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Original Article

Antinociceptive and anti-inflammatory effect of the *Scutia buxifolia Reissek* stem barks extract

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ABSTRACT

Background: Scutia buxifolia (Rhamnaceae) has been extensively studied for its phenolics groups, which are able to capture free radicals; being therefore, considered promising as an antioxidant in preventing diseases resulting from oxidative stress.
Hypothesis: Scutia buxifolia extract (SBE) presents antinociceptive and anti-inflammatory effect in mice.
Study Design: SBE (400–800 mg/kg) was tested in different pain models to investigate its antinociceptive and anti-inflammatory action.
Methods: It was carried out the abdominal writhing test, capsaicin test, thermal hyperalgesia and incisional pain. The inflamed tissue by carrageenan was used for the analysis of interleukins (IL), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), c-reactive protein (CRP), nitrite and nitrate (NOx) determination and myeloperoxidase (MPO) activity. Furthermore, we evaluate the possible action mechanism of SBE using naloxone in capsaicin test.
Results: SBE prevented the nociception caused by acetic acid, formalin and capsaicin test. However, neither the SBE prevented the thermal hyperalgesia in hot-plate test, nor the naloxone reversed the SBE antinociceptive effect in capsaicin test. Furthermore, the administration of SBE prevented significantly the increase of MPO activity, the NOx content, and the levels of IL-1, IL-6, TNF-α, INF-γ and CRP and was able to increase the IL-10 levels after the inflammation induced by carrageenan in mice. In addition, SBE prevented mechanical hyperalgesia in a postoperative pain model.
Conclusion: The SBE presents great antinociceptive and anti-inflammatory activity in mice but this effect not seem to have its action mechanism like opioids. It is possible that its antinociceptive effects are associated with levels decrease of inflammatory mediators.

Introduction

Inflammation can be caused by endogenous or exogenous stimuli, being intended to protect the body of the cause and the cellular damage consequences. Although the inflammation is essential and the physiological process are beneficial, if the damage is not recovered, it may be involved in the pathogenesis and progression of many inflammatory diseases, including postoperative pain, atherosclerosis and rheumatoid arthritis (Alessandri et al., 2013).

Abbreviations: S. buxifolia, *Scutia buxifolia Reissek*; SBE, *Scutia buxifolia* extract; CNS, central nervous system; COX, ciclooxigenase; PGs, prostaglandins; LOD, limit of detection; LOQ, limit of quantification; IL, interleukins; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; CRP, c-reactive protein; MPO, myeloperoxidase; NOx, nitrite and nitrate; ELISA, enzyme-linked immunosorbent assay; AST, aspartate aminotransferase; ALT, alanine aminotransferase; I_{max}, maximal inhibition; ID 50%, inhibitory dose 50%; NSAIDs, nonsteroidal anti-inflammatory drugs; NO, nitric oxide.

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The inflammatory pain occurs in response to tissue injury and inflammatory response. In an attempt to repair the affected part of the body, the sensory nervous system undergoes a change in its response capacity, where the stimuli that are normally harmless will produce pain responses (alodinia) and the response to noxious stimuli will be enlarged and extended (hiperalgesia) (Loeser and Treede, 2008; Woolf, 2010). The inflammatory pain results from activation of resident cells, infiltration of inflammatory cells, and the release of inflammatory mediators. These changes promote a threshold reduction and amplify the response of nociceptors that innervate the damaged tissue (Loeser and Treede, 2008; Woolf, 2010).

Scutia buxifolia Reissek (*S. buxifolia*; Rhamnaceae family) is popularly known as "coronilha". This plant is native to South America and is used as cardiotonic, hypotensive and diuretic in medical practice (Menezes, 1995). This species possess phenolics groups, including flavonoids, which are able to capture free radicals; being considered promising for use in preventing diseases resulting from oxidative stress. Recently, was observed that this plant has antiplatelet effect, which can be related to a possible anti-inflammatory action, since many anti-inflammatory drugs exert their action by inhibiting the enzyme cyclooxygenase (COX), which is responsible to produce prostaglandins (PGs), which cause pain, fever and inflammation, and thromboxane, which causes platelet aggregation (Alessandri et al., 2013; Boligon et al., 2014). Thus, is relevant to study the possible antinociceptive and anti-inflammatory potential of the *S. buxifolia Reissek* stem barks extract.

Materials and methods

The online supplementary material provides more detailed descriptions of all the procedures mentioned below.

Plant collection and extraction preparation

The stem barks of *S. buxifolia* were collected in Dom Pedrito (Rio Grande do Sul State of Brazil) in March 2013 (coordinates 30°59′09′′S and 54°27′44′′W). A dried voucher specimen is preserved in the herbarium of the Department of Biology at Federal University of Santa Maria (SMBD 12355). The plant stem barks were dried, powdered in a knife mill and obtained powder was macerated at room temperature with 70% ethanol for a week with a daily shake-up. After filtration, the extract was evaporated under reduced pressure to remove the ethanol, suspended in water and partitioned successively with ethyl acetate to obtain the *Scutia buxifolia* extract (SBE) and its yield is 5% from the amount of plant material. The extract was dissolved in saline and both the plant and the vehicle were administered at 10 ml/kg.

Quantification of compounds by HPLC-DAD

Quantification was carried out using the DAD-chromatograms obtained at 270 nm for gallic acid, 280 nm for caffeine, catechin, epicatechin and epigallocatechin, 327 nm for caffeic, ellagic and chlorogenic acids and 366 nm for flavonoids, by means of external standard calibration curves. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.020–0.250 mg/ml. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–600 nm). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and $10\sigma/S$, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (Boligon et al., 2013).

Animals

Adult male Swiss mice (25–35 g) were obtained from the animal house of the Federal University of Santa Maria (UFSM) and were randomly distributed into different experimental groups. The animals were housed in polypropylene cages at an ambient temperature of 25 °C ± 1 °C and 45–55% relative humidity, with a 12:12 h light/dark cycle, provided with commercial food pellets and water *ad libitum* and were adapted to local conditions for at least 72 h before the experiment. All experimental protocols were approved by the Ethics Animal Committee of Universidade Federal de Santa Maria (CEUA UFSM; Protocol 079/2012). The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the treatments.

Nociceptive parameters

Acetic acid-induced abdominal writhing

Abdominal constrictions were induced by intraperitoneal acetic acid injection (Oliveira et al., 2009).

Formalin test

The formalin test was carried out as described by Milano et al. (2008a).

Capsaicin test

For the capsaicin test, mice received an intraplantar capsaicin injection (Trevisan et al., 2012).

Hot-plate test

The hot-plate test was carried out according to Milano et al. (2008b).

Model of incisional pain

The incisional pain model was carried out as previously described by Oliveira et al. (2014).

Possible opioid action mechanism

To verify if SBE possess a similar action mechanism to opioids we followed the protocol preconized by Trevisan et al. (2013).

Inflammatory parameters

Carrageenan-induced peripheral inflammation

The carrageenan-induced peripheral inflammation model was used to determine anti-inflammatory activity (Winter et al., 1962). The right hind paw tissue was collected for the determination of enzyme myeloperoxidase (MPO) activity and it was expressed as optical density/g of protein (Oliveira et al., 2014). The measurement of protein levels was performed as described by Bradford, 1976. The serum was used for assay of serum nitrite and nitrate determination (NOx) was performed as Miranda et al. (2001), interleukins (IL), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ) and c-reactive protein (CRP). The cytokines measurement (IL-1, IL-6, IL-10, TNF-α, IFN-γ and CRP) was assessed by enzyme-linked immunosorbent assay (ELISA).