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Hydroalcoholic extract of Brazilian red propolis exerts protective effects on acetic acid-induced ulcerative colitis in a rodent model

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ABSTRACT

Ulcerative colitis (UC) is a common intestinal inflammatory disease with an etiology that is not well understood. Although the anti-inflammatory and anti-oxidant effects of the hydroalcoholic extract of Brazilian red propolis (HERP) have been reported in various experimental models, its protective effect in models of UC have not been evaluated. The purpose of this study was to investigate the chemopreventive effect of hydroalcoholic extract of Brazilian red propolis (HERP) in acetic acid-induced colitis (AAIC) using a rodent model. The HERP was chemically characterised by HPLC/DAD analyses. Male rats were randomly assigned into four groups: sham, vehicle (with AAIC, treated with vehicle), P10 (with AAIC, treated with 10 mg/kg HERP), and P100 (with AAIC, treated with 100 mg/kg HERP). Treatments were performed for 7 days, and colitis was induced on day seven. Animals were euthanized 24 h after colitis induction and body weight, colon length, gross and histological scores, malondialdehyde (MDA) and myeloperoxidase (MPO) concentrations in colon tissue, and the immunohistochemical expression of inducible nitric oxide synthase (iNOS) were assessed. The major compounds found in HERP were liquiritigenin (68.8 mg/g), formononetin (54.29 mg/g), biochanin A (30.97 mg/g), and daidzein (19.90 mg/g). Rats treated with 10 mg/kg HERP demonstrated significant decreases in MPO concentrations, gross and histological scores of tissue damage, and iNOS expression ($p < 0.05$). Similarly, rats treated with 100 mg/kg HERP demonstrated significant decreases in MPO levels ($p < 0.05$) and histological scores of tissue damage ($p < 0.05$). The results of this study indicate that oral administration of HERP attenuates AAIC in rats, which may be due to anti-inflammatory effects related to iNOS inhibition.

1. Introduction

Ulcerative colitis (UC) is a multifactorial intestinal disorder whose etiology is not fully understood. Recently, the occurrence of UC worldwide has increased, particularly in developing countries such as those in Latin America, Asia, and Eastern Europe, affecting approximately 4–64 individuals per 100.000 people [1]. In Brazil, UC represents approximately 40% of all intestinal inflammatory diseases, with approximately 13 cases per 100.000 habitants [2].

The physiological protective mechanisms involved in maintaining the integrity of the intestinal mucosa include endogenous antioxidant defense systems against the production of reactive oxygen species (ROS), such as superoxide dismutase (SOD) and catalase (CAT), and modulation of the inflammatory response [3]. Several studies have demonstrated that UC is related to the overproduction of ROS, leading to lipid peroxidation and downstream inflammatory responses [4]. Furthermore, increased production of pro-inflammatory cytokines and nitric oxide (NO) appear to exacerbate the inflammatory response. Inhibition of inducible

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nitric oxide synthase (iNOS) may have therapeutic promise in the treatment of UC, which suggests that NOS pathways of oxidative stress play a role in the pathogenesis of this disease [5].

To date, the treatment of UC is based on drugs with antioxidant activity or the ability to inhibit the production of inflammatory mediators. Currently, the pharmacotherapy of UC includes the use of aminosalicylates, antibiotics, steroids, and immunomodulators, but the efficacy and safety of these drugs have recently been questioned because of many adverse side effects as well as high costs [6].

Compounds with antioxidant and anti-inflammatory properties appear to be promising agents in the treatment of UC. Natural products containing flavonoids have demonstrated both activities and are considered an important alternative treatment to ameliorate ROS-induced tissue damage and to modulate UC-associated inflammatory processes [7–9].

Propolis has been used in folk medicine since ancient times, due to its many biological properties, such as anti-inflammatory [10,11], anti-oxidant [12], antibacterial [13], antitumor [14,15], antinociceptive [16] and immunomodulatory [17]. Biological activities of propolis are mainly attributed to the phenolic components such as flavonoids [18]. In this regard, there are many studies demonstrating the gastric anti-ulcer properties of flavonoids [19].

Isoflavonoids found in the hydroalcoholic extract of red propolis (HERP) possess potent antioxidant [20] and anti-inflammatory activity [21]. Because of these properties, HERP would be expected to reduce injury and/or improve tissue healing in models of induced UC. However, prior to this study, HERP had not been evaluated for potential protective effects in inflammatory bowel disease (IBD). In this study, we present the detailed investigation of the antioxidant and anti-inflammatory effects of HERP in an acetic acid-induced UC rat model.

2. Material and methods

A flow chart of the experiments involved in this study is shown in Fig. 1.

2.1. Preparation of the extract hydroalcoholic of red propolis and samples

Red propolis samples were collected in Marechal Deodoro, Alagoas state, Brazil, at coordinates 9° 44′36.84288″ S, 35° 52′3.632813″ W, in June 2011 from *Apis mellifera* bees. The propolis sample (1 g) was extracted with 12.5 mL of ethanol:water (70:30, v/v) for 1 h at room temperature in an ultrasound bath. After extraction, the mixture was filtered, and the solvent was evaporated to produce the HERP [22].

For HPLC analysis, the HERP was dissolved in methanol:water (50:50, v/v) (50 mg/mL) to a final concentration of 40.0 μg/mL and filtered through a 0.45 μm membrane (Millipore, Merck-Billerica, MA, USA). An aliquot of 2.0 μL of 1% HERP was injected into the HPLC. For in vivo studies, the extract was suspended in 5% Tween 80 and administered at a concentration of 10 and 100 mg/kg body weight.

2.2. HPLC instrumentation and chromatographic condition

The HPLC-DAD analysis were carried out using a Shimadzu system equipped with a LC-20AD HPLC pump, an DGU- 20A3 degasser, a SIL-20 autosampler, an SPD-M20avp diode array detector (DAD), and a CBM 20A controller, operated with the LC Solution data station software (Shimadzu, Tokyo, Japan). Concentrated acetic acid and methanol were HPLC grade. Mobile phases were filtered with nylon solvent filter (0.45 μm). The water used in experiments was obtained with the Millipore (São Paulo, Brazil) Milli-Q purification system.

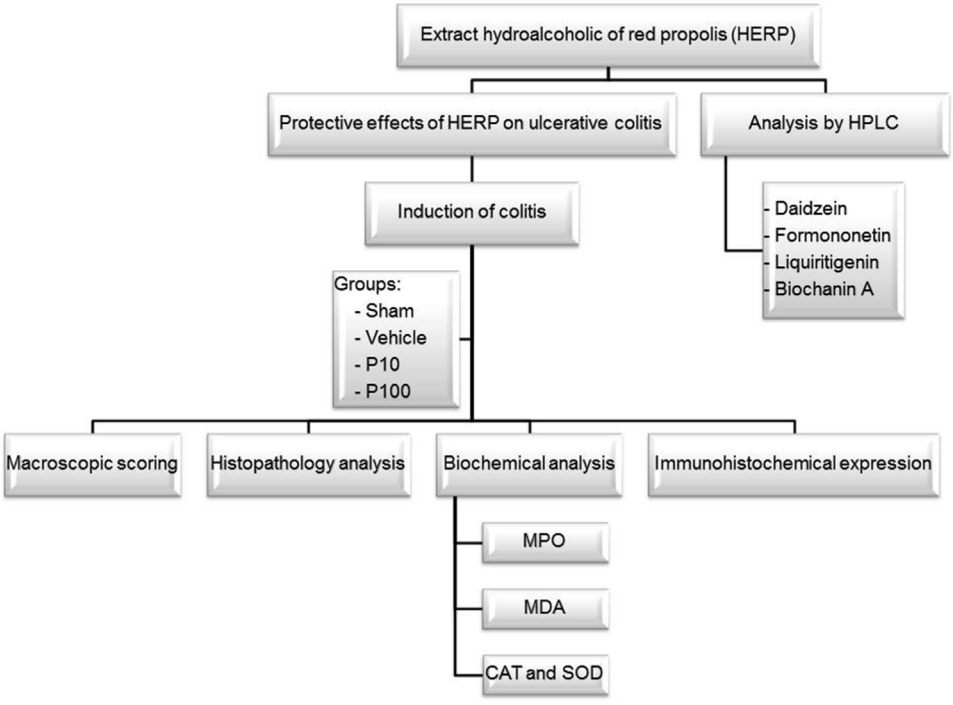

Fig. 1. Flow chart demonstrating the experimental approach.