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Original Research

Phytochemical Evaluation and Antinociceptive Effect of the Extract of *Ferula persica* Willd. Oleo-gum-resin

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Abstract

Ferula persica Willd. is a native plant of Iran known for its anti-spasmodic, analgesic, and anti-inflammatory properties in Persian medicine. This study investigated the antinociceptive effects of the oleo-gum-resin extract of *F. persica* in mice, as well as its chemical composition. The hydroalcoholic extract of *F. persica* oleo-gum-resin was prepared using the maceration method. Mice were randomly divided into six groups: a normal saline group (negative control), a dimethyl sulfoxide group, three groups receiving different doses of the hydroalcoholic extract (50 mg/kg, 100 mg/kg, and 200 mg/kg), and a sixth group (positive control) receiving Ketoprofen. To assess the analgesic effects of the extract, four tests were conducted: writhing, hot plate, tail flick, and formalin tests. Additionally, the chemical compounds, total phenolic content, and total flavonoid content of the oleo-gum-resin extract were determined. The findings demonstrated that the extract at doses of 50, 100, and 200 mg/kg effectively reduced pain and the analgesic effect was dose dependent. A significant difference was observed between the control and treatment groups across all four tests. The 200 mg/kg dose exhibited the greatest analgesic effect in both the acute and chronic phases of the formalin tests. Some chemical compounds of the gum such as flavonoids may be responsible for its analgesic effect. According to this study, *F. persica* gum extract can alleviate pain caused by thermal and chemical stimuli. These findings support the traditional use of *F. persica* gum in managing painful conditions. Further studies are needed to investigate effectiveness of *F. persica* oleo-gum-resin in pain management and the possible mechanisms of its analgesic effects.

Keywords: *Ferula persica;* Antinociceptive*;* Hot plate; Gum; Pain

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Introduction

Pain is an undesirable emotional and sensory experience that often serves as the primary symptom for diagnosing various diseases. It is associated with potential or actual tissue damage and prompts individuals to respond by eliminating the source of the pain causing stimulus [1,2].

Millions of people worldwide suffer from various types of pain and seek more effective medications with fewer side effects for relief [2]. Pain is categorized into two main types: fast (acute) and slow (chronic), and is considered one of the five vital signs, potentially leading to negative consequences. Opioids, combined pain relievers, non-steroidal anti-inflammatory drugs, and topiramate are among the most critical painkillers with adverse effects such as gastrointestinal irritation, resistance, and dependence [3-7]. Due to the potential side effects and lack of efficiency of standard treatments to control pain in some cases, the use of complementary medicines and herbal therapies is increasing [8]. There are some plants in traditional medicine which exhibit analgesic effects due to various chemical compounds, including flavonoids (like quercetin), phenolic compounds, alkaloids, and organic acids (such as caffeic and rosmarinic acid) [9-11].

Ferula persica Willd. (Umbelliferae family), particularly its oleo-gum-resin, has been used in Persian medicine for pain and inflammation. The genus *Ferula* includes 30 species, with 15 endemic plants in Iran. Various *Ferula* species, demonstrated anticonvulsant, analgesic, anti-inflammatory, and muscle-relaxing properties [12,13]. *F. persica*, known as "*Sakbinag*" in Persian medicine, is a perennial herb native to Iran, Türkiye, and Afghanistan, reaching heights of 1-2 meters and featuring large, delicate leaves [14]. It contains sesquiterpene coumarins, and active components like auraptene, umbelliprenin, and galbanic acid which showed antihypertensive, anti-inflammatory, anticancer, and antitumor effects [15,16]. Recent studies have reported its potential to reduce morphine side effects, as well as its antimicrobial, antitumor, and hypoglycemic properties [17,18]. Also, antinociceptive effect of the aerial part of *Ferula persica* has been investigated in animal study [19].

According to our investigations, there is no study on the antinociceptive effect of *Ferula persica* oleo-gum-resin in animal models. Given the traditional use of its gum in Persian medicine for treating pain and inflammation, along with the documented analgesic effects of the gum-resin from other *Ferula* species, the present study aims to investigate the antinociceptive effect of the oleo-gum-resin of *F. persica* in various pain models in mice . Also, the chemical composition of the *F. persica* oleo-gum-resin, along with the phenolic and flavonoid content of the extract, was evaluated.

Materials and Methods

Plant material

F. persica oleo-gum-resin was purchased from the medicinal plants market, and authenticated by Dr. G. Amin, Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, and deposited by voucher No. PMP-1858.

Preparation of the hydroalcoholic extract

F.persica oleo-gum-resin (400 g) was macerated with 70% ethanol (3 L ×4) at room temperature and concentrated by rotary evaporator in low temperature (40 °C). The dried extract (HEFG) was used for animal testing. The chemical compounds of *F. persica* oleo-gum-resin were investigated using phytochemical methods [16,20].

Phytochemical analysis

The phytochemical components of the hydroalcoholic extract of *F. persica* oleo-gum-resin were assessed using both qualitative and quantitative methods. This study evaluated the presence of steroids, triterpenoids, saponins, alkaloids, flavonoids, and tannins. Additionally, the total phenolic and flavonoid contents of the extract were measured [20-22]. Preliminary qualitative phytochemical screening was conducted using the methods of previous studies for terpenoids, alkaloids, saponins [20], flavonoids, tannins and cyanogenic glycosids [21,22]

Determination of total flavonoid content

The total flavonoid content (TFC) of HEFG was assessed according to aluminum chloride colorimetric assay. Quercetin was used as standard for the calibration curve. Briefly, 1 mL of quercetin as standard or other samples (dissolved in 90% ethanol), were thoroughly mixed with 0.2 mL of a 5% $NaNO_2$. After 5 min, 0.3 mL of 3% $AlCl_3$ solution and 2 mL of NaOH 2 M were added. The absorbance was determined after 30 min at 510 nm versus a blank. A linear calibration curve was drawn using quercetin ($y = 0.0169x + 0.3526$, $r^2 = 0.995$). It was used for calculating of TFC of each sample [23].

Determination of total phenolic content

Total phenolics content (TPC) of HEFG was calculated using Folin-Ciocalteu reagent [24]. The calibration curve was prepared by mixing 1 mL of HEFG or gallic acid solutions in concentrations of 75, 100, 150, and 200 mg/mL (50:50) with 5 mL Folin-Ciocalteu reagent (diluted 10-fold) and 4 mL sodium carbonate (75 mg/mL). The absorption was measured at 765 nm, after 30 min. The standard agent for the calibration curve was gallic acid. The same process and reagents were used for the gum extract (10 mg/mL) and the absorption

was measured. TPC was determined as mg gallic acid equivalents per gram of sample (mg/g).

Animals

Male albino mice (n=36, weight 25-30 g) were housed in animal unit of Iran University of Medical Sciences under standard laboratory conditions (temperature 23 ± 2 ˚C) with 12-h dark and 12-h light cycle. The animals were fed with standard diet and water as needed. The experiment was approved by the Ethics and Animal Care Committee of the Iran University of Medical Sciences (IR.IUMS.REC.1400.541).

Experimental studies

Total of 36 albino mice were randomly divided into 6 groups (n=6). In this study, the extract and drugs were injected intraperitoneally (i.p.). Negative control group received normal saline, dimethyl sulfoxide (DMSO) group in which animals were given DMSO as the solvent of the extract, groups 3-5 in which mice received 50, 100, 200 mg/kg of HEFG, and positive control group was treated by Ketoprofen 10 mg/kg.
The particular doses of HEFG and route of administration were selected according to a previous study with some modifications [14].

Hot plate test

The test was conducted as stated by Lanhers, (1992) and Williamson, (1996). A hot plate preserved at temperature of 50 ± 1°C was used. Thirty minutes after drug or extract injections, the mice were located on this plate [25,26]. The latency time for paw licking or jumping (pain reaction time in seconds) was recorded 30, 60, 120 and 180 minutes after injection for each mouse in different groups [27].

Acetic acid-induced writhing test

The test was done according to Koster et al. (1959) technique. In the groups one and two, mice were given normal saline and DMSO. Groups III, IV, and V received 50, 100, and 200 mg/kg of HEFG respectively, whereas the sixth group was given ketoprofen 10 mg/kg in i.p. Thirty minutes later, each mouse received 1% acetic acid (1 mL per 100 g) [28].
The number of abdominal constriction for each mouse was calculated after injection of acetic acid for ten minutes. Inhibition of writhing (%) was measured consuming the standard formula.

$$\text{Inhibition } \% = \frac{\text{Mean no of writhes (control)} - \text{mean no of writhes (test)}}{\text{Mean no of writhes (control)}} \times 100$$

Tail-flick test

Tail flick latency (TFL) test was done with an automatic analgesiometer (tail flick; Borj Sanat, Tehran, Iran). The TFLs were calculated as the period between tail contact to radiant heat and tail removal. The main reaction of animals to radiant heat is by inserting the tip (last 1 to 2 cm) of the tail. Withdrawing the tail from the radiant heat source is considered as the end point. The maximum time was 10 seconds to avoid damage to the animal's tail due to heat. The latent period of the tail flick response was determined 30, 45, 60, 75 and 90 minutes after drug administration [29].

Formalin test

In this test, 25 µL formalin (2.5%) is injected under the plantar surface skin of the right dorsal paw. Excessive licking or biting of the injected paw as the pain reaction time is recorded. This test has two phases, the first interval (0-15 minutes) regarding as the acute phase and the second interval (15-60 minutes after injection) as the chronic phase.
Pain reactions were scored as follows: 0, normal movement by injected mouse; 1, the injected foot has little or no weight moving on it; 2, the injected foot is raised by the mouse; and 3, the injected foot is being licked or bitten by the mouse [30]. The number of these quantitative data is counted in 12 blocks of 5 minutes. Data recording continued until 60 minutes after formalin injection.The average pain score in each block was calculated according to the following formula [31,32].

$$Pain\ score = \frac{0T0 + 1T1 + 2T2 + 3T3}{300}$$

Statistical analysis

One-way and Two-way analysis of variance (ANOVA) followed by Tukey's-Krammer, multiple comparison tests, were used to compare the differences between various treatment groups (treatment vs. time/ number/pain score) using GraphPad Prism 5.01 (San Diego, CA). The sample size was determined according to the following formula:
E=Nt-Ng (E: degree of freedom, Nt: the total number

of animals, Ng: the total number of groups. The goal is to retain E between 10 and 20 for an acceptable sample size.)[33].
As we had 6 mice in 6 groups, sample size of 36 has been adequate for this study. A statistical probability of $p<0.05$ was considered significant. Data were reported as Mean ± SEM.

Results

Phytochemical analysis results

Phytochemical studies of *F. persica* oleo-gum-resin showed the presence of flavonoids, cyanogenic glycosides, saponins, and steroids, but it did not contain any alkaloids and tannins.

Total phenolic and flavonoid content

The TPC was calculated using gallic acid and expressed as mg gallic acid equivalent (GAE)/g of extract . The TPC of *F. persica* oleo-gum-resin was 5.76±0.1 mg GAE/g of extract. TFC of the oleo-gum-resin extract was measured as mg quercetin equivalents (QE)/ mg of sample. The TFC of the extract was 1.21±0.5 g QE/g dried extract.

Effect of different doses of HEFG on hot plate-induced pain

In the hot plate test, Ketoprofen group revealed a significant difference ($p<0.0001$) with the control and DMSO groups at all times. The doses of 50, 100 and 200 mg/kg of HEFG had significant differences ($p<0.0001$ for 100 , 200 and $p<0.05$, $p<0.01$, $p<0.001$ for 50) at all times compared with the control group (Figure 1).

Effect of different doses of HEFG on acetic acid-induced writhing

The acetic acid writing test (Figure 2) showed that the Ketoprofen group had a significant difference from the control group ($p<0.0001$). Although, doses of 50, 100 and 200 mg/kg of HEFG had significant difference compared to the control group ($p<0.0001$). We also evaluated the pain inhibition percentage in different groups and compared with each other (Table 1).

Effect of different doses of HEFG on Tail flick latency

In the Tail Flick test, data showed that the Ketoprofen group had a significant difference with the control and DMSO groups at all times ($p<0.0001$). In the treatment groups, the doses of 50, 100 and 200 mg/kg showed significant differences at all times compared to the control group ($p<0.0001$) (Figure 3).

Table 1. Pain inhibitory percentage of *Ferula persica* extract and ketoprofen in acetic acid-induced writhing test

Pain inhibition (%)	Groups
2%	Control
87%	Ketoprofen
3%	DMSO
16%	Dose of 50 mg/kg
37%	Dose of 100 mg/kg
81%	Dose of 200 mg/kg

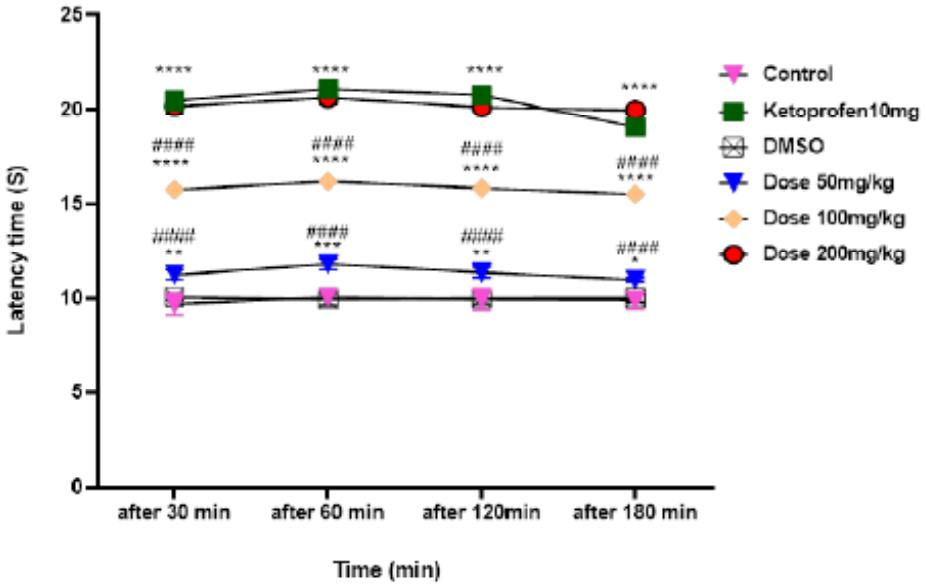

Figure 1. The effect of different doses of *F.persica* gum extract on Hot plate-induced pain test after 30, 60, 120, 180 min. The values were presented as Mean ± SEM. (*p<0.05,**p<0.01,***p<0.001, ****p<0.0001 compared with the control group, ####p<0.0001 compared to Ketoprofen).

Effect of different doses of HEFG on formalin-induced acute pain in male mice

In the acute-phase formalin test, it can be seen that the Ketoprofen group had a significant difference from the control group ($p<0.0001$). In the treatment groups, there was also a significant difference between the doses of 50, 100 and 200 mg/kg of HEFG and the control group ($p<0.0001$) (Figure 4).

Effect of different doses of HEFG on formalin-induced chronic pain in male mice

In the chronic-phase formalin test, it can be seen that the Ketoprofen group had a significant difference ($p<0.0001$) with the control group. The doses of 100 and 200 mg/kg of HEFG were significantly different from the control group ($p<0.0001$). Consequently,

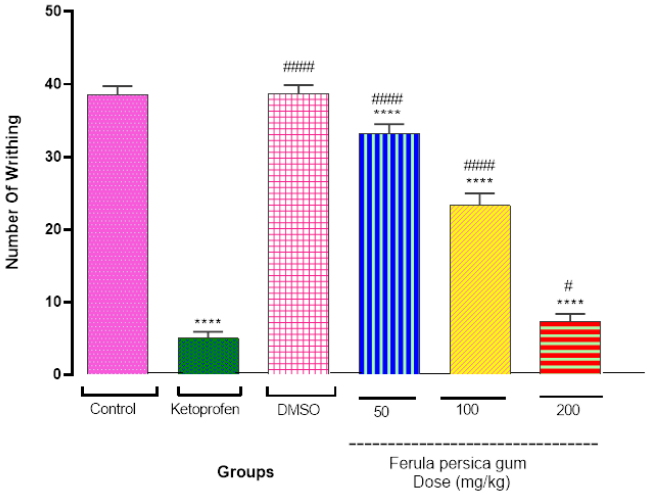

Figure 2. The effect of different doses of *F.persica* gum extract on Acetic acid-induced writhing. The values were presented as Mean ± SEM. (****p<0.0001 compared with the control group, #p<0.05, ####p<0.0001 compared to Ketoprofen).

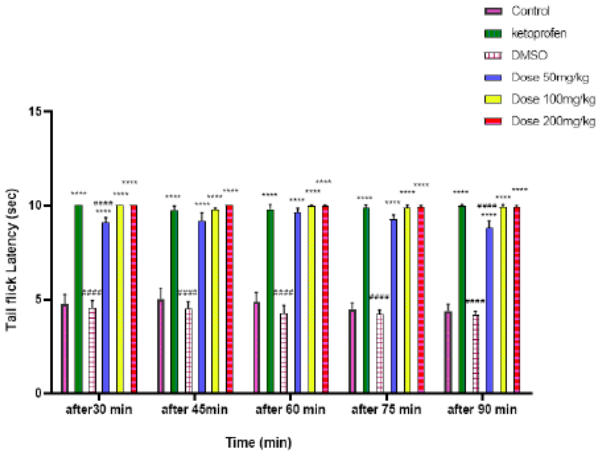

Figure 3. The effect of different doses of *F.persica* gum extract on Tail flick latency. The values were presented as Mean ± SEM. (****p<0.0001 compared with the control group, ####p<0.0001 compared to Ketoprofen).

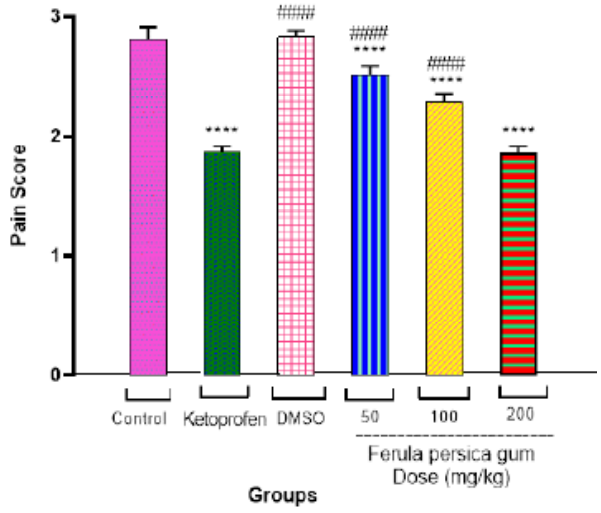

Figure 4. The effect of different doses of *F.persica* gum extract on Formalin-induced acute pain. The values were presented as Mean ± SEM. (****p<0.0001 compared with the control group, ####p<0.0001 compared to Ketoprofen).

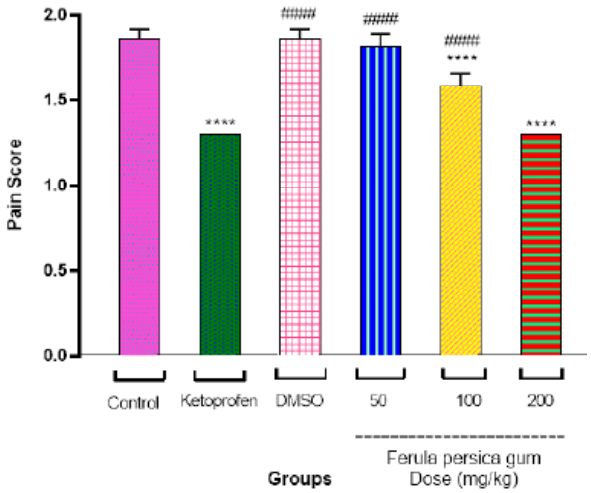

Figure 5. The effect of different doses of *F.persica* gum extract on Formalin-induced chronic pain. The values were presented as Mean ± SEM. (****p<0.0001 compared with the control group, ####p<0.0001 compared to Ketoprofen).

there was not a significant difference between the dose of 50 mg/kg of HEFG and the control group ($p>0.05$) (Figure 5).

Discussion

Due to side effects of analgesic drugs and efficacy of medicinal plants in pain and inflammation in traditional medicine, in the present study antinociceptive effect of *F. persica* oleo-gum-resin was investigated on different models of pain. The findings revealed that HEFG exerted significant analgesic effects in different doses (50, 100, 200 mg/kg) in comparison to the control group.

The hot plate test is a selective test for opioid-like compounds, but other drugs which act centrally, such as hypnotics and muscle relaxants, show similar effects in this test. This test examines the analgesic response to fast-acting stimuli; while the formalin test assessed the response to delayed pain stimuli and is closer to the clinical cases [34]. Also, the formalin test is widely used in rodents as a model of acute and chronic chemical pain to identify drugs with analgesic potential [31]. Toe licking induced by formalin is an acceptable two-phase chemical pain model: the first phase is neurogenic pain, which is caused by direct stimulation of sensory afferent fibers and is followed by the second phase, i.e., inflammatory pain, which is different due to the effect of inflammatory mediators [35]. Many studies have shown that both phases are sensitive to drugs with significant impacts, such as opioids. In addition, the second phase is delicate to non-steroidal anti-inflammatory drugs and corticosteroids [36].

Antinociceptive effect of HEFG was significant in hot plate and formalin test and it was consistent with a previous study on the analgesic effect of the aerial parts of this plant [14]. Interestingly, the decreased pain score (at early and late phase) by oleo-gum-resin extract in the present study was similar to the ketoprofen as a standard analgesic drug. These data specified that this extract might lessen the production and release of endogenous inflammatory intermediaries [37].

Furthermore, in the formalin test, HEFG reduced the pain score at the early phase (neurogenic phase), which showed that the drug activity was directly on nociceptors. In the same way, at the late phase (inflammatory phase), the oleo-gum-resin extract might act by inhibiting discharge of inflammatory intermediaries, practically the same as Ketoprofen. Reducing pain in hot plate and also both phases of formalin test is related to central and peripheral analgesic activity of this extract [37].

The tail flick method is also one of the standard methods of measuring acute pain in laboratory animals, which was used to determine the central analgesic effects of drugs and chemical compounds [38]. In fact, drugs that act on the central nervous system are sensitive to this test [39]. Due to significant analgesic effect of gum extract in this test (P-value < 0.0001), we can conclude that it worked through the central nervous system for analgesic activity. Writhing test is another test for investigation the peripheral antinociceptive activity of drugs and is considered as a chemical-induced pain model. In this model, the irritable compounds such as acetic acid or phenylquinone is injected into the mice [40]. Analgesic activity of HEFG in this test (P-value < 0.0001) which was consistent with the previous study on the aerial parts of this plant, showed that it could have peripheral analgesic effects too [41].

Different species in genus *Ferula* such as *F. assa foetida* and *F. gummosa* and its oleo-gum-resins showed antinociceptive activity in different models of pain [42]. Also, antinociceptive effect of the aerial parts of *F. persica* has been shown in a previous study and the analgesic effect of its oleo-gum-resin extract was presented in the current study [43].

Different chemical compounds in the *Ferula* species can be responsible for antinociceptive activity. Bagheri et al., suggested that the antinociceptive activity of *F. assa-foetida* might be due to some phenolic compounds such as ferulic acid which was present in high contents in this plant. [44]. Another study by Nasri et al., examined the antinociceptive effects of *F. persica* inflorescence, attributing these effects to its active sesquiterpenes and flavonoids [14].

Different studies have shown that some flavonoid compounds found in medicinal plants could be considered as candidates for new and natural analgesic drugs. Because of their anti-inflammatory and analgesic qualities, flavonoids—multi-target molecules—have drawn more and more attention.

It makes sense that flavonoids' analgesic effects are caused, at least in part, by their inhibition of Cyclooxygenase (COX)1,2. Furthermore, compared to single target medications that virtually completely eliminate a particular mechanism involved in disease and physiological processes, flavonoids' multitarget nature lessens their impact on physiological systems. So, flavonoids do not possess the typical side effects of Non-steroidal anti-inflammatory drugs (NSAIDs) even when COX is inhibited [45].

The nuclear factor-κB (NF-κB) inhibitory effects of flavonoids are linked, at least partially, to their analgesic and anti-inflammatory properties.

Flavonoids inhibit the production of inflammatory mediators like COX-2, Tumor necrosis factor alpha (TNF-α), Interleukin-1 beta (IL-1β), and nitric oxide (NO). They also suppress the expression of Vascular endothelial growth factor (VEGF) and Intercellular Adhesion Molecule 1 (ICAM-1) and activate the pathways for mitogen-activated protein kinase (MAP

kinases), Signal transducer and activator of transcription 3 (STAT3), NF-κB, and the NLR family pyrin domain containing 3 ($NLRP_3$) inflammasome [45].
Among these compounds, some key components such as quercetin, apigenin, luteolin, and rutin have been mentioned [46]. Quercetin had an analgesic effect in the formalin test's neurogenic and inflammatory phases, which was through mechanisms that interfere with L-arginine-nitric oxide, GABAergic, and serotonergic systems [47]. Luteolin inhibits neuropathic pain by activating GABA and μ-opioid receptors [48]. As previously mentioned, flavonoids are medications with a safe preclinical profile. For example, luteolin inhibits Prostaglandin I_2 (PGI_2) generated by COX-2 without causing the distinctive adverse effects of NSAIDs [45].
Although total phenolic content of *F. persica* oleo-gum-resin did not show high level in this study, the results of TPC and TFC of this extract was in consistent with previous studies on phenolic content of oleo-gum-resins of different *Ferula* species [43].
Another compound which may be responsible for antinociceptive activity of *Ferula* species is Umbelliprenin. It was one of the sesquiterpene coumarins and was found in different *Ferula* species, could inhibit the activity of 5-lipooxygenase and showed the anti-inflammatory action. [44]. Also, umbelliprenin in a single dose (0.01 mM) significantly reduced neuropathic pain, withour affecting acute pain comparing to diclofenac. So, it might be one of the possible mechanisms of action of active compounds of *Ferula* oleo-gum-resin [14,44].
All four pain tests which were employed in this study revealed that *F. persica* oleo-gum-resin extract could reduce pain caused by thermal and chemical stimuli. On the other hand, there was no significant difference between the results at various times of the experiment, so, it is concluded that the antinociceptive effects were in a dose-dependent manner, not time-dependent. Moreover, the data obtained revealed that *F. persica* oleo-gum-resin has the potential to act as an analgesic agent, functioning both peripherally and centrally. However, future experimental and clinical studies are necessary to identify the active principles of *F. persica* oleo-gum-resin and to elucidate its analgesic mechanisms. Further studies using different doses of *F. persica* oleo-gum-resin, as well as other extracts of *F. persica*, are suggested. These investigations should focus on identifying the components of *F. persica* oleo-gum-resin that may be responsible for the antinociceptive effects and determining their specific mechanisms of action. Additionally, *F. persica* oleo-gum-resin and its active analgesic components can be considered for further research in the development of analgesic drugs.

Conclusions
This investigation is the first to report the antinociceptive effects of *F. persica* oleo-gum-resin. The results confirm its traditional use in managing pain. This oleo-gum-resin may serve as an alternative to NSAIDs for treating inflammatory diseases and pain without the associated adverse effects. The pharmacology and pharmaceutical development of *F. persica* oleo-gum-resin are important topics. This study could serve as an introduction for conducting clinical studies to investigate the analgesic and anti-inflammatory effects of this plant's oleo-gum-resin in humans. Future studies can determine the mechanisms and active principles of *F. persica* oleo-gum-resin as an analgesic agent.

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Conflict of Interests
The funding source had no involvement in any part of the study, and there are no conflicts of interest to declare.

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References
[1] Farrar JT. Advances in clinical research methodology for pain clinical trials. Nat Med 2010;16:1284-1293.
[2] Taherian AA, Etemadi H, Sadeghi H. Assessment of aqueous extract of seed of Cuminum cyminum L. on neurogenic and inflammatory pain in mice. J Med Plants 2007;6:44-50.
[3] Cliff WH, Wright AW. Directed case study method for teaching human anatomy and physiology. Adv Physiol Educ 1996;270:S19.
[4] Sepehri G, Sheibani V, Pahlavan Y, Afarinesh Khaki M, Esmail Pour Bezenjani K, et al. Effect of interacerebroventricular injection of aqueous extract of Origanum vulgare L. ssp. viride on pain threshold in male rats. J Ardabil Uni Med Sci 2011;11:52-58.
[5] Roller L, Gowan J. Pain management and DMMRs. Aust J Pharm 2002:937-941.
[6] Eidi A, Eidi M, Mozaffarian V, Rustaiyan A, Mazooji A, et al. Antinociceptive and anti-inflammatory effects of ethanolic extract of Salvia syriaca L. in mice. Int J Pharmacol 2011;7:394-349.
[7] Smee D, Cooke J. Making it real: case-study exam model. HAPS Educator 2018;22:268-271.
[8] Lopes L, Pereira S, Silva L, Figueiredo K, Moura B, et al. Antinociceptive effect of topiramate in models of acute pain and diabetic neuropathy in rodents. Life Sci 2009;84:105-110.
[9] Ranjbar A. The effect of solenanthus circinnatus root extract on actue carrageenan–induced inflammation. J Isfahan Med School 2008;26:349-353.
[10] Hamidreza M-E, Sahand J, Paria S-B, Mohammad S, Mahdi V, et al. Antinociceptive effects of paeonia daurica subsp. macrophylla root extracts in mice. Trad Integr Med 2023;8(1).
[11] Nejad BN, Bamzadeh Z, Hejazi SH. Antimicrobial effects of ferula persica gum extract and gold nanoparticles on pseudomonas

aeruginosa. Avicenna J Clin Microbiol Infect 2016;4:36646.
[12] Panahi M, Rezaee M-B, Jaimand K. A review of phytochemistry and phylogeny that aid bio-prospecting in the traditional medicinal plant genus Ferula L.(Apiaceae) in Iran. J Med Plants By-Product 2020;9:133-148.
[13] Vosoughhosseini S, Aghbali A, Emamverdizadeh P, Razbani M, Mesgari M, et al. Effect of Ferula persica plant methanol extract on the level of Cox-2 in induced squamous cell carcinoma (SCC) in rat tongue. J Dental Res Dental Clin Dental Prospects 2018;12:91-96.
[14] Nasri S, Ghorbani Nohooji M, Amin GR, Sharifi A, Borbor M, et al. Phytochemical study of methanolic extract of ferula persica willd. inflorescence and its antinociceptive effect in male mice. J Med Plants 2018;17:136-144.
[15] Afifi F, Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J Ethnopharmacol 2000;72:101-110.
[16] Sattar Z, Iranshahi M. Phytochemistry and pharmacology of Ferula persica Boiss.: A review. Iran J Basic Med Sci 2017;20:1-8.
[17] Iranshahi M, Shahverdi AR, Mirjani R, Amin G, Shafiee A. Umbelliprenin from Ferula persica roots inhibits the red pigment production in Serratia marcescens. Z für Naturforsch C 2004;59:506-508.
[18] Ghanbari M, Zahedi Khorasani M, Vakili A. Acute and chronic effects of Ferula persica on blood pressure of hypertensive rats and its possible mechanism of action. J Med Plants 2012;11:62-68.
[19] Jadidi M, Vafaei AA, Miladi GH, Babaei SAA. The effect of Ferula persica L extracts,(sakbinag) on symptoms of morphine withdrawal and sleeping time in mice. Res Med 2010;34:225-230.
[20] Savithramma N, Linga Rao M, Ankanna S. Screening of traditional medicinal plants for secondary metabolites. Int J Res Pharm Sci 2011;2:643-647.
[21] Bhandary S, Kumari S, Bhat V, Sherly S, Bekal M. Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. Nitte University Journal of Health Science. 2012;2:34-38.
[22] De S, Dey Y, Ghosh A. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphophallus paeoniifolius (Araceae). Int J Pharm Biomed Res 2010;1:150-157.
[23] Abolmaali M, Motevalian M, Mehrzadi S, Shojaii A. Anticonvulsant effects of squill oxymel (a traditional formulation) in mice. Physiol Pharmacol 2022;26:1-6.
[24] Miliauskas G, Venskutonis P, Van Beek T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004;85:231-237.
[25] Lanhers M-C, Fleurentin J, Mortier F, Vinche A, Younos C. Anti-inflammatory and analgesic effects of an aqueous extract of Harpagophytum procumbens. Plant Med 1992;58:117-123.
[26] Abdulmalik I, Sule M, Yaro A, Abdullahi M, Abdulkadir M, et al. Evaluation of analgesic and anti-inflammatory effects of ethanol extract of Ficus iteophylla leaves in rodents. Afr J Tradit Complement Altern Med 2011;8:462-466.
[27] Moghadamnia AA, Afraz E. The effect of piperine on analgesia and naloxoane-inducd jumping in morphine dependent mice. 2000;2:17-24.
[28] Hajiallilo M, Abbasi-Maleki S. The antinociceptive effects of folic acid using formalin and acetic acid tests in male mice. J Shahrekord Uni Med Sci 2021;23:93-98.
[29] Taherian AA, Sameni HR, Sharifi S, Taherian MM, Taherian MH. Effects of hydroalchoholic extract of Iranian Propolis on acute, chronic and visceral pain in mice. Koomesh 2020;22:170-177.
[30] Erami E, Azhdari-Zarmehri H, Ghasemi-Dashkhasan E, Esmaeili M-H, Semnanian S. Intra-paragigantocellularis lateralis injection of orexin-A has an antinociceptive effect on hot plate and formalin tests in rat. Brain Res 2012;1478:16-23.
[31] Zarei M, Mohammadi S, Komaki A. Antinociceptive activity of Inula britannica L. and patuletin: In vivo and possible mechanisms studies. J Ethnopharmacol 2018;219:351-358.
[32] Tjølsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5-17.
[33] Pakgohar A, Mehrannia H. Sample size calculation in clinical trial and animal studies. Iran J Diabetes Obes 2024;16:42-50.
[34] Rosland JH, Tjølsen A, Mæhle B, Hole K. The formalin test in mice: effect of formalin concentration. Pain 1990;42:235-342.
[35] Parada C, Tambeli C, Cunha FdQ, Ferreira SH. The major role of peripheral release of histamine and 5-hydroxytryptamine in formalin-induced nociception. Neuroscience 2001;102:937-944.
[36] Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103-114.
[37] Elbadawy M, Abugomaa A, El-Husseiny HM, Mandour AS, Abdel-Daim MM, et al. The anti-nociceptive potential of tulathromycin against chemically and thermally induced pain in mice. Pharmaceutics 2021;13:1247.
[38] Taherian AA, Sameni HR, Sharifi S, Taherian MH. Effects of hydroalchoholic extract of Iranian Propolis on acute, chronic and visceral pain in mice. Koomesh 2020;22:170-177.
[39] Carlsson K-H, Jurna I. Depression by flupirtine, a novel analgesic agent, of motor and sensory responses of the nociceptive system in the rat spinal cord. Eur J Pharmacol 1987;143:89-99.
[40] Bakhtiarian A, Shojaii A, Hashemi S, Nikoui V. Evaluation of analgesic and antiinflammatory activity of Dorema ammoniacum gum in animal model. Int J Pharm Sci Res 2017;8:3102-3106.
[41] Jage J. Opioid tolerance and dependence–do they matter? Eur J Pain 2005;9:157-162.
[42] Kalam MA, Karim MS, Sofi G, Ahmad G. Evaluation of Anticonvulsant Activity of Aqer Qerha (Anacyclus pyrethrum DC) root in Experimental Animals. Hippocratic J Unani Med 2015;10:1-12.
[43] Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. J Ethnopharmacol 2011;134:1-10.
[44] Bagheri S, Dashti-R M, Morshedi A. Antinociceptive effect of Ferula assa-foetida oleo-gum-resin in mice. Res Pharm Sci 2014;9:207.
[45] Ferraz CR, Carvalho TT, Manchope MF, Artero NA, Rasquel-Oliveira FS, et al. Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. Molecules 2020;25:762.
[46] Xiao X, Wang X, Gui X, Chen L, Huang B. Natural flavonoids as promising analgesic candidates: a systematic review. Chem Biodivers 2016;13:1427-1440.
[47] Filho AW, Filho VC, Olinger L, de Souza MM. Quercetin: further investigation of its antinociceptive properties and mechanisms of action. Arch Pharmacal Res 2008;31:713-721.
[48] Hara K, Haranishi Y, Terada T, Takahashi Y, Nakamura M, et al. Effects of intrathecal and intracerebroventricular administration of luteolin in a rat neuropathic pain model. Pharmacol Biochem Behav 2014;125:78-84.