Original Article

Antinociceptive Effect of Seed's Essential Oil of *Ferula Assa-foetida* in Mice

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Abstract

Background and Aim: *Ferula assa-foetida* L. is distributed throughout Central Asia and Mediterranean area and grows wildly in Iran and Afghanistan. In Iranian traditional medicine, *F. assa-foetida* is considered to be sedative, analgesic, carminative, antispasmodic, diuretic, antihelminthic, emmenagogue, and expectorant. The aim of this study was to evaluate the antinociceptive effect of seed's essential oil of *F. assa-foetida* (SEOFAF) in mice. **Methods:** The analgesic activity of SEOFAF (2.5, 5, and 10 mg/kg) was compared with that of sodium diclofenac (30 mg/kg) or morphine sulfate (8 mg/kg) using hot plate and acetic acid-induced writhing tests. **Results:** In hot plate test, the percentage of maximum possible effect against the thermal stimulus at 15 min posttreatment time point for all doses of SEOFAF was significantly greater than control group. The number of writhes in all three doses of SEOFAF was significantly less than control group. **Conclusion:** According to our findings, SEOFAF exhibited a significant antinociceptive effect on chronic and acute pain in mice. These effects probably involve central opioid pathways and peripheral anti-inflammatory action.

Keywords: *Ferula assa-foetida*, hot plate, seed's essential oil, writhing test

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Introduction

Pain is a serious unpleasant sensation that limits productivity and diminishes the quality of life.[1] Despite the availability of effective and widely used analgesics, most of them exhibit many undesirable side effects which limit their clinical use.[2] Natural remedies and dietary traditions play an effective role in diminishing the patient's suffering, and plant products are usually considered to be less toxic than synthetic drugs, they can be an important source of new chemical substances with potential therapeutic effects.[3,4] The *Ferula* genuses from the *Umbelliferae* family have been found to be rich sources of bioactive natural products.[5] In Iranian folk medicine, *Ferula assa-foetida* is used as an antispasmodic, antihelminthic, and carminative agent.[6] It has also been used for the treatment of stomach ache, indigestion, bronchitis, asthma, and whooping cough.[5] In Nepalian traditional medicine, it is mainly considered as an aphrodisiac agent, and there are some reports indicating its potency to increase the sexual appetite.[7] There are a few studies about oil seed of *F. assa-foetida*. In a previous study, researchers reported the antifungal activity of oil seed against *Aspergillus niger* and *Aspergillus flavus*.[8] Kassis *et al*. examined ethanolic extract of seeds and roots of *F. assa-foetida* that was called masculine on male fertility and sexual functioning in rats and humans.[9] They showed that masculine exhibits a high level of safety in rats, humans, and cultures human fibroblasts, and increases erection in rats. Bagheri *et al*. reported that this essential oil has antispasmodic effect and this effect was stronger than oleo-gum-resin of *F. assa-foetida*.[10] Based on this evidence and extensive use of *F. assa-foetida* oil in Asia, in the present study, we have planned to determine the antinociceptive effect of this essential oil on acute and chronic pain in mice.

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Materials and Methods

Animals

Sixty male albino mice (25–30 g) 6–8 weeks old breeding in Shahid Sadoughi Medical School animal house were selected and were housed at controlled temperature (22°C ± 2°C) with a 12 h-light/dark cycle, with standard laboratory chow and tap water *ad libitum*. Each animal was used only once. The experiments reported in this study were carried out in accordance with the current ethical guidelines for the investigation of experimental pain in conscious animals.[11] The number of animals and intensity of noxious stimuli were considered in minimum necessary for demonstrating the consistent effects. Animals were randomly and equally divided into ten groups.

Plant material

Two hundred grams of *F. assa-foetida* seeds were gently grounded and mixed with 500 ml of double-distilled water. Then, it was extracted by steam distilled apparatus. The essential oil was separated from aqueous extract by a Soxhlet apparatus. The essential oil was separated from aqueous distillate of the crude extract by the Soxhlet apparatus. The concentration of essential oil in the extract was 1.5% V/V.

Drugs and extract administration

Morphine sulfate (Temad, Iran) in concentration of 8 mg/kg and sodium diclofenac (Pharma Chemie, Iran) in 30 mg/kg were used as positive control drugs, distilled water (as the drug's vehicle) as negative control, and seed's essential oil of *F. assa-foetida* (SEOFAF) in 2.5, 5, and 10 mg/kg as test agent. All administrations were used as intraperitoneal (i.p.) injection.

Hot plate test

The hot plate test was carried out according to the method previously described.[12] Mice were habituated to the cylinder of the apparatus for 5 min before the initiation of the experiment. The hot plate apparatus (Borj Sanat, Iran) was set to 54°C ± 0.1°C. Animals were placed on the hot surface inside the Plexiglas cylinder (20 cm in diameter), and the time (in seconds) spent to licking of their hind paws or jumping (whichever occurred first) was recorded as the pain response latency (reaction time). A 30-s cutoff was set to prevent tissue damage. After taking a baseline reaction time for each animal, mice immediately received their own administration (morphine sulfate, SEOFAF, or distilled water) and their reaction time to hot plate was evaluated after 15, 30, 45, and 60 min time points. The mean reaction time in each time point after drug administration was compared to the baseline reaction time in each group for evaluating the optimum response time. For between-group comparisons, the percentage of maximum possible effect (%MPE) against thermal stimulus in each time point for each animal was calculated by the following formula:

$$\%\text{MPE} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off} - \text{Control latency}} \times 100$$

Acetic acid-induced writhing test

The abdominal constriction test described by Collier *et al*.[13] was used with slight modification to measure the analgesic activity of SEOFAF. Male mice pretreated either with SEOFAF (2.5, 5, and 10 mg/kg), diclofenac sodium (30 mg/kg), or distilled water (10 ml/kg) were treated with i.p. injection of 0.6% acetic acid (10 ml/kg) after 15 min. Acetic acid induces a typical writhing response. Five minutes after acetic acid injection, mice were kept in individual cages, and the number of writhes (hind limb stretching and abdominal muscle contraction) of each mouse was counted for a period of 30 min by a blinded individual. The mean number of writhes was considered as a measure of analgesic effect in each group. The percentage of writhing inhibition was calculated using the following formula:

$$\%\text{Inhibition} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean number of writhes (control)}} \times 100$$

Statistical analysis

All data were expressed as mean ± standard error of the means. GraphPad PRISM 5 software (San Diego, CA, USA) was used to analyze the behavioral responses. Data were analyzed using repeated measure one-way ANOVA followed by the Tukey-Kramer posttest for multiple comparison. $P < 0.05$ was considered statistically significant.

Results

Hot plate test

Latency responses for animals in different groups are shown in Table 1. The latencies for time 0 (baseline latency) were statistically analyzed by one-way ANOVA, and there was no significant difference between groups. Despite the similarity

Table 1: Hot plate latency responses of animals in different groups ($n=6$)

Group	Latency time (s)				
	0	15	30	45	60
Control	9.7±2.1	11.3±3.2	9.6±1.6	9.2±2.8	9.1±2.5
SEOFAF 2.5 (mg/kg)	9.2±1.6	14.4±3.9*	11.4±2.6	10.3±2.4	10.3±2.3
SEOFAF 5.0 (mg/kg)	7.6±0.9	15.1±3.2*	11.9±2.5*	10.9±2.8*	9.1±3.1*
SEOFAF 10 (mg/kg)	8.6±1.5	15.3±3.4*	12.6±2.9*	11.2±1.7	9.9±2.1
Morphine 8 (mg/kg)	8.5±1.6	15.9±3.4*	16.9±4.2*	13.4±3.7*	11.8±4.8*

Data expressed are mean±SEM. The analysis of data was done by one-way ANOVA and *post hoc* by Tukey-Kramer test. The baseline time of each group is considered as control and other times were compared with baseline. *Comparison with baseline, $P<0.05$. SEOFAF: Seed's essential oil of *Ferula assa-foetida*, SEM: Standard error of the mean

in baseline latencies in different groups, for more accuracy, the %MPE against thermal stimulus was calculated in each time point after the treatment for each animal in each group. Fifteen minutes after the treatment, the analgesic effect of different doses of SEOFAF was obvious and declined for the next time points in all doses. The most effective dose of SEOFAF was 10 mg/kg and its maximum effect was observed 15 min after drug administration [Figure 1]. As shown in Figure 1, the %MPE at 15 min posttreatment time point for all doses of SEOFAF was significantly greater than control group.

Acetic acid-induced writhing test

The effect of different treatments on acetic acid-induced writhing is presented in Table 2. The percentage of writhing inhibition induced by different doses of SEOFAF was inversely dose dependent, and the number of writhes in all three doses of SEOFAF was significantly less than control group. Maximum inhibition percentage (63%) of writhes was observed by 10 mg/kg SEOFAF, which was statistically similar to that of 30 mg/kg sodium diclofenac.

Discussion

Our findings indicated that SEOFAF reduces the number of acetic acid-induced writhes in a dose-dependent manner. The analgesic effect of SEOFAF may be either due to its action on visceral receptors sensitive to acetic acid or due to the inhibition of production/action of prostaglandins.[14] In the other hands, hot plate test is one of the most common tests for monitoring the phasic nociceptive responses to a noxious heat stimulus of high intensity.[15] Pain induced by this thermal noxious stimulus is specific for centrally mediated antinociception[16] and is thought to involve opioid pain inhibitory pathways.[17] In the hot plate test, a significant effect of the SEOFAF at all doses was obtained 15 min after treatment, and the overall analgesic pattern of the most effective doses (10 mg/kg) was very similar to morphine sulfate. Therefore, the analgesic effect of SEOFAF in hot plate test may be related to the opioid pain inhibitory pathways. The fact that SEOFAF produced its analgesic action in both tonic and phasic nociceptive models is indicative that it may possess both central and peripheral antinociception. One possible mechanism of action for the active principles of this SEOFAF could be related to lipoxygenase and/or cyclooxygenase in the arachidonic acid cascade at the peripheral route. Phytochemical analysis showed that the essential oil *F. assa-foetida* seed contains monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and sulfur-containing compounds.[18] Sulfur compounds inhibited the production of nitric oxide and prostaglandin E_2 and the expression of the proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β, and interleukin-6.[19] Free radicals and related reactive species are strongly involved in several pathological and physiological processes, including cancer, cell death, inflammation, and pain.[12] Different studies indicated that essential oils extracted from *Ferula* species have antioxidant property.[18] In addition, terpene compounds in this oil could be another reason for its analgesic effect. It is demonstrated that terpenoids have anti-inflammatory and antinociceptive effects. Mandegary *et al*. attributed the anti-inflammatory and antinociceptive activity of *Ferula gummosa* to its terpenoids and alkaloids.[16]

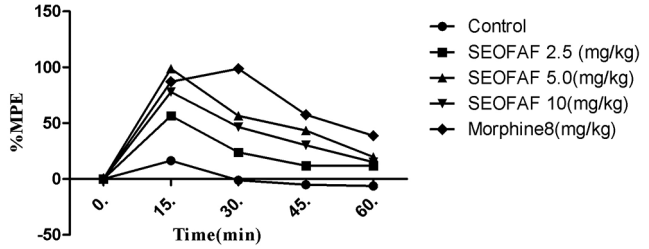

Figure 1: The percentage of maximum possible effect of different treatments on acute pain inhibition at different time points in hot plate test ($n = 6$). SEOFAF: Seed's essential oil of *Ferula assa-foetida*

Table 2: The effect of seed's essential oil of *Ferula assa-foetida* on acetic acid-induced writhing in mice ($n=6$)

Group	Number of writhing	Percentage of inhibition
Control	106.4±2.1	-
SEOFAF 2.5 (mg/kg)	59.9±1.6*	43.7
SEOFAF 5.0 (mg/kg)	45.8±1.5*	56.9
SEOFAF 10 (mg/kg)	38.9±4.1##	63.4
Sodium diclofenac (mg/kg)	35.9±2.9*	66.3

Data expressed are mean±SEM. The analysis of data was done by one-way ANOVA and *post hoc* by Tukey-Kramer test. *Comparison with control group, $P<0.05$, ##Comparison with sodium diclofenac group, $P<0.05$. SEOFAF: Seed's essential oil of *Ferula assa-foetida*, SEM: Standard error of the mean

Limitations of the study

In the present study, we have not assessed the active components of seed oil of Ferula Assa-foetida and its mechanism of action.

Conclusion

This study demonstrates the analgesic activity of SEOFAF, which is in accordance with the traditional use of this extract as an analgesic and anti-inflammatory medicine. However, its active components and their mechanism of actions need to be elucidated by further studies.

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Conflicts of interest

There are no conflicts of interest.

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