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Original article

Anti-edematogenic and anti-inflammatory activity of the essential oil from *Croton rhamnifolioides* leaves and its major constituent 1,8-cineole (eucalyptol)

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ABSTRACT

The species *Croton rhamnifolioides*, belonging to the *Croton* genus, is known in ethnomedicine as "quebra faca" and is used in the treatment of stomach pain, vomiting and fever. This study aims to evaluate the anti-edematogenic and anti-inflammatory effect of *Croton rhamnifolioides* leaf essential oil (OEFC) and its major constituent: 1,8-cineole (eucalyptol). The essential oil was extracted from fresh leaves through a hydrodistillation system. The chemical analysis was determined by gas chromatography-mass spectrometry (GC–MS). The acute anti-inflammatory activity was determined from the models of: ear edema by the single application of croton oil, paw edema induced by: carrageenan, dextran, histamine and arachidonic acid, while vascular permeability was determined by Evans blue extravasation and chronic anti-inflammatory activity by granuloma induction using the implantation of cotton pellets. The GC–MS results identified and quantified 11 constituents, with the major component being 1,8-cineole (41.33%). The OEFC (20 mg/mL) and 1,8-cineole (8.26 mg/mL) significantly reduced the edema induced by croton oil by 42.1 and 34.9%, respectively. The OEFC (25, 50, 100 and 200 mg/kg) and 1,8-cineole (10.33, 20.66, 41.33 and 82.66 mg/kg) statistically reduced paw edema induced by carrageenan, dextran as well as vascular permeability (protein extravasation). The OEFC (25 mg/kg) and 1,8-cineole (10.33 mg/kg) demonstrated efficacy in reducing edema induced by histamine and arachidonic acid and granuloma. In conclusion, the OEFC and 1,8-cineole have anti-inflammatory activity in the acute and chronic phase, suggesting therapeutic potential as a source for the development of new anti-inflammatory agents.

1. Introduction

Inflammation is considered a response or mechanism of tissue protection against cellular injury, which can be triggered from a variety of injurious, physical, chemical or biological agents [1]. In this process, a sequence of cellular and biochemical events occurs, among them: fluid extravasation, cell migration, release of reactive mediators responsible for tissue lysis and repair [2]. Such clinical manifestations result in external manifestations or classic signs, such as heat, redness (erythema), tumor (edema), pain and loss of tissue or organ function

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[3].

The inflammatory process is subdivided into an acute phase, with a relatively rapid duration. Its main features are structural changes (extravasation of plasma macromolecules) producing edema (exudate) and, finally, the migration of polymorphonuclear cells (neutrophils) into the inflamed tissue induced by the release of inflammatory mediators such as TNFα, IL-1, PGE and PAF [4]. However, in the chronic phase, the persistence of inflammation, tissue structural alterations resulting from macrophagic mononuclear infiltrates (lymphocytes, plasmocytes and macrophages), tissue destruction and fibrosis occurs [1].

Medicinal plants can be an important alternative used in the treatment of several inflammatory diseases [5,6]. Natural product of pharmaceuticals that got to the drugstore shelves have actually been developed from Cordia verbenacea DC. essential oil, as Acheflan®, that used topically for the chronic tendinitis and the myofascial pain [7]. This effect is attributed the presence of α-humulen [8,9]. Other species are marketed as herbal medicines such as Harpagophytum procumbens (Bioflan®) that indicated in the anti-inflammatory process[10], being indicated as an aid in the treatment of rheumatism, such as arthritis (inflammation of the joints), arthrosis and you actions is attributed the of harpagoside compound [11,12]. The genus *Croton* is one of the main genera of the Euphorbiaceae family, which is studied by virtue of its therapeutic potential [13], it belongs to the Crotoneae tribe and subfamily Crotonoideae [14]. In Brazil, it presents a wide distribution in the Northeast region of Brazil, being common in Ceará, Pernambuco, Maranhão, Piauí, Rio Grande do Norte, Paraíba, Bahia, Alagoas and Sergipe [15,16]. Some pharmacological activities stand out in relation to the genus: anti-inflammatory [17], antinociceptive [18], relaxant [19], gastroprotective [16], antioxidant [20,21], larvicide [22] and amebicide [23].

The species *Croton rhamnifolioides* belonging to the genus *Croton* is considered a sub-shrub or shrub [24] known in popular medicine in the Northeast region as “quebra faca” was used as anti-inflammatory [25]. The species is used in the treatment of stomach pain, gastric malaise, vomiting, diarrhea with blood and fever [26]. Several biological activities have been described, such as larvicidal activity on *Aedes aegypti* [27], gastroprotective [16] and antibacterial [28].

Considering the anti-inflammatory and antinociceptive activities described in the literature for the genus *Croton*, as well as the ethnopharmacological data that indicate the use of *Croton rhamnifolioides* in inflammation, pain and fever and finally the need for the search of agents that present better cost-benefit ratio stimulated us to investigate whether the essential oil from *Croton rhamnifolioides* (knife-breaker) leaves and its major constituent: 1,8-cineole (Eucalyptol) have anti-edematogenic and anti-inflammatory effects. Note that this is the first study that reporting anti-edematogenic and anti-inflammatory activity this species and its major constituent, however, other study conducted by our research group demonstrated in 2017 the potential gastroprotective effect of essential oil of *C. rhamnifolioides*.

2. Materials and methods

2.1. Collection and extraction of theCroton rhamnifolioides **leaf essential oil (OEFC)**

Leaves of *Croton rhamnifolioides* Pax. And K. Hoffm were collected in the morning (7:00 at 9:00) at the Caatingueira Creek Site (6°40′6″S and 40°10′51″ W – May/June 2014) situated in the municipality of Aiuaba – CE, Brazil, under the authorization of ICMBio (n°47705-1). The species (voucher number 12.062) was identified by Prof. Maria Arlene Pessoa da Silva in the Caririense Dárdano de Andrade Lima Herbarium of the Regional University of Cariri. After collecting the fresh leaves (7.512 g) was washed, crushed and the essential oil was extracted by means of a hydrodistillation system in Clevenger-type apparatus. The OEFC was conditioned under refrigeration until the time of the assays.

2.2. Essential oil chemical analysis

Analysis of the chemical composition of the OEFC was performed using a Shimadzu GC MS-QP2010 spectrometer (GC–MS system), Rtx-5MS capillary column (30 mx 0.25 mm, 0.25 μm film thickness), carrier gas: helium at 1.5 mL/min. Injector temperature: 250 °C, detector temperature: 290 °C, column temperature: 60–180 °C at 5 °C/min, then 180–280 °C at 10 °C/min (10 min). The reading speed was 0.5 scan/s from *m/z* 40 to 350, split ratio (1:200). A volume of 1 μL of [25 μL (essential oil)/5 mL $CHCl_3$] (1:200) was injected, whose solvent cut-off time: 2.5 min. The equipment was operated under ionization energy of 70 eV and the identification of the individual components was based on spectral fragmentation, using standards from the NIST 08 computer library, in addition to two other parameters: retention indices and comparison with literature data.

2.3. In vivo experimental protocols

2.3.1. Drugs

The compounds (arachidonic acid, histamine, carrageenan, 1,8-cineole, croton oil, dextran, Evans blue, indomethacin and dexamethasone) were purchased from Sigma-Aldrich (St. Louis, MO, USA), while xylazine and ketamine (Ceva Santé Animale, BR). All solutions were prepared immediately prior to use and administered by oral (v.o.), subcutaneous (s.c.) or intraperitoneal (i.p.) routes according to the animals weight (0.1 mL/10 g body mass) and specific experimental protocol. The control group was given distilled water, while the OEFC and 1,8-cineole were diluted in 1% Tween-80 aqueous solution.

2.3.2. Animals and ethical aspects of the study

For the *in vivo* assays, Swiss (*Mus musculus*) male mice with body mass (20–30 g) were used, kept in polypropylene cages and maintained in an environment temperature of 23 ± 2 °C, using a light/dark cycle of 12 h and having free access to potable water and rodent-specific food (Presence, Purina®), the animals were fasted (8–10 h) of solids before testing.

All the experimental procedures followed the norms of animal use, with the research being submitted and approved by the Animal Research Ethics Committee of the Regional University of Cariri (CEUA/URCA – n° 43/2015.1).

2.3.3. Determination of the OEFC average lethal dose (LD_{50})

Acute toxicity studies were performed with Swiss male mice, as described by OECD 425 [29], with slight modifications. The animals were randomly divided into two groups (n = 3) and fasted overnight with free access to water. The control group received water (0.1 mL/10 g/v.o.) and the group treated with OEFC using a single 2000 mg/kg dose by oral route. The animals were observed at 30, 60, 120, 180 and 240 min after oral treatment and daily for 14 days. Behavioral changes, weight, food and water consumption, clinical signs of toxicity or mortality were recorded daily. [30]

2.3.4. Anti-inflammatory activity

The acute anti-inflammatory activity was determined from the models of: ear edema by the single application of croton oil, paw edema induced by different phlogistic agents (carrageenan, dextran, histamine and arachidonic acid), vascular permeability by Evans blue extravasation, and chronic anti-inflammatory activity through the model of granuloma induced by implantation of cotton pellets. The animals (n = 6/group) were divided into groups: negative control (distilled water), positive control (indomethacin 10 mg/kg/s.c., dexamethasone 4 mg/mL/v.t., promethazine 6 mg/kg/v.o.), OEFC (25, 50, 100 and 200 mg/kg/v.o. or 2.5, 5, 10 and 20 mg/mL/v.t., values corresponding to, or less than, 10% of the OEFC LD_{50}), and 1,8-cineole (10.33, 20.66, 41.33 and 82.66 mg/kg/v.o. or 1.03, 2.06, 4.13 and 8.266 mg/mL/v.t., these values corresponding to the proportional dose of OEFC).