Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 102 (2005) 1–4
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Review

Anti-inflammatory activity of *Lippia dulcis*

S. Pérez [a,*], M. Meckes [b], C. Pérez [a], A. Susunaga [a], M.A. Zavala [a]

[a] *Depto Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, A.P. 23-181, México D.F., Mexico*
[b] *U.I.M. Farmacología de Productos Naturales, Hospital de Pediatría, CMN Nacional Siglo XXI, IMSS Col. Doctores, México D.F., Mexico*

Received 10 December 2003; received in revised form 25 April 2004; accepted 22 June 2005

Abstract

Lippia dulcis hexane and ethanol extracts were tested for its anti-inflammatory activity in several animal models. Hexane extract showed to be inactive, but the ethanol extract at doses of 400 mg/kg produced significant inhibition of carrageenan-induced paw oedema and reduced the weight of cotton pellet-induced granuloma, moreover, the topical application of 0.5 mg/ear of this extract inhibited the edema induced with TPA by 49.13%, an effect which is of less intensity than that produced by indomethacine at the same dose.

Keywords: *Lippia dulcis*; Anti-inflammatory; Medicinal plant

Contents

1. Introduction

Lippia dulcis Trav (Verbenaceae) is a medicinal plant commonly known in Mexico as “hierba dulce”. Aerial parts were collected in Oaxaca, Mexico. The species was authenticated by MsC Abigail Aguilar and a voucher specimen 14,417 was deposited in the Herbarium of Centro Medico Nacional Siglo XXI, IMSS.

This plant is commonly used in traditional medicine to treat inflammatory conditions (Argueta, 1994), an infusion or the decoction prepared with the aerial parts is orally administered. *Lippia dulcis* is also used to treat cough, diarrhoea and stomachache and the inhibitory effect produced by *Lippia* ethanolic extract on the growth of some enterobacteria has already been reported (Cáceres et al., 1993). Camphor, limonene, terpineol, α-pinene, pippiol (Pascual et al., 2001), α-copaene, δ-cadinene, (+) 4β-hydroxy-hernandulcine (Kaneda et al., 1992) and (+)-hernandulcine which is 1500 times sweeter than saccharose (Compadre et al., 1985) have been isolated from this plant. The present study was

* Corresponding author.
E-mail address: msperez@correo.xoc.uam.mx (S. Pérez).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.06.047

undertaken to evaluate its attributed anti-inflammatory properties.

2. Materials and methods

2.1. Preparation of the extracts

One hundred and sixty grams of dried powdered aerial parts of the plant were refluxed for 4 h with 500 mL of hexane or 80% ethanol and obtained extracts were filtered. The hexane extract was vacuum-dried (yield 2.5%), meanwhile the ethanol extract was concentrated under vacuum and the residual water was lyophilized (yield 9.2%).

A preliminary screening of the ethanol extract showed a positive $FeCl_3$ reaction for phenolic compounds (Domínguez, 1973). It also showed a positive Lieberman–Burchard, Tortelli–Jaffe and Tschugaeff test for terpenes (Domínguez, 1973), and Dragendorff, Wagner, Meyer, silicotungstic acid, Sonnenschein and Scheibler reactions for alkaloids (Domínguez, 1973).

2.2. Animals

Male Wistar rats (150–250 g) and BALB male mice (25–30 g), housed at 24 °C under a 12-h light/12-h dark cycle of 12:12, were maintained with food (purina) and water ad libitum.

All the experiments were performed according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation in conscious animals (Academia Nacional de Medicina, 1999).

2.3. Anti-inflammatory activity

The anti-inflammatory property of extracts of *Lippia dulcis* was investigated using the following animal models.

2.3.1. Carrageenan-induced edema

Groups of five rats were injected with 100 μL of 1% carrageenan solution into the sub-plantar region of the left hind paw. Test groups of rats were treated orally with 50, 100, 200 or 400 mg/kg of the extracts, 1 h prior to carrageenan injection.

At the same time, control group received p.o. the vehicle (10% Tween 80), while the reference group was treated with 8 mg/kg indomethacin. The paw volume was measured at 1.5, 3.0, 5.0 and 24 h after carrageenan administration by volume displacement method using a plethysmometer Ugo Basile according to the method described by Winter et al. (1962). For the dose of 400 mg/kg, the paw volume was also determined at 10, 20, 30, 60 and 120 min after carrageenan administration. Inhibitory activity was calculated according to Olajide et al. (2000):

$$\%\,\text{inhibition} = \frac{(C_t - C_0)\text{control} - (C_t - C_0)\text{treated}}{(C_t - C_0)\text{control}} \times 100$$

where C_t is displacement volume at t time after carrageenan administration and C_0 is displacement volume before carrageenan administration.

2.3.2. Cotton pellet-induced granuloma

Cotton pellets weighing 3 mg each were sterilized. Under anesthesia, the pellets were introduced subcutaneously through a skin incision at the sternum level of rats (Winter and Porter, 1957). The administration (p.o.) of 1 mL of water, 25 mg/kg of naproxen (as a positive control), and 200 and 400 mg/kg of the ethanol extract in 10% Tween 80 solution was followed 30 min after cotton pellet implantation. The edema was allowed to develop for 5 days, the animals were sacrificed with chloroform, the granuloma (diameter of 6 mm) and a plug of the same size of the opposite side of granuloma were removed, dried for 24 h at 60 °C and the dry weights determined. The difference between the weight of the granuloma, the plug without cotton pellet and the cotton pellet weight was considered in the determination of the amount of granulomatous tissue produced.

2.3.3. TPA-induced ear edema

A solution of 2.5 μg 12-*O*-tetradecanoylphorbol acetate (TPA) in 25 μL of acetone were topically applied to groups of BALB-c male mice (20–22 g) on both the inner and outer surfaces of the right (W) and the left ear (W') (Young and De Young, 1989). Topical administration of *Lippia dulcis* ethanol extract (0.25–3.00 mg/left ear) and indomethacin (0.5 mg/left ear) dissolved in acetone was performed 5 min after TPA. The extend of the inflammation in a control group was determined as a difference of the weight between the inflammed ear with TPA and the treated ear with the vehicle (W_o). In all groups, the edema was allowed to develop for 6 h, afterwards the animals were sacrificed and plugs (diameter of 6 mm) of the central portion were taken from both ears and weighed. The reduction of the edema in the ear treated with indomethacin or the extract was expressed as:

$$\%\,\text{inhibition} = \frac{(W' - W_o)}{(W - W_o)} \times 100 - 100$$

2.3.4. Statistical analysis

The results are expressed as mean ± S.E.M., statistical analysis was carried out by ANOVA followed by Dunnet's multiple comparison test. $P < 0.005$ or 0.01 were considered as indicative of significance.

3. Results

The effect of the hexane and ethanol extracts of *Lippia dulcis* on carrageenan-induced edema is shown in Fig. 1. The hexane extract was inactive in this model, but the ethanol extract at doses of 50–400 mg/kg, produced a significant effect against carrageenan-induced inflammation after 3.0 h of the administration. The dose of 400 mg/kg (Fig. 2) exhibited a significant inhibition of 43% after 1.5 h, the effect increased at 3.0 h (51.8%). Anti-inflammatory activity of the ethanol extract was significant and similar to that of indomethacin (8 mg/kg).

Values of the inhibitory effect produced by the ethanol extract on granuloma induced by pellet implantation indicated that at a dose of 400 mg/kg, the extract reduced 53.5 ± 6.8% the weight of cotton pellet-induced granuloma and 54.2 ± 4.7% with a