Pharmaceutical Sciences
December 2017, 23, 256-263
doi: 10.15171/PS.2017.38
http://journals.tbzmed.ac.ir/PHARM

Research Article

Wound Healing Potential of Methanolic Extract of *Scrophularia striata* in Rats

Ali Ghashghaii[1]*, Mohammad Hashemnia[2], Zahra Nikousefat[1], Mohammad Mahdi Zangeneh[2], Akram Zangeneh[2]

[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.*
[2]*Department of Pathobiology, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.*

Article Info

Article History:
Received: 13 April 2017
Accepted: 20 September 2017
ePublished: 30 December 2017

Keywords:
-Mmethanolic extract
-Rat
-Scrophularia striata
-Wound healing

ABSTRACT

Background: Scrophularia striata is a well-known plant in Iranian traditional medicine and its anti-oxidative and anti-inflammatory properties make it a logical adjuvant to improve wound healing. This study was designed to evaluate the wound healing potential of S. striata on cutaneous wounds in rat.
Methods: A full-thickness excisional wounds was induced on the back of 75 Sprague-Dawley rats. The animals were randomly allocated into five groups, treated with 1ml basal cream, 1ml tetracycline (3%), 1ml S. striata 5%, 1ml S. striata 10% and untreated (control). Five animals of each group were euthanized at each of 10, 20 and 30-days post-injury (DPI) and wounds were assessed through gross and histopathological analyses.
Results: Treated rats with S. striata showed a significant decrease in the wound area during the experiment compared to other groups. Additionally, treatment with S. striata decresed the number of lymphocytes and enhanced the number of fibroblasts at the earlier stages and increased number of fibrocytes at the later stages of wound healing. Other parameters such as alignment of the healing tissue, re-epithelilization and epithelial formation, enhanced maturity of the collagen fibers and fibroblasts and large capillary-sized blood vessels showed significant changes when compared to control. The best wound healing activity was observed with the high dose of S. striata.
Conclusion: The present study showed that application of S. striata extract on wounds induces considerable wound contraction and accelerates healing and it may be suggested for treating different types of wounds in animal and human beings.

Introduction

Wound is a physical injury that results in an opening and break of the skin that affects function and anatomy of the normal skin. The process of wound healing has several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and acquisition of wound strength.[1] Despite some recent developments in understanding its fundamental principles, healing of wound defects has also faced with considerable limitations, including scar tissue formation and cosmetic concerns.[2,3]

One of the developing fields in modern medical sciences is research on wound healing agents and the quest for more effective drugs is perhaps one of the important challenges for investigators. Medicinal plants are popular remedies used by many people throughout the world. The usefulness of medicinal plant in the treatment of diseases is unquestionable. The World Health Organization estimated that 80% of the people in many countries use plants as their primitive source of medication.

The medicinal plants have high content of tannins, flavonoids, saponins, alkaloids, naphthoquinone and triterpenes, hence they have been used for many years in the management of cutaneous wounds to increase the quality and rate of healing.[4-9]

Iran has a rich flora that are widely distributed throughout the country, particularly in the west. In Iranian traditional medicine, herbal medicines have been the source of treatment and cure for many diseases and physiological conditions. One of the most important herbal medicines, which is widely consumed in the west of Iran, is *Scrophularia striata* known as “Tashaneh Dari” from Scrophulariaceae

***Corresponding Author:** Ali Ghashghaei , E-mail: aghashghaii@razi.ac.ir

family.

This plant is annual or perennial herbs with five petals and zygomorphic flowers and corolla has lobe and the fruit is a capsule with many seeds.[10] Alkaloids, flavonoids, resin glycosides, iridoid and cryptophilic acid are active constitutes of *S. striata* that usually are found in some parts of plants such as stem, leave, bud, skin and scion.[11,12] *S. striata* has been reported to have some of pharmacological effects such as Analgesic, Anti-microbial, Nephroprotective, nitric oxide suppressive, antitumoral, hepatoprotective and anti-inflammatory properties.[11-15]

According to the literatures review, *S. striata* genus's anti-oxidative and anti-inflammatory properties make it a logical adjuvant to management wound healing. So, this study was designed to evaluate the dermal wound healing potential of *S. striata* after topical application of its methanolic extract on experimentally induced cutaneous wounds in rat models.

Materials and Methods

Plant Material and extract preparation

The aerial parts of *S. striata* were collected from Zagros mountain ranges (around the Kermanshah city) in April 2015 and the herbarium was prepared after they were identified and approved by the N. Eskandari (Code=SS405). The collected plant materials were first cleaned and then were dried at room temperature without exposure to direct sunshine. In the next step, small pieces of dried plants were provided by cutter and 300 grams were soaked in methanol for 72 hours and filtered subsequently. This process was repeated twice to ensure maximal extraction. After extraction, the solvent was filtered and then evaporated by Rotavapor®. The achieved extract was then stored at -20°C until being used in the experiment.

Animals

The experiments were performed in adult Sprague-Dawley rats of both sexes, weighing 200 to 220 g. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/dark cycle) and maintained with free access to water and *ad libitum* standard laboratory diet (70% carbohydrates, 25% proteins, 5% lipids).

Wound creation

The rats were weighed prior to the surgical procedure. The animals were anaesthetized by intramuscular injection of 1mg/kg xylazine HCl (Xylazine 2%; Alfasan) as premedication, and 60mg/kg ketamine HCl (Ketamine 5%; TRITTAU, Germany) for anesthesia. The backs of the animals, in the cervical region were surgically prepared for aseptic surgery. A square shape full thickness incision of 2×2 cm was made in skin and the incised piece was removed. The wound was left undressed and no local or systemic anti-microbial drugs were administrated.

Study design

After wound creation, the animals were randomly allocated into five main groups, each containing 15 animals, and three subgroups, representing days, 10, 20 and 30 after injury, each containing 5 animals. The subgroups were numbered as follows: control (1–3), basal cream (4–6), tetracycline (7–9), *S. striata* 5% (10–12) and *S. striata* 10% (13–15). No material was used in the wound area of rats in the control group. In the basal cream and tetracycline groups, the injured area was covered with 1ml basal cream (eucerin) and tetracycline (3%) daily, for 20-days post injury (DPI). In the group 4 and 5, the wound area was covered with 1ml *S. striata* 5% and 10% (5 and 10 g *S. striata* powder were suspended in 95 and 90 g eucerin) for 20 DPI, respectively. From each group, 5 animals were euthanized at each of 10, 20 and 30 DPI by chloroform inhalation. Samples from all these groups were collected and used for histopathological evaluation.

Measurement of wound area

The progressive changes in wound contraction were monitored planimetrically on days 10, 20 and 30 PI using the method as described by Oryan et al. (2010).[16]

Sample collection and histological evaluation

At the end of days 10, 20 and 30 postoperation, the animals were euthanized by chloroform inhalation and sampling was done. Full thickness skin samples from the wound area including dermis, epidermis and subcutaneous were carefully dissected and fixed in 10% neutral-buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 μm thickness, stained with hematoxylin and eosin and examined with a routine light microscope.

Histological examinations were performed by two pathologist with a procedure reported by Oryan et al. (2012)[8] with some modifications. The pictures were then captured by a digital camera (Dino capture; version 1.2.7) and transferred to the computer software (Photoshop CS-4; Adobe) for digital analysis. Five photomicrographs were selected from five microscopic fields of each tissue sample for histopathologic analysis. The parameters that were studied in histopathological sections were consisted of fibrin deposition, hemorrhage, polymorphonuclear and mononuclear cell infiltration, re-epithelialization, epithelium cornification, revascularizations, fibroblast and macrophage content, necrosis, presence of fibrocytes and maturation and organization of collagen.

Table 1. Mean ± SD of wound surface area (cm^2) in groups on different day post-injury (*n* = 15).

Day	Control	Basal cream	Tetracycline	*S. striata* (5%)	*S. striata* (10%)
Day 10	2.12 ± 0.05^{a}	2.05 ± 0.05^{ab}	1.95 ± 0.06^{abc}	1.91 ± 0.04^{bc}	1.77 ± 0.03^{c}
Day 20	1.29 ± 0.03^{a}	1.17 ± 0.05^{ab}	1.09 ± 0.03^{b}	1.05 ± 0.03^{b}	0.92 ± 0.02^{b}
Day 30	0.71 ± 0.02^{a}	0.63 ± 0.04^{a}	0.52 ± 0.02^{a}	0.29 ± 0.01^{b}	0.18 ± 0.01^{b}

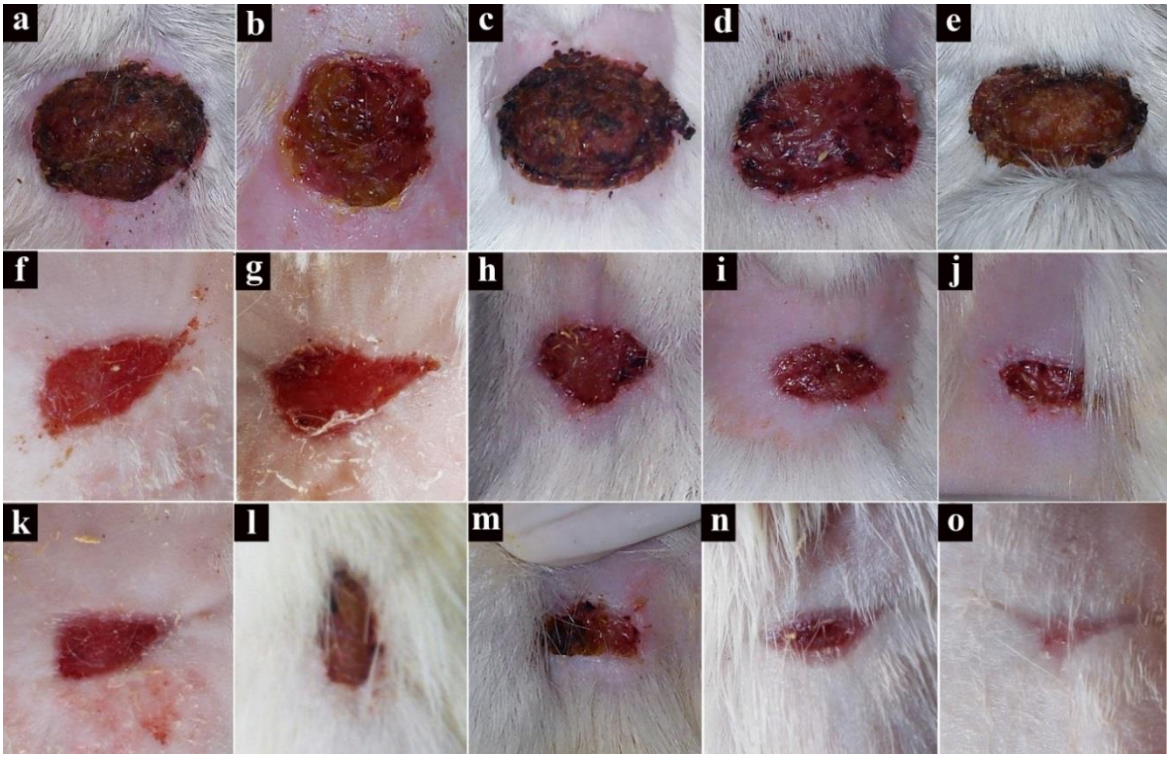

Figure 1. Macroscopic wound images of the control (a,f,k), basal cream (b,g,l), tetracycline (c,h,m), *S. striata* 5% (d,i,n) and *S. striata* 10% (e,j,o), on days 10, 20 and 30 post-injury.

Total cellularity (magnification ×200) and number of fibroblasts, fibrocytes, lymphocytes, macrophages, neutrophils and blood vessels (magnification×800) of the wound area were counted and their mean and standard deviations were calculated.

Statistical analysis

Descriptive statistics including the mean, standard error, median, minimum and maximum were calculated for all variables. For comparison of different parameters, the one-way ANOVA followed by Tukey post hoc test were used. The data were analyzed by SPSS software, version 22.0 (SPSS Inc., Chicago, IL, USA) and P<0.05 was accepted as statistically significant.

Results

The wound surface area was calculated and expressed in cm^2 as summarized in Table 1. A significant reduction was observed in the wound surface area of the low- and high-dose *S. striata* groups compared to those of the control group on days 10, 20 and 30 PI and also cream and tetracycline groups on day 30 PI (P<0.05) (Figure 1a-o).

Statistically significant differences were determined by ANOVA followed by post hoc tests comparing all groups. Means within a column with different superscript letters (a, b, c) denote significant differences. P<0.05 was accepted as statistically significant.

The data obtained from the histopathologic analysis are summarized in Table 2. At 10 DPI, treatment with low- and high-dose *S. striata* significantly reduced total cellularity compared with the cream, tetracycline and control groups (P<0.05; Figure 2a–e). Although the mean number of cells in low-dose *S. striata* group was lower than that in the high-dose *S. striata*, but this difference was not statistically significant (P>0.05). At 20 DPI, low- and high-dose *S. striata* significantly reduced the total cellularity compared with control and cream groups (P<0.05), but the differences were not significant as compared to the tetracycline group (P>0.05; Figure. 2f-j).

Five fields in each of five histopathologic sections were analyzed for each group.

Data are Mean ± SD, n = 15. Statistically significant differences were determined by ANOVA followed

by post hoc tests comparing all groups. Means within a column with different superscript letters (a, b, c) denote significant differences. P>0.05 was accepted as statistically significant.

Thirty days after injury, lower total cellularity was observed in high- and low-dose *S. striata* groups, respectively, but these differences were not statistically significant between groups at this stage (P>0.05; Figure 2k-o).

The vascular number were significantly increased in low and high-dose *S. striata* groups on day 10 PI compared with the other groups (P<0.05). At 30 DPI, except for high-dose *S. striata*, which showed a significant decrease in vascular number in comparison with the control and cream groups (P=0.001 and P=0.017), the differences for other groups were not significant.

At 10 DPI, low- and high-dose *S. striata* increased the mean number of fibrocytes compared to the other groups on day 10 PI, but these differences were not statistically significant (P>0.05). At 20 and 30 DPI, the number of fibrocytes was significantly higher in low- and high-dose *S. striata* groups compared with the control, cream and tetracycline groups (P<0.05). In comparison between treatment groups at 30 DPI, the high-dose *S. striata* group showed a higher number of fibrocytes in compared with the low-dose *S. striata* group (P<0.05).

The number of fibroblasts in the low and high-dose *S. striata* groups was higher than those in the other groups on day 10 PI, but these differences were not significant. At 20 and 30 DPI, the lowest number of fibroblasts was observed in the low- and high-dose *S. striata* groups, respectively, which this decrease in high-dose *S. striata* group was statistically significant when compared to the cream and control groups (P<0.05).

The number of lymphocytes was decreased in high-dose *S. striata* group compared with the other groups on day 20 PI, but this difference was not statistically significant (P>0.05). At 30 DPI, high-dose *S. striata* significantly decreased the number of lymphocytes compared to the control group (p=0.04).

Table 2. Histopathologic and histomorphometric analysis.

Day 10	**Control**	**Basal cream**	**Tetracycline**	***S. striata* (5%)**	***S. striata* (10%)**
Total cell	1323.30±68.44^{a}	1264.60±59.87^{a}	1424.70±21.96^{a}	852.80±103.08^{bc}	921.60±56.97^{bc}
Vascular no.	8.80±0.38^{a}	9.10±0.34^{a}	9.90±1.06^{a}	12.60±0.54^{b}	14.18±0.72^{b}
Fibroblast and fibrocytes	17.80±0.92^{a}	18.40±0.68^{a}	22.20±0.89^{a}	23.30±0.84^{a}	24.45±1.80^{a}
Fibrocytes	3.10±0.52^{a}	3.40±0.47^{a}	5.27±0.54^{a}	5.90±0.83^{a}	5.70±0.84^{a}
Fibroblasts	14.70±1.10^{a}	15.00±0.55^{a}	16.50±0.95^{a}	17.40±0.92^{a}	19.18±1.50^{a}
Ratio	0.23±0.04^{a}	0.21±0.03^{a}	0.37±0.07^{a}	0.36±0.06^{a}	0.28±0.03^{a}
Lymphocyte	21.70±1.04^{a}	20.90±1.13^{a}	22.10±3.30^{a}	23.50±1.31^{a}	22.18±1.06^{a}
Macrophage	19.10±0.94^{a}	21.20±1.31^{a}	20.70±0.94^{a}	22.20±1.51^{a}	21.63±0.84^{a}
Neutrophil	1.10±0.31	1.00±0.33	0.20±0.13	0.40±0.16	0.45±0.24
Day 20					
Total cell	1234.80±30.96^{a}	1253.40±54^{a}	814.50±38.54^{b}	709.70±73.41^{b}	770.50±38.46^{b}
Vascular no.	4.70±0.26^{a}	4.50±0.22^{a}	5±0.69^{a}	6.30±0.42^{a}	6.60±0.84^{a}
Fibroblast and fibrocytes	31.44±2.92^{a}	29.80±2.79^{a}	26.30±1.12^{a}	30.90±1.13^{a}	30.50±2.08^{a}
Fibrocytes	8.22±0.57^{a}	7.60±0.68^{a}	12.10±0.62^{a}	15.50±0.73^{b}	17.00±1.32^{b}
Fibroblasts	23.22±3.10^{a}	22.20±2.61^{a}	15.10±1.29^{ab}	15.40±1.40^{ab}	13.50±2.42^{b}
Ratio	0.43±0.08^{a}	0.38±0.05^{a}	0.80±0.08^{a}	1.10±0.14^{ab}	1.99±0.52^{b}
Lymphocyte	14.66±1.13	15.20±0.74	14.20±1.31	14.50±1.14	10.80±1.80
Macrophage	21.66±0.88^{a}	19.40±1.18^{a}	17.40±1.09^{a}	19.40±1.26^{a}	18.40±0.71^{a}
Neutrophil	0.22±0.14	0.30±0.30	0.20±0.13	0.10±0.10	0.00
Day 30					
Total cell	686.90±20.53^{a}	664.60±21.43^{a}	628.20±27.94^{a}	500.30±30.37^{a}	492.30±32.04^{a}
Vascular no.	6.70±0.53^{a}	6.10±0.34^{a}	5.10±0.58^{ab}	4.50±0.22^{ab}	3.30±0.33^{b}
Fibroblast and fibrocytes	31.20±1.73^{ab}	29.40±2.14^{a}	25.70±1.39^{a}	31.10±1.18^{ab}	37.60±1.48^{b}
Fibrocytes	13.90±0.92^{a}	12.50±0.74^{a}	13.30±1.44^{a}	21.20±1.05^{b}	28.80±0.85^{c}
Fibroblasts	17.30±1.85^{a}	16.90±1.68^{a}	12.40±0.88^{ab}	9.90±0.92^{ab}	8.80±0.91^{b}
Ratio	0.97±0.18^{a}	0.82±0.12^{a}	1.14±0.16^{a}	2.41±0.39^{b}	3.57±0.37^{c}
Lymphocyte	12.00±0.43	11.70±0.49	7.60±0.79	9.10±0.84	5.50±0.50
Macrophage	16.50±0.67^{a}	17.60±0.89^{a}	16.00±0.81^{a}	14.60±0.89^{ab}	10.80±0.85^{b}
Neutrophil	0.30±0.21	0.00	0.00	0.00	0.00

Treatment with high-dose *S. striata* significantly reduced the number of macrophages compared to the control (P=0.01), cream (P=0.001) and tetracycline groups (P=0.03) at 30 DPI; however, the animals in low-dose *S. striata* group revealed a reduction in the number of macrophages compared to other groups at this stage, but these differences were not significant (P>0.05).

At 10 DPI, a thick granulation tissue that covered the wounds area was observed in all rats. The newly synthesized collagens were still unorganized and had a randomly distributed pattern in all rats at this stage. The dilated blood vessels were observed within the granulation tissue area in all animals.

No regenerating epithelium was evident in control and cream groups, while animals in low- and high-dose *S. striata* groups showed minimal re-epithelization. In control, cream and tetracycline groups, the presence of neutrophils were prominent within the granulation tissue, however there are no signs of infection around the wound area.

At 20 DPI, more organized pattern in the collagen fibers and better tissue alignment were observed in low and high-dose *S. striata* groups when compared to the other groups. There was full thickness epidermal regeneration which covered completely the wound area. The keratin layer was thin, composed of orthokeratin. The epidermis was thick and disorganized, particularly as compared with the adjacent normal skin. The blood vessels of the treated wounds with *S. striata* were more than those of the untreated lesions and also their diameters were larger compared to the untreated lesions. In the control group, the dermis was cellular, and the presence of fibroblasts and disorganized and poorly oriented collagen fibers were prominent. In this stage, the evidence of pus accumulation, fibrin deposition, polymorphonuclear cells infiltration or edema were not seen in the lesions of animals in all groups.

At 30 DPI, the wounds treated with *S. striata* extract had a proper re-epithelilization and epithelial formation than other groups. Moreover, a number of lymphocytes and macrophages was decreased in the treated groups, and significantly greater tissue maturation and large capillary-sized blood vessels were observed compared to other groups. (Figure 2p-t).

Discussion

Wounds are referred to as a disruption of normal anatomic structure and function. Skin wounds could have made by several causes such as physical injuries resulting in opening and breaking of the skin.[17] Bleeding, heat and redness around the wound, loss of feeling or function below the wound site, painful or throbbing sensation, swelling of tissue in the area and pus like drainage are common symptoms of the wounds.[18]

Wound healing is a very complex, multifactor sequence of events that establish the integrity of the tissues. Regeneration and reconstruction of the interrupted anatomical continuity and functional status of the skin are the main aim in these processes.

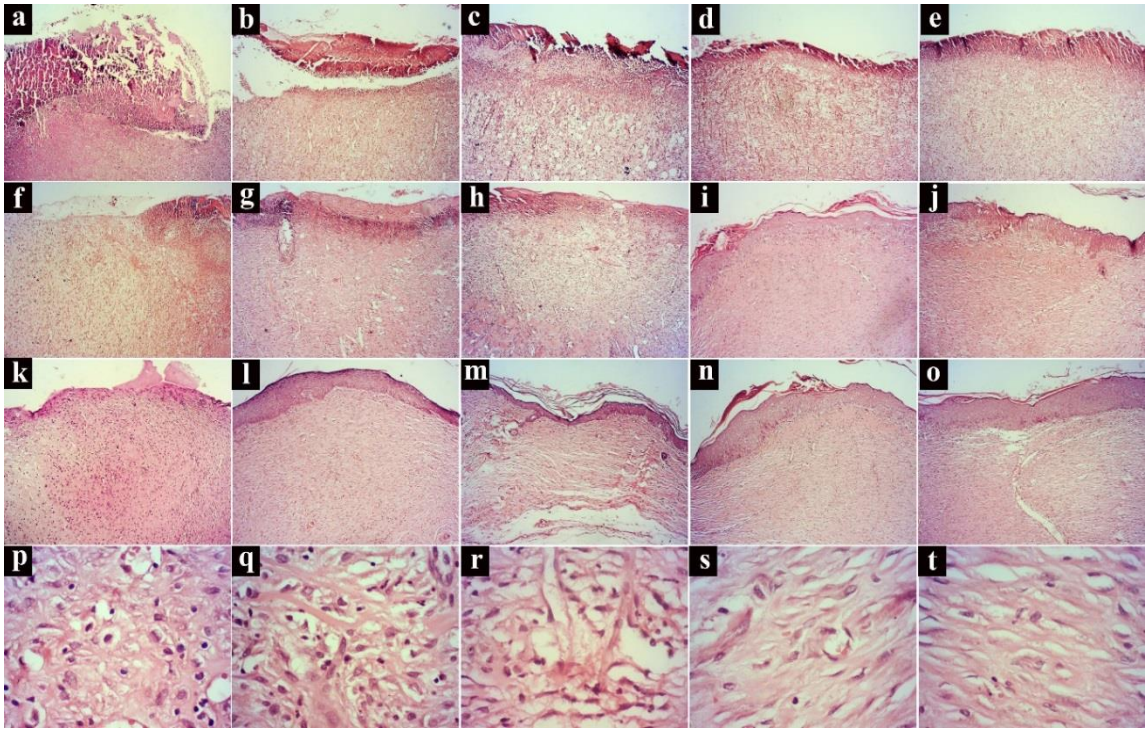

Figure 2. Longitudinal sections of the control (a,f,k,p), basal cream (b,g,l,q), tetracycline (c,h,m,r), S. striata 5% (d,i,n,s) and S. striata 10% (c,j,o,t), on days 10, 20 and 30 post-injury (H&E staining; magnification for a–o=×250, and for p–t=×1000).

Healing process initiates immediately after wounding and has four steps including coagulation, inflammation, re-epitheliasation and remodeling.[19]

In recent years, tendency of using natural sources as alternative medicine has been raised. Plants are candidate source of potentially valuable structures for achieving effective chemotherapeutic agents.[15] *S. striata* is used traditionally as a medicinal herb in Iran and some other Asian countries. Recently, some novel pharmacological actions of *S. striata* have been discovered, such as antimicrobial effects, inhibitory effect on matrix metalloproteinase (MMPs), suppression of NO production in activated murine peritoneal macrophages *ex-vivo* and stimulatory effects on human fibroblast cells proliferation and anti-tumor activity.[20] However, no study has been performed yet to investigate the wound healing activity of *S. striata*.

The results of this study established that the wound healing and repair is accelerated by topical application of *S. striata*. The enhanced capacity of wound healing with the *S. striata* could be explained on the basis of the anti-inflammatory properties of the plants that are well documented in the literature.[12,14,15] The inflammatory processes of the treated samples showed improved fibroplasia and remodeling stages of wound healing. Moreover, decrease in total cellularity and enhanced fibroblast maturation and differentiation were observed in the wound area.

The clear difference in the measured wound area between the rats treated with *S. striata* and other groups is supported by the above mentioned histological observations. Wound contraction is the procedure of mobilizing normal skin around the wound to cover the denuded area and contains complex and excellently coordinated interactions of cells, extracellular matrix and cytokines.[21] Probably, the anti-inflammatory effects of *S. striata* together with its effect on maturation and organization of the granulation tissue are responsible for the enhanced rate of wound closure and reduction in healing time in the treated rats.

Our histological findings on day 10 PI revealed that the original tissue regeneration is much better in skin wounds treated with *S. striata* than in control wounds. Although these results established the beneficial effects of the *S. striata* on the morphology of dermal wound healing, but the newly synthetized collagens were still unorganized and had a randomly distributed pattern in all rats at this stage.

During the early wound healing process, epithelial cells proliferate and migrate from the edges of the wounds and finally cover it. An adequate oxygen supply has an important role in the proliferation and migration of the epithelial cells and fibroblasts. This amount of oxygen may be provided from two ways; increasing the rate of blood flow in the existing blood vessels, or through the newly formed blood vessels.[8] Based on histopathological analysis, the treated lesions with *S. striata* extract were more vascular at this stage and it seems that the second mechanism is responsible for providing more blood and oxygen supply and therefore an improved wound healing outcome in the treated animals.

On day 20 PI, the presence of full thickness epidermal layer which covered completely the wound area, reduction in cellularity and also a greater degree of organization of the collagen orientation were an important histopathological features of the treated lesions with *S. striata* extract when compared to the other groups. These results can be due to the anti-inflammatory effects of this plant and also the presence of iridoid glycoside compounds of *S. striata* that can accelerate the fibroblast growth and causing more collagen synthesis and faster healing.[14]

The presence of the collagen fibrils with a greater degree of organization and a more normal alignment in the treated lesions may be due to a modification of the inflammatory reaction or organization of the fibrin network in the wound area at early stages of inflammatory phase of healing by the *S. striata* extract.[16]

On day 30 PI, the treated wounds with *S. striata* showed a proper re-epithelilization and epithelial formation when compared to other groups. In addition, the treated wounds with *S. striata* had a lower number of lymphocytes and macrophages and higher number of fibroblasts and fibrocytes, significantly better maturation, improved tissue alignment and large capillary-sized blood vessels than other groups.

The lower levels of inflammation in the *S. striata* groups can be due to the presence of phenyl propanoid glycosides in this plant species. This compound can inhibit the macrophages activities and production of chemical mediators; consequently, decrease inflammation and subsequently promote organization.[14,22]

Generally, the wounds treated with 10% *S. striata* showed a better healing than wounds treated with 5% *S. striata*. The reduction of the neovasculature and better organization of collagen fibrils in dermis observed in the treated lesions suggest that the high dose of *S. striata* accelerates the process of wound remodeling more than low dose of *S. striata*.

Due to some limitations, the biomechanical analysis was not performed in this experiment; certainly, along with histopathological examination, biomechanical analysis can be helpful in interpreting the results. To determine the methanolic extract properties of *S. striata*, its anti-oxidant effect and evaluate the expression of the related genes, more phytochemical studies are needed to characterize and identify the specific active compounds of this plant that are responsible for wound healing activity.

Moreover, the higher rate of the wound healing in rats, small population size, age and the anatomical and physiological variations between rats and humans should be considered as limiting factors. Therefore, this is beneficial to support the novel insight of this study with future clinical trials and to perform more *in vivo* mechanistic researches in different species of animals before suggesting this therapeutic regime for clinical practice.

Conclusion

The present study established that the methanolic extract of *S. striata* improve wound healing activity in animal as a preclinical study. The obtained results showed that application of *S. striata* extract on wounds induces considerable wound contraction and accelerates healing and it may be a good candidate for treating different types of wounds in animal and human beings.

Acknowledgement

We thank the authorities of Veterinary School of Razi University for their cooperation.

Conflict of interests

The authors claim that there is no conflict of interest. Int. j. appl. res. nat. prod

References

1. Ayyanar M, Ignacimuthu S. Herbal medicines for wound healing among tribal people in Southern India: Ethnobotanical and Scientific evidences. Int J Appl Res Nat Prod. 2009;2(3):29-42.
2. Ruszczak Z, Schwartz RA. Modern aspects of wound healing: An update. Dermatol surg. 2000;26(3):219-29. doi:10.1046/j.1524-4725.2000.09215.x
3. Stavrou D. Neovascularisation in wound healing. J wound care. 2008;17(7):298-302. doi:10.12968/jowc.2008.17.7.30521
4. Ohshima H, Yoshie Y, Auriol S, Gilibert I. Antioxidant and pro-oxidant actions of flavonoids: effects on DNA damage induced by nitric oxide, peroxynitrite and nitroxyl anion. Free Radic Biol Med. 1998;25(9):1057-65. doi:10.1016/s0891-5849(98)00141-5
5. Baie SH, Sheikh KA. The wound healing properties of *Channa striatus*-cetrimide cream-wound contraction and glycosaminoglycan measurement. J Ethnopharmacol. 2000;73(1-2):15-30. doi:10.1016/s0378-8741(00)00253-1
6. Nayak BS, Isitor G, Davis EM, Pillai GK. The evidence based wound healing activity of lawsoniainermis Linn. Phytother Res. 2007;21(9):827-31. doi:10.1002/ptr.2181
7. Tong M, Tuk B, Hekking IM, Pleumeekers MM, Boldewijn MB, Hovius SER, et al. Heparan sulfate glycosaminoglycan mimetic improves pressure ulcer healing in a rat model of cutaneous ischemia-reperfusion injury. Wound Repair Regen. 2011;19(4):505-14. doi:10.1111/j.1524-475x.2011.00704.x
8. Oryan A, Tabatabaiei Naieni A, Moshiri A, Mohammadalipoor A, Tabandeh MR. Modulation of cutaneous wound healing by Silymarin in rats. J Wound Care. 2012;21(9):457-64. doi:10.12968/jowc.2012.21.9.457
9. Hashemnia M, Javdani M, Nikousefat Z, Hoseinpour F Kakaei Sh. Evaluation of the wound healing activity of methanolic extract of *Tragopogon porrifolius* in rat. Res Opin Anim Vet Sci. 2014;4(8):446-52.
10. Sharafati-chaleshtori R, Rafieian-kopaei M. Screening of antibacterial effect of the *Scrophularia striata* against *E. coli in vitro*. J Herbmed Pharmacol. 2014;3(1):31-4.
11. Sofiabadi M, Azadmehr A, Hajiaghaei R, Rezazadeh Sh, Ajdari Zarmehri H. The effect of ethanolic extract of *Scrophularia striata* on pain in male rats. J Med Plants. 2012;2(42):113-9.
12. Bahrami AM, Ali V. Effects of *Scrophularia striata* ethanolic leaves extracts on Staphylococcus aureus. International Journal of Pharmacology. 2010;6(4):431-4. doi:10.3923/ijp.2010.431.434
13. Zaheri M, Ebrahimi S, Cheraghi J. Protective effect of aerial parts extract of *Scrophularia striata* on cadmium and mercury-induced nephrotoxicity in rat. Journal of Babol University of Medical Sciences. 2011;13(4):48-53.
14. Azadmehr A, Afshari A, Baradaran B, Hajiaghaee R, RezazadehS, Monsef-Esfahani H. Suppression of nitric oxide production activated murine peritoneal macrophages *in vitro* and *exvivo* by *Scrophularia striata* ethanolic extract. J Ethnopharmacol. 2009;124(1):166-9. doi:10.1016/j.jep.2009.03.042
15. Zamanian-Azodi M, Ardeshirylajimi A, Ahmadi N, Rezaee MB, Azizi Jalilian F, Khodarahmi R. Antibacterial effects of *Scrophularia striata* seed aqueous extract on *staphylococcus aureus*. J Paramed Sci. 2013;4(1):58-63. doi:10.22037/jps.v4i1.3936
16. Oryan A, Tabatabaei Naeini A, Nikahval B, Gorjian E. Effect of aqueous extract of Aloe vera on experimental cutaneous wound healing in rat. Veterinarski Arhiv. 2010;80(4):509-22.
17. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, Rodeheaver G, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol. 1994;130(4):489-93. doi:10.1001/archderm.1994.01690040093015
18. Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a

crude extract of *Portulaca oleracea* L. (growing in Jordan) in *Mus musculus* JVI-1. J Ethnopharmacol. 2003;88(2-3):131-6. doi:10.1016/s0378-8741(03)00194-6

19. Phillips GD, Whitehe RA, Kinghton DR. Initiation and pattern of angiogenesis in wound healing in the rats. Am J Anat. 1991;192(3):257-62. doi:10.1002/aja.1001920305
20. Salavati P, Ramezani M, Monsef-Esfahani HR, Hajiagha R, Parsa M, Tavajohi S, et al. Neuroprotective effect of total and sequential extract of *Scrophularia striata* boiss. in rat cerebellar granule neurons following glutamate-induced neurotoxicity: An *in-vitro* study. Iran J Pharm Res. 2013;12(2):389-94.
21. Kumari M, BR E, Amberkar M, Babu S, Rajshekar, Kumar N. Wound healing activity of aqueous extract of *Crotalaria verrucosa* in Wistar albino rats. Asian Pac J Trop Med. 2010; 3(10):783-7. doi:10.1016/s1995-7645(10)60187-3
22. Díaz AM, Abad MJ, Fernández L, Silván AM, De Santos J, Bermejo P. Phenylpropanoid glycosides from: *in vitro* anti-inflammatory activity. Life Sciences. 2004;74(20):2515-26. doi:10.1016/j.lfs.2003.10.008