

## The effect of aqueous and methanolic extracts of *Prangos ferulacea* on formalin-induced pain in mice

Emamghoreishi M<sup>\*1</sup>, Taghavi A<sup>1</sup>, Javidnia K<sup>2</sup>

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1. Dept. of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

2. Dept. of Pharmaceutical Chemistry, Research Center for Pharmaceutical and Herbal Chemistry, Shiraz University of Medical Sciences, Shiraz, Iran

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### Abstract

#### Introduction:

*Prangos ferulacea* has been used for GI disorders in traditional medicine. It has been known as an analgesic and anti-inflammatory herb among the communities. However, the analgesic effect of this plant has not been scientifically evaluated. Therefore, the aim of this study was to investigate the analgesic effect of *Prangos ferulacea* in mice.

#### Material and Methods:

30 minutes after intraperitoneal administration of different doses of aqueous or methanolic extracts of *Prangos ferulacea*, its analgesic effect was evaluated in mice using formalin test. The durations of paw licking and biting were recorded at 5 min. intervals for 30 min. after injection of formalin.

#### Results:

The aqueous extract at the dose of 100mg/kg reduced pain at phase 2 and at doses of 300, 900 and 1350 mg/kg it reduced pain at phases 1 and 2 of formalin test. The methanolic extract reduced pain at the dose of 200mg/kg at phase 2, and at doses of 400 and 800 mg/kg at phases 1 and 2 of formalin test.

#### Conclusion:

The results of this study showed that the aqueous and methanolic extracts of *Prangos ferulacea* have analgesic properties. Both extracts contain compounds which exert both peripheral and central analgesia. Future studies are needed to isolate the effective compound and to find the analgesic mechanism of this plant.

**Keywords:** *Prangos ferulacea*, Analgesics, Formalin Test, Plant Extracts

### Introduction:

Regarding the importance of pain in personal and social life of the people, pain relief has always been a concern by many physicians and the researchers in biological sciences. Considering the costs imposed to the society for pain relief treatments, and also having the knowledge about the numerous side effects of the available analgesics in the clinic, the need for new

analgesic drugs with higher efficacy and less side effects seems necessary. The use of medicinal plants has been common since ancient times in the ancient civilizations. Different forms of herbal therapy are nowadays common all around the world in either forms of herb products or pure extracts. Considering the vegetation diversity and history of traditional medicine in Iran, conducting researches on herbal

\* Corresponding author, Address: Shiraz, Zand Avenue, School of Medicine, Department of Pharmacology  
Phone: +98 711 2307591; Fax: +98 711 2307591; Email: emamm@sums.ac.ir

plants in order to achieve scientific evidence about their efficacy is necessary.

*Prangos ferulacea* is a genus of umbelliferous plants found in different regions of India, Afghanistan and Iran [1]. *Ferulacea* species which analgesic effect is assessed in this study is distributed in slopes of the Alborz and Kerman, Azerbaijani, Arak and Kermanshah provinces [2]. *Ferulacea* is used in the traditional medicine to treat digestive disorders, headaches and seizures [2]. Other properties of *Ferulacea* include increasing tolerance, opening the stenosis and obstructions of the lumens, breaking the kidney and bladder stones and reducing the swelling of the spleen [2]. The general population uses this herb as an analgesic drug and it is stated in traditional medicine that this herb is effective in relieving tooth pain, and eliminating the pain of joints, bruises, broken bones and flank [2]. Few scientific studies have been conducted on this plant species. For example, in a study in Shiraz it was shown that the aqueous and hydroalcoholic extracts of this plant increases the number of abortions in pregnant rats; however, this increase was not statistically significant and no association was observed between dose or type of extract and the number of abortions [3]. In other studies, antioxidant properties are mentioned for *Ferulacea* [4, 5]. In one of the recent studies the chemical compounds in the essential oil of this plant were identified and its antimicrobial properties have been shown [6]. As the analgesic effect of this herb has not been scientifically evaluated, the aim of this study was to assess the analgesic effect of aqueous and methanol extracts of *Ferulacea* in mice using formalin test.

### Material and methods:

#### Laboratory animals and classification

In this study, male mice weighing approximately 25 to 35 grams were used

which were provided by Animal House of Shiraz University of Medical Sciences. The animals were kept in a room with temperature of 2-22 ° C in groups of 10 in polycarbonate cages having free access to food and water.

In order to assess the analgesic effect of each of the aqueous extracts, 63 mice were randomly divided into seven equal groups. The study groups consisted of groups receiving different doses of aqueous extract (100, 300, 900 and 1350 mg / kg), the group receiving water (control) and the positive control groups (receiving 10 mg / kg of morphine sulfate and 200 mg/kg of sodium salicylate). To investigate the analgesic effects of methanol extracts, 72 mice were randomly divided into eight groups including groups receiving different doses of methanol extract (100, 200, 400 and 800 mg/kg), control groups (receiving water and 20% dimethyl sulfoxide, DMSO) and positive control groups (receiving morphine and sodium salicylate). The doses were selected based on the lethal doses of aqueous extract (1800 mg /kg) and methanol (900 mg /kg).

#### Collection and identification of the plant

*Prangos ferulacea* was collected at Derak Mountains near Shiraz and was identified and confirmed by the doctor Khosravi (Faculty of Science, Shiraz University); it was then kept in Medicinal Chemistry and Plant Research Center, Shiraz University of Medical Sciences with the number of 141-1-5 pc in barium.

#### Extracting the plants

##### Preparation of the aqueous extract by maceration method

After washing the plant, it was powdered using a mill. 100 grams of the powder was soaked for 24 hours in 300 ml of water and then filtered; the filtrate was concentrated using a Bain de Marie in 60-70° C temperature. The concentrated extract was

dried using a desiccator device. Extraction efficiency was 11.1 percent weight - weight.

#### Preparation of methanol extracts using maceration method

A total of 100 grams of plant powder was soaked in 400 ml of de petrole ether for 4 hours for defatting; it was then filtered and the remaining part on the filter paper was soaked in 500 mL of methanol 90% for 48 hours. After filtration, the filtrate was concentrated using a Bain de Marie in 60-70 ° C temperature and then dried in a desiccator device. Extraction efficiency was 6.6 percent weight - weight.

#### Phytochemical assessment of the aqueous and methanol extracts

To determine the type of basic compounds in the extracts, diagnostic testing was performed to detect alkaloids (Mayer's reagent), anthraquinone (Born-trager reaction), tannins (creating precipitate using lead acetate) and flavonoids (zinc / hydrochloric acid) on the dried extract [7].

#### Formalin test

To investigate the analgesic effect of plant extracts, the formalin test [8] was used. Thus, 30 min after injection of different doses of the extract or the carrier, 20 microliter of 5% formalin was injected into the right back paw of mice using a Hamilton syringe and the animal was immediately placed in the formalin test container. The time spent licking and biting the injected leg immediately after formalin injection was recorded at intervals of 5 minutes for 35 minutes. The time spent licking and biting the injected leg in the first 5 minutes was considered as the first phase of pain and during 15 to 30 minutes as the second phase. The time interval from 5 to 15 minutes is the painless phase of the formalin test.

#### Statistical Analysis

One-way ANOVA test was used to compare mean duration of licking and biting between the groups. In order to assess the difference in mean time of licking and biting between each test and control group, Dunnett test was used. Statistical analysis was performed using SPSS software version 11.5 and P-value less than 0.05 was considered as statistically significant.

#### Results:

The phytochemical assessments showed that the aqueous extract of *Ferulacea* contains nitrogen compounds, saponin and tannins. Methanol extract of *Ferulacea* also contains saponin, anthraquinone, tannins and flavonoids. The aqueous extract of *Ferulacea* at doses of 300, 900 and 1350 mg / kg caused a significant reduction in pain duration in the first phase of the formalin test which was 60.5%, 75% and 82.7%, respectively, compared to the control group ( $P < 0.05$ , Figure 1). Doses of 100, 300, 900 and 1350 mg / kg of the aqueous extract caused a significant reduction in pain duration in the second phase of formalin test which was 43.4%, 91.9%, 88.7% and 86.6%, respectively, compared to the control group ( $P < 0.05$ , Figure 1). Morphine also caused a significant reduction in pain duration in the first and second phases of formalin test which was 90.8% and 96.2%, respectively, compared to the control group ( $P < 0.05$ , Figure 1). Sodium salicylate caused a significant reduction of 83.6% in pain duration in the second phase of formalin test compared to the control group ( $P < 0.05$ , Figure 1).

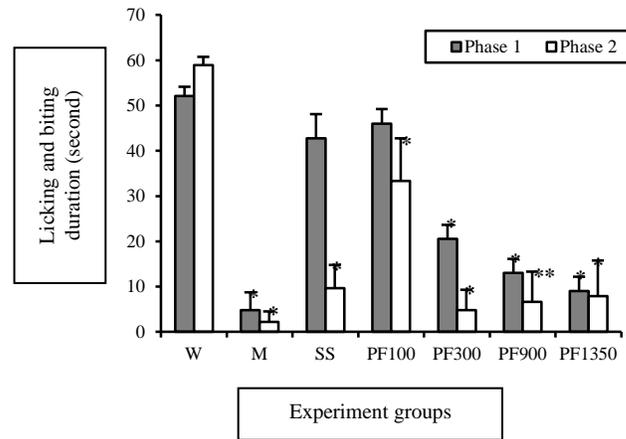


Figure 1: The effect of intra-peritoneal administration of different doses of the aqueous extract of *Prangos ferulacea* (PF) (100, 300, 900 and 1350 mg/ kg), sodium salicylate (SS) with dose of 200 mg/ kg, morphine (M) with dose of 10 mg/ kg, and water (W) on the licking and biting duration in the first and second phases of formalin test in male mice.

The columns represent the mean + standard error of the mean in each group (N = 9).

\* P <0.05 compared with the group receiving water.

\*\* P <0.001 compared with the group receiving water.

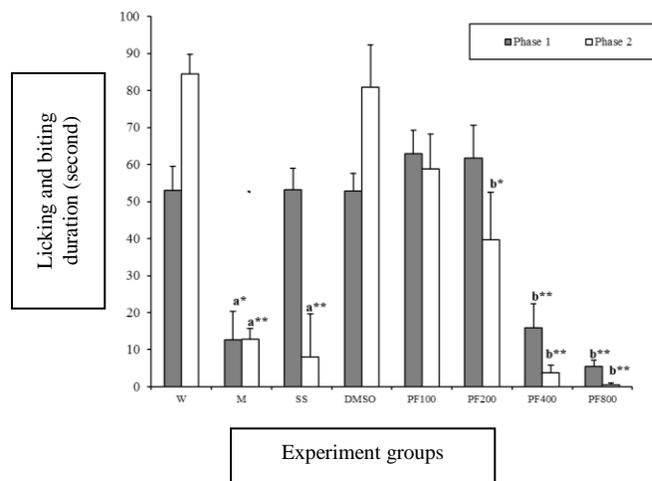


Figure 2: The effect of intra-peritoneal administration of 100, 200, 400 and 800 mg/ kg of methanol extract of *Prangos ferulacea* (PF), sodium salicylate (SS) with dose of 200 mg/ kg, morphine (M) with dose of 10 mg/ kg, water (W) and 20% DMSO on the licking and biting duration in the first and second phases of formalin test in male mice at 30 minutes after administration.

The columns represent the mean + standard error of the mean of licking and biting duration.

a\* P <0.05 significant difference compared with the group receiving water.

a\*\* P <0.001 significant difference compared with the group receiving water.

b\* P <0.05 significant difference compared with the group receiving DMSO.

b\*\* P <0.001 significant difference compared with the group receiving DMSO.

## Discussion:

Considering the use of Ferulacea plant as an analgesic in the traditional medicine, this study was conducted to investigate the analgesic effect of this plant. The results showed that the aqueous and methanol extracts of Ferulacea plant have analgesic effects.

In order to assess the analgesic effect of Ferulacea formalin test was used in this study [8]. The advantage of formalin test in comparison with other pain causing tests is that the induced pain is more continuous rather than being transient; hence, it is more similar to the clinical and inflammatory pains. The pain induced in the formalin test has two distinct phases. The first phase which begins immediately after formalin injection in the animal's paw and lasts for about 5 minutes is the result of direct stimulation of pain receptors and the activity of C nerve fibers while the second phase which lasts from the 15th to 30th minutes is due to an inflammatory process; however, the role of sensitization of central pain pathways should also be considered. This test can to some extent propose the probable mechanism of analgesic effect of the study materials. The opioid analgesics that often act centrally inhibit both phases of formalin by approximately the same intensity [8]. While nonsteroidal anti-inflammatory or aspirin-like drugs act preferably in the periphery, have the most effect in reducing inflammatory pain in the second phase of formalin test [8]. Sodium salicylate which is an aspirin like drug and was used as the positive control in this study, consistent with previous studies, resulted in a significant decrease in pain duration in the second phase of the formalin test. Morphine which is a well-known analgesic with central effect and was used as the positive control in this study reduced pain duration in the first and second phase of formalin test which is consistent with other studies.

The study of analgesic effect of Ferulacea showed the effect of aqueous and methanol extracts of this plant in reducing formalin-induced pain. The study of the effects of aqueous extract shows that the analgesic effect of the extract increases in the first phase of formalin test by increase in the dose, so that the analgesic effect of the extract at the highest dose which is 1350 mg/kg is close to the effect of 10mg/kg of morphine. The effect of aqueous extract in reducing pain in the second phase reaches its maximum at the dose of 300 ml / kg and then the increase in the dosage makes no change in the analgesic effect in this phase. The analgesic effect of the extract in the second phase is comparable with sodium valproate and morphine. According to these findings, it seems that the aqueous extract at low doses acts peripherally and in higher doses when higher amounts of its effective components enter the central nervous system, the effect of the extract in the first phase is increased. In other words, the analgesic effects of the extract occur with the central mechanisms by increased dosage. Thus, it could be proposed that the extract contains compounds with both peripheral and central analgesic effects.

The study showed that low doses of methanol extract of Ferulacea only have effects on the second phase as well and in high doses cause similar pain relief in both phases of formalin test. It had similar effects comparable to morphine even at the highest dose in the study. Therefore, it can be proposed that extracts provides both peripheral and central analgesic effects.

The results of this study showed that both aqueous and methanol extracts have analgesic effects. Both of the extracts act with central mechanism at the highest dose of the study and similarly reduce pain in both first and second phases. Since the central analgesic effect of methanol extract appeared at lower dosage (800 mg/kg) than

the aqueous extract (1350 ml / kg), it can be proposed that the level of the analgesic compounds acting centrally is higher in methanol extract while the components that act peripherally are more in the aqueous extract.

**Conclusion:** In a literature review, no information was found about the analgesic effects of this specie or other species of the *Prangos* genus to compare with the results of this study or attribute the observed effects to a specific probable combination. However, the phytochemical studies indicate the presence of saponin compounds, nitrogen groups and tannins in the aqueous extract

and saponin, anthraquinone, tannins and flavonoids in the methanol extract. According to the studies showing the analgesic effects of saponins compounds, tannins or flavonoids available in plants, it is possible that the analgesic effects of *Ferulacea* is due to tannins, saponins or flavonoids; however, further investigation are needed to provide more evidences [10-13].

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