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Antinociceptive and anti-inflammatory effects of Brazilian red propolis extract and formononetin in rodents

Rodrigo Lima Cavendish [a], Jandson de Souza Santos [b], Reinaldo Belo Neto [a], Ailma Oliveira Paixão [a], Juciele Valéria Oliveira [a], Edilson Divino de Araujo [c], Andresa Aparecida Berretta e Silva [d], Sara Maria Thomazzi [b,*], Juliana Cordeiro Cardoso [a], Margarete Zanardo Gomes [a]

[a] Instituto de Tecnologia e Pesquisa (ITP), Universidade Tiradentes, Av. Murilo Dantas, 300, Farolândia, CEP 49032-490 Aracaju, Sergipe, Brazil
[b] Departamento de Fisiologia, Universidade Federal de Sergipe, Av. Marechal Rondon, Cidade Universitária, CEP 49100-000 São Cristóvão, Sergipe, Brazil
[c] Departamento de Biologia, Universidade Federal de Sergipe, Av. Marechal Rondon, Cidade Universitária, CEP 49100-000 São Cristóvão, Sergipe, Brazil
[d] Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Av. do Café, CEP 14049-900 Ribeirão Preto, São Paulo, Brazil

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ABSTRACT

Ethnopharmacological relevance: Propolis has been used as a folk medicine for centuries around the world due to its wide spectrum of biological activities. The red propolis, a new Brazilian variety of this apimaterial, has presented an unusual chemical composition, including isoflavones such as formononetin and biochanin A. Since both the green and red varieties of propolis are traditionally used as medicine and commercialized with no label differentiation, the study of the activities of red propolis extract has become important in order to clarify whether this product has the same activities as commercial ones. In this work, we demonstrated the potential action of the hydroalcoholic extract of red propolis (HERP) and its biomarker, formononetin, as antinociceptive and anti-inflammatory drugs on experimental models.
Materials and methods: The HERP was chemically characterised by HPLC/DAD analyses. The biological activities of the HERP (3, 10, and 30 mg/kg) and formononetin (10 mg/kg) were evaluated using the antinociceptive (acetic acid, formalin, and glutamate injections) and anti-inflammatory (carrageenan-induced hindpaw oedema and peritonitis) models in mice after oral administration. The open field test was also performed.
Results: Formononetin, one of the main biomarker of red propolis, was identified in the HERP (21.62 mg/g). Pretreatment with the HERP (10 and 30 mg/kg) and formononetin (10 mg/kg) produced reduction ($P<0.001$) in the number of abdominal writhes, but the HERP was more effective ($P<0.001$) than formononetin. In the formalin test, all HERP doses (3, 10, and 30 mg/kg, $P<0.001$) inhibited the late phase (inflammatory pain) of formalin-induced licking, but the inhibition of neurogenic pain was observed only when the higher doses (10 and 30 mg/kg; $P<0.05$) were used. Formononetin caused inhibition ($P<0.001$) only in the second phase of formalin-induced nociception similarly at all HERP doses in the same phase of the test. The responses in glutamate-induced model presented crescent inhibition ($P<0.05$) with 10 and 30 mg/kg of HERP. Also, formononetin inhibited ($P<0.001$) the nociception induced by glutamate similarly to 30 mg/kg of HERP. There were no significant differences in the *open field* test after HERP administration, but formononetin decrease the spontaneous motor behaviour. Regarding the anti-inflammatory assessment, the HERP (10 and 30 mg/kg, $P<0.05$) and formononetin ($P<0.001$) treatments caused a significant inhibition of the oedema response. All doses of HERP (3, 10, and 30 mg/kg, $P<0.05$) and formononetin ($P<0.001$) also inhibited the carrageenan-induced leukocyte migration. In both cases, the results for the HERP at 30 mg/kg and formononetin were similar.

Abbreviations: ASA, acetylsalicylic acid; CI 95%, 95% confidence interval; Dexa, dexamethasone; ED_{50}, median effective dose; FOR, formononetin; HERP, hydroalcoholic extract of red propolis; IL, interleukin; Morph, morphine; NF-κB, nuclear factor-κB; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NSAID, nonsteroidal anti-inflammatory drug; ROS, reactive oxidative species; TNF, tumor necrosis factor; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1

* Correspondence to: Department of Physiology, Center of Biological and Health Sciences Federal University of Sergipe, 49100-000 São Cristóvão, SE, Brazil. Fax: +55 79 21056474.
E-mail address: sthomazzi@gmail.com (S. Maria Thomazzi).

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Conclusions: The HERP and formononetin presented significant anti-inflammatory activity. Moreover, the HERP presented antinociceptive action on inflammatory and neurogenic pain without motor side effects, possibly due to the action of other constituents present in the extract. These results, together, support the popular usage of this natural product.

1. Introduction

Pain states are related to a series of diseases and tissue injury, and usually appear as a sign of inflammatory conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation, but their long-term use is closely associated with complications of the gastrointestinal tract, including peptic ulcers (Tielemans et al., 2014). Therefore, new therapies for alleviating inflammatory response, pain and to avoiding side effects are required.

Natural products are, in turn, potential sources for new therapies. For example, propolis is a bee product that has been widely used from early times in folk medicine as an anti-inflammatory and antibacterial medicine (Toreti et al., 2013).

Propolis extracts have received great interest in medicine and various biological actions have been demonstrated, like antioxidant (Frozza et al., 2013), antinociceptive (Franchin et al., 2012), and anti-inflammatory (Búfalo et al., 2013; Szliszka et al., 2013). These activities are linked to its bioactive compounds and it is noteworthy that propolis from different geographic regions can vary significantly in its chemical composition (López et al., 2014; Piccinelli et al., 2011; Sawaya et al., 2011).

In the Northeast region of Brazil, the *Apis mellifera* (Apidae) bees produce red propolis from the botanical origin described as *Dalbergia ecastophyllum* (L.) Taub., Fabaceae (Daugsch et al., 2008). This red propolis presents new chemical substances (triterpenoids, isoflavonoids, prenylated benzophenones, and naphthoquinone epoxide) when compared to other samples (López et al., 2014; Morsy et al., 2013; Righi et al., 2011).

Although red propolis extracts and its chemical markers have demonstrated several biological properties, such as healing activity (Albuquerque-Júnior et al., 2009; de Almeida et al., 2013), cytotoxic activity toward tumour cells (Awale et al., 2008), antimicrobial (Righi et al., 2011), and potent antioxidant (Franchi et al., 2012; Righi et al., 2011) actions, the possible antinociceptive and anti-inflammatory effects of this product have not been explored so far.

This work aimed to assess the actions of hydroalcoholic extract of red propolis (HERP) and its main biomarker, formononetin, in experimental models of chemical-induced nociception and inflammatory responses.

2. Materials and methods

2.1. Drugs and reagents

The following drugs and reagents were used: acetylsalicylic acid (ASA), carrageenan, dexamethasone, L-glutamic acid hydrochloride, morphine hydrochloride, and formononetin, all obtained from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid was from Merck (Damstadt, Germany), formalin was obtained from Baker (Santo Amaro, SP, Brazil), and haloperidol was purchased from Janssen-Cilag Pharmaceuticals (São Paulo, SP, Brazil). All of the other reagents used were of analytical grade. The substances were dissolved in 0.2% Tween 80 in saline solution.

2.2. Red propolis collection and preparation of the hydroalcoholic extract

Red propolis was collected at Brejo Grande/Sergipe/Brazil (10°28'25''S, 36°26'12''W). The extraction was performed using propolis samples (2 g) and 70% ethanol (25 mL) at room temperature for 1 h in an ultrasound bath. After extraction, the mixture was filtered, and the solvent was evaporated to produce the HERP. The yield of the extraction process was estimated based on the percentage of dry mass obtained, which was calculated with reference to the initial mass of propolis before extraction.

2.3. High performance liquid chromatography-diode array detection analysis

The quantification of formononetin in the HERP was determined using HPLC analysis. A reverse-phase column C18 (Shimadzu Shim-Pack CLC-ODS (M) 250 × 4.6 mm^2, 5 μm particle size) with a diode array detector (Shimadzu Co. mod SPC-M20A) was used for chromatography analysis. The HERP was homogenised in methanol to a final concentration of 2.5 mg/mL. Ten μL of sample was injected into the HPLC system and the gradient started from 5% to 70% solvent A over a period of 170 min. The elution was performed in a linear gradient using solvent A (0.1% formic acid (v/v) in acetonitrile) and solvent B (0.1% formic acid (v/v) in water). The elution rate was 0.8 mL/min and the detection was recorded at 275 nm. The authentic standard of formononetin was used to prepare the standard curve.

2.4. Animals

Young adults Swiss mice (20–30 g) and Wistar rats (120–180 g) of both sexes were obtained from the Central Biotery of the Federal University of Sergipe (São Cristóvão, Brazil) and Central Biotery of the Tiradentes University (Aracaju, Brazil), respectively. Animals were maintained at controlled room temperature (21 ± 2 °C) with free access to food (Purina®) and water, under a 12 h light/dark cycle. The experiments were performed after approval of the protocols by the Institutional Ethics Committee (011213 and 250608) of the Tiradentes University (Aracaju, Brazil) and were carried out in accordance with the current guidelines for the care of laboratory animals. The protocols used in the antinociceptive study were conducted in accordance with the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

2.5. Acetic acid-induced abdominal constriction test

Abdominal writhes were induced by intraperitoneal (i.p.) injection of acetic acid (0.6%, 0.1 mL/10 g) in mice (Koster et al., 1959). Animals were pretreated orally (p.o.) with HERP (3, 10, or 30 mg/kg), formononetin (10 mg/kg), acetylsalicylic acid (ASA, 300 mg/kg), or vehicle (0.2% Tween 80 in saline solution, 0.1 mL/10 g) 60 min before initiating the algesic stimulation (n=6/group). The abdominal writhes were observed for a period of 20 min and began 5 min after injection of the nociceptive agent.