Journal of Ethnopharmacology 151 (2014) 975–983

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Anti-inflammatory and anti-hypersensitive effects of the crude extract, fractions and triterpenes obtained from *Chrysophyllum cainito* leaves in mice

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ARTICLE INFO

Article history:
Received 12 September 2013
Received in revised form
22 November 2013
Accepted 7 December 2013
Available online 14 December 2013

Keywords:
Chrysophyllum cainito L.
Triterpenes
Hypersensitivity
Inflammation
Carrageenan
Mice

ABSTRACT

Ethnopharmacological relevance: *Chrysophyllum cainito*, popularly known as "star apple", caimito, "abiu-roxo" or "abiu-do-Pará", is a tree of about 25 m in height. Besides its culinary use, it is also used in folk medicine for the treatment of diabetes *mellitus* and several inflammatory diseases.
Materials and methods: The crude methanolic extract (CME) was submitted to phytochemical studies for obtaining fractions and isolated compounds. They were monitored by thin-layer-chromatography (TLC). The biological activity was evaluated in mice using the carrageenan-induced mechanical hypersensitivity and paw oedema. Biochemical assays, such as myeloperoxidase (MPO) and activity and cytokines levels quantification, were carried out to analyse the involvement of neutrophil migration and IL-1β and TNFα production. Some adverse effects were investigated using the open-field and rota-rod tests, and it was also measured the rectal temperature.
Results: This study demonstrates, for the first time, the anti-hypersensitivity and anti-inflammatory effects of CME, fractions and two isolated triterpenes obtained from the leaves of *Chrysophyllum cainito* on carrageenan-induced hypersensitivity and paw-oedema. The mice treated with CME or chloroform fraction ($CHCl_3$) presented reduction in mechanical hypersensitivity. The effect of the CME seemed to be partially related to the anti-inflammatory activity, as the paw-oedema and MPO activity were also significantly inhibited. The isolated compound Lup-20(29)-en-3β-O-hexanoate demonstrated more reduction of the hypersensitivity than 3β-Lup-20(29)-en-3-yl acetate, suggesting that this molecule might be partially responsible for the biological effects obtained with CME and $CHCl_3$ fractions. Finally, animals treated with CME and $CHCl_3$ did not present changes in locomotor activity, motor performance or body temperature.
Conclusions: Our data demonstrates, for the first time, that the crude extract, fractions and pure compounds obtained from the *Chrysophyllum cainito* leaves possess important anti-hypersensitive properties against inflammatory pain in mice. The mechanisms through which *Chrysophyllum cainito* exerts its anti-hypersensitive actions are still unclear, and require further investigation; however, this could well constitute a new and attractive alternative for the management of persistent inflammatory and neuropathic pain in humans.

1. Introduction

Data from World Health Organisation (WHO; 2012) demonstrates that symptoms of acute and chronic pain are considered a public health problem. Chronic pain affects approximately 100 million U.S. adults, reducing their quality of life and enhancing the costs to society (IOM, 2011) and this scenario is growing increasingly worse (Woolf, 2010). Conventional analgesics, such as non-steroidal anti-inflammatory drugs, opioids or adjuvant therapies (tricyclic anti-depressants, and anti-convulsants, etc.) are clinically ineffective in attenuating chronic pain, or else they exhibit a wide spectrum of adverse effects which limits their continual use (Garg and Adams, 2012).

Natural products, especially those obtained from higher plants, have been an important source of substances or prototypes with

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http://dx.doi.org/10.1016/j.jep.2013.12.014

relevant therapeutic potential. Few studies about the biological activity of species belonging from Sapotaceae family have been reported. There are several reports from folk medicine indicating that the tree bark, leaves, fruits and seeds of the *Chrysophyllum* genus present pronounced biological activity spectrum (Lemmens, 2007; Morton, 1987; Obasi, 1991; Tassara and Silva, 2005). *Chrysophyllum cainito*, popularly known as "star apple", caimito, "abiu-roxo" or "abiu-do-Pará", is a tree of about 25 m in height. Besides its culinary use, it is also used in folk medicine for the treatment of diabetes *mellitus* and several inflammatory diseases (Obasi, 1991). Luo et al. (2002) have isolated from the ethyl acetate fraction of *Chrysophyllum cainito* fruit, nine known polyphenolic antioxidants, (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, quercetin, quercitrin, isoquercitrin, myricitrin, and gallic acid, emphasising the antioxidant activity of the compound quercetin. Recently, Bastos et al. (2010) demonstrated that the ethanolic extract of this plant exhibited anti-helminthic activity.

In the present study, we evaluate the anti-hypersensitive and anti-inflammatory effects of the crude methanolic extract (CME), fractions, and two isolated compounds (triterpenes) obtained from *Chrysophyllum cainito* leaves, on mechanical hypersensitivity and paw oedema induced by carrageenan in mice. Experiments were carried out with the aim of discarding the interference of non-specific effects, such as changes in locomotor activity, motor performances and body temperature, in the biological effects observed with *Chrysophyllum cainito* treatment.

2. Material and methods

2.1. Plant material

Leaves of *Chrysophyllum cainito* were collected at Epagri, in Itajaí, in the State of Santa Catarina (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.

2.2. Extraction and isolation

Dried and powdered leaves (A, 880 g) were extracted with methanol (MeOH) at room temperature for approximately one week. The solvent was then evaporated under reduced pressure to give crude methanolic extract (CME), which was submitted to biological assays and phytochemical studies. The CME was dissolved in MeOH/H_2O and partitioned respectively with hexane (Hx), chloroform ($CHCl_3$), and ethyl acetate (EtOAc).

The bioactive fraction ($CHCl_3$; 10.5 g) was purified through a silica gel column with Hx and mixtures of Hx with increasing amounts of 10% chloroform, to obtain 18 fractions (CCZ-3 [1–18]). The fractions and compounds were monitored by thin-layer-chromatography (TLC). Spots were visualised with an anisaldehyde sulphuric reagent solution.

Fraction CCZ-3 [10] (116 mg) resulted to be a pure compound, identified as 3β-Lup-20(29)-en-3-yl acetate or lupeol acetate (**1**), a common triterpene (Fig. 1A).

Fraction CCZ-3 [8] (345.40 mg) was purified through a silica gel column eluted with Hx:Dichloromethane. Two hundred fractions were collected and combined into four final fractions (CCZ-4 [1–4]. Fraction CCZ-4 [4] was then purified by flash column and eluted with Hx:CHCl3 furnishing CCZ-5 [73–85] as a pure compound (14 mg), identified as Lup-20(29)-en-3β-O-hexanoate (**2**), a rare natural triterpene (Fig. 1B). The structural elucidation of both compounds was determined by infrared spectroscopy and nuclear magnetic resonance experiments in comparison with authentic samples and data reported in the literature by one of us (Brum et al., 1998).

2.3. Pharmacological studies

2.3.1. Animals

Female and male Swiss mice (20–28 g) were used throughout this study. The animals were housed under conditions of optimum light, temperature and humidity (12 light–dark cycle, 22 ± 1 °C), with food and water provided ad libitum. Swiss mice were obtained from the vivarium of University of Vale do Itajaí (UNIVALI, Itajaí, Santa Catarina, Brazil). The in vivo experiments mice were evaluated by an observer blinded to treatment and all procedures used in the present study followed the "Principles of Laboratory Animal Care" of NIH publication No. 85-23, and were approved by the Animal Ethics Committee of the UNIVALI (Protocol numbers 008/2010 UNIVALI). The number of animals and the intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects.

2.3.2. Mechanical hypersensitivity induced by carrageenan.

For the induction of inflammatory pain, the mice were treated intraperitonealy (i.p.) with the CME (0.3, 1, 3, 10 or 30 mg/kg), fractions (3, 10 or 30 mg/kg) or isolated compounds (0.1, 0.3, 1 or 3 mg/kg). Next, they received an i.pl. injection of 50 μL of λ-carrageenan (300 μg/paw) into the surface of the right hindpaw 30 min after the treatment (Manjavachi et al., 2010). The control group was treated with the vehicle (10 mL/kg, 1% Tween 80 in 0.9% NaCl solution). The mechanical hypersensitivity of all the groups was assessed using an electronic anesthesiometer, for up to 48 h after carrageenan administration.

2.3.3. Paw-oedema induced by carrageenan

The animals were treated i.p. with the CME (0.3, 1, 3, 10 or 30 mg/kg), fractions (3, 10 or 30 mg/kg) or vehicle (10 mL/kg, 0.9% NaCl solution) 30 min before the i.pl. injection of saline 0.9% containing λ-carrageenan (300 μg/paw, 50 μL) into the right hindpaw (Manjavachi et al., 2010). The contralateral (left) hindpaw received 50 μL of saline and was used as control. Oedema was measured using a plethysmometer (Ugo Basile) at different time points after carrageenan injection for up to 48 h after carrageenan administration. Oedema is expressed in μL as the difference between the right and left paws. Indomethacin (5 mg/kg, i.p., 30 min; purity ≥ 95%) was used as positive control.

2.3.4. Mechanical withdrawal threshold evaluation

Mechanical hypersensitivity was evaluated using the electronic anesthesiameter. The test consisted of evoking a hindpaw flexion reflex with a hand-held force transducer (electronic anesthesiometer; IITC Life Science, Woodland Hills, CA) adapted with a 0.5-mm^2 polypropylene tip. After paw withdrawal, the intensity of the pressure was automatically recorded, and the final value for the response was obtained by averaging three measurements. The animals were tested before and after treatments (Cunha et al., 2004).

2.4. Biochemical assays

2.4.1. Myeloperoxidase (MPO) activity

Neutrophil recruitment to the mouse paw was assessed indirectly by means of tissue MPO activity, according to the method described beforehand (Cunha et al., 2005). For this purpose, the animals were treated with the CME or $CHCl_3$ (3, 10 or 30 mg/kg, i.p.) 30 min before the i.pl. injection of carrageenan, as described above. The mice were euthanized 6 h after the treatment and the right hindpaw tissue was collected. The hindpaw tissue was homogenised at 5% (w/v) in EDTA/NaCl buffer (pH 4.7) and centrifuged at 10,000 rpm for 15 min at 4 °C. The pellet was