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Pereskia aculeata Miller leaves present *in vivo* topical anti-inflammatory activity in models of acute and chronic dermatitis

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5280794
Phytol
PubChem CID
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ABSTRACT

Ethnopharmacological relevance: The leaves of *Pereskia aculeata* Miller (Cactaceae), known as Barbados gooseberry, are used in Brazilian traditional medicine as emollients and to treat skin wounds and inflammation. This study investigated the topical anti-inflammatory activity of the hexane fraction (HF) obtained from the methanol extract of the leaves of this species in models of acute and chronic ear dermatitis in mice.

Material and methods: Mice ear edema was induced by topical application of croton oil, arachidonic acid, capsaicin, ethyl-phenylpropiolate and phenol; and by subcutaneous injection of histamine. Ear biopsies were obtained to determine the levels of IL-1β, IL-6 and TNF-α cytokines by ELISA assay. Histopathological analysis was also performed to evaluate the HF activity in croton oil multiple application test. In addition, acute dermal irritation/corrosion test in rats was accomplished. HF chemical characterization was performed by GC–MS analysis.

Results: HF intensively reduced the inflammatory process induced by all irritant agents used, except for arachidonic acid. This activity is related, at least in part, to the reduction of IL-6 and TNF-α cytokines levels. Moreover, when the glucocorticoid receptor antagonist mifepristone was used, HF failed to respond to the croton oil application.The results strongly suggested a glucocorticoid-like effect, which was reinforced by the presence of considerable amounts of sterol compounds identified in HF. The acute dermal irritaton/corrosion test showed no signs of toxicity.

Conclusions: This study showed that the acute and chronic anti-inflammatory activity of *P. aculeata* leaves is very promising, and corroborates to better understand their ethnopharmacological applications.

1. Introduction

The skin, the largest organ of the human body, plays an essential role as a protective barrier against microorganisms, and chemical and physical injuries from the external environment. This physiological barrier consists of a complex network of structural, cellular and molecular components which provide an effective defensive immunological reaction against harmful stimuli (Nestle et al., 2009; Bangert et al., 2011). Nevertheless, in some circumstances, alterations in the skin immunological balance may elicit inappropriate defensive responses which lead to an

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inflammatory process or chronic skin disorders, including psoriasis, eczema, atopic dermatitis and allergic contact dermatitis (Lee and Hwang, 2012). The current therapeutic agents available to treat skin inflammation are mainly glucocorticoids, which often exhibit adverse reactions that affect the hypothalamic–pituitary axis, the lipids, carbohydrates, proteins and bone metabolisms, and the organs related to the immune system (Poetker and Reh, 2010; Sarnes et al., 2011; Rang et al., 2012).

In this context, plant extracts and natural substances remain as an alternative in the search for new safer and more effective topical anti-inflammatory drugs (Newman and Cragg, 2012). There are reports that leaves of *Pereskia aculeata* Miller (Cactaceae), known as Barbados gooseberry, are used in folk medicine as emollients and to treat skin wounds and inflammatory process (Duarte and Hayashi, 2005; Sartor et al., 2010; Pinto and Scio, 2014). *P. aculeata* is a climbing cactus shrub distributed in Central and South America, covering mainly from south to northeast of Brazil, where its leaves are also used by natives as a vegetable in traditional cuisine (Takeiti et al., 2009; Rosa and Souza, 2003; Paterson et al., 2009). However, there are only few studies about *P. aculeata* leaves phytochemicals and their therapeutic potential (Pinto et al., 2012; Pinto and Scio, 2014). Recently, Carvalho et al. (2014) reported the *in vitro* wound healing activity of the mucilage extracted from the leaves of this species, and Pinto et al. (2015) showed that the hydromethanolic fraction from the crude extract of the leaves presented central and peripheral antinociceptive activity in mice.

Preliminary studies performed by our group showed that, among the different fractions obtained from the methanol extract of *P. aculeata* leaves by solvent partition, the hexane fraction showed the most remarkable anti-inflammatory potential (data not shown).

For that reason, in order to contribute to the search for new plant extracts or natural substances with anti-inflammatory potential, and for better understanding the ethnopharmacological relevance of *P. aculeata*, this study was conducted to further investigate the topical anti-inflammatory activity of the hexane fraction (HF) obtained from the methanol crude extract of the leaves of this species in models of acute and chronic ear dermatitis in mice, using different phlogistic agents. In addition, an established acute dermal irritation and corrosion test in rats was accomplished to predict a possible dermal toxicity of HF. Chemical characterization of HF was also performed.

2. Materials and methods

2.1. Plant material

The plant material was collected in Juiz de Fora (MG, Brazil) in August 2010, in the morning. A voucher specimen (No. 57539) was deposited in the Herbarium Leopoldo Krieger of the Federal University of Juiz de Fora for future evidence.

The leaves were air-dried in a well-ventilated place at room temperature (25 °C) for 15 days. Once dried, the material (approximately 1 kg) was powdered using a knife mill and then extracted by maceration with methanol until exhaustion. The extract was concentrated on a rotary evaporator to obtain the crude methanol extract (140 g), which was dissolved in methanol/water (8:2 v/v) and then fractionated with hexane by solvent partition. The hexane fraction (38 g) was stored in a refrigerator at 4 °C.

2.2. Chemicals

Croton oil, arachidonic acid (AA), capsaicin, ethyl-phenylpropiolate (EPP), phenol, histamine, indomethacin, dexamethasone, mifepristone, bovine serum albumin (BSA), phenylmethylsulphonyl fluoride (PMSF), benzethonium chloride, ethylenediaminetetracetic acid (EDTA) and aprotinin were purchased from Sigma-Aldrich® (St. Louis, MO, USA). Ketamine chloride and xylazine chloride were obtained from Syntec® (Hortolândia, SP, Brazil). Animal commercial chow was from Nuvitalis® (Colombo, PR, Brazil). All other reagents were of the highest quality available.

2.3. Pharmacological assays

2.3.1. Animals

Male Swiss mice (20–30 g) and Male Wistar rats (160–200 g) bred in the Center of Reproductive Biology (Federal University of Juiz de Fora, Brazil) were used. The animals were kept under standard temperature (22 °C), 12/12 h light/dark cycle, and had food and water *ad libitum*. The groups consisted of 6–8 animals. All experimental procedures are in accordance with the Ethical Principles of Animal Research adopted by Brazilian College of Animal Experimentation (COBEA – Protocols no 013/2013, 021/2013, 016/2013 and 028/2014).

2.3.2. Topical anti-inflammatory activity on acute inflammation

2.3.2.1. Croton oil single application-induced ear edema test. This test was carried out in accordance to the method described by Schiantarelli et al. (1982). Each mouse was immobilized by hands, and 20 μL of a fresh solution of croton oil 2.5% (v/v) were topically applied on the inner surface of the right ear and the same volume of acetone (vehicle) on the inner surface of the left ear. Immediately after the application of the phlogistic agent, the animals received topical treatment with 20 μL of HF (0.1, 0.5, and 1.0 mg/ear), dexamethasone (reference drug) 0.1 mg/ear, or acetone. Four hours after the topical applications, the animals were euthanized and 6 mm diameter ear punch biopsies were collected and subjected to ear edema measurement. Subsequently, the fragments obtained from the right ears of dexamethasone, vehicle and HF 1.0 mg/ear groups were stored at −80 °C in order to measure interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) cytokines levels.

2.3.2.2. AA, EPP, capsaicin and phenol-induced ear edema tests. Each mouse was immobilized by hands. Then, 20 μL of fresh solutions of AA 2.0 mg/ear (Young et al., 1984), EPP 1.0 mg/ear (Giner et al., 2000), capsaicin 1.0 mg/mL (Gábor and Razga, 1992), or phenol 10% (v/v) (Gábor, 2000) were topically applied on the inner surface of the right ear, and 20 μL of acetone (vehicle) on the inner surface of the left ear. Immediately after the application of the phlogistic agents, the animals received topical treatment with 20 μL of HF 1.0 mg/ear, indomethacin 0.5 mg/ear (reference drug for AA test), dexamethasone 0.1 mg/ear (reference drug for EPP, capsaicin and phenol tests) or acetone. One hour after topical applications of AA, EPP and phenol, and 30 min after topical application of capsaicin, the animals were euthanized and 6 mm diameter ear punch biopsies were subjected to ear edema measurement.

2.3.2.3. Histamine-induced ear edema test. The animals were treated topically with 20 μL of HF 1.0 mg/ear, dexamethasone 0.1 mg/ear (reference drug) or acetone on the inner surface of the right ear and the same volume of acetone on the inner surface of the left ear. After 20 min, mice were anesthetized by intraperitoneal application of ketamine 80 mg/kg and xylazine 15 mg/kg. Thirty minutes after the topical treatments, 10 μL of histamine dihydrochloride 0.1 mg/μL diluted in NaCl 0.9% were applied subcutaneously with a 29-G needle syringe in the right ear of each mouse, whereas only NaCl 0.9% was injected in the left ear. Two hours after the treatments applications, the animals were euthanized and 6 mm diameter ear punch biopsies were collected for