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Antinociceptive, anti-inflammatory and acute toxicity effects of *Zataria multiflora* Boiss extracts in mice and rats

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Abstract

Antinociceptive, anti-inflammatory and acute toxicity of the aqueous infusion and ethanolic maceration extracts of the aerial parts of *Zataria multiflora* Boiss were studied in mice and rats. Antinociceptive activity was done using hot-plate and writhing tests. The effect of the extracts against acute inflammation was studied by vascular permeability increased by acetic acid and xylene induced ear edema in mice. The activity of the extracts against chronic inflammation was assessed by the cotton pellet test in rats. LD50 of the infusion and maceration extract was 3.85 and 3.47 g/kg, respectively. Intraperitoneal injection of both extracts in mice showed significant and dose-dependent antinociceptive activity. Naloxone, an opioid antagonist, pretreatment inhibited the antinociceptive activity of the extracts. The extracts exhibited antinociceptive activity against acetic acid induced writhing which was partially blocked by naloxone. Both extracts did not show remarkable activity against acute inflammation induced by acetic acid in mice. In the xylene ear edema, *Z. multiflora* Boiss extracts showed significant activity in mice. In the chronic inflammation the efficacy of the extracts was more than in acute inflammation. It is concluded that aqueous infusion and maceration of the aerial parts of *Z. multiflora* Boiss have antinociceptive effect and this may be mediated by opioid receptors. The extracts showed also anti-inflammatory effect against acute and chronic inflammation.

Keywords: *Zataria multiflora* Boiss; Antinociceptive activity; Anti-inflammatory activity; Herbal medicine

1. Introduction

Labiatae are generally known for their multiple pharmacological effects such as analgesic and anti-inflammatory activity (Hernandez-Perez et al., 1995), antioxidant (Cuppett and Hall, 1998), hepatoprotective (Wasser et al., 1998) and hypoglycemic action (Hosseinzadeh et al., 1998). *Zataria multiflora* Boiss is a plant belonging to the labiatae family that geographically grows only in Iran, Pakistan and Afghanistan. This plant with the vernacular name of Avishan Shirazi (in Iran) has several traditional uses such as antiseptic, anaesthetic and antispasmodic (Zargari, 1990). This study has initiated since to best of our knowledge there has been no report on antinoci-

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ceptive and anti-inflammatory activity effect of *Z. multiflora* Boiss.

2. Materials and methods

2.1. Animals

Male and female albino mice weighing 25–30 g and Wistar rats weighing 150–210 g were obtained from a random bred colony maintained on a special diet (Khorassan Javane, Mashhad, Iran) in the animal house of Mashhad University of Medical Sciences. The animals were housed in a colony room with a 12/12 h light/dark cycle at 21 ± 2°C.

2.2. Plant material

The plant was collected 120 km north of Bandarabas, Iran, and dried in shadow followed by grinding. Ferdowsi University properly identified the *Z. multiflora* Boiss and voucher samples were preserved for reference in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad (153-2613-2).

2.3. Phytochemical procedures

2.3.1. The preparation of extracts

Powder of the plant was extracted using aqueous infusion and maceration with alcohol. In the infusion method 100 g of the plants was added to 1 l hot water for 15 min and then filtered with cloths. The extract was then concentrated under reduced pressure to the desired volume. In the maceration method the powdered plants (200 g) were macerated in 500 ml ethanol (85%, v/v) for 3 days and, subsequently, the solution was filtered and concentrated in a rotaevaporator at 50°C. The ethanolic extract was emulsified by Tween-80 in saline.

2.3.2. Preliminary chemical tests

Phytochemical screening of the extract was performed using the following reagents and chemicals (Trease and Evans, 1983).

Alkaloids with Dragendorff's reagent, flavonoids by the use of Mg and HCl; tannins with 1% gelatin and 10% NaCl solutions and saponins with ability to produce suds.

2.4. Acute toxicity

Different doses of extracts were injected intraperitoneally into groups of six mice. The number of deaths was counted at 48 h after treatment. LD50 values were calculated by the logit method.

2.5. Antinociceptive study

2.5.1. 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.

2.5.2. Writhing test

One hour after the administration of the extract, the mice were given an intraperitoneal injection of 0.7% (v/v) acetic acid solution (volume of injection 0.1 ml/10 g). The number of writhes produced in these animals was counted for 30 min.

2.6. Anti-inflammatory study

2.6.1. Xylene-induced ear edema

The mice were divided into groups of seven. Thirty minutes after i.p. injection of the extract, diclofenac and dexamethasone, 0.03 ml of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application the mice were sacrificed and both ears 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.

2.6.2. Vascular permeability increased by acetic acid in mice

The mice were divided into groups of seven. Thirty minutes after i.m. injection of the extract and diclofenac, the mice received an i.v. injection of 0.5% Evan's blue solution (5 ml/kg). Five minutes 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 absorbance at 610 nm.

2.6.3. 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) anesthesia, two cotton pellets were implanted subcutaneously in the groin region of the rats, one on each side. The extract and 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.

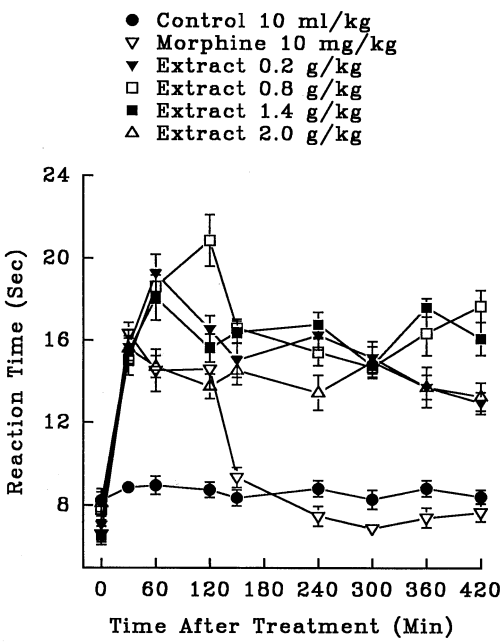

Fig. 1. Effect of the aqueous extract of *Z. multiflora* Boiss and morphine on the pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of reaction time for $n = 10$ experiments on mice. After 30 min all doses of the extract compared to control were significant ($P < 0.001$, Tukey–Kramer test).

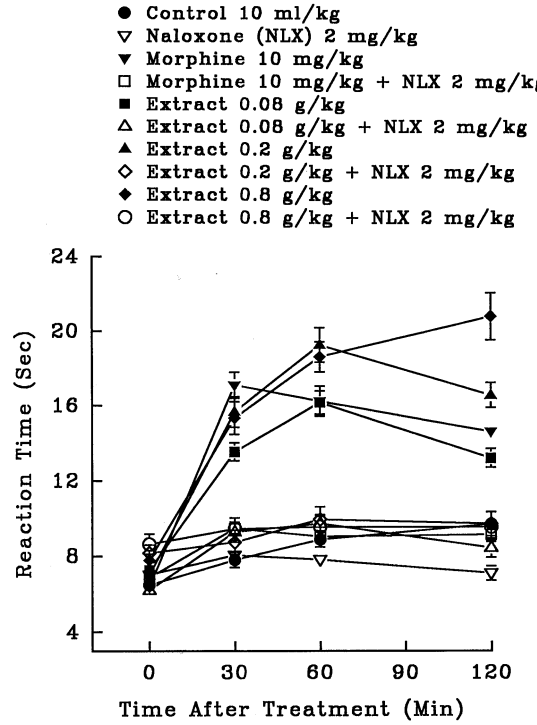

Fig. 2. Effect of naloxone on the aqueous extract of *Z. multiflora* Boiss and morphine antinociceptive activity in mice (hot-plate test). Each point represents the mean ± S.E.M. of the reaction time for $n = 10$ experiments on mice. Naloxone completely inhibited the effect of the extracts and morphine.

2.7. Statistical analysis

The data were expressed as mean values ± S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey–Kramer.

3. Results

Preliminary phytochemical tests indicated that ethanol extract of *Z. multiflora* contains alkaloids, flavonoids and tannins.

LD50 of the infusion and maceration extracts was 3.85 and 3.47 g/kg, respectively, and the maximum non fatal doses were 2.2 and 2 g/kg, respectively.

In the hot plate test administration of the aqueous and ethanolic extracts showed antinociceptive activity that was dose-dependent with at least 420 min duration of action. The time latency of the high doses of the extracts was more than morphine (Figs. 1 and 3). The peak effect of the lowest and the highest doses of the ethanolic extracts were reached after 60 and 150 min, respectively. Naloxone, (2 mg/kg, s.c.) pretreatment after i.p. injection of the extracts and morphine (10 mg/kg), inhibited the antinociceptive activity of both extracts and morphine (Figs. 2 and 4).

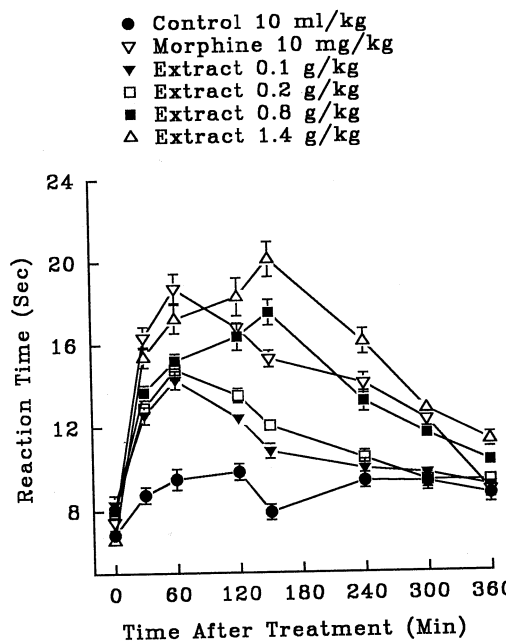

Fig. 3. Effect of the ethanolic extract of *Z. multiflora* Boiss and morphine on pain threshold of mice in the hot-plate test. Each point represents the mean ± S.E.M. of the reaction time for $n = 10$ experiments on mice. After 30 min all doses of the extract compared to control were significant ($P < 0.001$, Tukey–Kramer test).

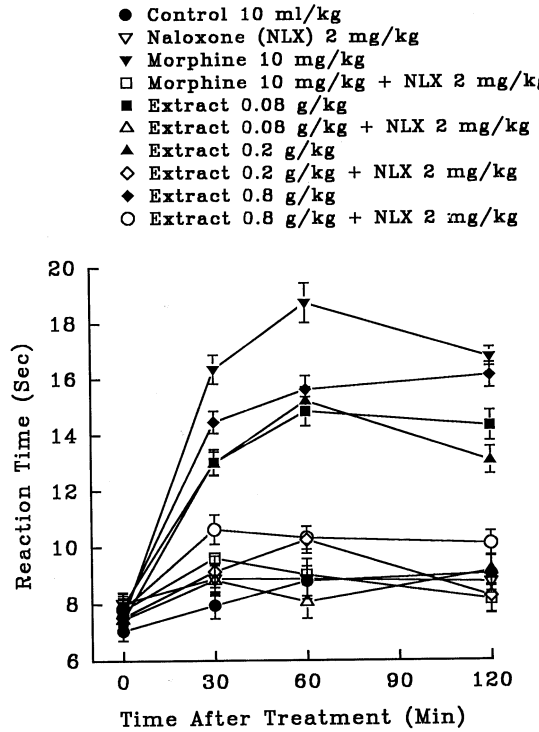

Fig. 4. Effect of naloxone on the ethanolic extract of *Z. multiflora* Boiss and morphine antinociceptive activity in mice (hot-plate test). Each point represents the mean ± S.E.M. of the reaction time for $n = 10$ experiments on mice. Naloxone completely inhibited the effect of the extracts and morphine.

The aqueous and ethanolic extracts of *Z. multiflora* showed significant antinociceptive activity in the writhing test. Naloxone, (2 mg/kg, s.c.) pretreatment after i.p. injection of the extract partially inhibited the antinociceptive activity of both extracts (Tables 1 and 2).

In the xylene induced ear edema the ethanolic extract showed more anti-inflammatory activity than the aqueous extract (Table 3).

The aqueous and ethanolic extracts showed activity against acute inflammation induced by acetic acid with higher doses (Table 4).

In chronic inflammation (cotton-plate) both extracts exhibited significant anti-inflammatory activity. (Table 5).

4. Discussion

The present results indicate that the extracts of aqueous and ethanolic of the aerial part of *Z. multiflora* Boiss have markedly central and peripheral antinociceptive activities The extracts also showed activity against acute and chronic inflammation.

Table 1
Effect of subcutaneously injection of naloxone on antinociceptive effect of the aqueous extract of *Z. multiflora* Boiss on acetic acid-induced writhing test in mice[a]

Treatment	Number of writhing
Control (10 ml/kg)	71 ± 1.7
Naloxone (NLX) (2 mg/kg)	82.0 ± 2.5***
Morphine (10 mg/kg)	5.8 ± 0.8***
Morphine (10 mg/kg)+NLX (2 mg/kg)	69.7 ± 2.19
Diclofenac (10 mg/kg)	13.9 ± 1.6***
Extract (0.04 g/kg)	20.0 ± 1.3***
Extract (0.04 g/kg)+NLX (2 mg/kg)	37.9 ± 1.2***
Extract (0.08 g/kg)	14.6 ± 0.9***
Extract (0.08 g/kg)+NLX (2 mg/kg)	30.5 ± 1.3***
Extract (0.16 g/kg)	7.7 ± 1.2***
Extract (0.16 g/kg)+NLX (2 mg/kg)	19.1 ± 1.4***

[a] The values are the mean ± S.E.M. for ten mice
*** $P<0.001$, compared to control, Tukey–Kramer.

Table 2
Effect of subcutaneously injection of naloxone on antinociceptive effect of the ethanolic extract of *Z. multiflora* Boiss on acetic acid-induced writhing test in mice[a]

Treatment	Number of writhing
Control (10 ml/kg)	66.1 ± 1.6
Naloxone (NLX) (2 mg/kg)	80.4 ± 3.1***
Morphine (10 mg/kg)	5.5 ± 1.1***
Morphine (10 mg/kg)+NLX (2 mg/kg)	62.5 ± 1.7
Diclofenac (10 mg/kg)	14.3 ± 1.7***
Extract (0.1 g/kg)	19.7 ± 1.2***
Extract (0.1 g/kg)+NLX (2 mg/kg)	39.8 ± 1.2***
Extract (0.2 g/kg)	12.3 ± 0.9***
Extract (0.2 g/kg)+NLX (2 mg/kg)	23.9 ± 1.3***
Extract (0.8 g/kg)	8.4 ± 0.9***
Extract (0.8 g/kg)+NLX (2 mg/kg)	18.3 ± 0.9***

[a] Values are the mean ± S.E.M. for ten mice.
*** $P<0.001$, compared to control, Tukey–Kramer

Table 3
Effect of the intraperitoneal doses of the aqueous and ethanolic extracts of *Z. multiflora* Boiss on xylene-induced ear swelling in mice[a]

Treatment (dose)	Ear swelling (mg)	Inhibition (%)
Control (10 ml/kg)	10.2 ± 0.8	–
Diclofenac (15 mg/kg)	6.5 ± 03*	36.27
Dexamethasone (15 mg/kg)	6.5 ± 0.4***	36.27
Aqueous extract (0.2 g/kg)	7.9 ± 0.9	22.55
Aqueous extract (0.8 g/kg)	5.5 ± 1.1***	46.08
Aqueous extract (1.4 g/kg)	6.1 ± 0.9*	40.20
Ethanolic extract (0.2 g/kg)	6.8 ± 0.4***	33.33
Ethanolic extract (0.8 g/kg)	4.2 ± 0.6***	58.82
Ethanolic extract (1.4 g/kg)	2.9 ± 0.5***	71.57

[a] Values are the mean ± S.E.M. for seven mice.
* $P<0.05$, compared to control, $n=7$, Tukey–Kramer.
*** $P<0.001$, compared to control, $n=7$, Tukey–Kramer.

In respect to LD50 values, the ethanolic extract was more toxic than the aqueous extract. Compared with a toxicity classification (Loomis, 1968), these extracts are relatively toxic.

The present results indicated that the extracts of *Z. multiflora* Boiss exhibited central and peripheral antinociceptive activities. These effects were inhibited by naloxone, an opioid antagonist. Opioid agents exert their analgesic effects via supraspinal (μ_1, κ_3, δ_1, σ_2) and spinal (μ_2, κ_1, δ_2) receptors (Reisine and Pasternack, 1996). The aqueous and ethanolic extracts showed antinociceptive activity in the hot plate test and this effect was inhibited by naloxone. The hot plate test is a specific central antinociceptive test (Parkhouse and Pleuvry, 1979). Therefore, it is possible that the extract exerted its effect through central opioid receptors or promoted release of endogenous opiopeptides.

Antinociceptive activity of opioid agonist, opioid partial agonist, non-steroidal anti-inflammatory agents can be determined by the writhing test

(Vogel and Vogel, 1997). As the antinociceptive activity of the extracts were partially inhibited by naloxone, thus the extracts likely acts on spinal opioid receptors such as μ_2, κ_1 and δ_2 receptors, although, other mechanism of action such as inhibition of cyclo-oxygenase is also possible.

In comparison to the lowest dose the peak effect of the highest dose of ethanolic extract appeared with a delay. This may relate to the high viscosity of the extract which affects the absorption of it.

In vascular permeability and xylene induced ear edema tests, mediators of inflammation are released following stimulation. This leads to the dilation of arterioles and venules and to increased vascular permeability. (Vogel and Vogel, 1997). The extracts had significant anti-inflammatory effects in these tests, thus it may have a membrane-stabilizing effect that reduces capillary permeability and/or has inhibitory effects on mediators. The effects of the extracts in the acute inflammatory test were not remarkable.

Table 4
Effect of the intramuscular doses of the aqueous and ethanolic extracts of *Z. multiflora* Boiss on vascular permeability increase induced by intraperitoneal 0.7% acetic acid in mice[a]

Treatment (dose)	Evan's blue (μg/ml)	Inhibition (%)
Control (10 ml/kg)	4.9 ± 0.5	–
Diclofenac (15 mg/kg)	1.8 ± 0.1***	63.27
Dexamethasone (15 mg/kg)	2.1 ± 0.2***	57.14
Aqueous extract (0.8 g/kg)	4.0 ± 0.3	18.36
Aqueous extract (1.4 g/kg)	3.1 ± 0.2***	36.73
Aqueous extract (2.0 g/kg)	3.8 ± 0.2	22.45
Ethanolic extract (0.8 g/kg)	4.4 ± 0.4	10.20
Ethanolic extract (1.4 g/kg)	3.8 ± 0.4	22.45
Ethanolic extract (2.0 g/kg)	3.5 ± 0.3*	28.57

[a] Values are the mean ± S.E.M. for seven mice.
* $P<0.05$, compared to control, $n=7$, Tukey–Kramer.
*** $P<0.001$, compare to control, $n=7$, Tukey–Kramer.

Table 5
Effect of the intraperitoneal doses of the aqueous and ethanolic extracts of *Z. multiflora* Boiss (consecutive for 7 days) on the weight of granuloma in the rat[a]

Treatment (dose)	Cotton pellet (mg)	Inhibition (%)
Control (10 ml/kg)	102.3 ± 3.7	–
Diclofenac (15 mg/kg)	31.3 ± 4.5***	69.40
Dexamethasone (15 mg/kg)	35.3 ± 2.8***	65.49
Aqueous extract (0.8 g/kg)	50.6 ± 3.8***	50.54
Aqueous extract (1.4 g/kg)	47.7 ± 4.6***	53.37
Aqueous extract (2.0 g/kg)	45.7 ± 3.8***	55.33
Ethanolic extract (0.2g/kg)	58.0 ± 1.9***	43.30
Ethanolic extract (0.8 g/kg)	52.6 ± 5.3***	48.58
Ethanolic extract (1.4 g/kg)	62.8 ± 4.2***	38.61

[a] Values are the mean ± S.E.M. for seven mice.
*** $P<0.001$, compared to control, Tukey–Kramer.

The repair phase of the inflammatory process begins with the 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 inflammation.

In some experiments relatively high doses (such as 1.4 g/kg (Fig. 3, Tables 4 and 5)) have been tested. The isolation of active constituents may increase the potency of the extracts, although in low doses the extracts were significantly effective (such as 80 mg/kg (Fig. 2) and 40 mg/kg (Table 1)).

It is concluded that the aqueous and ethanolic extracts of aerial parts of *Z. multiflora* Boiss have central and peripheral antinociceptive effects. Opioid receptors and/or inhibition of cyclo-oxygenase enzyme may mediate these effects. The extracts have also activity against acute and especially chronic inflammation.

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