

Analgesic and Anti-Inflammatory Activities of *Teucrium stocksianum*

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Abstract

Plants belonging to *Teucrium* sp. (Lamiaceae) are traditionally used to treat painful and inflammatory conditions. In the present study, *Teucrium stocksianum*, which is found in Arabia, was evaluated for its analgesic and anti-inflammatory properties. Analgesic activity of 10% ethanol extract was evaluated using hot plate method, tail-flick method and acetic acid writhing method. Anti-inflammatory activity was studied using carrageenan induced rat paw edema, cotton-pellet method, and by topical application of the extract on edema. *T. stocksianum* showed significant ($p < 0.05$) analgesic and anti-inflammatory activities in all the models studied. Topical application of the extract was also shown to be anti-inflammatory. Results support the traditional use of the plant in the treatment of painful, inflammatory conditions.

Keywords: *Teucrium stocksianum*, analgesic, anti-inflammatory, topical.

Introduction

Certain species of *Teucrium* (Lamiaceae) are used traditionally to treat inflammation, rheumatism, diabetes and ulcers. The anti-inflammatory properties of *T. polium* and *T. buxifolium* have already been reported (Tariq et al., 1989; Fernandez et al., 1997). The aim of the present investigation is to study the possible analgesic and anti-inflammatory activity of another species of *Teucrium*, viz. *Teucrium stocksianum*, a perennial herb distributed throughout Arabia, which has not been scientifically evaluated for these activities.

Materials & methods

Animals

Sprague-Dawley rats (either sex) weighing 200–250 g and Albino mice weighing 30–40 g purchased from the Harlan, UK and bred in the Animal Unit of our Institute were used for the experiments. All animals had access to standard rat chow and water *ad libitum* and were kept at a light-dark cycle of 12 h each.

Preparation of the extract

The plant was authenticated and collected from U.A.E. by the taxonomists of our Institute. A specimen of the plant is kept in the herbarium. The aerial parts of the plant were dried under shade and powdered using a comminuting mill. The powder was exhaustively extracted in a Soxhlet apparatus with 10% ethanol. The extract was evaporated under vacuum using a Buchi Rotary Evaporator until dried. The dried extract was dispersed in distilled water for administration in the animals.

Carrageenan-induced paw edema

Inflammation was induced in the hind paw of the rats by injecting 0.05 ml of 1% suspension of carrageenan in normal saline subcutaneously in the subplantar region of the right hind paw according to the method of Winter et al. (1962). Two acute doses (400 and 800 mg/kg) of the *Teucrium* extract were administered orally in two different groups of rats 1 h

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before carrageenan injection. Another group received 400 mg/kg of the extract intraperitoneally (i.p.), 30 min before carrageenan injection. The control groups received only the vehicle either orally or intraperitoneally. The hind paw volume was measured plethysmometrically in all groups before and after the carrageenan challenge (Burch et al., 1990) at 1 h intervals for 6 h and then at 24 h.

Cotton pellet-induced granuloma formation

Pellets of cotton weighing 50 ± 1 mg were sterilized in an a hot air oven at 120°C for 2 h and then implanted bilaterally in groin regions of the rat under light ether anesthesia according to the method of Goldstein et al. (1976). The two treated groups of animals were given the extract at a dose of 100 mg/kg either orally or intraperitoneally, throughout the experiment period of 7 days, whereas the control group received only the vehicle. On the last day of the treatment, the pellets were dissected out under light anaesthesia, dried overnight at 70°C and weighed after cooling.

Hot plate test

The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at $55 \pm 0.5^\circ\text{C}$. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test group received 400 mg/kg of the extract intraperitoneally, and reaction times were determined before and at 1, 2, 4 and 6 h after the treatment. The control group received only vehicle and reaction times were determined as in the treated group.

The tail-flick test

The method described by D'Amour and Smith (1941) and modified by Gray et al. (1970) was followed. The rat's tail was placed at the window of the tail flick apparatus (UGO Basile, Italy) where radiant heat from an infrared source is emitted. A built-in timer on the apparatus was started simultaneously when the lamp was turned on. When the rat felt the pain and flicked the tail, the heat source and the timer were automatically stopped and the reaction time was displayed. The intensity of the radiation was adjusted to give a reaction time of 5–10 sec. Test animals were given the extract (400 mg/kg, i.p.) and the control animals were given the vehicle. Reaction times were determined before and at 1, 2, 4 and 6 h in both treated and control groups.

Acetic acid writhing test

Method described by Nakamura et al. (1986) was followed. Mice were injected intraperitoneally with 0.6% v/v of acetic acid and time of onset of writhing was recorded. The number of writhings in the first 25 min of acetic acid injection in time

intervals of 5 min were also recorded. Animals were either treated with the extract (400 mg/kg, p.o.) or with the vehicle. The writhing was induced after 1 h of drug administration and number of writhings in both treated and control groups were recorded.

Topical anti-inflammatory test

Inflammation was induced in the hind paw of the rats by injecting 0.05 ml of 1% suspension of carrageenan in normal saline subcutaneously in the subplantar region of the right hind paw according to the method of Winter et al. The hind paw volume was measured plethysmometrically (Burch et al., 1990) in all groups, 30 min after the carrageenan challenge. The animals were restrained in such a way that their hind limbs were outside the restrainer. *Teucrium* extract was formulated as a topical application in two different concentrations, viz. 1% w/w and 5% w/w, in carboxymethylcellulose (CMC) gel base. The *Teucrium* gels (1 and 5%) were applied to the inflamed paws of the rats in two separate groups and the gel base was applied to the inflamed paws of the control animals. The paw volumes in all groups were measured at 3 and 6 h after the application of the gel.

Chemicals

Carrageenan and diclofenac were obtained from Sigma Chemical Co, St. Louis, U.S.A.

Statistical analysis

The results are expressed as mean \pm S.E.M. Student's *t*-test for samples was used to determine the statistical significance at $p < 0.05$.

Results

Acute intraperitoneal administration of *Teucrium* extract in rats at the dose of 400 mg/kg significantly reduced the increase in hind paw volume induced by carrageenan, compared to the vehicle treated control group (Table 1).

Subacute treatment using the cotton pellet method showed anti-inflammatory activity in the intraperitoneal group, but not in the orally treated group (control = 122.5 ± 17.3 ; *Teucrium*, 100 mg/kg, i.p. = $76.03 \pm 6.63^*$; *Teucrium*, 100 mg/kg, p.o. = 123.1 ± 18.4 ; diclofenac, 4 mg/kg, i.p. = $42.95 \pm 4.68^*$, values representing the percentage increase in the weight of cotton pellets from their initial weight; * statistically significant at $p < 0.05$).

The hot plate reaction time was increased by *Teucrium* extract at 1st, 2nd, 4th and 6th h after the administration of the extract, but the increases were statistically significant only at 2nd and 6th h only (Table 2).

Tail flick reaction time was significantly increased by *Teucrium* extract at the dose of 400 mg/kg, i.p. and p.o. In the intraperitoneal administered group, the effect was

Table 1. Percentage increase in hind paw volume in the control, *Teucrium* extract and diclofenac treated animals at different time intervals after induction of edema using carrageenan.

Time (h)	0	1	2	3	4	5	6	24
Control	0	69 ± 5.6	109 ± 6.7	116 ± 5.1	109 ± 6.0	116 ± 5.8	114 ± 6.1	80 ± 7.2
Ext. 400 mg/kg., i.p.	0	18 ± 3.6*	20 ± 4.5*	20 ± 2.9*	28 ± 4.7*	32 ± 3.9*	34 ± 5.1*	35 ± 2.9*
Ext. 400 mg/kg. p.o.	0	9 ± 3.4*	62 ± 11.3*	63 ± 12.1*	67 ± 12.3*	71 ± 11.2*	67 ± 11.6*	35 ± 6.3*
Diclofenac 4 mg/kg., i.p.	0	10.5 ± 2.3	32 ± 4.5*	–	63 ± 7.5*	–	73.6 ± 8.2*	56.6 ± 7*

* Significantly different from control, $p < 0.05$, Student's *t*-test.

Table 2. Effect of *Teucrium* extract (400 mg/kg., i.p.) on the hot plate reaction time in mice, expressed as a percentage of the initial response.

	Time (h)				
	0	1	2	4	6
Control	100	117.5 ± 12.9	102.9 ± 6.7	116.8 ± 17.5	119.6 ± 8.7
<i>Teucrium</i>	100	134.4 ± 12.7	163.9 ± 14.7*	155 ± 15.8	164.1 ± 11.4*

* Significantly different from control, $p < 0.05$, Student's *t*-test.

Table 3. Effect of *Teucrium* extract (400 mg/kg., i.p.) on the tail-flick reaction time in mice, expressed as a percentage of the initial response.

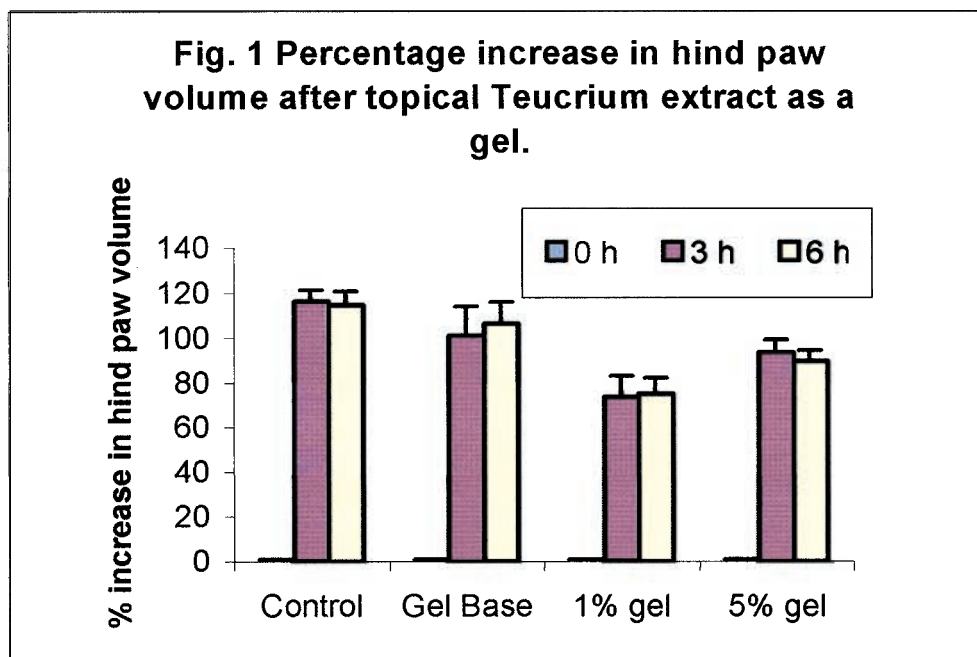
	Time (h)				
	0	1	2	4	6
Control	100	104 ± 9.2	110.2 ± 16.4	112.8 ± 10.8	107.7 ± 12.0
<i>Teucrium</i>	100	180.6 ± 19.9*	187.4 ± 22.9*	193.4 ± 21.2*	200.4 ± 25.8*

* Significantly different from control, $p < 0.05$, Student's *t*-test.

Table 4. Effects of different doses of *Teucrium* extract on the number of acetic acid induced writhing in mice.

Time interval (min)	<i>Teucrium</i>		
	Control	200 mg/kg., p.o.	400 mg/kg., p.o.
0–5	6.8 ± 1.20	3.3 ± 0.29*	2.67 ± 1.01*
5–10	19.4 ± 1.18	12.1 ± 1.58*	10.7 ± 1.66*
10–15	17.2 ± 1.27	6.5 ± 0.96*	9.58 ± 1.50*
15–20	12.2 ± 1.02	4.5 ± 0.99*	5.42 ± 0.98*
20–25	8.10 ± 1.09	3.17 ± 0.77*	3.08 ± 0.70*
Total	63.7 ± 4.09	29.6 ± 4.41*	31.4 ± 4.86*

* Significantly different from control, $p < 0.05$, Student's *t*-test.



significant at 1st, 2nd, 4th and 6th h after treatment, whereas in the orally treated group, the effect was significant only at 2nd h (Table 3).

The number of writhings induced by acetic acid were significantly reduced in the animals treated with the extract at both the doses studied compared with the vehicle treated control group (Table 4).

The carrageenan induced increase in the paw volume was prevented by 1 and 5% *Teucrium* gels. The gel base did not inhibit the carrageenan induced inflammation. One percent gel showed better anti-inflammatory activity compared to the 5% gel (Fig. 1).

Discussion

The results from the rat paw edema experiment and the cotton – pellet method indicate significant anti-inflammatory activity for *T. stocksianum* extract in the doses studied. *Teucrium* extract did not show any anti-inflammatory effect when given orally in the sub-acute study using cotton – pellet method but was effective orally in acute study using rat paw edema. This could be due to the low dose employed in the sub-acute study (100 mg/kg., p.o.) compared to the higher dose (400 mg/kg., p.o. and i.p) used in the acute study.

The finding that *Teucrium* extract can elicit anti-inflammatory activity when applied topically indicates that the anti-inflammatory constituent(s) present in *Teucrium* can be absorbed percutaneously to bring about the anti-inflammatory effect.

In all the studies carried out to assess the analgesic activity of *Teucrium* using different methods, it was shown that

Teucrium extract possess analgesic activity. The hot plate method and acetic acid induced writhing method are useful in detecting centrally acting analgesics whereas the tail flick method is useful to detect peripheral analgesic effects. The fact that *Teucrium* extract showed analgesic activity in all the models studied, indicate that the analgesic effect of *Teucrium* could possess two components, viz., central and peripheral (Panthong et al., 1998).

The results of this study support the traditional use of *Teucrium* spp. in painful and inflammatory conditions like rheumatism and other similar diseases.

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