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SHORT COMMUNICATION

Antinociceptive, Antiinflammatory and Acute toxicity Effects of *Salvia leriifolia* Benth. Seed Extract in Mice and Rats

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The antinociceptive and antiinflammatory effects as well as the acute toxicity of *Salvia leriifolia* aqueous seed extract were studied in mice and rats. Antinociceptive activity was assessed using the hot-plate and tail flick tests. The effect on acute inflammation was studied using vascular permeability increased by acetic acid and xylene-induced ear oedema in mice. The activity against chronic inflammation was assessed using the cotton pellet test in rats. The LD_{50} of the extract was found to be 19.5 g/kg (i.p.) in mice. The aqueous seed extract showed significant and dose-dependent (1.25–10 g/kg) antinociceptive activity over 7 h, and was inhibited by naloxone pretreatment. Significant and dose-dependent (2.5–10 g/kg) activity was observed against acute inflammation induced by acetic acid and in the xylene ear oedema test. In the chronic inflammation test the extract (2.5–5 g/kg) showed significant and dose-dependent antiinflammatory activity. The aqueous seed extract of *S. leriifolia* may therefore have supraspinal antinociceptive effects which may be mediated by opioid receptors, and showed considerable effects against acute and chronic inflammation.

Keywords: Salvia leriifolia; antinociceptive activity; antiinflammatory activity; herbal medicine.

INTRODUCTION

The genus *Salvia*, Labiatae, is generally known for its multiple pharmacological effects including analgesic and antiinflammatory activities (Hernandez-Perez *et al.*, 1995; Hosseinzadeh and Yavari, 1999), antipyretic (Maklad *et al.*, 1999), antioxidant (Cuppett and Hall, 1998; Wang *et al.*, 1996), hepatoprotective (Wasser *et al.*, 1998) and hypoglycaemic actions (Hosseinzadeh *et al.*, 1998), antiepileptic, antiulcerogenic, as well as tranquillizing activities, besides increasing the bleeding time (Maklad *et al.*, 1999).

S. leriifolia has an effect on morphine dependence (Hosseinzadeh and Lari, 2000) and hypoglycaemic effects (Hosseinzadeh *et al.*, 1998). Antiinflammatory activities have also been reported for this species (Hosseinzadeh and Yavari, 1999). In this study, the antinociceptive and antiinflammatory activities as well as the acute toxicity of *S. leriifolia* seed were evaluated.

MATERIALS AND METHODS

Animals. Male and female albino mice 25–30 g and Wistar rats weighing 150–210 g were used. Animals had free access to water and food.

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Plant material. Seeds were collected in Khorassan province and shade dried then ground. Voucher samples were preserved for reference in the herbarium of Department of Pharmacognosy, School of Pharmacy, Mashhad (Haddad, 153-1912-1).

The preparation of extracts. 100 g dried and ground seeds was extracted in hot water for 15 min and filtered. The extract was then concentrated under reduced pressure (yield: 16.6%).

Antinociceptive study

Hot-plate test. The hot-plate test was assessed on male mice. The temperature of the metal surface was maintained at 55° ± 0.2 °C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 40 s.

Tail-flick test. The tail-flick test was assessed on male mice. The base line and cut-off time were 2–3 s and 10 s, respectively.

Antiinflammatory study

Xylene-induced ear edema. Mice were divided into groups of ten. Sixty minutes after i.p. injection of the extract or diclofenac, 0.03 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as a control. Two hours after xylene application, mice were killed and both ears

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were removed. Circular sections were taken using a cork borer with a diameter of 7 mm, and weighed. The increase in weight caused by the irritant was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections.

Vascular permeability increased by acetic acid in mice. Mice were divided into groups of seven. Thirty minutes after i.m. injection of the extract or diclofenac, mice received an i.v. injection of 0.5% Evan's blue solution (5 mL/kg). Five min later, each mouse was given an i.p. injection of 0.7% acetic acid solution 10 mL/kg. Thirty minutes after i.p. administration of acetic acid, the mice were killed. The concentration of Evan's blue in the fluid of the peritoneal cavity was measured by the absorbance at 610 nm.

Cotton pellet granuloma in rats. Pellets of dentistry cotton weighing 30 mg were sterilized in an air oven at 121 °C for 20 min and impregnated with 0.4 mL of an aqueous solution of ampicillin. Under thiopental (10 mg/kg) anaesthesia, two cotton pellets were implanted subcutaneously in the groin region of rats, one on each side. The extract or diclofenac were given once daily for 7 days. On day 8, the rats were killed and the pellets and surrounding granulation tissues were dried at 60 °C for 24 h. The weight of granuloma was determined.

Acute toxicity. Different doses of extracts were injected intraperitoneally to separate groups of six mice. The number of deaths was counted at 48 h after treatment. LD_{50} values were calculated by logit method.

Statistical analysis. The data were expressed as mean ± SEM and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer.

RESULTS

The LD_{50} of the intraperitoneal injection of the aqueous seed extract was 19.5 g/kg and the maximum non-fatal dose was 10 g/kg.

In the hot-plate test, the intraperitoneal injection of the aqueous seed extract showed a significant and dose-dependent (1.25–10 g/kg) antinociceptive activity with a duration of action of 420 min (Fig. 1). Pretreatment with naloxone (0.5 mg/kg, i.v. or 1 mg/kg, s.c.) inhibited the antinociceptive activity of the extract (5 g/kg, i.p.) and morphine (10 mg/kg, i.p.). Thirty minutes after injection, the time latency of the antinociceptive effect of the extract was similar to morphine (Fig. 2). While the aqueous seed extract (1–5 mg/kg) had no antinociceptive activity in the tail-flick test, morphine (10 mg/kg, i.p.) showed a significant antinociceptive effect (data not shown).

In the xylene-induced ear oedema test, the extract (2.5–10 mg/kg, i.p.) showed significant antiinflammatory activity (Table 1).

The aqueous seed extract showed significant and dose-dependent (2.5–10 mg/kg, i.p.) activity against acute inflammation induced by acetic acid. In this experiment, a dose of 5 g/kg of the extract showed antiinflammatory effect similar to diclofenac (10 mg/kg) (Table 2).

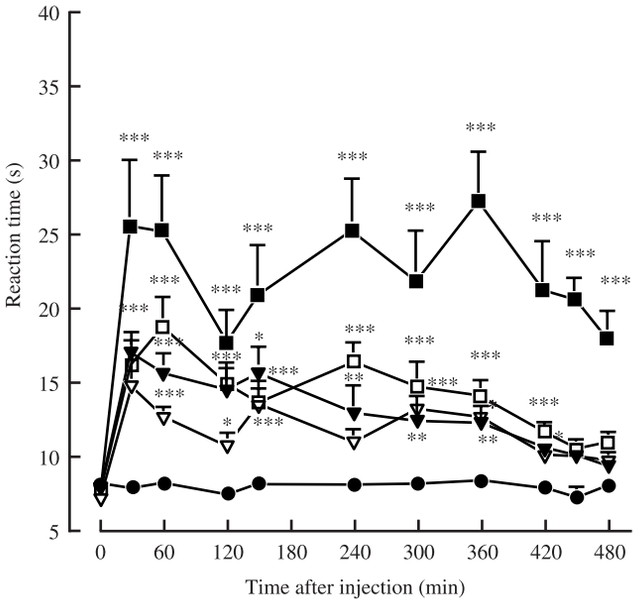

Figure 1. Effect of intraperitoneal doses of *Salvia leriifolia* aqueous seed extracts on the pain threshold of mice in the hot-plate test. Each point represents the mean ± SEM of reaction time for $n = 10$ experiments on mice. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with saline, Tukey-Kramer test. ● saline; ▽ extract 1.25 g/kg; ▼ extract 2.5 g/kg; □ extract 5 g/kg; ■ extract 10 g/kg.

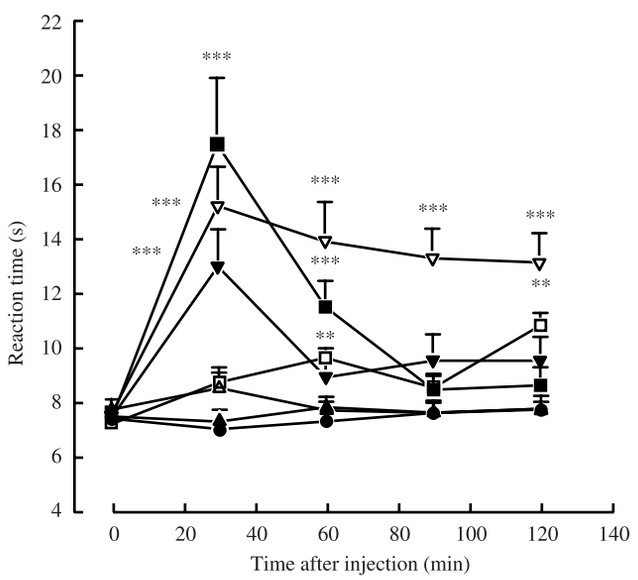

Figure 2. Effect of naloxone on *Salvia leriifolia* aqueous seed extracts and morphine antinociceptive activity in mice (hot-plate test). Each point represents the mean ± SEM of reaction time for $n = 8$ experiments on mice. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with saline, Tukey-Kramer test. ● saline i.p.; ▽ extract 5 g/kg i.p.; ▼ extract 5 g/kg i.p. + naloxone 1 mg/kg s.c.; □ extract 5 g/kg i.p. + naloxone 1 mg/kg i.v.; ■ morphine 10 mg/kg i.p.; △ morphine 10 mg/kg i.p. + naloxone 1 mg/kg s.c.; ▲ naloxone 1 mg/kg s.c.

In the chronic inflammation (cotton-plate), the extract (2.5–5 g/kg) showed significant and dose-dependent antiinflammatory activity. A dose of 5 g/kg showed a higher effect than diclofenac 5 mg/kg (Table 3).

Table 1. Effect of the intraperitoneal doses of *Salvia leriifolia* aqueous seed extracts on xylene-induced ear swelling in mice

Treatment	Dose	Ear swelling (mg)	Inhibition (%)
Control	10 mL/kg	9.21 ± 0.5	–
Diclofenac	10 mg/kg	2.56 ± 0.3[a]	72.2
Aqueous extract	2.5 g/kg	7.77 ± 0.59	15.6
Aqueous extract	5 g/kg	7.79 ± 0.4	15.4
Aqueous extract	10 g/kg	4.74 ± 0.5[a]	48.5

Values are mean ± SEM, $n = 10$, [a]$p < 0.001$ compared with control, Tukey-Kramer test.

Table 2. Effect of the intramuscular doses of *Salvia leriifolia* aqueous seed extracts on vascular permeability increase induced by intraperitoneal 0.7% acetic acid in mice

Treatment	Dose	Evans blue (μg/mL)	Inhibition (%)
Control	10 mL/kg	2.85 ± 0.3	–
Diclofenac	10 mg/kg	0.954 ± 0.2[b]	63.27
Aqueous extract	2.5 g/kg	1.54 ± 0.2[a]	45.8
Aqueous extract	5 g/kg	1.02 ± 0.2[b]	64.8
Aqueous extract	10 g/kg	1.13 ± 0.2[b]	60.345

Values are mean ± SEM, $n = 7$, [a]$p < 0.01$ and [b]$p < 0.001$ compared with control, Tukey-Kramer test.

Table 3. Effect of the intraperitoneal doses of *Salvia leriifolia* aqueous seed extracts (consecutive for 7 days) on the weight of granuloma in rats

Treatment	Dose	Cotton pellet (mg)	Inhibition (%)
Control	10 mL/kg	82.3 ± 1.8	–
Diclofenac	5 mg/kg	63.06 ± 3.1[a]	23.4
Aqueous extract	1.25 g/kg	67.65 ± 2.1[a]	17.8
Aqueous extract	2.5 g/kg	56.38 ± 1.9[a]	31.5
Aqueous extract	5 g/kg	50.37 ± 1.9[a]	38.8

Values are mean ± SEM, $n = 6$, [a]$p < 0.001$ compared with control, Tukey-Kramer test.

DISCUSSION

The present results indicate that the aqueous extract of *S. leriifolia* seed has central antinociceptive activity, because it showed a significant antinociceptive effect in the hot-plate test and also its effect was inhibited by naloxone, a specific antagonist of opioid receptors. The extract also showed activities against acute and chronic inflammation.

With respect to the LD_{50} value and in comparison with a toxicity classification (Loomis, 1968), the extract was of low toxicity.

The inhibitory effect of naloxone on the antinociceptive activity of extract suggests a morphine-like activity profile for *S. leriifolia*. In the tail-flick test, the extract had no antinociceptive effect. As the tail-flick test has a spinal mechanism (Mohrland, 1982), the antinociceptive effect of extract is not mediated by a spinal mechanism.

In vascular permeability and xylene-induced ear oedema tests, mediators of inflammation are released following stimulation. This leads to dilation of arterioles and venules and to increased vascular permeability (Vogel and Vogel, 1997). The extracts had significant antiinflammatory effects in these tests, thus it may have a membrane-stabilizing effect that reduces capillary permeability and/or has inhibitory effects on mediators.

The repair phase of the inflammatory process begins with proliferation of fibroblasts as well as multiplication of small blood vessels. Such proliferating cells penetrate the exudate producing a highly vascularized reddened mass known as granulation tissue (Swingle, 1974). The extract effectively and significantly reduced cotton pellet-induced granuloma, thereby suggesting its activity in the proliferative phase of the inflammation.

Antinociceptive and/or antiinflammatory activities have been reported for some *Salvia* genera such as *S. hemaematodes* (Akbar *et al.*, 1984), *S. aethiopis* (Hernandez-Perez *et al.*, 1995) and other genera (Zargari, 1990). This study and other research on *S. leriifolia* leaf (Hosseinzadeh and Yavari, 1999) also confirm that *Salvia* genera are good candidates for antiinflammatory and analgesic uses.

It is concluded that the aqueous seed extract of *S. leriifolia* has a central (no spinal) antinociceptive effect and this may be mediated by opioid receptors. The aqueous seed extract showed also considerable activity against acute and chronic inflammation.

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