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Anti-inflammatory and antinociceptive activities of *Campomanesia adamantium*

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ABSTRACT

Ethnopharmacological relevance: *Campomanesia* species are used in folk medicine as anti-inflammatory, anti-rheumatic, anti-diarrheal and hypocholesterolemic.
Aim of the study: The present study investigated the *in vivo* anti-inflammatory and antinociceptive properties of ethyl acetate (AE) and aqueous (Aq) extracts from leaves of *Campomanesia adamantium* and *in vitro* anti-inflammatory activity of AE and its isolated flavonols, myricitrin and myricetin.
Materials and methods: The antinociceptive activity of AE and Aq was evaluated using acetic acid-induced writhing and formalin methods. The *in vivo* anti-inflammatory effect of AE and Aq was evaluated using carrageenan-induced paw oedema in mice. AE, myricitrin and myricetin were evaluated for their abilities to modulate the production of NO, TNF-α and IL-10 in LPS/IFN-γ stimulated J774.A1 macrophages.
Results: It was found that orally administrated AE and Aq (125 and 250 mg/kg) inhibited carrageenan-induced paw oedema in mice. AE (125 and 250 mg/kg) and Aq (125 mg/kg) reduced the time to licking at the second phase of the formalin method *in vivo* in mice. AE (250 mg/kg) and Aq (125 mg/kg) also reduced the number of writhes. AE, myricitrin and myricetin inhibited NO (320 μg/mL and 6.25–100 μM, respectively) and TNF-α production by macrophages (320 μg/mL for AE, 100 μM for myricitrin and 25–100 μM for myricetin). AE (160 and 320 μg/mL), myricitrin (50 and 100 μM) and myricetin (25–100 μM) increased IL-10 production by macrophages.
Conclusions: The ethyl acetate and aqueous extracts from *Campomanesia adamantium* showed anti-nociceptive and anti-inflammatory effects supporting the use of the plant in folk medicine. The results suggest that anti-oedematogenic effect promoted by aqueous extract involves several anti-inflammatory mechanisms of action. The antinociceptive effect shown by aqueous extract can be due to the modulation of release of inflammatory mediators involved in nociception. The anti-inflammatory effects of AE and of its isolated flavonols may be attributed to inhibition of pro-inflammatory cytokines production, TNF-α and NO and to the increased of IL-10 production.

1. Introduction

Campomanesia adamantium (Myrtaceae) is popularly known as gabiroba and used in folk medicine to treat inflammation and rheumatism (Lorenzi, 2000; Ballve Alice et al., 1995).

The inflammation process presents the signs of redness, heat, pain and oedema (Vane and Bolting, 1995) involves events such as enzymes activation, mediators release, fluid leakage, cell migration, tissue damage (Hayden et al., 2006) and can start by various stimuli such as tissue injury, viral or microbial infection, chemical or toxins irritation (Lin et al., 2008). Thus, macrophages play a central role in the inflammation process, since their activation leads to pro-inflammatory cytokines production such as tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6), inflammatory mediators generated by inducible enzymes nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) activation (Medzhitov and Janeway, 1997; Walsh, 2003; Kim et al., 2005). These mediators and cytokines induce the immune cells recruitment such as neutrophils and T lymphocytes (Walsh, 2003; Kim et al., 2005). The second phase of macrophages activation is responsible for preventing the tissue damage and involves the production of interleukin 10 (IL-10), an anti-inflammatory cytokine

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(Sabat et al., 2010). The aim of the present study was to evaluate the anti-inflammatory and antinociceptive effects of ethyl acetate and aqueous extracts from *Campomanesia adamantium* and to evaluate the ability of ethyl acetate extract and its isolated flavonols to modulate the production of NO, TNF-α and IL-10 in LPS/IFN-γ stimulated J774.A1 macrophages.

2. Materials and methods

2.1. General experimental procedures

Analytical high performance liquid chromatography (HPLC) was carried out on Waters Alliance 2695 equipped with vacuum degasser, quaternary pump, and diode array detector (DAD 2996), the analyses were performed on Shimadzu ODS column (250 mm × 4.6 mm × 5 μm). Semi-preparative HPLC was carried out on Shimadzu Liquid Chromatograph LC-6AD with photodiode array detector (SPD M20A). The analyses were performed on Shimadzu ODS column (250 mm × 20 mm × 5 μm). The chromatography columns (CC) were carried out on Polyamide (Fluka), Sephadex LH-20 (GE) and silica gel 60 Merck (0,063–0,200 mm), the fractions were monitored by thin-layer chromatography (TLC) on silica gel 60 G Merck coated plates. Tween-80 was obtained from U.S.P. Dimethylsulphoxide (DMSO) P.A., from Nuclear. Carrageenan, indomethacin, MTT (3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide), non-essential amino acids and sodium pyruvate and lipopolysaccharide (LPS) were purchased from Sigma. Interferon-γ (IFN-γ) was obtained from RD Systems and foetal bovine serum from LGC Biotecnologia. Digital caliper rule from Starret.

2.2. Plant material

Leaves of *Campomanesia adamantium* (Cambess.) O. Berg. were collected in Ouro Preto, MG, Brazil, in October, 2007, with permission of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA-license no. 17021-4). The plant was identified by Dr. Marcos Eduardo Guerra Sobral of Departamento de Botânica of Universidade Federal de São João Del Rei. A voucher specimen was deposited at Herbarium of Instituto de Ciências Exatas e Biológicas of Universidade Federal de Ouro Preto, reference number OUPR25911.

2.3. Extraction, isolation and HPLC analysis

The leaves were dried at 40 °C, reduced to powder (688.0 g) and were submitted to exhaustive percolation with hexane, ethyl acetate and methanol. The solvents were evaporated under reduced pressure resulting in dried hexane (6.4 g), ethyl acetate (AE, 9.7 g) and methanol (85.7 g) extracts, respectively. AE (5.0 g) was fractionated by CC on Polyamide eluted with water, methanol, ethyl acetate and hexane, with gradual reduction of the polarity, resulting in eleven fractions (F1–F11). F5 [eluted with H_2O:MeOH (10:90)] was fractionated by CC on Sephadex LH-20 using methanol, resulting in ten fractions (F5.1–F5.10). F5.8 (eluted with MeOH) yielded solid identified with myricitrin (0.095 g, yellow solid, m.p. 190.4–191.3 °C). F5.10 (eluted with MeOH) was purified on semi-preparative HPLC with water (A)—methanol (B) gradient, 0–15 min (50%–100% B); 15–16 min (100% B) and 16–20 min (100%–50% B), resulting in quercetin isolation (0.006 g, yellow solid). The flow rate was of 8 mL/min, the column temperature was set at 25 °C, the volume of the sample injection was of 2 mL, and detection wavelength was in 254 nm. F7 (eluted with MeOH) was submitted on CC over silica gel eluted with hexane, dichloromethane, ethyl acetate and methanol gradient resulting in five fractions (F7.1–F7.5). F7.5 [eluted with AcOEt:MeOH (80:20)] was purified on semi-preparative HPLC employing the same conditions described above resulting in the isolation of myricetin (0,012 g, yellow solid). Isolated compounds were characterized using spectroscopic techniques.

AE and its isolated compounds were individually analyzed by analytical HPLC. AE (5 mg) and 1 mg of myricitrin, quercetin and myricetin were dissolved in MeOH (1 mL). After injection of 25 μL of each sample, the gradient elution was carried out with mixture of water (A) and methanol (B). The elution was initiated with 80% A and 20% B, taking 55 min to reach 100% B, flow rate of 0.8 mL/min, wavelength detection 254 nm and column temperature at 25 °C. The isolated compounds were identified and compare using their time retention.

The leaves of *Campomanesia adamantium* (300.0 g) were extracted with 3 L of distilled water by percolation. 200 mL were lyophilized resulting in 500 mg of dried aqueous extract (Aq).

2.4. Animals

The experiments were conducted on male Swiss mice (25–35 g) supplied by Universidade Federal de Ouro Preto (UFOP). The animals received standard chow and water *ad libitum* with light/dark period of 12 h. All experimental procedures were approved by the Ethical Committee of Universidade Federal de Ouro Preto, Brazil (no. 2010/61 and 2012/45) and were carried out in accordance with international guidelines for the care and use of laboratory animals, published by the US National Institute of Health (NIH Publication, revised in 1985).

2.5. Preparation of test samples and drugs

Indomethacin and morphine were dissolved in distilled water and Tween-80 (95:5). Acetic acid and carrageenan were dissolved in distilled water just before use. AE was solubilized in Tween-80, DMSO and distilled water (1:1:8) and Aq was solubilized in distilled water. Morphine (10 mg/kg) and indomethacin (10 mg/kg) were orally administered (0.2 mL) and used as reference drugs. The control group received vehicle (Tween-80, DMSO and distilled water 1:1:8 for AE and distilled water for Aq).

2.6. Carrageenan-induced paw oedema assay

The anti-oedematogenic effect was evaluated by the carrageenan-induced paw oedema method in mice, according to previously described (Winter et al., 1962) with modifications. The animals were treated by oral route with vehicle, indomethacin (10 mg/kg) and both doses (125 and 250 mg/kg) of AE and Aq. Half an hour after administration of the various agents, oedema was induced by injection of carrageenan (20 μL, 0.1%, w/v) into the sub-plantar tissue of the right hind paw. Only needle introduction was performed into the left paw, corresponding damage induced by mechanical perforation. Paws thickness were measured with a caliper rule before and 1, 2, 3, 4, 5, and 6 h after carrageenan injection. Inflammatory swelling was expressed as thickness variation (Δ). Indomethacin was used as reference drug while control group received the vehicles that were used to dissolve AE and Aq. The group treated with vehicle was considered as maximum of inflammation and all others treatments were compared to this group.

2.7. Acetic acid-induced writhing method

This test was performed by using the modified method described by Koster et al. (1959). Animals were treated by oral