

Stereological and molecular studies on the effects of *Ferula persica* extract on wound healing in rats

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Abstract

Background: *Ferula persica* is one of the most important traditional medicinal plants that is used to treat various diseases such as diabetes, backache and rheumatism. The aim of the present study was to evaluate the anti-inflammatory and wound healing potential of *F. persica* using stereological and molecular methods in experimental models.

Methods: In the present study, two wound models (circular excision and linear incision) were used. Male Wistar rats were divided into four groups ($n = 16$), including control, vehicle treated, treated with *F. persica* extract ointment (5% w/w) and treated with the reference drug (Madecassol). All the animals were treated topically once a day. The circular and linear wounds were treated for 9 and 17 days, respectively. At the end of the study, samples from wounds area were harvested for histology, stereology, immunohistochemistry and molecular assessments to determine the in vivo healing potential and anti-inflammatory activity.

Results: We observed significant recovery in macroscopic evaluation of wound healing in the *F. persica* extract treated group compared with the control and vehicle treated groups ($p < 0.05$). Histological and stereological assessments showed complete repair of the epidermal layer, increasing fibroblast cells and collagen density, decreasing inflammatory cells and a remarkable degree of neovascularization by determining length density of blood vessels in the extract group, which were significant as compared to the control and vehicle treated groups ($p < 0.05$). Expressions of TNF- α and TGF- β were found to be decreased and increased ($p > 0.05$, $p < 0.05$, respectively), in the extract treated group as compared to the control and vehicle treated groups. Also, greater COX-2 expression could be detected in the control and vehicle treated groups, which was significantly attenuated in the extract group.

Conclusion: Our results confirm that the *F. persica* extract is a valuable source of antioxidant and anti-inflammatory activity and can allow damaged tissue in wounds to recover markedly.

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KEYWORDS

anti-inflammation, circular excision, *Ferula persica*, linear incision, stereology, wound healing

1 | INTRODUCTION

Wound healing is a complex physiological process that occurs in the skin and the tissues under it (Nguyen et al., 2009). The wound healing process depends on cellular and molecular mechanisms and divided into three overlapping and interdependent phases, including inflammatory phase (consisting of homeostasis and inflammation), proliferative phase (consisting of granulation, contraction and epithelialization) and remodelling phase (Ximenes et al., 2013). In acute wounds, such as burns and shear, maintaining the integrity of the skin to protect against dehydration, bleeding and preventing of microorganisms is vital (O'Mathúna, 2016). Acute wounds are usually superficial and completely recover in 8–12 weeks (Monaco & Lawrence, 2003). However, the infections remain one of the major causes of mortality (Bowler, 2002). Another destructive factor in wound healing is prolonged inflammation in the wound site (Landén et al., 2016). Prolonged inflammation is capable of inducing apoptosis in principal dermal cells, like fibroblasts, and delaying wound healing by excessive synthesis of proteins such as cyclooxygenase-2 (Nasiry et al., 2017) and the expression of the TNF- α inflammatory gene (Dong et al., 2016). On the other hand, some growth factors are useful in healing. For example, the expression of TGF- β gene by macrophages is considered as an important factor in the migration and differentiation of keratinocytes and fibroblast cells in the proliferative phase (El Gazaarly et al., 2013). This agent is also reduced in chronic wounds and prolonged inflammation. Therefore, the rapid and optimal repair of wounds, along with the reduction of complications, has always been one of the priorities of the treatment teams (Nasiry et al., 2017). Besides, there is a continuous search for an optimal treatment, which is cost-effective with minimal side effects (Aghbali et al., 2016). For many years, herbal medicine has a special place in the treatment of various diseases (Maver et al., 2015; Nasiry et al., 2019). It is believed that treatment with medicinal herbs is less toxic and safer. Medicinal herbs do not have side effects, and therefore have a remarkable impact on chemical drugs (Pal & Shukla, 2003). In Iran, the consumption of medicinal plants traditionally has a long history. Because of its favourable climatic and geographical conditions, there are a wide range of medicinal plants (Sadighi et al., 2004). One of these plants is *Ferula persica*. It belongs to the Umbelliferae family and comprises 53 species that grow wild in all the way through the Mediterranean zone and central Asia, especially in Iran where 16 species are endemic (Afifi & Abu-Irmaileh, 2000; El-Razek et al., 2001; Mandegary et al., 2004). *Ferula persica* is traditionally used as carminative, anti-hysterical, anti-spasm, anti-convulsion, anti-bacterial, anti-swelling, laxative and expectorant and also for the treatment of diabetes, backache, lowering of blood pressure and rheumatism (Bagheri et al., 2010; Ghanbari et al., 2012; Sahebkar & Iranshahi, 2010). In recent years, some studies have been reported to isolate, purify and elucidate the structure of *F. persica* compounds (Iranshahi

et al., 2003, 2004, 2008). Also, some studies revealed that *F. persica* contains biologically active sesquiterpene coumarins including umbelliprenin (Mahran et al., 1973), farnesiferols A (Mozaffarian, 1996) and B (Sahebkar & Iranshahi, 2010). On the other hand, Umbelliprenin has shown some properties including anti-inflammatory (Iranshahi et al., 2015), anti-apoptotic (Barthomeuf et al., 2008) and anti-pigmentation (Iranshahi et al., 2004). Regarding the aforementioned properties and their accessibility, the aim of the present study was to evaluate the anti-inflammatory and wound healing potential of *F. persica* using stereological and molecular methods in experimental models.

2 | MATERIALS AND METHODS

2.1 | Plant material and preparation of plant extract

Ferula persica aerial parts were collected from Firoozkooh, Iran in summer 2017. Samples were dried at room temperature and coarsely ground before extraction. Dried powder (100 g) was extracted by maceration using methanol (300 ml) for 24 h at room temperature. The extract then was separated from the sample residue by filtration through Whatman No. 1 filter paper. This procedure was repeated thrice. The extract was filtered and concentrated under reduced pressure at 35°C using a rotary evaporator until a crude solid extract was obtained, which then was freeze-dried for complete removal of the solvent (yield, 15.2%). A 5% ointment was prepared in Vaseline.

2.2 | Determination of total phenolic compounds and flavonoid contents and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Total phenolic compound and flavonoid contents were determined by using the Folin-Ciocalteu method (Ebrahimzadeh et al., 2010). The extract sample (0.5 ml) was mixed with 2.5 ml of Folin-Ciocalteu reagent (0.2 N) for 5 min, and 2.0 ml of 75 g/L sodium carbonate was then added. The absorbance of reaction was measured at 760 nm by spectrophotometer (UV-Visible EZ201; Perkin Elmer, Norwalk, Connecticut) after 2 h of incubation at room temperature. The results were expressed as gallic acid equivalents (GAE). Total flavonoids were estimated using our recently published papers (Ebrahimzadeh et al., 2010). Briefly, 0.5 ml solution of extract in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer. Total flavonoid contents were calculated as quercetin equivalents (QE) from a calibration curve. The

stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the extract. Different concentrations of extract (50, 100, 200, 400 and 800 $\mu\text{g/ml}$) were added, at an equal volume, to methanolic solution of DPPH (100 μM). After 15 min at room temperature in the dark, the absorbance was recorded at 517 nm. The experiment was repeated three times. Butylated Hydroxyanisole (BHA) was used as standard control. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals (Ebrahimzadeh et al., 2010).

2.3 | Animals

Male adult Wistar rats (200–250 g) were housed at room temperature ($22 \pm 2^\circ\text{C}$) and maintained on a 12-h light/dark cycle. Rats were granted free access to standard feed and water. Animals were kept for at least 7 days before and over the course of the experiment. Each sample that had history of surgery or medical intervention was excluded. All experimental procedures and protocols were conducted in accordance by ethical committee of Health Sciences, Mazandaran University of Medical Sciences (Ethics approval number: IR.MAZUMS.REC 1397-1353) to minimize the animal's suffering. These guidelines were in accordance with the NIH guidelines of the Care and Use of Laboratory Animals.

2.4 | Experimental groups

The animals were randomly allocated in four groups ($n = 16$; 10 and six rats for circular and linear wound models, respectively), including (I) control group which non-treated; (II) vehicle group, which topically treated with ointment base (Vaseline); (III) extract group, which topically treated with 5% concentration of *F. persica* extract ointment (1 g); (IV) Madecassol group, which treated with 1 g of Madecassol (Bayer, 00001199) as a reference drug. The dose of *F. persica* was determined by the previous study (Bagheri et al., 2010). To assess gene expression and immunohistochemistry in study groups, five animals of each group in circular excision wound model were euthanized, and skin samples were harvested on day 7. Five other animals of each group were euthanized, and skin samples were harvested on day 17 for histological evaluation.

2.5 | Circular excision wound model

The rats were anesthetized with anesthetic solution containing ketamine (80 mg/kg) and xylazine (15 mg/kg) before and during infliction of experimental wounds. The hairs on the dorsal interscapular area of the animals were shaved, and ethanol 70% was applied on the region before making the wound. A full-thickness circular excision of about 20 mm in diameter was made with a sterile scalpel No. 15 blade. Treatments were topically applied once in a day at the same time and continued for 17 days. The progressive changes in wound area were monitored by a camera (S20 Pro; Fuji, Japan), and macroscopic healing rate

TABLE 1 System for scoring the histological features of wound tissue samples

Score	Epithelial regeneration	Granulation tissue thickness
1	Little epithelial organization	Thin granular layer
2	Moderate epithelial organization	Moderate granular layer
3	Complete epithelial organization	Thick granular layer
4	Complete epithelial organization	Very thick granular layer

was evaluated by using ImageJ software (National Institutes of Health, MD, USA) on a personal computer. Wound contraction was calculated as the percentage reduction in wounded area as follows:

$$\text{Percentage wound contraction} = ((A_0 - A_n)/A_0) \times 100,$$

where A_n is wound area on n th day and A_0 is wound area on 0 day.

2.6 | Linear incision wound model

Animals were anesthetized and the hairs on the dorsal part of the rats were shaved and cleaned with ethanol 70%. Two 4 cm-longitudinal full-thickness incisions were made with a sterile scalpel No. 15 blade at a distance of 2 cm from the dorsal midline on each side. The wounds were closed with interrupted sutures at a distance of 1 cm apart. Treatments were topically applied once in a day at the same time and continued for 9 days. The progressive changes in wound length were measured by caliper on the first, third, fifth, seventh and ninth day after surgery.

2.7 | Histological and stereological analyses

For sampling, the animals had been sacrificed at 18th day of the study and full-thickness of the skin tissue including the wound and adjacent skin was harvested. The tissue samples were processed, embedded in paraffin, and serial sections with 5 μm (to determine the total volumes of new epidermis or new dermis and collagen deposition) or 20 μm (to determine the numerical density of cells and length density of the blood vessels) thickness were prepared. For each sample and each staining method, 10 sections throughout the wound bed with equal distances from each other were selected. All stereological measurements were done according to the method reported by Howard and Reedas (2004). To evaluate histological changes (epithelialization and granulation tissue formation), we used a standard scoring system (Table 1) (Nasiry et al., 2017). To calculate the collagen deposition in healing dermis and to compare it between groups, we used Masson's trichrome staining. Ten sections with equal distances from each other were selected from each rat. A total of 25 randomly picked micrographs were captured from each sample using a 40 \times magnification objective lens. The collagen percentage was calculated by dividing the total blue-coloured area by the total areas of the micrographs using ImageJ software (National Institutes of Health) (Nasiry et al., 2020).

2.7.1 | Estimation of numerical cell density

The optical dissector method was performed to determine the numerical density (N_v) of the polymorphonuclear and mononuclear leukocytes cells (neutrophil and lymphocyte) and fibroblasts in the healing bed, using the following equation: $N_v = \frac{\Sigma Q}{\Sigma P \times h \times \frac{a}{f}} \times \frac{t}{BA}$, where ΣQ is the number of nuclei, h (μm) is the height of the dissector, ΣP is the total number of the counted frames, a/f (mm^2) is frame area, BA (μm) is the block advance of the microtome (set at 20 μm) and t (μm) is real sectional thickness (Figure S1).

2.7.2 | Estimation of the length density of the blood vessels

The formula $L_v = \frac{2 \Sigma Q}{\Sigma P \times \frac{a}{f}}$ was used for measuring length density of the blood vessels, where ΣP is the total number of the counted frames, ΣQ is the total number of the blood vessel profiles counted per skin and a/f (mm^2) is counting frame area (Figure S1).

2.8 | Immunohistochemistry

In order to evaluate the anti-inflammatory activity of *F. persica* extract, cyclooxygenase-2 (COX-2) was taken by immunohistochemistry (Nasiry, Khalatbary, & Ebrahimzadeh, 2017; Nasiry, Khalatbary, Ahmadvand, et al., 2017). After the rehydration tissue sections, they were incubated in goat normal serum (in order to block non-specific site), and then with anti-COX-2 rabbit polyclonal antibody (1:100 in phosphate-buffered saline (PBS), v/v, Abcam) overnight at 4°C. After washing with PBS, samples were incubated with secondary antibody conjugated with horseradish peroxidase (goat anti-rabbit IgG, Abcam) and in order to detect the reactions, diaminobenzidine tetrahydrochloride was added for 5 min. Finally, they were dehydrated and mounted. To quantitatively analyze, five photographs of each sample (from all rats in each experimental group) were taken and assessed by densitometry using MacBiophotonics ImageJ software (National Institutes of Health). Data were expressed as percentages of total tissue area.

2.9 | Quantitative RT-PCR

The extraction of the total RNA in harvested tissue samples on day 7, after homogenizing using Lyser device, was extracted using TRIzol (Invitrogen, USA). The quality of the extracted RNA was confirmed using a nano-spectrophotometer and 1% gel agarose electrophoresis (Figure S2). The cDNA was reverse-transcribed from 1 μg of the total RNA in a 20 μl reaction mixture based on Takara Bio cDNA synthesis kit protocol (Takara Bio, Inc., Kusatsu-Shi, Japan). The ratio of the absorbance at 260 and 280 nm (A260/280) for all samples was about 1.808–2.014, and A260/230 ratios were about 0.3–0.7. Then, qRT-PCR reactions were performed for three biological replicates to

assess the gene expressions of TGF- β , and TNF- α was performed on the real-time PCR system (Applied Biosystems StepOne instrument) using SYBR Green Master Mix and sets of primers (Table 2). The results were analyzed using StepOne software (version 2.1; Applied Biosystems). The expression level of each target gene was normalized to its internal control (β -actin), and the final analyses were performed using the comparative CT method ($2^{-\Delta\Delta\text{ct}}$) (Raofi et al., 2021)

2.10 | Statistical analysis

Results were presented as mean \pm SD. The K-S test was used to evaluate the normality. One-way ANOVA followed by Tukey's post hoc tests were used to compare each two groups and data among the groups, respectively. All statistical analyses were performed using SPSS version 15.0. p values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Phenol and flavonoid contents of extract and antioxidant activity

Total phenolic content was reported as GAE (105.3 ± 4.91) by reference to standard curve ($y = 0.0058x$, $r^2 = 0.989$). The total flavonoid contents (20.4 ± 1.37 QE) were reported by reference to standard curve ($y = 0.0064x - 0.0076$, $r^2 = 0.998$). IC50 for DPPH radical-scavenging activity was 422.6 ± 13.5 mg/ml. IC50 of BHA was 53.96 ± 3.1 $\mu\text{g}/\text{ml}$.

3.2 | Wound closure rate

The rate of wound contraction in the present study (from day 1–17) is shown in Figure 1. Furthermore, the photographs from the wounds in four experimental groups at the end of study are shown in Figure 2 (A1–D1). Treatment with *F. persica* extract showed reduction of wound diameter compared to the control and vehicle groups in four points of the experiment (on day 3rd, 7th, 10th and 14th), and this difference was statistically significant ($p < 0.5$). Wound closure area observed in the extract treated group reached about 100%, as compared to about 89% and 90% in the control and vehicle groups, after 17 days, respectively. Furthermore, difference in wound closure between extract and Mades-cassol was not statistically significant ($p > 0.05$).

3.3 | Wound length and newly formed tissue

The results of the wound length and newly formed tissue examination are presented in Table 3. The length of wounds on different days showed reduction in the extract group compared to the control and vehicle groups ($p < 0.5$). In the extract treated group, after 9 days, the length of wound reached about 100% (0.1 ± 0.07) as compared

TABLE 2 The initial sequence (forward and reverse), product size and polymerase chain reaction (PCR)

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
TNF- α	AGCCTCTTCTCATTCTGCTC	GTTTGCTACGACGTGGGCTAC
TGF- β	GGCTGAACCAAGGAGACGGA	CCATGAGGAGCAGGAAGGGT
β -Actin	CCCATCTATGAGGGTTACGC	TTTAATGTACGCACGATTC

TABLE 3 The compare length of wound in different days

Group	Length of wound (cm)				
	Day 1	Day 3	Day 5	Day 7	Day 9
Control	3.97 \pm 0.02	3.85 \pm 0.06	2.17 \pm 0.2	1.05 \pm 0.05	0.45 \pm 0.1
Vehicle	3.92 \pm 0.04	3.6 \pm 0.2	2.07 \pm 0.3	0.85 \pm 0.2	0.3 \pm 0.04
Extract	3.86 \pm 0.04	2.47 \pm 0.37 [*]	1.3 \pm 0.25 ^ˆ	0.38 \pm 0.11 [*]	0.1 \pm 0.07 [*]
Madecassol	3.95 \pm 0.05	2.52 \pm 0.4 [*]	0.6 \pm 0.16	0.00 \pm 0.00	0.00 \pm 0.00

Values are expressed as mean \pm SD.

^{*} $p < 0.01$ versus control and vehicle groups.

^ˆ $p < 0.05$ versus control and vehicle groups.

[#] $p < 0.001$ versus other groups.

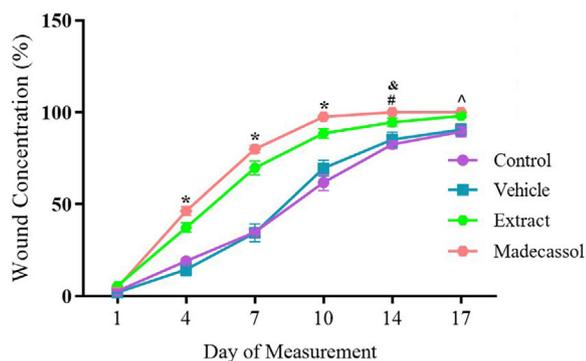


FIGURE 1 The impact of treatment regimens on wound closure. The percentage of wound closure during the experiment period with respect to the initial wound area. Data are represented in mean \pm SD. ^{*} $p < 0.001$ extract versus control and vehicle groups. [#] $p < 0.01$ extract versus control group. ^ˆ $p < 0.05$ extract versus vehicle group. [^] $p > 0.05$ extract versus control and vehicle groups

to the control and vehicle groups (0.45 \pm 0.1 and 0.3 \pm 0.04, respectively). Also, healing rate in the Madecassol group compared to the other groups was faster and these differences were statistically significant ($p < 0.001$).

3.4 | Histological evaluation

Histological photomicrographs of the wound area are shown in Figure 2 (A2-D2). In the present study, we used a standard system for scoring epithelialization and granulation tissue formation to evaluate the histological changes (Table 1). A significant increase was noted in epithelialization and granulation tissue formation scores in the extract

group compared to the control and vehicle groups ($p < 0.05$). To evaluate the amount of collagen deposition in the healing dermis, Masson's trichrome staining (Figure 2; A3-D3) and quantification were performed on the tissue sections (Figure 3). The results demonstrated that collagen deposition in the extract (both, $p < 0.001$) and Madecassol (both, $p < 0.01$) groups was significantly higher in comparison to the control and vehicle groups. Moreover, difference between the extract and Madecassol groups was not statistically significant ($p > 0.05$).

3.5 | Stereology

The graphs for the evaluated stereological parameters are shown in Figure 4. The numerical density of inflammatory cells (neutrophil and lymphocyte) was significantly lower in the extract group compared to the control, vehicle and Madecassol groups ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively). Comparison of fibroblasts showed that the numerical density increased significantly in the extract group compared to the control, vehicle and Madecassol groups ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). Considering the length density of blood vessels, we found that the extract group had significantly more blood vessels, compared to control, vehicle and Madecassol groups ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively).

3.6 | Immunohistochemical assessment

Figure 2 (A4-D4) shows the immunohistochemical staining against COX-2 in the wound bed. Quantification of COX-2 positive cells in the regenerating dermis showed that administration of the extract significantly decreased the expression of COX-2 as compared to the control and vehicle groups (both, $p < 0.001$). Also, the COX-2 expression in the

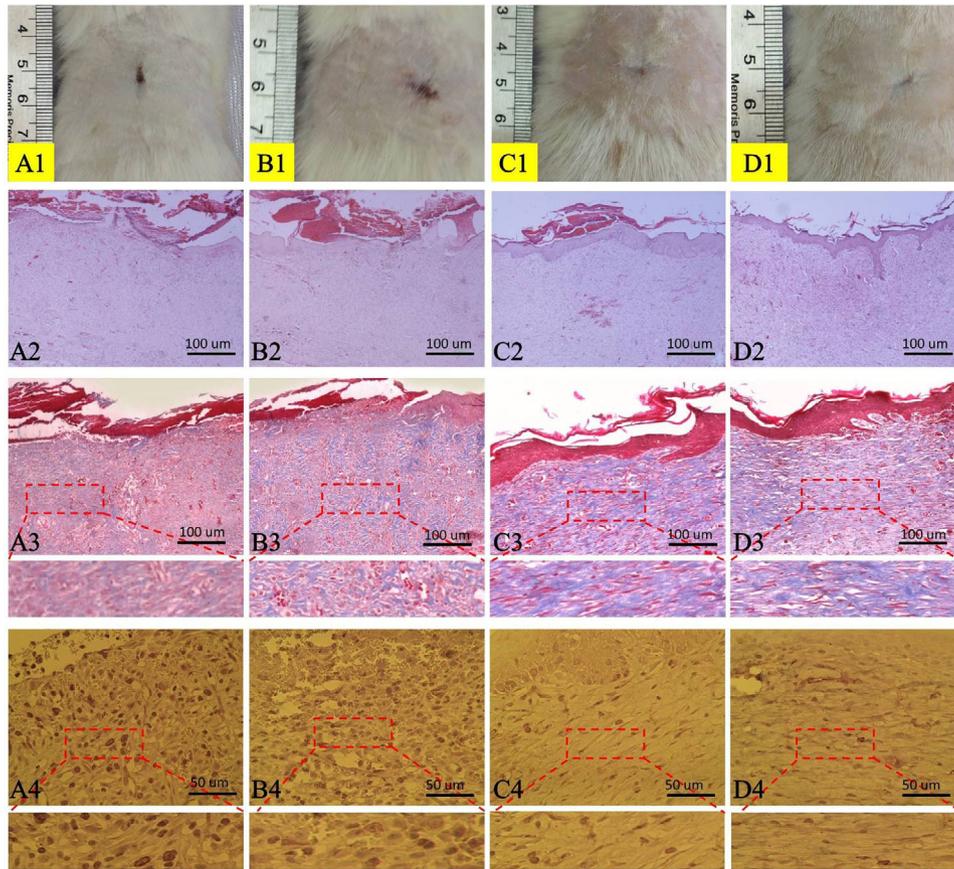


FIGURE 2 Wound closure, histological and immunohistochemical view of wound on day 17. A1-D1: The photographs represent healing of the treatment wounds at the end of the study compared with the other two experimental groups; A2-D2: Representative micrographs of the healing wound tissues stained by H & E; A3-D3: Representative micrographs of the healing wound tissues stained by trichrome masson methods to show collagen in blue colour; A4-D4: Representative micrographs from immunohistochemistry against COX-2 protein in the healing wound tissues that COX-2-positive cells represented by dark brown. (a) Control group; (b) vehicle group; (c) extract treated group and (d) Madecassol group

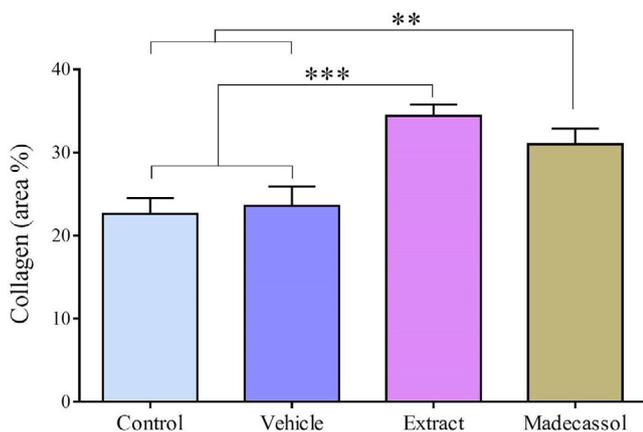


FIGURE 3 The impact of treatment regimens on collagen deposition in the dermis of healing wound. The quantitative analysis of the amount of collagen deposition in the dermis of healing wound. Data are represented in mean \pm SD, ** $p < 0.01$; *** $p < 0.001$

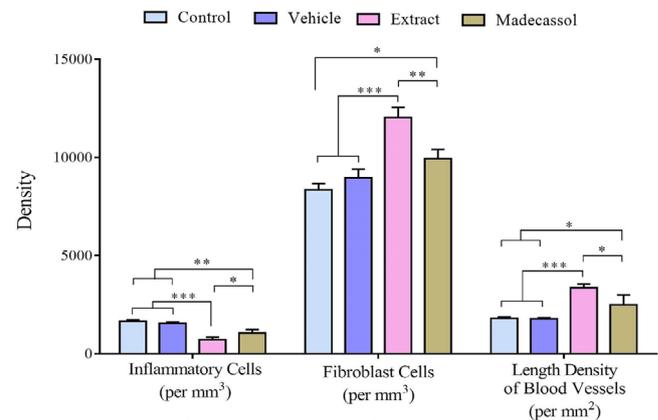


FIGURE 4 The impact of treatment regimens on stereological parameters. Numerical density of inflammatory cells, fibroblasts and length density of blood vessels in the wound bed. Data are represented in mean \pm SD, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

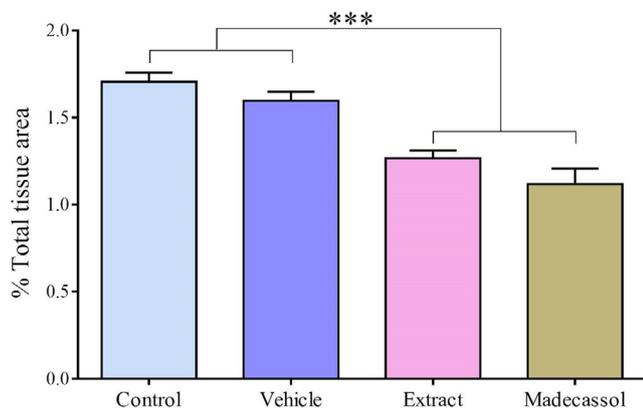


FIGURE 5 The impact of treatment regimens on COX-2 protein expression in the dermis of healing wound. The quantitative examination of COX-2-positive cells in the dermis of healing wounds. Data are represented in mean \pm SD, *** $p < 0.001$

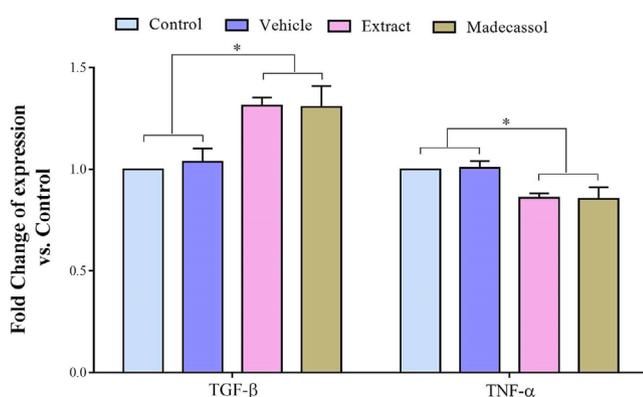


FIGURE 6 The impact of treatment regimens on gene expression of healing wound. The quantity of transcripts for a gene contributing to regeneration (TGF- β) and a gene involved in inflammation (TNF- α) were analyzed using qRT-PCR. Data are represented in mean \pm SD, * $p < 0.0$

Madecassol group was significantly lower than the control and vehicle groups (both, $p < 0.001$) (Figure 5).

3.7 | qRT-PCR gene expression analysis

To evaluate the impact of *F. persica* extract treatment on wound healing at molecular level, the amount of transcript for a gene contributing in regeneration (TGF- β) and a gene involved in inflammation (TNF- α) were analyzed (Figure 6). The results demonstrated that the transcript for TGF- β in the extract (both, $p < 0.05$) and Madecassol (both, $p < 0.05$) groups significantly increased compared to the control and vehicle groups. On the other hand, compared to the control and vehicle groups, significant downregulation was observed for TNF- α in the extract (both, $p < 0.05$) and Madecassol groups (both, $p < 0.05$).

4 | DISCUSSION

As other herbs whose wound healing potential isolated from popular knowledge has been demonstrated experimentally (Arunachalam & Parimelazhagan, 2013; Fikru et al., 2012), the present study proved the effectiveness of *F. persica* ointment in rat skin wound healing, according to macroscopic repair of wounds after 17 days, alongside its effects in the anti-inflammatory activity, elevate in re-epithelialization, angiogenesis, cell proliferation, and extracellular matrix remodelling by histology, immunohistochemistry and molecular assessments. We used two different models of excision and incision wounds because the use of a single method was not adequate to evaluate the wound healing process (Mukherjee et al., 2013). Also, in the present study, we reported antioxidant properties of *F. persica* for the first time. In order to evaluate the effect and compare treatment regimens in wound healing, we investigated the results in two time points, including day 7 (the interval between the two inflammation and proliferation phases) and day 17 (the interval between the two proliferation and remodelling phases) (Han & Ceilley, 2017; Milan et al., 2016).

Our results in linear incision model revealed the significant effect of *F. persica* extract on wound repair and recovery acceleration on day 3, in contrast to the control and vehicle treated groups. Also, macroscopic evaluation of healing in circular model showed similar results. Therefore, in the circular wound, the fastest and most complete healing (100% wound contraction) was observed within 17 days in the extract and Madecassol treated groups, whereas in the control and vehicle groups, wound area was still open at the end of the study. In our previous study about *Cantharellus cibarius* extract effects in wound healing, the major reason for similar results in macroscopic healing was due to the anti-oxidative and anti-inflammatory properties (Nasiry, Khalatbary, & Ebrahimzadeh, 2017). It seems to be the reason for this study as well.

As mentioned, one of the major challenges in wound healing is prolonged inflammation in wound area (Koh & DiPietro, 2011; Nasiry, Khalatbary, & Ebrahimzadeh, 2017). In wound healing phases, after assembly of the haemostatic clot at the wound site, some cytokines secreted from platelets and cells into region, such as TNF- α and IL-1 β , which stimulate leukocyte infiltration into the region and initiating the inflammatory response (Gushiken et al., 2017; Reinke & Sorg, 2012). Previous studies documented that this factor is involved in the mechanisms that contain the three phases of wound healing process (Ahanger et al., 2010; Kieran et al., 2013; Lim et al., 2006; Mori et al., 2002). On the other hand, continuous secretion of cytokines and proinflammatory factors by leukocytes and upregulation of inflammatory mediators of COX-2 in lesion cells lead to chronic inflammation and delaying the healing mechanism and finally, loss of tissue function (Khalatbary et al., 2017). Study of Siqueira et al. (2010) showed that blocking TNF- α gene caused a decreased in apoptosis and increased proliferation fibroblast cells and subsequently accelerated the wound healing. Also, in our previous study, there was a significant relationship between reducing COX-2 and wound healing (Nasiry, Khalatbary, & Ebrahimzadeh, 2017). In the present study, to evaluate anti-inflammatory potential of

TABLE 4 The mean scores for the histological features of the wound tissue samples

Wound healing ^a scaling group	Epithelial regeneration	Granulation tissue thickness
Control	1.5 ± 0.44	1.2 ± 0.56
Vehicle	1.5 ± 0.73	1.75 ± 0.3
Extract	4.0 ± 0.0 ^c	3.5 ± 0.49 [#]
Madecassol	4.0 ± 0.0 ^c	3.5 ± 0.22 [#]

Data are represented as mean ± SD.

^a*p* < 0.001 versus control and vehicle groups. [#]*p* < 0.001 versus the control group.

^c*p* < 0.01 versus the vehicle group.

F. persica, we used three different methods including stereology (inflammatory cells), immunohistochemistry (COX-2) and reverse transcription polymerase chain reaction (TNF- α). The results showed a considerable decrease in the expression of TNF- α in the extract treated group as compared to the control and vehicle groups. Our immunohistochemical assessments showed increased COX-2 expression in the control and vehicle groups, while significantly attenuated in the *F. persica* extract treated group. Also, this result was consistent with the inflammatory cells count.

Furthermore, at the same time with haemostatic clot formation and inflammation, the cells of the wound site synthesize some growth factors (e.g. TGF- β , EGF, and FGF), which stimulate cells migration, proliferation, and improved the synthesis of provisional extracellular matrix that led to new formation of vessels in the granulation tissue, modulated collagen deposition and remodelling of the permanent extracellular matrix (El Gazerly et al., 2013; Gushiken et al., 2017). In the present study, according to the molecular survey results of TGF- β , significant expression was observed in the extract treated group as compare to the control and vehicle groups. Therefore, it can significantly contribute to the wound healing activity of *F. persica*. Also, the comparison of fibroblast cells number in stereological assessment between extract with control and vehicle groups showed a significant increase. One of the most important products in fibroblast cells is collagen (Nasiry, Khalatbary, & Ebrahimzadeh, 2017). In the present study, we evaluated and compared collagen density between groups using Masson's trichrome staining (Figures 2 and 3). As shown, the extract treated group was significantly higher than the control and vehicle groups. This is probably due to stimulating the collagenesis and fibroblast proliferation of this extract. It can be said, probably *F. persica* stimulate the TGF- β gene expression and then proliferation of fibroblast cells, and ultimately, these cells, by producing collagen and contraction, accelerate the wound healing.

In the histological examination, the scoring system (epithelialization and granulation tissue formation) was evaluated in the experimental groups (Tables 1 and 4). Our results showed a significant difference in epithelialization and tissue granulation between the extract treated with control and vehicle treated groups. Moreover, the angio-

genesis factor in the present study was evaluated by length of blood vessels in stereological method. The angiogenesis plays an important role in newly formed blood vessels, comprising 60% of the repaired tissue (Ehrlich et al., 1972). Neovascularization helps hypoxic wounds to attain normal conditions of oxygen use.

5 | CONCLUSION

To the best of our knowledge, the present study was the first report on generation and application of *F. persica* extract to enhance healing of wound in the animal model. The *F. persica* extract showed a high anti-inflammatory activity by decreasing inflammatory cells and down-regulation of TNF- α gene and COX-2 protein. Also, our results revealed that the present extract is able to increase the fibroblasts, collagen density and up-regulation of TGF- β gene. However, in all cases, the extract was even better than Madecassol drug. Generally, all data suggest that the *F. persica* extract can be based on its traditional uses supported by evidence based on experimental data. To study the safety and effectiveness of the *F. persica* extract in human wounds, further investigations are needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Lei Huang: Methodology, Project administration, Writing-review & editing. Mengting Wang: Investigation, Validation, Visualization. Mohammad Ali Ebrahimzadeh: Data curation, Formal analysis, Methodology, Project administration. Aref jafari: Data curation, Methodology. Kejjiao Jiange: Data curation, Investigation, Methodology, Project administration, Validation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants performed by any of the authors. The authors declare their consent to participate for this article

DATA AVAILABILITY STATEMENT

The authors stated supporting data will be made available to others on request.

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