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Anti-inflammatory Properties of *Salvia connivens*

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ABSTRACT

The anti-inflammatory properties of chloroform, methanol and aqueous extracts of the leaves of *Salvia connivens* Epling were investigated. It was found that aqueous extract on carraggenan induced edema at dose of 400 mg/kg had no activity. Methanol extract at the same dose inhibited the edema 64.3 ± 18% (1 h), however the effect decreased after this time. Chloroform extract showed at this dose an inhibition of 73.8 ± 10.6% (4 h), and the effect observed for this extract at dose of 200 mg/kg was 87.76 ± 7.6% (5 h). Indomethacin was used as a positive control and inhibited edema by 73.2 ± 3.5% at a dose of 8 mg/kg. Tests demonstrated that the chloroform extract inhibited 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced ear edema in mouse by 50.7 ± 2.1%; The chloroform extract inhibited pellet implantation-induced granuloma formation by 39.34 ± 5.36% at a dose of 200 mg/kg, a result comparable to that exhibited by naproxen at a dose of 50 mg/kg (40.81 ± 4.78%). These results suggest that *S. connivens* chloroform extract has anti-inflammatory activity, supporting its folkloric use for treatment of inflammatory diseases.

Keywords: carrageenan, granuloma test, TPA

INTRODUCTION

Salvia is a genus of plants in the mint family, Lamiaceae. This genus includes approximately 700 to 900 species of shrubs, herbaceous perennials, and annuals with almost world wide redistribution. Some of them are reported to possess a wide range of biological activities as antimicrobial, antitumor (Ulubelen 2003), anti-inflammatory (Benrezzouk *et al.* 2001) and ulcerogenic (El-Sayed *et al.* 2006). *Salvia connivens* Epling is a small shrub with blue flowers. In Mexico its seeds are known as *chia* and are used in a sweetened decoction for sore throat and fevers, while a cold decoction is applied to inflamed eyes. The plant is also used as an antipyretic, to combat malaria, and to treat inflammatory conditions (oral information).

No reports on chemical or pharmacological studies were found in the literature, thus, the objective of this research trial was to validate the use of *S. connivens* in traditional medicine and to explore, more specifically, its anti-inflammatory properties.

MATERIALS AND METHODS

Plant material

The aerial parts of *S. connivens* were collected in the municipality of Guadalcazar, San Luis Potosi State, México in January 2006, and were identify by José García Pérez, a taxonomist. A voucher specimen (SLPM43013) is kept at the Herbarium, Isidro Palacios of the Universidad Autónoma de San Luis Potosí.

Reagents

Carrageenan, TPA, indomethacin and acetone were purchased from Sigma-Aldrich. Methanol and chloroform RA from Merck.

Extract preparation

The powdered leaves (400 g) were extracted with 2.5 L of chloroform, methanol or water under reflux for 4 h. The extracts were filtered. Solvents were removed under reduced pressure using a rotatory evaporator and water was lyophilized, with the following yields: chloroform extract (4.49%), methanol extract (5.7%) and aqueous extract (7.1%). The dried extracts were suspended in 10% Tween 80 for administration to the experimental animals.

Animals

CD1 male mice (20-25 g) and male Wistar rats (200-250 g) from the Universidad Autónoma Metropolitana animal facility were housed in isolated cages under standardized conditions (dark/light, 12 h/12 h) at 24°C and 50-55% relative humidity; they were supplied with purina chow 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 (Guia para el cuidado y uso de animales de laboratorio, Edición Mexicana, Academia Nacional de Medicina Academy Press, 1999).

Anti-inflammatory evaluation

The anti-inflammatory property of extracts of *Salvia connivens* was investigated using the following three animal models:

1) 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema (Young *et al*. 1989).

Irritant dermatitis was induced on the right ear by topical application on both the inner and outer surfaces of 2.5 µg TPA in 25 µL of acetone in CD1 male mice (20-22 g). Administration of the experimental extract (2.0 µg/ear) or indomethacin (2.0 µg/ear) dissolved in acetone were performed 30 minutes after TPA application topically to the right ear (W). The left ear was treated with the vehicle (acetone) (Wo) only. The extent of inflammation in the control group was determined as a difference of the weight between the inflamed ear with TPA (W') and the treated ear with the vehicle (W'o). In all groups the edema was allowed to develop for 6 hours; 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 mice treated with indomethacin or the extract was expressed by:

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Short Communication

$$\% \ inhibition = \frac{(W - Wo)}{(W' - W'o)} \times 100$$

2) Carrageenan-induced rat paw edema

100 µL/paw of a 1% carrageenan solution was injected into the sub-plantar region of the left hind paw. The foot volume of the animals was determined by the displacement method using an Ugo Basile plethysmometer according the method described by Winter *et al*. (1962) at 1, 2, 3, 4 and 5 h after carrageenan administration. A reference group was administered orally (p.o.) with indomethacin (8 mg/kg). The extract was administered at doses of 400 mg/kg p.o. before carrageenan administration. The control group received vehicle only. The percent edema inhibition was calculated for each animal group in comparison to group treated with vehicle (10% Tween 80) according to the following formula (Olajide *et al*. 2000):

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

where C_t = paw volume displacement at time t, C_o = paw volume displacement before any treatment.

3) Granuloma induction

Three groups of rats (n = 8) were used. Cotton pellets 6 mm in diameter and weighing 3 mg each were sterilized. Animals were anaesthetized, and the pellets were subcutaneously introduced through an abdominal skin incision. Each group was treated daily for five consecutive days with 1 mL of water, 50 mg/kg of naproxen (positive control), or 200 mg/kg of the chloroform extract in 10% Tween 80 solution were administered 30 min after cotton pellet implantation. On the sixth day, the animals were sacrificed, the pellets dissected and a plug of tissue removed at the site of implantation. A plug of the same size was removed from the opposite side of the abdomen for comparison. The plugs were dried at 60°C overnight to determine the dried weight. The difference between the weight of the granuloma, the plug without cotton pellet and the cotton pellet weight was used to determine the amount of granulomatous tissue produced (Winter *et al*. 1957).

Housing conditions and all *in vivo* experiments followed the guidelines established by the Ethics Committee for Research and Ethical Issues of IASP (1983) and NOM-062-Z00-1999 on animal care.

Statistical analysis

Statistical analysis was performed using Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison test. $P < 0.05$ or 0.01 were considered as indicative of significance.

RESULTS AND DISCUSSION

We evaluated the anti-inflammatory activity of chloroform, methanol and aqueous extracts of *Salvia connivens* at dose of 400 mg/kg in a carrageenan-induced edema test. The results of evaluation of the three extracts (**Fig. 1**) showed that the aqueous extract had no effect on this model, methanol extract had effect after one h of carrageenan administration (64.3 ± 18.0), and this effect decreased after 2 h, while chloroform showed a good anti-inflammatory activity with respect to the positive control.

It was evident that the chloroform extract at doses of 100-400 mg/kg (**Table 1**) produced a significant effect against carrageenan-induced inflammation 2 h after administration. At dose of 100 mg/kg the inhibition was 54.8 ± 3.3% which decreased and after 5 h there is not effect, however the dose of 200 mg/kg exhibited significant inhibition (69.8 ± 7.8%) after 2 h and an increased effect at 5 h (87.4 ± 6.6%) was observed. At dose of 400 mg/kg the effect of chloroform extract was similar to that obtained with the dose of 200 mg/kg, but after 5 h the activity significantly decreased (45 ± 6.8%). Anti-inflammatory activity of the chloroform extract was significantly higher than that of indomethacin (72.3 ± 3.5% after 5 h).

Carrageenan-induced edema has been commonly used as an experimental model of acute inflammation and it believed to be biphasic. The early phase (1-2 h) in this model is mediated by histamine, serotonin and increased synthesis of prostaglandins, followed by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins in the later phase (Brito *et al*. 1998). The extract showed effect against this model, suggesting that its anti-inflammatory activity is possibly backed by its anti-serotonin activity (Perazzo *et al.* 2005)

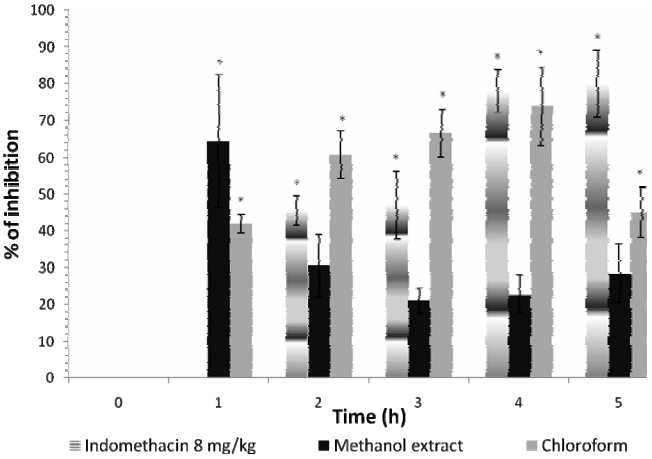

Fig. 1 Comparison between percentage of paw edema inhibition at 400 mg/kg with different extracts of *S. connivens*. Results are expressed as percentage of inhibition and are the mean of six determinations ± SE. ANOVA one way test, $*p < 0.05$ for comparison of extract and indomethacin-treated groups with negative control. Aqueous extract had no effect.

Table 1 Anti-inflammatory effect of *S. connivens* chloroform extract on carrageenan-induced edema test.

Time (h)	(% inhibition)			
	Indomethacin (mg/kg)	Chloroform extract of *S. connivens* (mg/kg)		
	8	100	200	400
1	33.3 ± 3.5	30.0 ± 13.0	49.3* ± 6.3	41.9* ± 2.5
2	58.5 ± 8.9	54.8 ± 3.3	69.8* ± 7.8	60.7* ± 6.5
3	67.7 ± 6.0	44.2 ± 4.5	62.6* ± 8.1	66.6* ± 6.4
4	71.1 ± 2.1	32.9 ± 6.3	85.0* ± 11.9	73.8* ± 10.6
5	73.2 ± 3.5	N.I.	87.4* ± 6.6	45.0* ± 6.8

Results are expressed as percentage of inhibition and mean of eight determinations ± SE. ANOVA one way, $*p < 0.05$ for comparison of extract and indomethacin treated groups with negative control according to Student's *t*-test. N.I. no inhibition

TPA significantly increased the weight of the right ear to a mean value of 11.8 ± 0.5 mg (n = 8) in the control group. **Table 2** shows that the chloroform extract of *S. connivens* at a dose of 2 mg/ear inhibited TPA-induced ear mouse inflammation by 50.7 ± 2.1%. The effect of this extract was similar to that of indomethacin (66.9 ± 6.3).

Acute inflammation caused by topical application of TPA seems to depend primarily on leukotrienes, which are synthesized by lipoxygenase enzymes (Lloret *et al*. 1995), and is accompanied with free radical generation, enhancement of prostanoid synthesis, and an increase in vascular permeability (Murakami *et al*. 2000). Chloroform extract was also active in the TPA model, and its inhibitory potency was similar than that of a synthetic cyclooxygenase inhibitor, indomethacin, suggesting it contains active inhibitors of leukotriene and PGE_2 synthesis (Berger *et al*. 2002).

Values of the inhibitory effect produced by the chloroform extract on granuloma induced by pellet implantation are shown in **Table 3**. The results indicate that at a dose of 200 mg/kg, the extract reduced the weight of pellet-induced granuloma by 39.34 ± 5.36%. The effect of this extract was similar to that of naproxen (40.81 ± 4.78%) at a dose of 50 mg/kg.

It is known that inflammatory granuloma is a typical

Table 2 Effects of the topical application of indomethacin and *S. connivens* chloroform extract on TPA-induced ear model in mice.

Group	Edema (mg)	% Edema inhibition
Control	11.8 ± 0.5	0
S. connivens 2 µg/ear	5.8 ± 0.4	50.7 ± 2.1*

Results are means ± S.E.M.
* extract and the reference p< 0.01 vs control according to Student's *t*-test.

Table 3 Effect of the chloroform extract of *S. connivens* (consecutive for 5 days) on the weight of granuloma in rats.

Treatment (dose)	Cotton pellet (mg)	Inhibition (%)
Vehicle	18.42 ± 3.32	0
Naproxen 50 mg/kg	10.90 ± 0.95	40.81 ± 4.78
S. connivens 200 mg/kg	12.50 ± 0.63	39.34 ± 5.36

Results are expressed as percentage of inhibition and mean of eight determinations ± SE. Student's *t*-test *$p < 0.05$ for comparison of extract and naproxen treated groups with negative control.

reaction in the chronic inflammatory process, and it has been established that the dry weight of implanted pellets correlates with granulomatous tissue (Olajide *et al*. 2000). The chloroform extract of *S. connivens* showed a significant anti-inflammatory effect in this test. This fact is related to the capacity of the extract to inhibit the proliferative events of granulation tissue formation (Ratnasooriya *et al.* 2005).

The present study demonstrated the anti-inflammatory activity of chloroform extract of the leaves of *S. connivens* in the three models used.

Several species of the genus *Salvia* possess anti-inflammatory properties, for example the chloroform extract of *Salvia hierosolymitana*, showed an inhibition of 70% of the croton oil-induced ear edema in mice at dose of 300 µg/ear and its effects is due to the presence of triterpenoids (De Felice *et al.* 2006). Chloroform extract of *Salvia trilova* at dose of 25 mg/kg exhibited the highest antiinflamatory activity in both acute and chronic models, probably due to tre presence of triterpenoids (El-Sayed *et al.* 2006).

Based on the results obtained in this study, we can suggest that the chloroform extract of *S. connivens* leaves possess an anti-inflammatory property, and is possible to suggest that this activity might be due to the presence of triterpenoids as in other *Salvia* spp. These results also support the claims by traditional medicine practitioners regarding the usefulness of *S. connivens* in inflammatory diseases. More detailed phytochemical studies are necessary to identify the active compound(s) and exact mechanism of action.

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