseases and the glucose metabolism in cholesterol-fed rats. Therefore, hypercholesterolemic rats (1% cholesterol) were orally treated with *C. iguanaea* extract (C-150, CI-300, or CI-600 mg/kg) or simvastatin (4 mg/kg) ($n = 6$) once a day (30 days) with a hypercholesterolemic diet. A control group (C) was given saline. *C. iguanaea* extract showed significant decreases in serum levels of total cholesterol, LDL-cholesterol, HMG-CoA-reductase, interleukin-1 and 6, TNF-α and IFN-γ when compared to group C ($p < 0.001$). Hypoglycemic effects were observed along with a decrease of the activity of sucrase (CI-600), maltase (CI-150, CI-300), and an increase in muscle glycogen levels (CI-300). Antioxidant effects were observed in plasma by the decrease of TBARS and increase of nonprotein thiols levels (CI-600). The histopathological analysis showed a significant decrease in the liver fat area for *C. iguanaea* extract compared to group C ($p < 0.001$). Our results suggest that the biological effects of *C. iguanaea* extract could be related to the flavonoids that possibly exert antioxidant, enzymatic inhibitory, and insulin-mimetic effects.

Introduction

Cardiovascular diseases (CVD), a group of disorders of the heart and blood vessels, are considered the first cause of death globally (WHO, 2016a). The risk for developing CVD such as atherosclerosis increases significantly with elevated total cholesterol and LDL-cholesterol and decreased HDL-cholesterol values (American Heart Association, 2017). In addition to hypercholesterolemia, oxidative stress plays an important role in the progress of atherosclerosis. Excessive production of reactive species contributes to converts LDL-cholesterol in oxidized LDL (oxLDL-C) that is recognized by macrophages. Macrophages activated by oxLDL-C induce further oxidative stress, which in turn, contributes to inflammatory response by secreting pro-inflammatory cytokines (Chávez-Sánchez et al., 2014).

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Other risk factor for the development of CVD is diabetes, a chronic disease that occurs when the body is unable to produce or effectively use the hormones responsible for regulating blood glucose levels (WHO, 2016b). Diabetes can contribute to the occurrence of CVD such as hyperlipidemias, characterized by an excess of lipids, mainly cholesterol, triacylglycerides (TG), and low-density lipoprotein (LDL) (Aronow, 2013; Nirosha et al., 2014; WHO, 2016a).

For the treatment and prevention of hyperlipidemia, statins are the drugs of choice. However, these molecules can cause several side effects (Stroes et al., 2015). Various plants, which contain substances such as saponins, polyphenols, and flavonoids, have shown good results in reducing plasma lipid levels (Shimoda et al., 2006), and recently, advances have been made in the search for natural products able to reduce hyperlipidemias with fewer side effects (Koriem, 2014; Mohamed et al., 2014; Waltenberger et al., 2016).

Celtis iguanaea (Jacq) Sarg., Cannabaceae, is popularly known as "esporão-de-galo", "taleira", "sarã" and "gurrupiá", according to the region where it is found in Brazil, and may develop in temperate or tropical regions (Silva and Proença, 2008; Martins and Pirani, 2009; Paula et al., 2010). Previous studies showed the traditional use of leaves of *C. iguanaea* in the treatment of body pain, rheumatism, asthma, cramping, dyspepsia, urinary infections, and for the control of diabetes mellitus (Hernandez-Galicia et al., 2002; Tene et al., 2007; Silva and Proença, 2008; Paula et al., 2010; Martins et al., 2015).

Recently, a gastro-protective effect of the hexane fraction of the ethanolic extract was observed using different models of gastric ulcers (Sousa et al., 2013; Martins et al., 2014a,b). The extracts administered to mice and *Artemia salina* demonstrated no cytotoxic or genotoxic effects (Trevisan et al., 2012; Borges et al., 2013). Among the chemical constituents, the presence of pentacyclic triterpenes of type friedelano, friedelin, and epifriedelinol is reported (Trevisan et al., 2012). Preliminary phytochemical analyses of leaves and stems of *C. iguanaea* also revealed the presence of coumarins, mucilage, and flavonoids (Paula et al., 2010).

Flavonoids have demonstrated multiple pharmacological effects, including anti-inflammatory, antioxidant, anti-cancer, hypoglycemic, and hypolipidemic activities (Lago et al., 2014; Zhang et al., 2016; Li et al., 2016). However, to *C. iguanaea* have not been performed studies evaluating protection against the risk factors for CVD and the hypoglycemic effects. Therefore, the present study aimed to investigate the chemical composition and to evaluate the effects of *C. iguanaea* on markers of lipids and glucose metabolism in cholesterol-fed rats.

Materials and methods

Solvents and chemicals

The solvents MeOH and acetic acid used for HPLC analysis were purchased from Merck® (São Paulo; SP, Brazil). Ethanol for the production of extracts was purchased from Vetec® (Rio de Janeiro; RJ, Brazil). Nylon membrane filters (0.45 mm) were purchased from of Flow Supply®, and the water was purified using a Milli-Q plus system from Millipore®.

Plant material

The leaves of *Celtis iguanaea* (Jacq) Sarg., Cannabaceae, were collected in Chapecó (SC), Brazil (27° 01′ 55.14′ S – 52° 47′ 29.42′ O) in September 2015, and authenticated by Professor Adriano Dias de Oliveira of the Community University of the Region of Chapecó (Unochapecó), where a voucher specimen is deposited (#3463).

Production of hydroalcoholic extract of Celtis iguanaea

The leaves of *C. iguanaea* were dried at room temperature (25 ± 5 °C), pounded in a knife mill (Ciemlab®, CE430), selected in a sieve (425 μm; 35 Tyler/Mesch), identified, and stored with protection from light. The extracts were produced via maceration (5 days) at room temperature using dry-milled leaves of the plant (100 g) and ethanol 70% (1:20, w/v). After filtration through Büchner funnel, the hydroalcoholic extract of *C. iguanaea* was concentrated via evaporation under reduced pressure, lyophilized, weighed, and stored at −20 °C.

Chemical analysis of Celtis iguanaea

To increase the phytochemical study of *C. iguanaea* in addition to the extracts, a dichloromethane extract of the leaves (1:20 w/v) was prepared via maceration (5 days). Both the extracts were analyzed via liquid chromatography tandem mass spectrometry. The samples (5 mg) were dissolved in methanol (3 ml) and filtered in a Sepak RP-18® cartridge, and then through a nylon membrane (Flow Supply®) with a 22.25 mm diameter and a 0.22 μm pore size.

HPLC-ESI-IT-MS^n analyses

An aliquot of CI and dichloromethane extract were analyzed separately via in-line HPLC-ESI-IT-MS^n, using a SURVEYOR MS micro system coupled in-line to an LCQ Fleet ion-trap mass spectrometer (Thermo Scientific). HPLC separation was conducted on a chromatographic column (250 × 4.6 mm i.d. 5 micron) using a gradient mobile phase with a flow rate of 0.8 ml/min of water and MeOH plus 0.1% acetic acid. Initial conditions were 5% MeOH increasing to reach 100% MeOH and hold at 100% MeOH at 80 min and held at 100% MeOH for 10 min. Both extracts were analyzed by ESI-MS^n in negative ion mode with a LCQ Fleet ion-trap instrument from Thermo Scientific. The capillary voltage was set at −20 kV, the spray voltage at −5 kV and the tube lens offset at 100 V, sheath gas (nitrogen) flow rate at 80 (arbitrary units) and auxiliary gas flow rate at 5 (arbitrary units). Data were acquired in MS1 and MS^n scanning modes. The capillary temperature was 275 °C. Xcalibur 2.1 software (Thermo Scientific) was used for data analysis.

ESI-MS^n analysis

For analysis via mass spectrometry, CI and dichloromethane extract (5 mg) were dissolved in methanol (3 ml) and filtered in a Sepak RP-18 cartridge, and then through a nylon membrane (Flow Supply®) with a 22.25 mm diameter and a 0.22 μm pore size. The samples were analyzed online via the LCQ Fleet, Thermo Scientific® mass spectrometer, equipped with a direct sample insertion device for streaming injection analysis (FIA). The samples were ionized via electrospray ionization (ESI) and fragmentations into multiple stages (MS^n) were held in an Ion-Trap (IT) interface. The negative mode was chosen for the generation and analysis of all the spectra, and the experimental conditions were as follows: capillary voltage −35 V, spray voltage −5000 V, capillary temperature 350 °C, drag gas (N_2) and flow rate 60 (arbitrary units). The acquisition track was m/z 100–2000, with two or more scan events held simultaneously in the spectrum. The experiment was performed by Laboratory of Bioprospecting of Natural Products (LBPN), UNESP, Coastal Campus IB-CLP.

Animals

The International Guidelines for Care and Use of Laboratory Animals were followed for all experiments, and the experimen-

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