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Chrysophyllum cainito leaves are effective against pre-clinical chronic pain models: Analysis of crude extract, fraction and isolated compounds in mice

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ABSTRACT

Ethnopharmacological relevance: *Chrysophyllum cainito* L. (Sapotaceae), commonly known as caimito or star apple, is a neotropical tree valued for its ornamental quality and edible fruits. Besides its culinary use, the leaves are also popularly used to treat diabetes mellitus and several inflammatory diseases.
Aim of this study: This study aimed to complement previous data obtained about the anti-hypersensitivity effects of the crude methanol extract (CME), $CHCl_3$ fraction and isolated compounds obtained from *C. cainito*.
Materials and methods: The CME, $CHCl_3$ fraction and two isolated triterpenes identified as 3β-Lup-20(29)-en-3-yl acetate (1) and Lup-20(29)-en-3β-O-hexanoate (2) were evaluated regarding their effects using clinical pain models, such as post-operative, inflammatory and neuropathic pain. Acute inflammatory pain models induced by PGE_2, epinephrine, LPS and CFA were also used to improve the knowledge about the mechanism of action.
Results: The animals treated with the CME and submitted to PGE_2, epinephrine, LPS or CFA had the mechanical hypersensitivity significantly reduced. When repeatedly administered, the CME enhanced the mechanical withdrawal threshold of mice submitted to post-operative pain model, CFA-induced chronic inflammatory pain and two different models of neuropathic pain. In turn, the $CHCl_3$ fraction presented anti-hypersensitivity effect against epinephrine- or LPS-induced hypersensitivity, with a more prominent activity in both the neuropathic pain models. The compound 1 seems to present the same profile of the $CHCl_3$, whereas compound 2 exhibited activity similar to the CME.
Conclusions: This data suggests that the CME effect involves interference in the production, release or action of some chemical mediators, such as PGE_2, sympathetic amines, cytokines, etc. Part of these effects was observed with the CHCl3 fraction, emphasizing the prominent inhibition of neuropathic pain. The results also demonstrated that part of the CME effects are due to the presence of the triterpenes 1 and 2, but it is important to mention that we cannot discard the effects of countless other compounds presented in the crude extract, acting in a synergic way.

1. Introduction

Chrysophyllum cainito L. (Sapotaceae), commonly known as caimito or star apple, is a neotropical tree valued for its ornamental quality and edible fruits (Morton, 1987). The species is usually considered native to the Greater Antilles and naturalized in Central and South America (Pennington, 1990). Less commonly, the species is considered as native to Panama (Correa et al., 2004). In Panama, both cultivated and wild *C. cainito* trees are found growing in close proximity and exhibit high levels of intraspecific variability for fruit traits such as fruit and seed size, sugar content, and levels of polyphenols. These differences between cultivated and wild individuals suggest that the cultivated trees are semi-domesticated (Parker et al., 2010).

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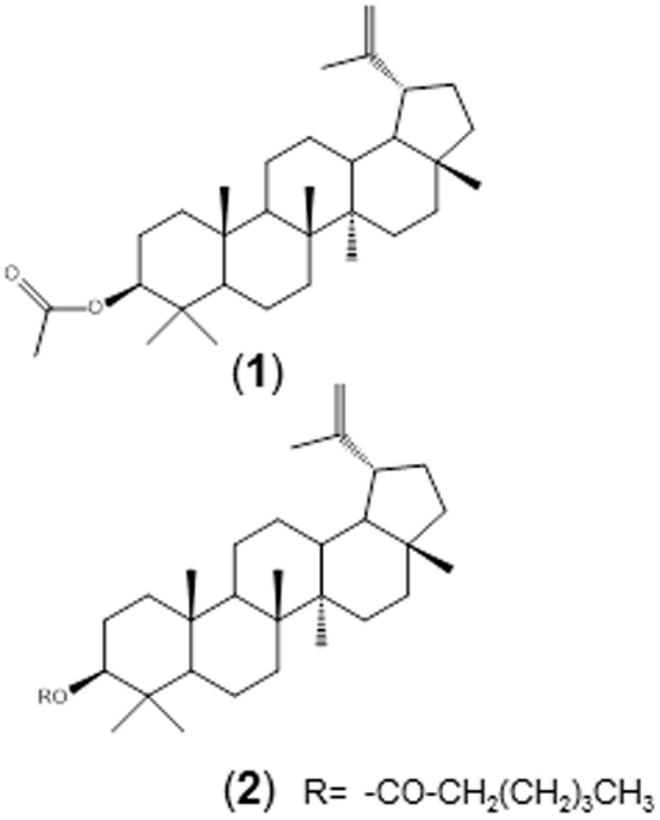

Fig. 1. The molecular structures of the triterpenes **1** (3β-Lup-20(29)-en-3-yl acetate) and **2** (Lup-20(29)-en-3β-O-hexanoate) isolated from *Chrysophyllum cainito* leaves.

Besides its culinary consumption, this plant is also used in folk medicine for the treatment of diabetes mellitus and several inflammatory diseases, such as rheumatoid arthritis (Obasi, 1991). Despite the existence of popular use, there are few studies scientifically proving its biological effects. Our group has recently shown that the crude extract, fractions and pure compounds (**1** and **2**; Fig. 1A and B) obtained from the *C. cainito* leaves presented important anti-hypersensitive properties against acute inflammatory pain induced by carrageenan in mice without interference in locomotor or exploratory activity (Meira et al., 2014). Considering that chronic pain remains among the most difficult symptom to be relieved, this study aimed to add to previously published data scientific evidences for the use of the crude methanol extract (CME) to treat persistent pain diseases (inflammatory and neuropathic pain). We also performed experiments with the $CHCl_3$ fraction and both isolated compounds (triterpenes) obtained from *C. cainito* leaves, once they have shown positive results in our previous study. Besides the persistent pain models, different algogenic substances, such as lipopolysaccharide (LPS), complete Freund adjuvant (CFA), prostaglandin E_2 (PGE_2) and epinephrine were also used to better understand the anti-hypersensitive effect of the extract and its fraction.

2. Materials and methods

2.1. Drugs and reagents

The following drugs were used: prostaglandin E_2 (PGE_2), epinephrine, lipopolysaccharide (LPS), complete Freund's adjuvant (CFA) all came from Sigma Chemical Company (St Louis, MO, USA), gabapentin (Ranbaxy Laboratories Limited, Paonta Sahib, India).

2.2. Plant material and compound isolation

Leaves of *C. cainito* were collected at Epagri, in Itajaí, in Santa Catarina State (Brazil), in October 2009, and identified by Prof. Oscar B. Iza (Universidade do Vale do Itajaí). A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí-SC) under number VC Filho 087.

Compounds **1** (3β-Lup-20(29)-en-3-yl acetate) and **2** (Lup-20(29)-en-3β-O-hexanoate) were isolated and identified as previously described (Meira et al., 2014).

2.3. Animals

Female Swiss mice (25–30 g) or Wistar rats (180–200 g) obtained from Universidade do Vale do Itajaí(UNIVALI, Itajaí, Brazil) were used throughout this study. The animals were housed under optimum conditions of light and temperature (12/12 h light-dark cycle; 22 ± 1 °C temperature). Food and water were provided *ad libitum* and all the procedures were approved by The Animal Ethics Committee of UNIVALI (Protocol numbers 008/2010 UNIVALI). Experiments were conducted in accordance with the guidelines for the care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals suggested by Zimmermann (1983). The number of animals (5–8 per group) and the intensity of noxious stimuli used were the minimum to demonstrate consistent effects. All the experiments were repeated at least twice when the results were not convincing and the number of animals were 5, considering the need of respecting the 3R' rules, that emphasize the reach of consistent data with the lesser number of animals as possible.

2.4. Mechanical hypersensitivity induced by epinephrine or PGE_2

In this set of experiments we investigated the possible mechanism of the anti-hypersensitivity effect of the CME, $CHCl_3$ fraction and isolated compounds from *C. cainito* leaves using as irritant agents PGE_2 (0.1 nmol/paw; Kassuya et al., 2007) and epinephrine (100 ng/paw; Khasar et al., 2005). Mice were pre-treated with the CME (3–30 mg/kg, i.p.), $CHCl_3$ fraction (3–30 mg/kg, i.p.), compound **1** or **2** (1 mg/kg, i.p.), or vehicle (10 mL/kg). Then they received a 0.05 mL intraplantar (i.pl.) injection of PGE_2 or epinephrine into the right hindpaw and the mechanical hypersensitivity was evaluated using an electronic anesthesiometer, as described later.

2.5. LPS-induced hyperalgesia

Mice were pre-treated with CME (3-30 mg/kg, i.p.), $CHCl_3$ fraction (3-30 mg/kg, i.p.), compound **1** or **2** (1 mg/kg, i.p.) or vehicle (10 mL/kg, i.p.) 30 min before the i.pl. injection of 0.05 mL of lipopolysaccharide (LPS; 100 ng/paw; Santodomingo-Garzón et al., 2006) into the right hindpaw. The behavior evaluation was performed in several time points (1, 2, 4, 6, 24 and 48 h) using the electronic anesthesiometer.

2.6. CFA-induced hypersensitivity

2.6.1. Preventive protocol

To produce a persistent inflammatory response, mice received an i.pl. injection of 0.02 mL of CFA (1 mg/mL heat-killed and dried *Mycobacterium tuberculosis*; each mL of vehicle contained 0.85 mL paraffin oil plus 0.15 mL mannide monooleate) into the plantar surface of the right hindpaw (Quintão et al., 2005). The mice were treated with CME (3–30 mg/kg, i.p.), $CHCl_3$ fraction (3–30 mg/kg, i.p.), compound **1** or **2** (1 mg/kg, i.p.), or vehicle (10 mL/kg, i.p.) 30 min before the CFA injection. The mechanical hypersensitivity