ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF THE SEED AND ROOT EXTRACTS OF *FERULA GUMMOSA* BOISS IN MICE AND RATS

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ABSTRACT

Ferula gummosa Boiss (Apiaceae) has been used in Iranian traditional medicine for the relief of stomach pain. In this study, effects of aqueous, methanolic and acetone extracts of the seed and root of this plant in experimental models of acute pain (Tail-flick=TF), chronic pain (Formalin test=FT) and inflammation (Cotton pellet granuloma=CPG) was investigated. The results showed that the highest non-sedative dose of each of these three extracts had no effect in TF. Among the extracts, only the acetone extract of the root could reduce licking and biting time in the late phase of FT, although this effect might be to some extent due to the solvent (Tween 80). None of the extracts had anti-inflammatory effect in CPG. Preliminary phytochemical analysis of methanol and acetone extracts showed presence of terpenoids and alkaloids and small amounts of cardenolids. The results of our study suggest further evaluation of antinociceptive and anti-inflammatory effects of other kinds of extracts in order to determine the best extract with highest efficacy and lowest side effects.

Key words: *Ferula gummosa* Boiss, Antinociceptive, Anti-inflammatory, Formalin test, Tail-flick, Cotton pellet granuloma

INTRODUCTION

The gum of *Ferula gummosa* Boiss (Apiaceae) has a vast folkloric application in Iran. *Ferula gummosa* is a perennial plant, indigenous to Iran and Central Asia and blooms once in its several years' life (1). In Iranian traditional medicine, the gum obtained from the aerial parts of this plant has been used for the treatment of stomach pain, chorea, epilepsy and as a wound-healing remedy (1, 2). In some recent studies the presence of compound(s), affective through opioid receptors, in aerial parts of this plant has been reported (3, 4). Also, spasmolytic activity for essential oil and various extracts of this plant has been reported (5). More recently, anticonvulsant effect of the seed essence (6) and the acetone extract of the seed and root of *F. gummosa* was reported (7, 8). Composition of the essential oil of the fruit of the plant has been determined and it has been shown that terpenoid compounds like alpha-pinene, beta-pinene, 3-carene, alpha-thuujene and sabinene are abundant in this plant (6).

Neurotoxicity and lethality of the acetone extracts of the seed and root have been assessed and it has been shown that they produced locomotor's impairment in the rotarod ataxia test (TD_{50}) at approximately 376 and 546 mg/kg and killed 50% of mice (LD_{50}) in the acute lethality test at approximately at 1720 and 1251 mg/kg, respectively (7, 8). There are some reports that plants of the genus *Ferula*, including *F. gummosa* are a rich source of terpenoid compounds (9). Also, there are some reports regarding the antinociceptive, anti-inflammatory and antipyretic effects of some terpenoid compounds isolated from the plants of *Ferula* genus (9, 10) and some terpenoid-producing plants, such as *Eupatorium formosanum*, have been used as anti-inflammatory and antipyretic herbal medicine (11). These evidences provided reliable reasons for investigation of the effects of aqueous, methanolic and acetone extracts of seed and root of *F. gummosa* on pain and inflammation in rodents. In this study, the antinociceptive effects of various extracts of this plant in mice using the tail-flick test were investigated. Also anti-inflammatory activity in an acute and a chronic inflammatory process was determined by the formalin test and the cotton pellet granuloma in rats, respectively.

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MATERIALS AND METHODS

Plant materials

Seeds and root of *F. gummosa* were collected from Polour (90 Km northeast of Tehran) in May 2001 and authenticated by M. Kamalinejad, Department of Pharmacognosy, Shaheed Beheshti University of Medical Sciences, where a voucher specimen (No. 563) was deposited.

Extract preparations

Seeds and root of *F. gummosa* were dried at the lab temperature for a week. Aqueous and methanolic extracts were obtained by decoction. For the preparation of acetone extract, the seeds and root were macerated in acetone (2L), in portions of 200 and 300 g, respectively, for 3 days. After filtration of mixtures, filtrates were concentrated by a rotary evaporator apparatus. The residues were then dried at room temperature. The final weight of crude acetone extracts was approximately 13.5 g which maintained at 4º C throughout the experiments.

Chemicals

Morphine sulphate and piroxicam were purchased from Sigma (Poole, UK). Acetone, methanol, tween 80, ether and formalin were purchased from Merck (Darmstadt, Germany). Ketamine and xylazine were prepared from Rotex. Aqueous extract was provided in distilled water. Acetone and methanol extracts were prepared in 5% v/v tween 80 in distilled water. Piroxicam (Sigma) was dissolved in a mixture of benzyl alcohol, propylene glycol and distilled water (3:3:4 V/V). All drugs and extracts were 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 NMRI mice (18-28 g) were purchased from Institute Pasteur of Iran. The animals were housed in standard cages with controlled temperature (23 ± 3.0 ºC) and a 12-h light/dark cycle (Light from 6:00 a.m. till 6:00 p.m.) with free access to food (Standard laboratory rodent's chaw) and water. All efforts were made to minimize animal suffering and to reduce the number of animal used.

Methods

The doses which were used in this study were up to the highest non-sedative doses of the extracts and were 300 and 500 mg/kg for the acetone extract of the seed and root of *F. gummosa*, respectively (8, 9).

Tail-flick test

Groups of mice, including 12 mice in each group were used. The mice were stimulated by the concentrated Infra- Red light from the tail-flick apparatus (P-102, Pooya-e-Armaghan, Iran) at the one third terminals of their tails. Maximal time for stimulation without any tissue injuring (Cut off Point) was 10 s. Latency time (the time that mouse take its tail away from the stimulating light) was obtained for every mouse. Later, mice were treated i.p. with the aqueous extract of the seed (3000 mg/kg), methanolic extract of the seed and root (500 mg/kg), acetone extract of the seed (300 mg/kg), acetone extract of the root (400 mg/kg), Morphine sulphate (as positive control, 10 mg/kg) and tween preparation (as control, 10 ml/kg). After 30 min they were tested with tail-flick again. Analgesia Index (AI) was calculated as follow (12):

$$\%AI = \frac{\text{Test Latency (s)} - \text{Baseline (s)}}{\text{Cut Off Point (s)} - \text{Baseline (s)}}$$

Formalin test

Each animal of the nine groups of 7-8 rats pretreated i.p. with the acetone extract of the root (200, 300, 400 and 500 mg/kg), methanolic extract of the seed (300 mg/kg), acetone extract of the seed (400 mg/kg), piroxicam (5 mg/kg, as positive control), tween preparation and saline (10 ml/kg, as control). After 30 minutes, 50µl of 2.5% formalin in saline was injected s. c. into the planar surface of the left hind paw of the rats (13) and the time spent for licking and biting of the injected paw was recorded (14, 15).

Cotton pellet granuloma in rats

Animals were anaesthetized by i.p. Ketamine/Xylazine solution(2 / 0.25 v/v) and 2 centimeters incision were made on the rat's nape skin, and the sterile compressed cotton pellets (Weighing 50 mg) were implanted (16). Each of the five groups of 5-7 rats, were treated with acetone extract of the root (500 mg/kg), piroxicam (5 mg/kg, as positive control), sodium salicylate (350 mg/kg), distilled water (10 ml/kg, as control) and tween preparation (10 ml/kg, as control) once a day for a week. After sacrificing animals by ether, pellets were extracted and dried in an oven at the 55 ºC for 24h. Differences between initial and ultimate weights showed the intensity of inflammation.

Preliminary phytochemical analysis

Acetone extract of the seed and root of *F. gummosa* were screened for alkaloids, coumarins, amino acids, terpenoids, salicin, potassin and cardenolids by the reported method (17).

Data analysis

The data were expressed as Mean ± S.E.M. and tested by analysis of variance followed by

multiple comparison test of Tukey-Kramer post hoc for formalin and cotton pellet granuloma tests. Data which were obtained in the tail flick test were analyzed using Student's pair t-test. Statistical significance was considered as P-values < 0.05 for all tests.

RESULTS AND DISCUSSION

In this study antinociceptive and anti-inflammatory effects of aqueous, methanolic and acetone extracts of the seed and root of *Ferula gummosa* Boiss was evaluated in experimental models of pain and inflammation. The results showed that the maximum non-sedative doses of aqueous, methanolic and acetone extracts of *F. gummosa* (7, 8) had no significant effect in pre and post latency time in TF which both of them were between 3-4 s (data not shown here), while morphine (10 mg/kg) increased latency time more than Cut Off Point (10 seconds). These results are in contrast to the results of a report that showed chloroform extracts of the aerial parts of *F. gummosa* had antinociceptive activity in hot plate test that was in part mediated by opioid receptors (3). This inconsistency might result from differences in extraction methods or parts of plant that were used. TF and hot plate tests are most common tests of nociception that are based on a phasic stimulus of high intensity. The nociceptive experience is short-lasting and it is well accepted that agonists of mu-opioid receptors produce analgesia in acute pain models (18). Therefore, it is believed that substances that are effective in TF exert their effects predominantly through mu-opioid receptors. These findings indicate that these kinds of extraction methods may not extract sufficiently opioid-like compound(s) out of the plant.

According to the results presented in table 1, aqueous, methanolic and acetone extracts of the seed had no prominent effect in formalin test. The acetone extract of the root could dose-dependently reduce the time of licking and biting of the injected paw just in the late phase of FT. This reduction was significant when compared with saline group (P< 0.05) at the dose of 500 mg/kg but not with vehicle group (Tween preparation). These findings show that the effect of extract in late phase is somewhat due to the solvent (tween 80).

Table 1. Effect of root and acetone extract of the seeds of *Ferula gummosa* Boiss. on licking and biting time in formalin test in rats

Treatment	Dose	Time of licking and biting (s)	
		Early phase(0-5′) (Mean ± SEM)	**Late phase(15-0′)** (Mean ± SEM)
Saline 0.9%	10 ml/kg	62 ± 5.8	244.6 ± 44.5
Tween 80, 5% v/v	10 ml/kg	53 ± 6.7	205.5 ± 24.8
Aqueous extract of the seed	3000 mg/kg	55 ± 4.8	210 ± 19.2
Methanolic extract of the seed	300 mg/kg	42.3 ± 6.6	152 ± 20.5
Acetone extract of the seed	300 mg/kg	43.5 ± 11.7	196.8 ± 23.4
Acetone extract of the root	300 mg/kg	38.8 ± 9.3	144.8 ± 35.5
Acetone extract of the root	400 mg/kg	35.5 ± 9.5	129.8 ± 25.3
Acetone extract of the root	500 mg/kg	41.8 ± 0.6	86.5 ± 12.2 **
Piroxicam	5 mg/kg	49.4 ± 5.8	116.11 ± 12.4 *

Data are presented as the mean ± S.E.M. (n= 7-8)
* P< 0.05 and ** P< 0.01 compare to saline group.

Table 2. Effect of the acetone extract of the root of *Ferula gummosa* Boiss. (Administered i.p. for 7 consecutive days) on cotton pellet granuloma in rats

Treatment	Dose	**Cotton pellet weight (mg)** (Mean ± SEM)
Saline 0.9%	10 ml/kg	134.8 ± 5.8
Tween 80, 5% v/v	10 ml/kg	120.28 ± 4.8
Acetone extract of the root	500 mg/kg	117.6 ± 2.1
Piroxicam	5 mg/kg	91.38 ± 6.1**
Sodium salicylate	350 mg/kg	116.4 ± 2.5

Data are presented as the mean ± S.E.M. (n= 5-7)
** p< 0.01 compare to other groups.

An important feature of the formalin test in rodents is that the animal show two phases of nociceptive behavior which possibly involves two distinctly different stimuli. The first phase starts immediately after injection of the formalin and lasts for 3-5 min. Evidences show that effect on the opioid receptors is one of the main ways involved in exertion of antinociceptive effects in this phase (13). None of the *F. gummosa* extracts was effective in the first phase of FT. This is consistent with results obtained from TF and both of them confirm lack of opioid-like compounds or others that can affect opioid receptors. There are evidences that suggest peripheral inflammatory processes are involved in the second phase and are blocked by non-steroidal anti-inflammatory drugs (NSAIDs) while the first phase seems unaffected (13). This is consistent with our results that show effectiveness of piroxicam (as positive control in this study) in late phase but not in early phase. As it was mentioned earlier, only acetone extract of the root (500 mg/kg, i.p.) was effective in late phase. It can be assumed that compound(s) are present in the root extract that exert their anti-inflammatory effects through mechanisms similar NSAIDs. However, determination of implicit mechanism needs more studies to be done.

Cotton pellet granuloma (CPG) is a chronic inflammatory test that has been used for studying the effects of compounds on inflammation. As represented in table 2, only piroxicam (as positive control) could significantly ($p< 0.001$) reduce the weight of cotton pellet compared to other groups and there was no significant difference between the extract (500 mg/kg, i.p.) and tween group (as control). Unlike the previous report (14), from our results it may be concluded that anti-inflammatory effect of the acetone extract of the root of *F. gummosa* is not mediated by the proliferative phase of inflammation.

Preliminary phytochemical analysis of the seed and acetone extract of the root of *F. gummosa* showed the presence of large amounts of terpenoids and alkaloids as well as small amounts of cardenolids. These results are consistent with the results of the previous works on this plant and other species of *Ferula* genus (4, 6, 9). The most prevalent terpenoids in Ferula genus are alpha- and beta-pinene and 3-carene (6, 9) that have already been reported to have anti-inflammatory activities (19, 20, 21). Therefore, the anti-inflammatory activity shown by the acetone extract of the root might be in part due to the major component of extract, namely terpenoids. More works are required to be done in order to better elucidate the mechanism(s) and the compound(s) involved in therapeutic and toxic effects of *ferula gummosa* extract

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