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Lipid-lowering effects of standardized extracts of *Ilex paraguariensis* in high-fat-diet rats

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ABSTRACT

Mate (*Ilex paraguariensis* A. St.-Hil) is a native species of South America used to prepare traditional beverages. Recently a possible effect of its infusion on oxidative stress found in dyslipidemias has been reported. The main compounds related to these activities are phenolic compounds derived from chlorogenic acid. This study aimed to determine the anticholesteremic effect of the hydroethanolic extract (HEIP) and its n-butanolic fraction (n-BFIP), with standardized content of phenolic compounds derived from chlorogenic acid, in rats treated with high-fat diet (HFD). The contents of these compounds in the ethanol extract and n-butanol fraction were respectively two and three times higher than in traditional infusion with predominance of dicaffeoylquinic derivatives. The extracts were able to reduce serum triglycerides and cholesterol and decrease the atherogenic index in treated animals. These results support a potential effect of the mate extract in cardiovascular disease.

1. Introduction

The prevalence of overweight and obese individuals worldwide has increased dramatically due to, in large part, the over consumption of a high-fat diet (HFD) [1,2]. A HFD has been shown to cause an elevation of plasma lipids, including cholesterol levels [3]. The two most common components of cholesterol are low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and elevated serum levels of LDL cholesterol and reduced levels of HDL cholesterol are important independent risk factors for cardiovascular disease (CVD) [4]. Therefore, the control of postprandial LDL and HDL has been shown to be important in the treatment of CVD and in the prevention of atherosclerosis. Several publications have reported that the natural products have a beneficial effect on CVD and atherosclerosis by regulating multiple epigenetic mechanisms, including the levels of HDL and LDL cholesterol [5]. These reports have demonstrated a reduction in the incidence of CVD and atherosclerosis associated with obesity by natural product therapy.

Mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) is a native species of the subtropical regions of South America. The leaves are used to prepare traditional beverages (chimarrão, mate and tererê) especially in Brazil, Argentina, Uruguay and Paraguay [6]. Recently it has been used in the development of food and cosmetic products and a review of the importance of this species was published [7,8].

Previous work [9] about the chemical composition of this species showed the presence of methylxanthines — caffeine and theobromine, the average content of caffeine in mate ranged from 0.315 to 0.667% and theobromine of 0.037 to 0.237%. In addition, there is the presence of numerous triterpenic saponins derived from ursolic and oleanolic acids. Different saponins (triterpene oligoglycosides) were identified

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in the methanol extract of leaves of *I. paraguariensis*, and several of these compounds showed inhibitory activities on pancreatic lipase [10]. The presence of flavonoids and vitamins such as B_1 (thiamine), B_2 (riboflavin), B_5 (pantothenic acid), C, E, β-carotene, sucrose, fructose, folic acid, trigonelline and choline has also been reported [7].

In recent years, the importance of phenolic compounds in biological activity of *I. paraguariensis* has been highlighted. Phytochemical analyses performed by different methods have shown average levels of phenolic compounds could vary between 7.9 and 9.6% [9,11–16]. The phenolic compounds of major importance in mate refer to caffeoyl derivatives, mainly monocaffeoyl quinic isomers (3-*O*-caffeoyl quinic or neochlorogenic acid, 5-*O*-caffeoyl quinic or chlorogenic acid and 4-*O*-caffeoyl quinic or cryptochlorogenic acid) and dicaffeoyl quinic isomers (3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid). Moreover, the levels of phenolic compounds are modified according to extractive methods, [11,14,17], processing, genetic and environmental variability, and harvest time [18].

Mate infusion has been used in folk medicine for the treatment of arthritis, rheumatism and other inflammatory diseases, headache, obesity, hypertension, and digestive disorders [6]. Several biological activities of mate are associated with the phenolic compounds and some researchers have shown the inhibition of LDL oxidation *in vitro* and *in vivo* models [19].

Mate extracts inhibited the enzymatic and nonenzymatic lipidic peroxidation as well as it was effective in other methods to evaluate the *in vitro* antioxidant effect [20–23]. Studies demonstrate that an aqueous extract of *I. paraguariensis* and *l. brasiliensis* protects the myocardium against ischemia–reperfusion injury and attenuates oxidative damage. These effects may be attributed to the potent antioxidant properties of the extract [24,25]. Furthermore, *I. paraguariensis* aqueous extract effectively inhibited the progression of atherosclerosis in cholesterol-fed-rabbits [6] and reduced the body weight, visceral fat, serum lipids, glucose, leptin and insulin levels in HFD-fed-rats [26]. Recent studies indicate the chlorogenic acid as one of the main responsible for this activity [27,28].

Considering the data presented above and the importance of phenolic compounds in the cardioprotective effects of mate, we propose to develop and standardize a fraction enriched in phenolic compounds from the ethanol extract of *I. paraguariensis* and investigate their effectiveness on body weight and serum lipid levels of rats submitted to high-fat diet.

2. Materials and methods

2.1. Drugs

Caffeine, theobromine, chlorogenic acid and cholic acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Cholesterol was obtained from Vetec Fine Chemicals (São Paulo, Brazil). Simvastatin from Sigma (St. Louis, MO, USA) was used as the reference hypolipidemic drug. All other drugs and reagents used were purchased from J. T. Backer (USA) and Vetec.

2.2. Collection of plant material

Mate leaves (*I. paraguariensis* St. Hil.) were obtained from experimental cultivated progenies to guarantee the content of methylxanthines and phenolic compounds as previously described [18]. The cultivation is located in the municipality of Ivai/PR, Brazil, which is located at 650–750 m altitude above the sea level (S 25°01′–W 50°47′).

2.3. Preparation of I. paraguariensis extracts

Mate leaves' powder was dried in a forced draft oven (45 °C, 48 h). The infusion of the leaves (0.1 g:10 ml) was obtained using aqueous extraction. Another fraction of the sample was extracted with 70% ethanol (1:2.5 w/v), filtered and evaporated to dryness, to obtain the crude extract (HEIP). The HEIP was fractionated by liquid/liquid partitioning (n-hexane, chloroform, ethyl acetate and n-butanol), obtaining the n-butanol fraction (n-BFIP). These extracts (HEIP and n-BFIP) were used to evaluate the pharmacological activity.

2.4. Phytochemical analysis

2.4.1. Apparatus and chemicals

The analysis was performed by a Shimadzu (Mod. SCL-10A) high performance liquid chromatography coupled with diode array detector (HPLC-DAD) consisting of a SIL-20AHT injector, a LC-20AT pump, an FCV-10AL mixer, a DGU-20A5 degasser and an injector valve, a 20 μl sample loop. A SPD-M20A DAD detector coupled to the chromatograph and an interphase module Shimadzu CBM-20A. A 5 μm Phenomenex C-18, 4.6×250 mm analytical column was used. The column was maintained at 30 °C using the CTO-20A HPLC integrated oven.

2.4.2. Procedure

Sample of infusion (0.1 g/10 ml) [9], ethanol extract (0.05 g/10 ml) and n-butanol fraction (0.05 g/10 ml) were filtered with 0.45 μm nylon filters and the solvent system consisted of (A) acidulated water purified by the Milli-Q system (Milipore Milford, MA), with 0.3% acetic acid, and (B) methanol. The solvents were run at a flow rate of 1.0 ml min^{-1} using the following linear gradient: 15% B to 20% B for 20 min, 20% B to 40% B for 25 min, 40% B to 85% B for 50 min, and 85% B to 15%B for 10 min. Detection was monitored at 265 nm for caffeine and theobromine, and at 325 nm for chlorogenic acids. All samples were run in duplicate. Chromatographic peaks were identified by comparing retention times with those of caffeine, theobromine and chlorogenic acid standards recorded in the same conditions. Calibration curves were obtained with the mentioned standards after dilution in the mobile phase. Linearity was determined by regression and precision and accuracy was determined using the variation coefficient (CV<3%). The correlation coefficients obtained were as follows r2 = 1.0 for caffeine, r2 = 0.9942 for theobromine and r2 = 0.9999 for chlorogenic acid. The contents of the caffeoyl derivatives were expressed in (5-*O*-caffeoyl quinic) chlorogenic acid absorbance (mg/g).

2.5. Pharmacologic activity

2.5.1. Animals

Male Wistar rats (8 weeks-old, weighting between 250 and 300 g) from the colony of the Universidade Paranaense (UNIPAR, Paraná, Brazil) were used. All animals were maintained under standard laboratory conditions, with a constant 12 h light/dark