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Antioxidant extracts of coffee leaves and its active ingredient 5-caffeoylquinic acid reduce chemically-induced inflammation in mice

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ABSTRACT

Leaves from *Coffea arabica* L. have been considered an agricultural residue of little value; however, these leaves are a potentially sustainable source of phenolic compounds. Investigation of the properties of these leaves is a crucial strategy for identifying agents that may be beneficial to the health. This study evaluates the antioxidant and anti-inflammatory activity of extracts of *C. arabica* leaves and 5-caffeoylquinic acid (5-CQA) in a mouse model. Hexane (HFCAL), dichloromethane (DFCAL), ethyl acetate (EFCAL), and butanol (BFCAL) fractions were obtained by partition of the methanol extract (MECAL). High performance liquid chromatography with UV-diode array detection (HPLC-UV-DAD) and spectrophotometric methods were used to identify and quantify the chemical constituents. The antioxidant activity of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and β-carotene/linoleic acid assays. Ear edema was induced in Swiss mice by the topical application of Croton oil, phenol, or histamine. Tissues were analyzed histopathologically, and myeloperoxidase and *N*-acetyl-β-D-glucosaminidase activities were assessed. 5-CQA and mangiferin were identified in MECAL, EFCAL, and BFCAL. The total phenolic and flavonoid contents ranged from 1.65 to 20.60 g gallic acid equivalents/100 g of extract and 0.26 to 14.96 g of rutin equivalents/100 g of extract, respectively. The content of 5-CQA ranged from 2.29 to 5.50 g/100 g of extract, while that of mangiferin was 1.46–4.00 g/100 g of extract. The IC_{50} values ranged from 7.47 ± 0.12 to 122.76 ± 1.38 μg/mL as determined by DPPH assay and from 4.67 ± 0.02 to 71.90 ± 0.22 μg/mL as determined by FRAP. The greatest inhibitor of lipid peroxidation was HFCAL (49.19 ± 1.10%). The MECAL and 5-CQA (0.1, 0.5, and 1.0 mg/ear) reduced the edema thickness and weight induced by croton oil and phenol. After histamine application, the edema thickness and weight were inhibited by MECAL but not by 5-CQA. Treatment with either MECAL or 5-CQA decreased inflammatory parameters and the activity of myeloperoxidase and *N*-acetyl-β-D-glucosaminidase. The results suggest that 5-CQA and extracts from coffee leaves (*C. arabica*) possess antioxidant and anti-inflammatory properties, indicating new possibilities for the treatment of disorders involving oxidative and cutaneous damage.

1. Introduction

Arabica coffee (*Coffea arabica* L.) originated in the tropical forests of Ethiopia, Kenya, and Sudan. This product is now one of the major Brazilian agricultural commodities on the international market because of its extensive cultivation, export, and consumption (Mussatto et al., 2011). While the coffee fruit is of the greatest commercial interest, parts of the plant that are commonly discarded, such as leaves and branches, hold promise in the search for new therapeutic agents (Mussatto et al., 2011; Palomino García and Del Bianchi, 2015).

The oral administration of coffee leaves is traditionally used to treat diarrhea, bleeding accompanying abortion, migraine, body pain, headache, intestinal and stomach pain, as cough suppressant, to manage HIV/AIDS, decrease fever, stimulate prolactin production, and as a laxative (Patay et al., 2016). Phenolic acids such as caffeic, chlorogenic, p-coumaric, ferulic, and sinapic acids, and flavonoids (rutin, quercetin, kaempferol, and isoquercitrin) have been identified in the leaves of Arabic and Bengal coffee (Patay et al., 2016). These compounds are reported to have anti-cariogenic (Ferrazzano et al., 2009), antimicrobial, anti-inflammatory, anti-mutagenic and anti-

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carcinogenic (Naveed et al., 2018), hepatoprotective (Dickson et al., 2015), antioxidant (Murthy and Naidu, 2012), and neuroprotective (Campos-Esparza et al., 2009) properties. Mangiferin, present in the leaves and fruits, is a phenolic compound that possesses antiviral, hepatoprotective (Pardo-Andreu et al., 2008), antibacterial (Campa et al., 2012), analgesic, anti-inflammatory, antioxidant (Campa et al., 2012; Campos-Esparza et al., 2009), and immunomodulatory activities. The chemical composition and biological activity of the leaves of *C. arabica* require further study, as previous studies have investigated a range of plant parts and coffee varieties (Domingues et al., 2012).

The search for antioxidative agents with action against free radicals has increased in recent years. Free radicals are independent molecules that have atoms with one or more unpaired electrons. These chemically reactive molecules are highly unstable and have very short half-lives (Ahmadinejad et al., 2017). Peroxidation-induced free radicals have gained much attention because of the involvement in disorders such as atherosclerosis, inflammation, and cancer (Biswas et al., 2017). The components of free radicals include superoxide ($O2^{\cdot}-$), hydroxyl ($OH^{\cdot}$), hydroperoxide ($HO2^{\cdot}$), nitric oxide ($NO^{\cdot}$), and nitrogen dioxide ($NO2^{\cdot}$) (Ahmadinejad et al., 2017). Of these reactive species, the hydroxyl radical is the most reactive in damaging intracellular molecules. Hydrogen peroxide can cross the nuclear membrane and damage DNA (Ahmadinejad et al., 2017; Biswas et al., 2017). Reactive species can also cause tissue damage through lipid peroxidation, which stimulates the production of inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase and induces leukocytes (monocytes and macrophages) to release proinflammatory cytokines and activate nuclear factor kappa beta (NF-κB) (Biswas et al., 2017). New therapeutic options are needed for the treatment of diseases associated with oxidative stress and inflammatory processes.

The investigation of the chemical and biological potential of natural products is a crucial strategy for identifying agents that may be beneficial to the health of people and animals while minimizing negative environmental impacts. Accordingly, the objective of this study is to identify and quantify chemical compounds in *C. arabica* leaves and to evaluate the antioxidant and anti-inflammatory activities using *in vitro* and *in vivo* assays.

2. Materials and methods

2.1. Plant material and extraction

In May 2015, leaves of *Coffea arabica* L. were collected in the Medicinal Garden of the Faculty of Pharmacy, Federal University of Juiz de Fora (UFJF), Juiz de Fora city, Minas Gerais State, Southeast Region, Brazil. A voucher specimen (no. 66.873), identified by Fátima Regina Gonçalves Salimena, was deposited in the Herbarium Leopoldo Krieger (CESJ) of the Institute of Biological Sciences, UFJF, Brazil.

After being dried and powdered, the leaves (411.13 g) were extracted with methanol (6.0 L) by static maceration for 6 weeks at room temperature, with renewal of the solvent every 2 days until exhaustion of the material as determined by color. The methanol extract from *C. arabica* leaves (MECAL) was filtered and evaporated under a rotary vacuum evaporator (Rotavapor RII, Büchi, Flawil, Switzerland) at controlled temperature (50 °C). This material was placed in a desiccator with silica to yield 110.65 g of crude extract. The MECAL (30 g) was suspended in water:ethanol (9:1), followed by liquid/liquid partition with organic solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, and butanol) to yield fractions from each (Cechinel Filho and Yunes, 1998). The MECAL and/or fractions were analyzed using chemical and biological assays.

2.2. Chemicals

The drugs and reagents used in this study are as follows: Croton oil, arachidonic acid, phenol, dexamethasone, indomethacin, rutin hydrate, trichloroacetic acid, ascorbic acid, gallic acid, Folin-Ciocalteu reagent, butylated hydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid and β-carotene (Sigma-Aldrich Co., USA), pyridine, ethanol, sodium carbonate, aluminum chloride, potassium ferrocyanide, phosphate buffer, tween 20, ferric chloride, dimethyl sulfoxide, acetic acid, hexane, dichloromethane, chloroform, ethyl acetate, butanol and acetone (Vetec Química Farm Ltda, Brazil), and ketamine chloride and xylazine chloride (Syntec, Brazil). HPLC-grade methanol (JT Baker, Philipsburg, NJ, USA), HPLC-grade acetic acid (Merck, Darmstadt, Germany), and ultrapurified water were used as the mobile phase in chromatographic analysis. Standards of chlorogenic acid 5-CQA (purity ≥ 95%) and mangiferin (purity ≥ 99%) were purchased from Sigma-Aldrich (Sigma-Aldrich Co., USA).

2.3. Total phenolic content determination

The total phenolic content was quantified by spectrophotometry using the Folin-Ciocalteu method and gallic acid (10–60 μg/mL) as a standard (Sousa et al., 2007). Stock solutions of EFCAL (100 μg/mL), MECAL (500 μg/mL), BFCAL (500 μg/mL), HFCAL (1000 μg/mL), and DFCAL (1000 μg/mL) were prepared. To perform the assay, 1 mL of each stock solution was reacted with 5 mL of 10% Folin-Ciocalteu reagent (v/v) and 4 mL of sodium carbonate (7.5%, w/v) at room temperature (24 ± 2 °C) for 2 h. The Folin-Ciocalteu reagent oxidizes phenolic compounds, generating a blue color. After the reaction, the absorbance was measured at 763 nm in a spectrophotometer (SHIMADZU, UV-1800, Tokyo, Japan). All analyses were carried out in triplicate, and the average is presented as the gram equivalents of gallic acid (g GAE/100 g of extract).

2.4. Total flavonoid content determination

As described by Sobrinho et al. (2008), the aluminum chloride colorimetric method was used to determine the total flavonoid content using rutin (2–30 μg/mL) as a standard. Semi-purified solutions of MECAL and fractions (HFCAL, DFCAL, EFCAL and BFCAL) (500 μg/mL) were prepared using 5 mL aliquot of each sample added to chloroform (2 mL) in test tubes. After centrifugation, 400 μL of the samples were reacted with 8% aluminum chloride (500 μL) in the presence of acetic acid (120 μL), 20% pyridine (2 mL, v/v), ethanol (500 μL), and distilled water (adjusted to 5 mL) at room temperature (22 ± 2 °C) for 30 min. The absorbance then was measured at 420 nm using a spectrophotometer (SHIMADZU, UV-1800, Tokyo, Japan). The results, assayed in triplicate, are reported as the gram equivalents of rutin (g RE/100 *g* of extract).

2.5. Identification of 5-caffeoylquinic acid and mangiferin by HPLC-UV-DAD

The amount of 5-caffeoylquinic acid and mangiferin in MECAL, EFCAL, and BFCAL was determined by HPLC using a modified method developed by Campa et al. (2012). The extracts were dissolved in water/methanol (95:5 v/v) and filtered (0.45 μm filter) before injection into a modular HPLC (Waters, USA) composed of a binary pump (Waters1525), photodiode array detector (Waters 2998) and autosampler (Waters 2707). The chromatographic software Empower 3 was used for data collection and processing. Chromatography was performed on a reversed phase C18 silica column (model Promosil - Agela Technologies) (4.6 × 150 mm; 5 μm of particle size). The mobile phase was composed of ultrapure water and acetic acid (98:2; Solvent A), and methanol, water and acetic acid (90:5:5; Solvent B). Before injection, 18% eluent B was used to equilibrate the column for 2 min. After injection (50 μL), MECAL, EFCAL, BFCAL and standards were eluted in a gradient in which the concentration of eluent B was increased from 18% to 25% in 2 min, followed by a 13-minutes gradient increase from 25% to 36%, and returning to 18% in 10 min. The final gradient