ANTINOCICEPTIVE EFFECTS AND TOXICITY OF *FUMARIA PARVIFLORA* LAM. IN MICE AND RATS

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ABSTRACT

In this study the antinoiciceptive and histopathological effects of the methanolic extract of *fumaria parviflora* Lam. in animal models was investigated. For this purpose, the antinociceptive effects of percolated and soxhlet extracts were evaluated in mice subjected to acute thermal (hot-plate) and persistent chemical (formalin) pain stimuli. Intraperitoneal (i.p.) injection of the percolated extract evoked significant antinociceptive effects at a dose of 100 mg/kg in the second phase of formalin test. The maximum antinociceptive effect was induced by the dose of 300 mg/kg that was significant in both phases of formalin test. Dose of 400 mg/kg of the percolated extract induced acute adverse effects such as diarrhea, polyurea, malasia and hyperventilation in mice. Soxhlet extract of *F. parviflora* (300 mg/kg, i.p.) also showed significant effects in the both phases of formalin test. The most effective dose of the percolated extract as well as the soxhlet extracts (300 mg/kg, i.p.) were evaluated in hot plate test. The results showed that only percolated extract had significant antinociceptive effect in hot-plate. Pretreatment of mice with naloxane, an opioid antagonist, did not change antinociceptive effect of percolated extract in formalin test, but in hot-plate it increased extract's effect after the first 15 minutes. In histopathological evaluation of liver, toxic dose of percolated extract (400 mg/kg) caused degeneration, necrosis and regeneration of hepatic cells. The study of ulcerogenic effects of oral percolated extract on stomach in rats showed that this adverse effect was significantly lower in comparison with the same dose of indomethacin. These results showed *F. parviflora* is relatively safe for use and it is a good candidate for further studies to determine its effective and probable toxic compound(s).

Keywords: *Fumaria parviflora* Lam, Antinociceptive, Histopathology, Ulcerogenic effect, Formalin test, Hot-plate

INTRODUCTION

The management and treatment of pain is probably one of the most common and yet the most difficult aspects of medicinal practice. Analgesic therapy is currently dominated by two major classes of analgesic drugs; namely opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious side effects, such as gastrointestinal disturbances, renal damages (with NSAIDs drugs), respiratory depression and possibly dependence (with opioids) (1, 2). It is obvious that the design of analgesic agents with fewer side effects is desirable. One of the ways to achive this aim is the use of medicinal plants which are a rich source of new potentially effective compounds.

Fumaria parviflora Lam. (Fumariaceae) is an herbaceous plant that grows in wide variety parts of Iran and has been used in Iranian folk medicine in dermatological diseases, for stimulation of liver function and gall bladder and as antiscabies, antiscorbite, antibronchite, diuretic, expectorant, antipyretic, diaphoretic, appetizer and antineoplastic (3-5). A review of literatures reveals similar effects for *F. officinalis* (another species of genus fumaria) including ''colicky pain affecting the gallbladder and billiary system, together with the gastrointestinal tract'' (6). In respect to these similarities in their activities and lack of any pharmacological studies in literature about the antinociceptive activity of *fumaria parviflora* Lam, it was decided to investigate the antinociceptive activity of this plant in animal models of pain. Phytochemical analyses of some plants of genus fumaria, including *F. parviflora* has indicated presence of isoquinoline alkaloids namely protropine, cryptopine, sinactine, stylopine, biculine, adlumine, parfumine, fumariline, fumarophycine, fumaritine, dihydro-fumariline, perfumidine and dihydrosanguirine in these plants (7).

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Significant oral antipyretic activity has been shown by hexane- chloroform- and water-soluble extracts of *F. parviflora* in rabbits (8). In another study, an aqueous-methanolic extract of this plant (500 mg/kg, orally twice daily for 2 days) prevented the paracetamol-induced hepatotoxicity, but had no effect against hepatotoxicity which was induced by CCl_4 (9). Hepatoprotective effects of several other species of fumaria have also been demonstrated (10, 11). There are some contradictory reports about toxic effects of *F. parviflora* extracts; while no obvious toxic effects for hexane-, chloroform and water-soluble extracts of *F. parviflora* up to dose of 1.6 g/kg have been reported (8), there is a report that dose of 500 mg/kg (p.o.) of aqueous-methanolic extract caused significant prolongation in pentobarbital-induced sleep as well as increased strychnine-induced lethality in mice (9). In the present study, the antinociceptive effects of percolated and soxhlet extracts of *F. parviflora* using acute thermal (mouse hot-plate) and chemical persistent (mouse formalin-test) pain stimuli models were investigated. The analgesic effects of extract were compared with those of morphine and acetyl salicylic acid (A.S.A) and ulcerogenic effects of percolated extract were also assessed and compared with those of indomethacin. Histopathological effects of the percolated extract on liver were also assessed.

MATERIALS AND METHODS

Extract preparation

Fumaria parviflora Lam. was collected in July 1999 and authenticated by the School of Agricultural Sciences, Shaheed Bahonar University, Kerman, Iran. The whole parts of plant were air-dried in shadow followed by grinding. Extraction was performed with methanol by percolation and soxhlet methods. The obtained extracts were concentrated by rotary evaporator apparatus and air dried at room temperature for 2-3 days. After dissolving 1g of the dried extracts in saline, the total volume was adjusted to 10 ml (stock solution; 100 mg/ml) and from these stock, different concentrations were prepared.

Chemicals

Morphine sulphate and indomethacin were purchased from Sigma (Poole, UK). Methanol, diethylether, formalin, naloxane and acetyl salicylic acid were prepared from Merck (Germany). All drugs and extracts were dissolved in physiological saline (0.9% sodium chloride) and administered intraperitoneally (i.p.) in volume of 0.1 ml/10g of the animal body weight.

Animals

Male wistar rats (180-280 g) and male albino mice (18-28 g) were obtained from the Neuroscience Research Center, Kerman University of Medical Sciences. Animals were housed in standard cages with controlled temperature (23 ± 3.0 ºC) and a 12-h light/dark cycle (Light from 6:00 am till 6:00 pm) with free access to food (standard laboratory rodent's chew) and water. All efforts were made to minimize animal sufferings, and to reduce the number of animals.

Antinociceptive tests

Formalin test

The formalin test was based on the modified method (12) of Dubisson and Dennis (13), and carried out in an open Plexiglas, with a mirror placed under the floor to allow an unobstructed view of the paws. Mice were allowed to acclimate for 15 min in the test cages before formalin injection. Seven animals were used for each dose and animals were then pretreated i.p. with saline (10 ml/kg, as control), morphine sulphate (2.5 mg/kg, as positive control), A.S.A (300 mg/kg, as positive control), percolated extract (100, 200, 300 and 400 mg/kg), naloxane + percolated extract (4 and 300 mg/kg, respectively; naloxane was administrated 15 min before extract administera-tion) and soxhlet extract (300 mg/kg). Each animal was injected 25µl of 0.5% formalin in the intraplanar region of the right hind paw. Mice were then observed for 30 min. The time (sec.) spent on licking and biting the injected paw during successive period of 5 min was measured as an indicator of the pain (14).

Hot-plate test

The method used was a modification of previously reported method (10). Mice were placed into a 10 cm wide glass cylinder on a hot plate maintained at 55 ºC. Control latency was determined for each mouse. To minimize tissue damage, a maximum latency of 30 sec was imposed (cut off point). The normal latency (reaction time) was 3-5 second. Antinociceptive responses were calculated as percent maximum possible effect (MPE%, where MPE% = [(test latency – control latency) / (cut off point – control latency) × 100]. The reaction time was recorded when animals jumped or licked their paws. Seven mice per dose were injected i.p. with saline (10 ml/kg, as control), morphine sulphate (2.5 mg/kg, as positive control), A.S.A (300 mg/kg, as positive control), percolated extract (300 mg/kg), soxhlet extract (300 mg/kg) and naloxane + percolated extract (4 and 300 mg/kg, respectively; naloxane was administrated 15 min before extract administration) and tested at various times (15, 30, 45, 60, 75, 90, 105 and 120 min) thereafter to establish a time course.

Gastric ulcerogenecity in rats

Twenty four hours before the tests, rats were kept in the mesh cages (to prevent them from eating

their faeces) and had only free access to water. Five groups of rats, each five rats, were pretreated orally with saline (10 ml/kg, as control), indomethacin suspensions (200 and 400 mg/kg, as control) and percolated extract (200 and 400 mg/kg). After 4h, animals were sacrificed with ether, their stomach were removed, inflated by injection of 10 ml of 2% formalin to fix the inner layers of the gastric walls, opened along the greater curvature and lesions in the glandular portion were evaluated.

The ulcer index was calculated using the j-score (15,16). The erosions were classified in size order: 0-1 mm in diameter = 1; 1-2 mm = 2; greater than 2mm in diameter = 3. The sum of points in each animal was defined as the ulcer index.

Data analysis
All data were expressed as mean ± S.E.M. Statistical analysis of data was evaluated by analysis of variance (ANOVA), followed by multiple comparison test of Newman-Kauls test and a p-value less than 0.05 was considered statistically significant.

RESULT

Formalin test
Effects of F. parviflora percolated extract
The effects of systemic administration (i.p.) of the percolated extract on formalin induced biphasic response were investigated 15 min after injection. As shown in figure 1, the painful effect was decreased between 10-15 min and reached a second peak at 20-30 min (second phase) after formalin injection. Dose-response relationships were established for different doses of extract, but only the 300 mg/kg dose could significantly reduce the time of pain response in the first ($p < 0.05$) and second ($p < 0.01$) phases of formalin test

Effects of the soxhlet extract of F. parviflora
In figure 2 effects of soxhlet and percolated extracts in comparison with controls (saline, morphine and A.S.A) have been demonstrated. These results show that the doses of 300 mg/kg of both extracts have significantly antinociceptive effects in both phases of formalin test but percolated extract has greater effect than soxhlet extract. A.S.A had only significant antinociceptive effect after 20-25 and 25-30 min; whereas morphine had significant effect at all times of the formalin test.

Involvement of opioid receptors
As assessment of the role of opioid receptors in antinociceptive activity of the percolated extract of *F. parviflora*, a group of mice were pretreated with this extract (300 mg/kg) + naloxane (4 mg/kg) and results were compared with the percolated extract

and saline groups. The results showed that naloxane has no significant effect on the antinociceptive effect of percolated extract in formalin test (Data are not shown).

Hot-plate test
Effects of F. parviflora extracts
The results presented in figure 3 show that percolated extract of *F. parviflora* has greater maximum possible effect (MPE%) than soxhlet extract in hot-plate test and this effect is significant in comparisson with saline group at 15 and 30 min after injection. Morphine has significant MPE% and this effect reduces within 120 min.

Involvement of opioid receptors: As shown in figure 4, pretreatment with naloxane significantly increases antinociceptive effect of percolated extract at times of 30, 45, 90 and 120 min in hot-plate test.

Assessment of extract toxicity
Figure 5 shows the ulcerogenicity of percolated extract in comparison with indomethacin. Doses of 200 and 400 mg/kg, p.o. of extract have significantly less ulcer index than corresponding doses of indomethacin ($p < 0.05$).

Adverse effects
The dose of 400 mg/kg of the percolated extract induced acute adverse effects such as diarrhea, polyurea, malaise and hyperventilation in mice.

Histopathological studies in rats
Histopathological studies of *F. parviflora* percolated extract in rat showed the presence of hepatic injuries like degeneration, necrosis and regeneration of hepatic cells.

DISSCUSION

The present study was carried out to evaluate antinociceptive activity of *fumaria parviflora* Lam. (Fumariaceae) extracts in different models of pain. Data demonstrate that *F. parviflora* percolated and soxhlet extracts elicited antinociceptive effects in mice subjected to chemical persistent (formalin) pain stimuli; but only percolated extract had significant effect on acute thermal pain stimuli (hot-plate)
As shown in figure 1, percolated extract of *F. parviflora* dose-dependently reduced the time that animals spent on licking and biting of the injected paw in first (0-5 min) and late phases (10-30 min) of formalin test. Among the different doses of the extract, only dose of 300 mg/kg had significant effect in both phases of formalin test; therefore, it was considered for the next experiments. Then the effect of this dose of percolated and soxhlet extracts were evaluated in formalin test (figure 2). Results showed that there are no dominant differences between effects of this dose in formalin test.
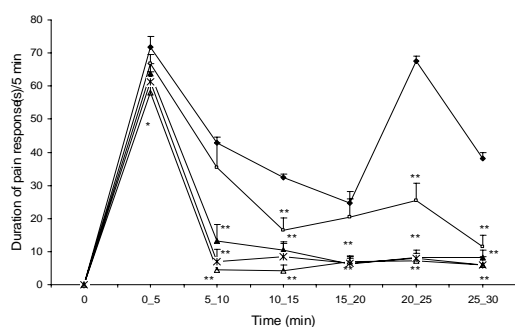
Fig. 1. Time course of pain response induced by formalin in mice and effects of saline (♦; 10 ml/kg) or different doses of percolated extract of *F. parviflora* (□) 100, (▲) 200, (△) 300 and (×) 400 mg/kg. Each point is the mean ± S.E.M. of 7 experiments.
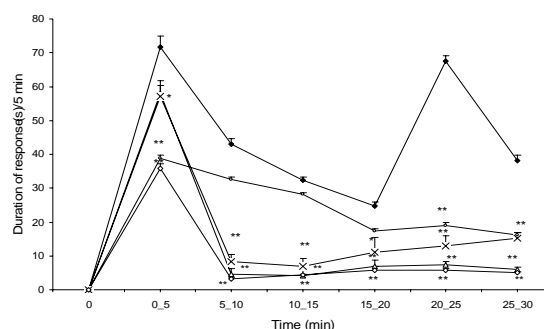* p< 0.05 and ** p< 0.01 compare with saline-injected group.

Fig. 2. Time course of pain response induced by formalin in mice and effects of saline (♦; 10 ml/kg), percolated extract of *F. parviflora* (△; 300 mg/kg), soxhlet extract of *F. parviflora* (×; 300 mg/kg), A.S.A (∘; 300 mg/kg) and morphine (◇; 2.5 mg/kg). Data are represented as mean ± S.E.M (n= 7). * p< 0.05 and ** p< 0.01 compared with saline-control group.
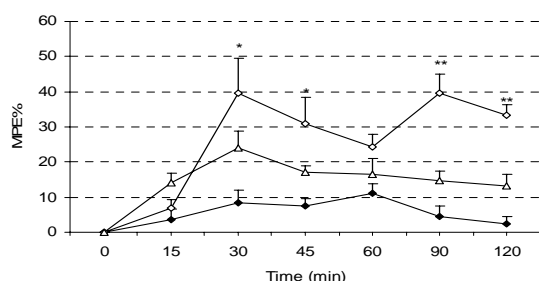

Fig. 3. Mean (± S.E.M.) degree of antinociception (MPE%) vs. time curve achieved using the hot plate test following single dose i.p. administration of saline (♦; 10 ml/kg), percolated extract of *F. parviflora* (△; 300 mg/kg), soxhlet extract of *F. parviflora* (×; 300 mg/kg), A.S.A (∘; 300 mg/kg) and morphine (◇; 2.5 mg/kg) 15 min before test in mice. * p< 0.05 and ** p< 0.01 compare with saline-control group.
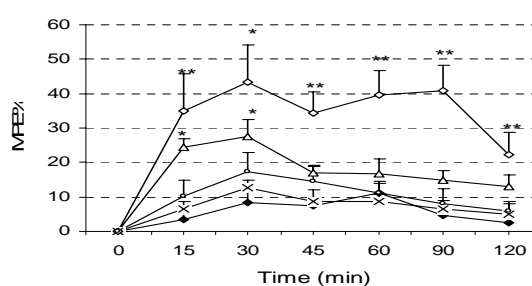

Fig. 4. Effect of naloxane (an opioids receptor antagonist) on antinociceptive effect of percolated extract of *F. parviflora* in hot plate test in mice. Saline (♦; 10 ml/kg), percolated extract of *F. parviflora* (△; 300 mg/kg), percolated extrac of *F. parviflora* t + naloxane (◇; 300 and 4mg/kg, respectively). Data are represented as mean ± S.E.M. (n= 7). * p< 0.05 and ** p< 0.01 compared with saline-control group

From these results it might be concluded that compound(s) responsible for antinoception effect are extracted in both methods.

Evaluation of *F. parviflora* extracts (300 mg/kg) in hot-plate, an animal model for acute thermal pain stimuli, showed a significant MPE% for percolated extract at 15 and 30 min, but soxhlet extract had no significant MPE% at any time of the test (figure 3). An important feature of the formalin test in rodents is that animals show two phases of antinociceptive behavior which seem to be involved by two distinctly different stimuli (17,18).

The first phase starts immediately after injection of formalin and lasts for 3-5 min. This phase is largely due to direct chemical stimulation of nociceptores. It is believed that the first phase modulated by CNS and blocked more intensively by opiates, whereas NSAIDs have weak effect in this phase (18). The second phase starts approxi

mately 15-20 min after formalin injection and reach to a peak at 20-30 min. Evidences suggest that peripheral inflammatory processes are involved in the late phase and NSAIDs; such as indomethacin and piroxicam reduce nociceptor behaviors during the second phase, while the first phase seems to be unaffected (18). These evidences are consistent with results from A.S.A (figures 2 and 3) that has no effect in the first phase in hot-plate whereas it is effective in the second phase of formalin test. From these findings it might be concluded that *F. parviflora* percolated extract has a more prominent peripheral effect than central effects. This inference is supported by results obtained by the testing the extract with a CNS opioids receptor antagonist (naloxane) that couldn't reverse antinociceptive effects of extract in formalin and hot-plate tests (figures 3).
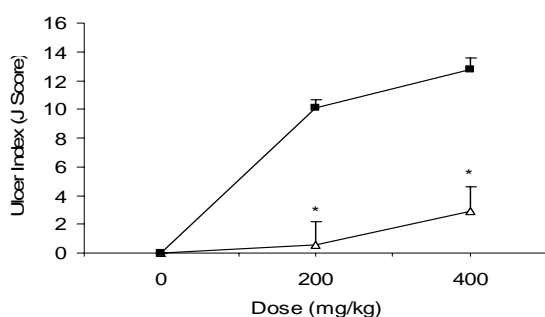
Fig. 5. Comparison of ulcer indices of doses of 200 and 400 mg/kg of percolated extract of *F. parviflora* (△) and indomethacin (■) in rats (n= 6). Data values are expressed as mean ± S.E.M. * $P < 0.05$ vs. indomethacin group. See text for explanation of ulcer index.

An interesting result in this study was potentiation of antinociceptive effects of percolated extract by naloxane (figure 4); and as a result pretreatment with naloxane in hot-plate significantly increased extract effects in comparison with the use of extract by itself. Results of this study revealed the hepatotoxicity of percolated extract (400 mg/kg) which appeared by degeneration and necrosis of hepatic cells. This result is in contrast with those of previous studies on this plant and other species of fumaria (8, 10). This controversial might arise from differences in method of extract preparation, animals and experimental methods. Comparison of ulcerogenecity of percolated extract of *F. parviflora* with corresponding doses of indomethacin (200 and 400 mg/kg, p.o.) showed extract had significantly less ulcer indexes (figure 5).

In summary, the results of this study show that percolated and soxhlet extracts of *F. parviflora* have antinociceptive effect in formalin test, especially at the late phase and hot-plate test, and this effect may not be mediated by opioids receptors. It is proposed that this plant is a good candidate for further investigation in other models, especially models of inflammation.

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