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RESEARCH ARTICLE

# Effect of Afghan Ferula assa-foetida L. oleo Gum Resin Aqueous Extract on Withdrawal Signs in Morphine-Dependent Rats

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## ABSTRACT

*Aim:* To investigate the effect of the aqueous extract of Afghan Ferula assa-foetida L. oleo gum resin on the withdrawal signs in morphine-dependent rats.

*Methods:* This study was carried out in 2018. Rats were divided into five groups (one Normal Saline, one Morphine, and three Extract groups). The rats that received morphine and extract became morphine-dependent by subcutaneous (s.c.) administration of morphine hydrochloride for 7 days (2.5, 2.5, 5, 10, 20, 40 mg/kg doses twice daily from first to sixth day, respectively, and a single dose of 50 mg/kg on seventh day). The extract groups received 50, 75, and 100 mg/kg doses of the Ferula assa-foetida L. oleo gum resin aqueous extract by intraperitoneal (i.p.) administration simultaneously with morphine. Naloxone (3 mg/kg, i.p.) was injected 2 h after the administration of the last dose of morphine, and withdrawal signs were noted for 30 min.

*Results:* The results showed that the administration of the Ferula assa-foetida L. oleo gum resin aqueous extract (50, 75, and 100 mg/kg) significantly decreased most of the naloxone induced withdrawal signs in morphine-dependent rats ($P < 0.05$).

*Conclusion:* Afghan Ferula assa-foetida L. oleo gum resin aqueous extract even in the lowest doses can alleviate the withdrawal signs in morphine-dependent rats.

KEYWORDS:
Afghanistan; Ferula assa-foetida L.; morphine dependence; oleo gum resin; substance withdrawal signs

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## INTRODUCTION

The use of medicinal plants in the opioid dependency and withdrawal signs treatment is considered as a reasonable option.[1,2] The efficacy of numerous plants, such as Ferula assa-foetida in the alleviation of morphine withdrawal signs is proved. Studies showed that different parts of Ferula assa-foetida, including aerial parts, flower, and root can decrease the withdrawal signs in morphine-

dependent mice[3]. However, despite the presence of effective compounds in Ferula assa-foetida oleo gum resin and its therapeutic properties,[4] there is no evidence of the effect of Ferula assa-foetida oleo gum resin on morphine withdrawal signs. This study aimed to evaluate the effect of aqueous extract of Afghan Ferula assa-foetida oleo gum resin on withdrawal signs in morphine-dependent rats, using the behavioral method.

Opioid dependency and its consequences are usually considered as the most important social and health problems[5]. Repetitive consumption of opioid drugs such as morphine, for alleviating pain or for pleasure, activates motivation and rewards related mechanisms in the central nervous system. These mechanisms lead to long-term adaptive changes in brain nuclei and neurons. Tolerance and dependency are the consequences of these adaptive changes.[5–7]

Although the molecular mechanisms of morphine dependence and withdrawal signs are still unknown, it has been proposed that repetitive use of morphine causes adaptation in multiple neurotransmitter systems across several brain regions, thereby developing behavioral and neurochemical consequences of them.[8] For example, we can point to dopaminergic,[9] glutamatergic,[10] nitric oxide,[7] orexinergic,[11] GABAergic,[12] and serotonergic systems.[13]

From the past until now, the treatment of dependency has been proposed as a serious problem. Medicinal plants have attracted a wide range of attention in the treatment of drug dependency because of their fewer side effects and costs rather than synthetic drugs.[1,14,15] Ferula assa-foetida is a herbaceous, perennial, and monocarpic plant from the Apiaceae family, found in Kashmir, Iran, and Afghanistan.[4,16] Its oleo gum resin is found in two forms: tears and mass form, however, the mass form is common.[4] The important constituents of Ferula assa-foetida oleo gum resin are resin (40–46%), gum (25%), and essential volatile oils (10–17%). The resin fraction includes ferulic acid and its esters, coumarins, sesquiterpene coumarins, and other terpenoids. The gum includes glucose, galactose, L-arabinose, rhamnose, glucuronic acid, polysaccharides, and glycoproteins. The volatile fraction contains sulfur-containing compounds, monoterpenes, and the other volatile terpenoids.[4,17]

Ferula assa-foetida has multiple uses in traditional and modern medicine.[4] The pharmacological effects of Ferula assa-foetida oleo gum resin includes anti-hyperglycemic,[18] anti-hyperlipidemic,[19] anti-cancer,[20] anti-oxidant, anti-epileptic,[21] enhancing kidney functions,[22] anti-nociceptive, and anti-inflammatory.[17,23]

The previous study has evaluated the effect of different parts of Ferula assa-foetida, including aerial parts, flower, and root on morphine withdrawal signs.[3] However, despite the presence of effective compounds in Ferula assa-foetida oleo gum resin and its therapeutic properties,[4] there is no evidence of the effect of Ferula assa-foetida oleo gum resin on morphine withdrawal signs. This study aimed to evaluate the effect of aqueous extract of Afghan Ferula assa-foetida oleo gum resin on withdrawal signs in morphine-dependent rats, using the behavioral method of counting the naloxone-induced withdrawal signs.

## MATERIALS AND METHODS

The required materials and methods of this study are as the following.

**Materials:** The main materials and instruments of this study include morphine hydrochloride (idol ilaç Dolum San. and Tic. A.Ş. Topkapı-Istanbul), Naloxone hydrochloride (toliddaru Pharma. Co. Tehran-Iran), Ferula assa-foetida oleo gum resin (Temran, Daikundi, Afghanistan), and glass cylinders.

**Animals**. The study subjects were, 30 adult Wistar rats weighing between 150 and 200 g, randomly selected from Khatam Al-Nabieen University Research and Technology Center (KNURTC). They were housed in plexy-glass cages with free access to food and water. Animals were kept under stable room temperature (23 ± 2°C) for a 12-h light/dark cycle (the light period started at 7 a.m.). The experimental protocol was approved by the ethic research board of Khatam Al-Nabieen University and was conducted in accordance with the ethical guidelines set by the eighth edition of the National Institute of Health (NIH) guide for the care and use of laboratory animals. Rats were carefully handled to minimize unwanted stress during housing and experiments.

**Experimental groups.** The study was carried out in 2018. The rats were randomly divided into 5 groups (n = 6). Group 1, saline-treated rats; Group 2, morphine-treated rats received naloxone to evaluate morphine withdrawal signs; In Groups 3–5, rats received Ferula assa-foetida oleo gum resin extract (50, 75 and 100 mg/kg doses) by intraperitoneal (i.p.) administration simultaneously with morphine. They also received naloxone 2 h after the last morphine administration.

**Preparation of extract:** The mass form of Ferula assa-foetida oleo gum resin was collected from Temran, Daikundi province of Afghanistan in the early summer of 2018. The oleo gum resin was powdered and 10 mg of its powder was soaked in 100-ml distilled water overnight, then filtered.[17]

**Induction of morphine dependency.** To induce morphine dependency, rats received a subcutaneous injection of morphine hydrochloride for 7 days in 13 times. The doses of morphine were 2.5, 2.5, 5, 10, 20, and 40 mg/kg twice daily on first to sixth day, respectively, and a single dose of 50 mg/kg on seventh day.[24]

**Induction and monitoring of morphine-withdrawal signs.** Morphine withdrawal signs were induced by injection of Naloxone (3 mg/kg, i.p.) 2 h after the last morphine injection. Thereafter, six distinct withdrawal signs (jumping, teeth chattering, rearing, paw tremor, chewing, and penis licking) were monitored in a glass cylinder during a 30-min period. After the placing of each rat in the cylinder, the intra-cylinderal sawdust was changed to remove all smell cues.

**Statistical analysis.** The statistical data analysis was performed using Graph Pad Prism 6.07 software. The morphine withdrawal signs recorded in different experimental groups

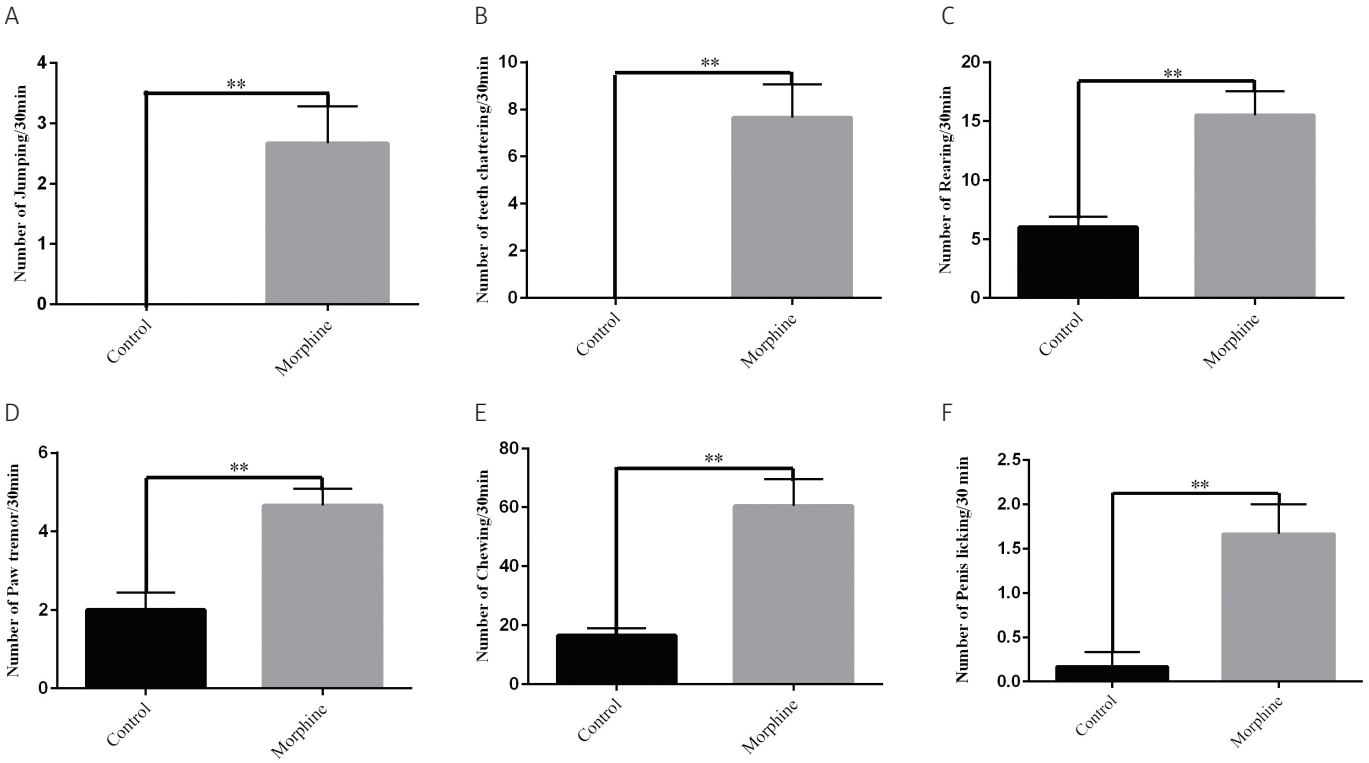


**Figure 1** Naloxone-induced morphine withdrawal signs in morphine-dependent rats. The number of various withdrawal signs, including jumping, teeth chattering, rearing, paw tremor, chewing, and penis licking (A–F) was significantly increased in the morphine group as compared with the control group. Data were expressed as mean ± SEM. **: $p < 0.01$.

of animals were statistically analyzed by unpaired, two-tailed t-test or the non-parametric Mann–Whitney U and Kruskal–Wallis tests when counts were not normally distributed. Multiple comparisons performed by the One-Way ANOVA test. The difference among means was considered statistically significant if $P < 0.05$. The results are expressed as mean ± SEM.

## RESULTS

In this study, morphine-treated rats showed morphine withdrawal signs following the intraperitoneal injection of naloxone hydrochloride (3 mg/kg). There was a significant difference in the number of jumping, teeth chattering, rearing, paw tremor, chewing, and penis licking among control and morphine groups (Figure 1). Also, the number of jumping, teeth chattering, rearing, paw tremor, and chewing was significantly decreased in each 50, 75, and 100 mg/kg extract groups as compared with the morphine group. However, there is no significant difference between the number of penis licking in 50 and 75 mg/kg extract groups and morphine groups, whereas the number of this sign was significantly decreased in 100 mg/kg extract group as compared with morphine group (Figure 2).

## DISCUSSION

In this study, the effect of aqueous extract of Ferula assa-foetida oleo gum resin on withdrawal signs in morphine-dependent rats was evaluated. First, the results showed that the number of some distinct withdrawal signs including jumping, teeth chattering, rearing, paw tremor, chewing, and penis licking was significantly increased in the morphine group. That is, the injection of morphine hydrochloride for a 7 days period, induced morphine dependency in rats. In addition, the aqueous extract of Ferula assa-foetida oleo gum resin could significantly decrease the withdrawal signs in morphine-dependent rats. The 50, 75, and 100 mg/kg doses of the extract could decrease the most withdrawal signs including jumping, teeth chattering, rearing, paw tremor, and chewing. However, the 50 and 75 mg/kg doses did not have a significant effect on the number of penis licking. Whereas, the 100 mg/kg dose of the extract could significantly decrease the number of penis licking as well. Therefore, one can conclude that even the lowest doses of the aqueous extract of Ferula assa-foetida oleo gum resin are effective in the reduction of morphine withdrawal signs. But its high dose (100 mg/kg) is the most effective dose in the reduction of naloxone induced withdrawal signs in morphine-dependent rats.

Previous studies have shown that the different parts of Ferula assa-foetida including aerial parts, fruit, and root can decrease the morphine withdrawal signs. It is concluded that this effect is related to the presence of different constituents such as ferulic acid, cafeic acid, α- and β-pinenes and other terpenoids and their effects on inhibitory neurotransmitter systems.[3]

It is well known that the Locus ceoroleus (LC) in the most sensitive site for the induction of motor aspects of opioids withdrawal as the activity of LC neurons aggregates during opioid withdrawal. Inhibition of LC neurons activity leads to a reduction of withdrawal signs.[11,25,26] Also, studies have shown that free radicals, including nitric oxide, are considered as endogenous modulators of long-term opioid effects. There

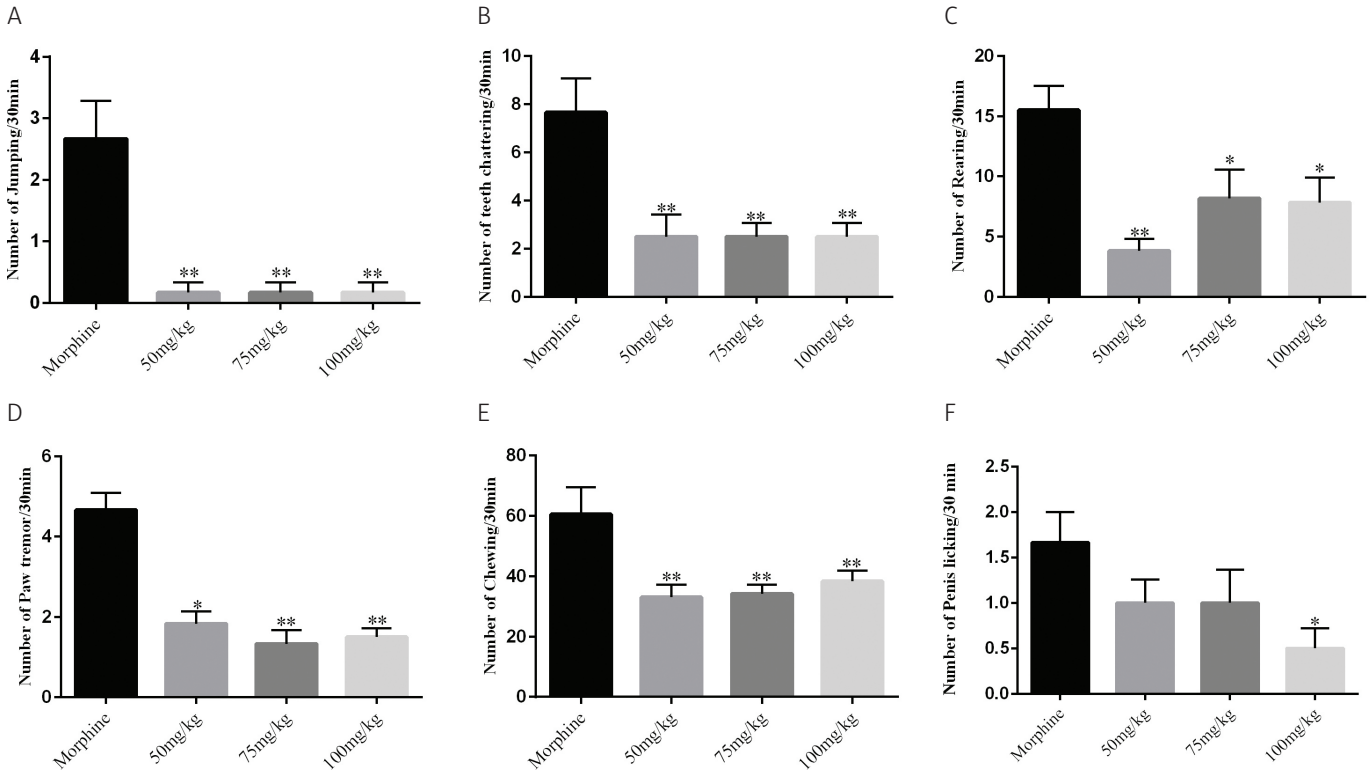


**Figure 2** Effect of Ferula assa-foetida oleo gum resin aqueous extract (50, 75, and 100 mg/kg) on the naloxone-induced withdrawal syndrome in morphine-dependent rats. The number of various withdrawal signs, including jumping, teeth chattering, rearing, paw tremor, chewing, and penis licking (A–F) was significantly decreased in extract groups, as compared with the morphine group. Data were expressed as mean ± SEM. *: $p < 0.05$, **: $p < 0.01$.

are three isoforms of nitric oxide synthase which form nitric oxide: endothelial (eNOS), neuronal (nNOS), and inducible nitric oxide synthase (iNOS). There are some reports shown that nitric oxide synthase inhibitors may have potential therapeutic effects in the opioid withdrawal of humans. LC has significant amounts of nitric oxide signaling proteins and its activity is modulated by nitric oxide pathway as the administration of nitric oxide synthase inhibitors in LC leads to a reduction in opioid withdrawal signs.[27-29]

In addition, there is an interaction between the GABAergic system and the LC.[30] LC is under inhibitory control of GABA, so agonists of both GABA-A and GABA-B receptors reduce the opioid withdrawal signs. The agonist of the GABA-B receptor has a greater effect on the reduction of withdrawal signs.[26]

On the other hand, studies have shown that ferulic acid can inhibit nNOS and iNOS.[31] Also, this compound can increase the expression of GABA-B receptor subunit 1.[32] The cafeic acid isolated from Ferula assa-foetida, can inhibit the LPS-induced nitric oxide production.[33]

As the large percent of Ferula assa-foetida oleo gum resin is constituted by resin compounds such as ferulic acid and also cafeic acid,[4,16] it is probable that the positive effects of Ferula assa-foetida oleo gum resin extract like the other parts of the plant, maybe due to these compounds. Therefore, one can conclude that Ferula assa-foetida oleo gum resin extract by inhibition of nitric oxide synthase and inhibition of nitric oxide production, as well as effects on GABAergic system, can inhibit the hyperactivity of LC neurons during morphine withdrawal. As a result, the morphine withdrawal signs decreased significantly following the administration of Ferula assa-foetida oleo gum resin extract.

In addition, many studies have shown that inflammatory processes and oxidative stress are greatly aggregated during morphine withdrawal. Also, various medicinal plants decrease the withdrawal signs, because of their anti-inflammatory and anti-oxidative properties.[1,34] As it has been shown that Ferula assa-foetida oleo gum resin and its constituents, especially ferulic acid have anti-inflammatory and anti-oxidative properties.[21,23] Therefore, probably the effect of Ferula assa-foetida oleo gum resin aqueous extract on the reduction of withdrawal signs in morphine-dependent rats also can be due to the effect of Ferula assa-foetida oleo gum resin aqueous extract and its constituents on inflammatory processes and oxidative stress.

## CONCLUSION

In summary, one can conclude that aqueous extract of Afghan Ferula assa-foetida oleo gum resin reduces the withdrawal signs in morphine-dependent rats. The positive effects of this extract may be due to the effect of its constituents on nitric oxide and GABAergic systems as well as inhibition of inflammatory and oxidative stress processes.

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