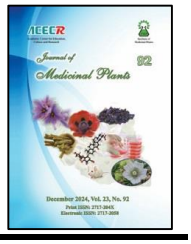
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Research Article

Evaluation analgesic and anti-inflammatory effects of *Rosa×damascena* Herrm. fixed oil (Persian medicine preparation)

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ABSTRACT

Background: Historical medical and pharmacological manuscripts describe various pharmaceutical applications of medieval Persian medicine. One of the known forms of application for therapeutic purposes via topical or systemic administration is herbal oils. "Rose fixed oil" (*Rosa×damascena* Herrm.) has traditional applications in the management of inflammation and pain due to ailment conditions such as inflammatory bowel disease, hemorrhoid, earache, and gastro-esophageal reflux diseases topically or orally. **Objective**: This study evaluated mechanism of analgesic and anti-inflammatory effects of "Rose fixed oil" by animal models. **Methods**: 50 grams of dried powdered petals of *R.×damascena* were weighed and soaked with 800ml distilled water for 24 hours. The mixture was heated for 70 minutes. Writhing, Tail flick, Hot plate, and carrageenan tests were used for the evaluation of the anti-inflammatory and analgesic effects of rose oil. The level of TNF-α and MPO activity in serum was measured. **Results**: "Rose fixed oil" 400mg/kg in all tests have been shown a significant effect. "Rose fixed oil" could decrease TNF-α and MPO. Previous studies demonstrated Rose extract can inhibit inflammation in animal models and clinical trials. "Rose fixed oil" in some studies has been shown an anti-inflammatory effect. The results of this study confirmed anti-inflammatory and analgesic effects of "Rose fixed oil" as a Persian medicine preparation. **Conclusion**: The main finding of this study was that "Rose fixed oil" *as* a Persian medicine preparation, possesses analgesic and anti-inflammatory effects through peripheral and central analgesic activity, and modulation of acute and chronic inflammation mediators in a dose-dependent manner.

Abbreviations: TM, Traditional medicine; PM, Persian medicine; ITM, Iranian traditional medicine; RFO, Rose fixed oil; MPO, Myeloperoxidase; MPE, Maximum possible effect; GAE, gallic acid equivalent

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1. Introduction

Traditional medicine (TM) is the oldest form of health care in the world, and is used in the prevention, and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of health- and life-threatening diseases. TM is also variously known as complementary and alternative, or ethnic medicine, and it still plays a key role in many countries today. Iranian traditional medicine (ITM), also known as Persian medicine (PM) and familiar to some as Unani medicine, is a holistic school of medicine, founded on a philosophical theory with a categorical framework [1]. Historical medical and pharmacological manuscripts describe various pharmaceutical applications of medieval Persian medicine. One of the known forms of application for therapeutic purposes via topical or systemic administration are medicinal oils prepared from numerous medicinal herbs [2].

The formulation and preparation of herbal oils, known as ‘‘Dohn’’ or the plural term‘‘Adhaan’’ are described in a series of historical Persian pharmaceutical manuscripts, namely ‘‘Qarabadin’’ (pharmacopeia) [2]. One of these oils is Rose oil. Rose oil is produced by *Rosa* × *damascena* Herrm., and a base oil like sesame oil or olive oil. *R.*×*damascena*. belongs to the family Rosaceae. The commercial products of this plant in the world include Rose essential oil, rose water, rose concrete, rose absolute, rose hips, and dried flowers. In this study, it’s named Rose fixed oil (RFO). The RFO’s extraction method and its chemical profiles are different from rose essential oil. RFO has traditional applications in the management of inflammation, and pain due to ailment conditions such as inflammatory bowel disease, hemorrhoid, earache and gastro-esophageal reflux diseases topically or orally [3]. Some studies have reported the anti-inflammatory and analgesic effects of RFO in humans [4-6] but in this study, we want to examine the anti-inflammation and analgesic effects of RFO by animal models.

2. Methods and Materials

The study protocol was approved by the ethics committee of the medical school of Shahid Sadoughi University of Medical Sciences in Yazd with license number IR.SSU.AEC.1401.023.

2.1. Plant identification

The dried petals of *R.*×*damascena* were purchased from a local herbalist shop in Kashan. The identification and affirmation of specimens of *R.*× *damascena* (Voucher spiceman NO. SSU0097) was done by Department of Pharmacognosy, faculty of Pharmacy, Shahid Sadoughi University of Medical Science, Yazd, Iran.

2.2. Preparation of fixed oil

The aqueous extract was prepared by diffusion method; 50 grams of dried powdered petals of *R.*×*damascena* were weighed and soaked with 800ml distilled water for 24 hours. The mixture was heated for 70 minutes, while its temperature was kept at 85-90°C. As the extract cooled, it was filtered with the help of filter paper. 400ml of aqueous extract was mixed with sesame oil at a 1:1 ratio. Then the mixture was heated for 4 hours, while its temperature was kept at 90-95°C [2].

2.3. Standardization

2.3.1. Quantification of total phenol of oil

Two grams of prepared oil were added to 8 ml of methanol 80% in a balloon and shaken. After centrifuging for 30 minutes and separating in two phases, the upper phase as methanolic extract was

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collected. To ensure of separation of methanolic extract, methanolic extract was centrifuged in 5000 rounds for 30 minutes three times [7].

The aqueous extract was measured as phenol content by Folin-Ciocalteus method [8] Among 5, 12, 25, 50, 100, 150, and 200µg/ml concentrations of gallic acid were prepared. Out of 2 ml Folin-Ciocalteus reagent was added to 0.4 ml of each prepared gallic acid concentrations, 0.4 ml RFO and 0.4 ml methanol 80 in separated tubes. After 3-8 minutes, 1.6 ml sodium bicarbonate 7.5% was added to tubes. The tubes were kept in a dark room for 30 minutes then the absorbance at 760nm of the RFO and gallic acid concentrations were measured.

2.3.2. Quantification of total flavonoid

The methanolic extract was measured as flavonoid content by the aluminum chloride colorimetric method [9]. concentrations of 10, 25, 50, 75, and 100 µg/ml concentrations of quercetin were prepared. To prepare the test balloon, 300 µl sodium nitrite 5% with 4 ml distilled water were added to 1ml methanolic extract. After 5 minutes, 300 µl aluminum chloride 10% was added. Also, a blank balloon was prepared by adding 300 µl sodium nitrite 5% with 4 ml distilled water to 1 ml methanol; after 10 minutes 300µl distilled water was added. After adding 2ml NaOH 2 N, each solution made up to 10 ml with distilled water. Out of 1 ml of each solution was diluted 10 times by distilled water. The absorbance at 415nm of the test solution and quercetin concentrations were measured.

2.4. Chemicals and reagents

Vehicle oil (Barooj, Iran); Methanol (Merck, Germany); Normal saline (Daroopakhsh, Iran); Morphine (Daroopakhsh, Iran); Indomethacin (Hakim, Iran); Acetic acid 0.6% (Sigma-Aldrich, Germany); Formalin 2.5% (Sigma-Aldrich, Germany); Carrageenan 1% (Sigma-Aldrich, USA); Ketamine (Alfasan, The Netherlands); Xylazine (Alfasan, The Netherlands);

2.5. Experimental animals

Male NMRI mice, aged 6-8 weeks and weighing between 25 and 30 g, were purchased from Royan Infertility Center (Tehran, Iran) and were quarantined and acclimated for 1 week. The animals were housed in the animal room, which was maintained at a temperature between 21 and 25°C. Lighting was controlled to give a 12-hour light cycle. All mice had free access to water and a pellet diet during the time of the experiment.

2.6. Experimental design

Animals were randomly divided into 7 experimental groups (n = 6) as follows:

Group I, and II were administered with sesame oil and normal saline

Group III, and IV received standard drugs (ie, 10mg/kg of morphine, and indomethacin (25 mg/kg)

Groups V, VI, and VII were given 100 mg/ml, 200mg/ml, and 400mg/ml *R.* × *damascena* fixed oil.

2.7. Evaluation of analgesic activities of the extract

2.7.1. Acetic acid-induced writhing test

A writhing test was conducted to evaluate the analgesic effect of RFO on visceral pain. All of the groups were pretreated with proper doses of vehicle, RFO, and negative and positive controls intraperitoneally. After 30 minutes, visceral pain was induced by an intraperitoneally injection of (10 mg/kg i.p.) acetic acid 0.6% (Sigma-Aldrich, Germany). The number of writhes was recorded over 30 minutes, starting 10 minutes after the administration of acetic acid. Writhes are defined as stretching at least one of the posterior organs or abdominal contraction [10].

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/2.7.2. Tail flick test

The analgesic effect of RFO in acute pain was measured using the Tail Flick Test. The test was performed with a tail flick test device in which radiant heat was focused on a spot 2 cm from the tip of the tail. A cut-off time of 10 seconds and 90% severity of radiation was selected to avoid any tissue damage in the animal. Wagging and pulling the tail were considered the mice's responses to pain induction by radiation. The test was repeated for each group before injection, 30, 60, and 90 minutes after injection, twice. The latency was recorded from the onset of stimulation to withdrawal of the tail. Maximum possible effect was calculated (MPE) as below: [11].

$$\% \text{ MPE} = \frac{Test\ Latency - Base\ Line \times 100}{Cut\ Off - Base\ Line}$$

2.7.3. Hot plate test

The hot-plate tests were conducted with a hot plate test device with 55°C±1 temperature and 40 seconds as cut-off time. Licking one of the paws or jumping to the posterior legs is considered a mice's response to pain. The latency time was recorded from the onset of the test to the mice's response. The latency time was recorded for each group before injection, and at 30,60,and 90 minutes after injection [12].

2.8. Anti-inflammatory activity

2.8.1. Carrageenan-induced paw edema

The Carrageenan test was conducted with the aim of evaluating inflammation by measuring the mice's paw changes caused by edema. At first, the volume of mice's paw was measured by plethysmograph. Thirty minutes after the last intraperitoneal administration of RFO, 100 µl of a 1% Carrageenan solution was injected S.C. by Hamilton syringe. Volumes of mice's paws were recorded 1, 2, 3, 6 and 24 h after injection

2.8.2. TNF-α

The level of TNF-α in serum samples was measured by the ELISA method. Following the instructions of the TNF-α kit (Karmania pars gene, Iran) the solutions for the washing buffer and HRP-Avidin were prepared. Washing buffer was diluted to a 1:10 ratio with distilled water. To prepare HRP-Avidin, for each eight-well row, 416 µl HRP-Avidin were mixed with 41 µl HRP. To evaluate TNF-α levels, the first 1-4 wells were filled with 50 µl of 1 to 4 standard solutions respectively. The other wells were filled with serum samples. Then the kit was incubated for 60 minutes while shaking at a rate of 200 rpm rate. By washing the wells 3 times with washing buffer, 50 µl conjugated antibodies were added to all of the wells and incubated for 60 minutes while shaking at a rate of 200 rpm rate. After washing the wells 3 times with washing buffer, 50 µl HRP-Avidin solution was added to all of the wells and incubated for 30 minutes while shaking in 200 rpm rate. Plates were washed 5 times with buffer solution then 50 µl substrate were added to all of wells and incubated for 15 minutes. After adding 25 µl of stopping solution to wells, the absorbance of the samples was measured at 450 nm using an ELISA reader (Biotech, USA).

2.8.3. MPO

The activity of the MPO enzyme in serum samples was measured by the ELISA method. Following of the recipe of the MPO kit (Navand salamat, Iran).

2.9. Statistics

Data obtained are expressed as mean ±SEM. Data analysis was performed using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Significance was defined at $P < 0.05$ level.

3. Results

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3.1. Total phenol and flavonoid amount

Total phenol and flavonoid concentrations of extract were standardized as 142.58 mg/ml and 195.46 mg/ml respectively.

3.2. Writhing test

Fig. 1 presented the number of writhes of RFO in comparison with indomethacin and normal saline groups. Through the time, the number of writhes has decreased in all groups. The RFO 400 mg/kg group showed a significant decrease in the number of writhes compared to the RFO100 mg/kg and normal saline group ($P < 0.05$); but didn't show significant change in comparison with the indomethacin group ($P > 0.05$). No stretching or writhing were observed in the morphine group.

3.4. Tail flick test

As shown in Table 1, during all periods after injection, all RFO groups differed significantly ($P < 0.05$) from the morphine group. No significant difference between 400 mg/kg RFO and the indomethacin group was observed ($P > 0.05$). in first 30 min, 400 mg/kg of RFO produced high MPE% with significant difference compared to vehicle group ($P < 0.05$).

3.5. Hot plate test

Through all periods, The RFO groups (100 and 400 mg/kg) differed significantly ($P < 0.05$) from the normal saline group, there was a significant difference ($P < 0.05$) between the 100 and 400mg/kg RFO groups and the indomethacin group. No significant difference between the 400 mg/kg RFO group and indomethacin was observed ($P > 0.05$) (Table 2).

3.6. Carrageenan test

Until one hour after injection, none of the groups differ significantly ($P > 0.05$). Two hours after the injection, RFO 200 mg/kg and 400 mg/kg groups showed a significant difference with the normal saline group ($P < 0.05$); but RFO 200 mg/kg group didn't have the significant difference with indomethacin and morphine groups ($P > 0.05$) (Fig. 2).

Although there were significant differences between RFO 200mg/kg and 400mg/kg groups and the normal saline group ($P < 0.05$); there were no statistically significant difference with the indomethacin group ($P > 0.05$).

During six hours after injection, all of the RFO group had significant differences with the normal saline group, and RFO 400 mg/kg had significant difference with vehicle ($P<0.05$); however, there was no statistically significant difference between the RFO 400 mg/kg group and the indomethacin group ($P> 0.05$).

Within 24 h after injection, RFO 200 mg/kg and 400 mg/kg groups had significant differences from the normal saline ($P<0.05$); but there was no statistically significant difference between RFO 400 mg/kg and indomethacin groups ($P > 0.05$). The higher dose of oil (400 mg/kg) showed a significant difference compared to the vehicle ($P < 0.05$).

3.7. TNF-α, MPO test

Fig. 3 shows that TNF-α levels in the RFO 400 mg/kg group significantly differ from the vehicle ($P < 0.05$); however, there was no significant difference with normal saline, morphine, and indomethacin groups ($P > 0.05$).

Fig.4 suggests RFO 400 mg/kg group significantly differs from the vehicle ($P < 0.05$); however, there is no significant difference from morphine, and indomethacin groups ($P > 0.05$).

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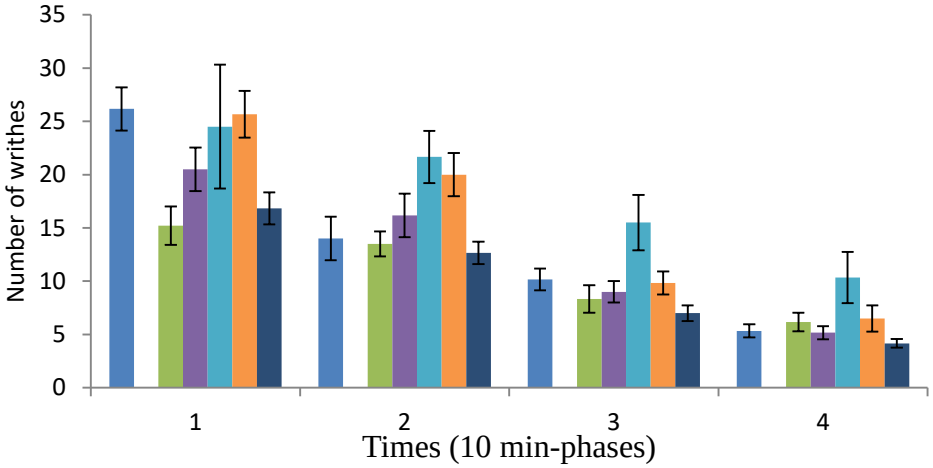


Fig. 1. Analgesic effects of *R.* × *damascena* fixed oil, indomethacin, and normal saline groups in writhing test. Morphin group shown different significance with Normal saline group in all phases (P < 0.05)

Table 1. Analgesic effects of *R.* × *damascena* fixed oil, positive and negative control groups in tail flick test

Groups	MPE% after 30 min	MPE% after 60 min	MPE% after 90 min
Normal Saline	0.2 ± 5.1	-0.31 ± 6.8	-2.5± 2.7
Morphine	92.6 ± 4.3*****	82.8 ± 8.9*****	65.9 ± 5.6*****
Indomethacin	45.7 ± 8.5	22.9 ± 8.1	9.1 ± 4.8
Vehicle	-9.1 ± 10.7	-5.1 ± 5.5	-16.9 ± 12.2
RFO 100mg/kg	7.9 ± 5.9	6.7 ± 4.3	2.8 ± 5.9
RFO 200mg/kg	5.7 ± 3.02	2.6 ± 3.3	4.8 ± 6.9
RFO 400mg/kg	28.9 ± 8.3$^{\#}$	13.1 ± 10.02	6.02 ± 7.2

*: Significant difference with Normal saline group (P < 0.05), #: Significant difference with Vehicle group (P < 0.05)

Table 2. Analgesic effects of *R.* × *damascena* fixed oil, positive and negative control groups in hot plate test

Groups	MPE% after 30 min	MPE% after 60 min	MPE% after 90 min
Normal Saline	3.02 ± 0.9	7.64 ± 1.3	5.71± 1.7
Morphine	54.11 ± 5.4*#	45.80 ± 4.4*	34.35 ± 4.7*#
Indomethacin	20.54 ± 1.2*#	19.19 ± 2.0	17.75 ± 2.0#
Vehicle	5.41 ± 4.4	8.68 ± 4.1	9.92 ± 3.1
RFO 100mg/kg	9.11 ± 2.9	9.76 ± 2.1	8.59 ± 4.4
RFO 200mg/kg	1.61 ± 2.7	7.89 ± 3.6	3.31 ± 3.7
RFO 400mg/kg	19.01 ± 1.3*	16.83 ± 3	12.41 ± 1.8

*: Significant difference with Normal saline group (P < 0.05), #: Significant difference with Vehicle (P<0.05), MPE: Maximum Possible Effect

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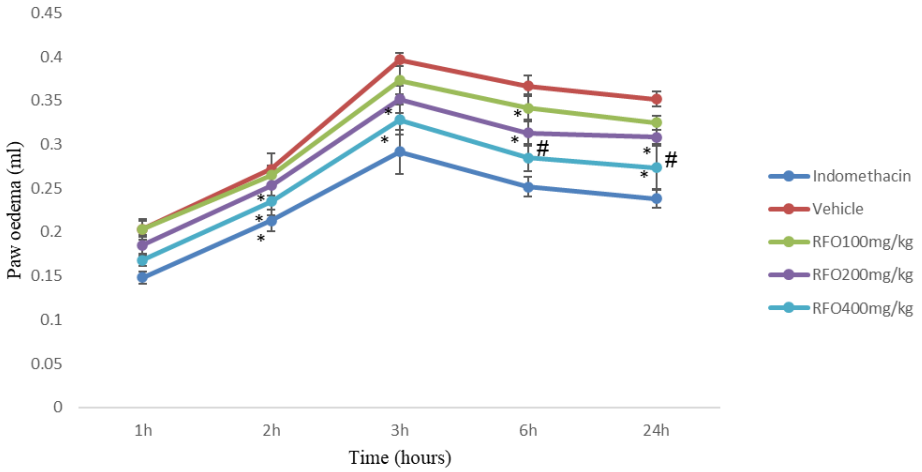


Fig. 2. Anti-inflammatory effects of RFO, positive and the negative control groups in Carrageenan test
*: Significant difference with Normal saline group (P < 0.05), #: Significant difference with Vehicle group (P < 0.05)

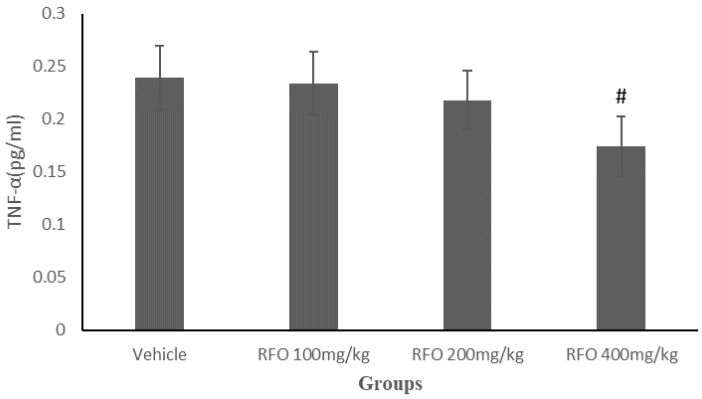


Fig. 3. TNF-α levels in groups; #: Significant difference with Vehicle group (P < 0.05)

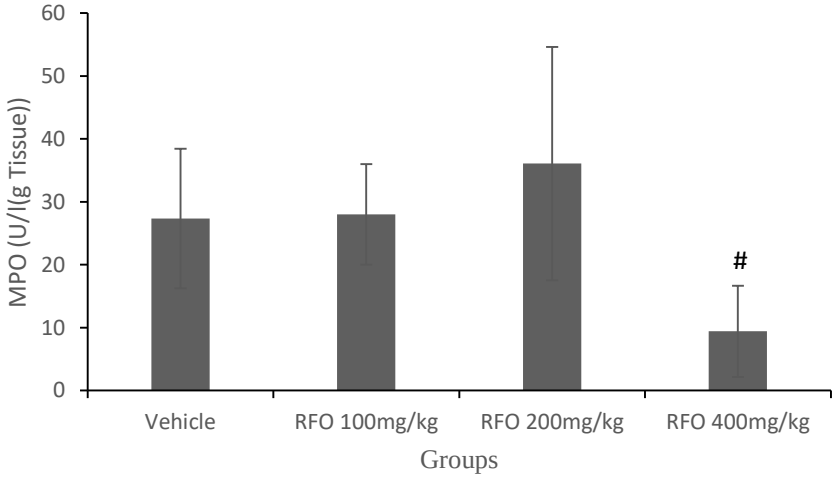


Fig. 4. MPO levels in groups, #: Significant difference with Normal saline group (P < 0.05)

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4. Discussion

RFO has a long history of usage especially as an analgesic medicine for oral and topical use in PM. In this study, we evaluated the anti-inflammatory and analgesic effects of RFO in mice through behavioral tests and biochemical markers. RFO showed an inhibitory effect in some of the experiments.

RFO reduced the number of muscle twitches in the abdomen in writhing test. The writhing test is used extensively for the evaluation of peripheral analgesics and is composed of the release of prostaglandins and phlogistic mediators like PGE2 and PGE2 alpha. Acetic acid acts indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics. The observed results suggested RFO has antinociceptive activity in the acetic acid-induced writhing test. Given that the difference between indomethacin and RFO was not significant, peripheral antinociception might be related to the mechanism like peripherally acting drugs such as NSAIDs [13, 14].

The results of a study by Hasheminejad et al about the antinociceptive and anti-inflammatory effect of the hydroalcoholic extract of *R.*×*damascena* confirmed our findings. They measured the analgesic effects of hydroalcoholic extract and essential oil of *R.*×*damascena* through formalin, tail flick, and writhing tests; the data indicated that hydroalcoholic extract of *R.* × *damascena* (1000 mg/kg) showed the analgesic properties in a dose-dependent manner, whereas no antinociceptive activity of essential oil was observed [15].

The results of the tail-flick test, as a central selective model of opioid-derived analgesics, supposed that 400 mg/kg RFO has analgesic properties [16].

During the hot plate test a noninflammatory and acute nociceptive response was produced and appeared as paw-licking and jumping behaviors occurring at the supraspinal level. Therefore, a drug or substance possesses centrally mediated activity as opioids if its paw withdrawal threshold increased. The RFO significantly increased the MPE%, probably through central mechanisms involving these receptor systems [13, 14, 16-18].

In the carrageenan test as an *in vivo* model for predicting anti-inflammatory efficacy, 400mg/kg RFO reduced carrageenan-induced edema by inhibiting the acute inflammation's mediators [19]. Carrageenan can cause localized edema and infiltration of white blood cells, increase the levels of local PGE2, and stimulate the secretion and release of some cytokines such as IL-1β and TNF-α. IL-1β, IL-6, and TNF-α are important inflammatory cytokines, which play important roles in regulating and mediating inflammation. A previous study evaluated the anti-inflammatory potency of *R.*×*damascena* against acetic acid-induced colitis in rats demonstrating the therapeutic potential of hydroalcoholic extract containing 15.7 ± 0.2 g of GAE/100 g as an anti-inflammatory agent in IBD management [20].

The laboratory tests of measuring the level of TNF-α and MPO activity showed that 400 mg/kg RFO possessed an anti-inflammatory value to decrease the level of TNF-α and MPO activity as inflammatory biochemical markers. citronellol as a main constituent in *R.*×*damascena* essential oil, exerts anti-inflammatory effects against lung injury in the cecal ligation and puncture model by diminishing the levels of biochemical parameters such as MPO [21].

Some trial studies evaluated the analgesic properties of *R.*×*damascena.* In a clinical study with preemptive administration capsules; which contained 15% of dried 70% hydroalcoholic

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R. × *damascena* extract, a significant decrease in postoperative pain compared with the control group was reported [22]. Another clinical trial illustrated pain relieving properties of *R.* × *damascena* extract capsules on primary dysmenorrhea [9]. A randomized clinical study demonstrated that topical administration of rose oil could effectively reduce pregnancy-related back pain with low intensity. They prepare rose oil by soaking rose petals in almond oil. They used almond oil as base oil instead of vehicle oil. [23] Another double-blind clinical study revealed the efficacy of RFO in migraine headaches, especially in hot type. Vehicle oil was used to prepare fixed oil but the oil was made by soaking not heating. Identified components of RFO are as follows: oleic acid, linoleic acid, palmitic acid, s stearic acid, α-linolenic acid, γ-linolenic acid, citronellol and geraniol [4]. In this study, RFO prepared by heating an aqueous extract of rose in vehicle oil. The data of standardization of RFO estimate its valuable phenol and flavonoid contents. previous studies demonstrated sesamin, the major component of vehicle oil, possesses anti-inflammatory and antinociceptive activity in an *in vivo* model [24]. Soaking petal rose in a base oil causes essential oil extraction but in heating and evaporating method, chemical constituents in the aqueous phase become trapped in the oil phase [2]. Obviously, different methods of preparing oils cause the components of oils to differ from each other, but we see anti-inflammatory and anti-nociceptive effects from both, the mechanism of effect and length of effect may be different.

A case cohort study depicted the therapeutic value of co-administration of RFO capsules with omeprazole in the healing of gastritis symptoms especially belching and abdominal pain as well as increasing the efficacy of omeprazole[25] .

5. Conclusion

The main finding of this study is that RFO, as a PM preparation, possesses both analgesic and anti-inflammatory effects through peripheral and central analgesic mechanisms, as well as through of the modulation of the mediators of acute and chronic inflammation in dose-dependent manner, respectively.

Author's contributions

S. J. and R. Z. participated in the design, data collection, and drafting of the article. E. A. was involved in data collection and drafting. H. M. and M. Gh. participated in data analysis and drafting.

Conflict of interests

The authors declare that they have no competing interests.

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