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Research Article

The Effect of Aqueous-Alcoholic Extract of Toothbrush Tree (*Salvadora persica*) on the Healing of Second-Degree Skin Burns in BALB/c Mice

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Abstract

Background: Researchers have mentioned many beneficial effects for the compounds present in the toothbrush tree (Miswak) (*Salvadora persica*: SP); such as anti-inflammatory, antioxidant and antimicrobial activities. The current study aimed to evaluate the effect of aqueous-alcoholic extract of toothbrush tree on the wound healing of second-degree skin burns in BALB/c mice.

Methods: In this study, 60 mature mice (8 weeks) were used. The mice were divided into 5 groups of twelve. Groups 1 and 2 were respectively treated with concentrations of 5% and 10% of aqueous-alcoholic extract of the toothbrush plant, group3 was treated with silver sulfadiazine ointment (positive control), group4 was treated with Vaseline (negative control), and group 5 (sham) received no treatment. A second-degree circular burn wound with a diameter of 1 cm was made on the back of the animal. The first to fourth groups were dressed twice a day. On days 4, 7, 10 and 14, sampling was performed from the wounded site and wound healing was evaluated histopathologically.

Results: Inflammation and infiltration of neutrophils and lymphocytes, being compared to the negative and sham control groups, were significantly reduced in the group treated with 10% SP extract ($P<0.01$); besides, on days 10 and 14 in the group treated with 10% and 5% SP extracts, the number of fibroblasts, followed by collagen production, epithelialization and formation of new hair follicles in the wound margins significantly increased compared to the negative control and sham group ($P<0.05$). The number of fibroblasts and collagen fiber density in the group treated with 10% SP extract, compared to the 5% extract group and silver sulfadiazine, showed a significant increase ($P<0.05$).

Conclusion: The findings showed that using extract of toothbrush plant accelerates the healing process of burn wounds.

Introduction

Skin as a physical barrier separate the outside from the inside the body, protects body from microorganisms, pathogens and other chemicals; besides, skin help prevent water loss . thus the skin indispensably needs to maintain its integrity.[1] Skin consists of three layers i.e. the epidermis with ectoderm origin having epithelial structure, the dermis with mesoderm origin being made up of connective tissue specially collagen fibers, and the hypodermis (subcutaneous tissue), a layer beneath the dermis with a fatty tissue structure.[2]

Burn wounds are caused by heat, chemicals, electricity, sunlight or nuclear radiation. Skin acts as a protective barrier against the environment; burn injuries, locally and systemically decrease skin resistance and also reduce its strength as an immune organ. Burns with regard to the depth and penetration into the skin layers and lower parts are divided into 4 types. In second-degree burns, the epidermis and dermis are entirely damaged. Following burns, a set of physiological responses are occurred; including changes in cellular protection mechanisms, systemic and local inflammation.[3] The skin healing process is a systematic and gradual process that includes 4 stages of homeostasis, inflammation, proliferation and remodeling.[4,5]

The wound healing quality depends on many features as to the wound type, the general condition of the patient and related diseases, the wound size, the infection (due to the production of toxins and free radicals), the presence of a foreign body and microorganisms, the blood flow

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to the site, hypoxia, as well as other factors such as age, health status, and nutrition.[6,7] The perfect treatment and protection of these wounds till tissue integrity is restored, increase the healing speed, reduce scar formation and also prevent them from being chronic ~~conditions~~ and infectious, which have always been seriously considered by researchers.[8,9]

Today, many therapeutic drugs have natural origins. Herbal remedies have a special position in traditional medicine and are used for treatment of many diseases.[10,11] in ancient traditional medicine, the use of medicinal plants and their derivatives has been prevalent for wound healing; interestingly, owing to their antioxidant and anti-inflammatory properties, and enhancing collagene synthesis, in new studies the effectiveness of these compounds in wound healing process has been confirmed.[8,12,13] Herbal medicines are more affordable and also more compatible with body's physiology; because they are less likely to poison human being and rarely cause side effects.[14,15] *Salvadora persica* (SP), belonging to the Salvadoraceae family is a plant that is called "Miswak or toothbrush" in Arabic. for many centuries, Muslims have used the twig of the plant as toothbrush for brushing their teeth.[16] Some of the most important components extracted from the plant are salts (namely chlorides), salvadourea, salvadorine, alkaloids, fluoride, silica, sulfur, vitamin C, resin, tannins, saponins, flavonoids, cyanogenic glycosides, and benzyl isothiocyanate. With these ingredients, SP has several pharmacological properties, including antibacterial and antifungal activities,[17] anti-inflammatory and sedative,[18] antioxidant,[19] anti-plaque formations, anti-hyperlipidemia and hypoglycemic.[20] Among the studies carried out on the healing process of gastrointestinal ulcers in rats, it was indicated that the extract of this plant has anti-inflammatory (by inhibiting the NF-kB pathway) and anti-ulcer properties.[21,22] In another study, the effect of *Salvadora persica* extract on excision wounds in rats was investigated, which showed that the wounds were thus closed and healed in a shorter time;[23] moreover, a cellular study showed that the aqueous extract of SP increased the number of fibroblasts in vitro.[24]

Based on the anti-inflammatory, antioxidant, and antimicrobial effects of SP, it seems that the extract of this plant is effective in the process of wound healing, and as very few studies have been done on wound healing, the present study investigates the effects of the aqueous-alcoholic extract of SP on the healing process of skin burn wounds in BALB/c mice.

Materials and Methods

Experimental animals

In this study, 60 mature male BALB/c mice (8 weeks old) weighing 25±5 g, were provided from the Experimental Medicine Research Center of Birjand University of Medical Sciences. Mice, having free access to food and water, were kept in separate clean cages. Environmental conditions were controlled by a 12:12 h light-dark cycle, 22-23°C temperature, and air humidity of 45 to 50%.

The ethical standards related to working with laboratory animals were conducted based on the protocol of work with laboratory animals approved by the Research Ethics Committee at Birjand University of Medical Sciences with the approved code 455460 and ethics code: IR.BUMS.REC.1397.89.

Mice were randomly divided into 5 groups of twelve, the first group received an ointment containing 5% SP aqueous-alcoholic extract, the second group received an ointment containing 10% SP aqueous-alcoholic extract,[23] The third group though received standard treatment with silver sulfadiazine as a positive control, and the fourth group received Vaseline dressing as a negative control. The fifth group (sham) did not receive any treatment.

Initially, mice were anesthetized with an intraperitoneal injection of ketamine 70 mg/kg and xylazine 2 mg/kg.[25,26] After shaving the hair of dorsal thoracic skin, a superficial second-degree burn wound (1 cm diameter), including the thickness of the dermis was made using a flat circular metal stamp. The flat head of the device was held on an alcohol lamp for 3 minutes, and then that was placed in contact with the mouse skin for 10 seconds.[8] the Mice with deep wounds getting beyond the hypodermis and even into the muscles were excluded from the study. Finally, for macroscopic comparison photographs were taken from the wound site using a digital camera. The wounds of each group were dressed topically using ointment twice a day at 8: am and 8: pm for 14 consecutive days. Three mice from each group were randomly selected on days 4, 7, 10 and 14, and after complete anesthesia with ketamine-xylazine, samples were taken from the burn wound.[27,28]

Preparation of plant extract

To prepare the aqueous-alcoholic extract of the mentioned plant, after collecting the plant for approval, the sample was identified by an experienced botanist and was kept in the herbarium of the Faculty of Natural Resources and Environment of Birjand University (Herbarium code: 2891). Sixty gram of the plant was stirred with 600 mL of 80% methanol for 48 hours at room temperature, and then the material was filtered via Whatman paper. The concentrated extract was dried using a freeze-dryer and was stored at -20°C until being used; ultimately, the extract yield was calculated. Using powder aqueous-alcoholic extracts of freeze-dryer, SP ointment with two different concentrations was mixed in sterile Vaseline, and *Salvadora persica* 5% and 10% was prepared; then they were kept in the refrigerator. each time a thick layer of the ointment was placed topically at the burn sit, and dressing was performed.[8]

Histological tests

Samples taken from the wound site on different study days were fixed with 10% formalin. Then after performing tissue passage steps on them, including dehydration with excessive concentration of alcohol, they were cleared in

xylene. After the sections were embedded in paraffin, 5 µm sections of specimens were prepared by rotary microtome. Later, to evaluate the cell, epithelialization, and hair follicles, they were stained with hematoxylin and eosin either. Specific staining of Masson's trichrome method was used to determine the general density of collagen fibers.[26] Several stained sections covering the entire wound were specifically selected for examination. A number of photographs were taken randomly from the selected slides by the Olympus SZX research microscope equipped with a camera (Euromex-CMEX-10).-Finally, cell count, collagen fiber density, epithelialization and a number of hair follicles were evaluated using ImageJ software.[28]

Statistical analysis

The normal distribution of numerical data was assessed by Shapiro-Wilk and Kolmogorov-Smirnov tests. Different parameters in groups were compared with each other by One-way ANOVA test with post hoc Tukey. Analysis of data was done by SPSS software version 22. Data were tabulated as Mean ± SD. The significance threshold was considered less than 0.05.

Results

In this study, various parameters i.e. the number of inflammatory cells, including neutrophils, lymphocytes, dermal fibroblasts, in addition to epithelialization, collagen fiber density and the number of hair follicles on different days were investigated. The gathered results are presented in Tables 1 and 2 below.

Evaluation of the parameters on day 4

On this day, the number of neutrophil cells in the groups treated with 10% SP extract (P<0.01) and silver sulfadiazine (P<0.01) was significantly decreased compared to the sham group. The epithelial coverage on both sides of the wound was far from each other than usual, and the wound surface was covered with fibrin, so other parameters were not examined.

Evaluation of the parameters on day 7

The number of neutrophils and lymphocytes in the group treated with 10% SP extract (P<0.01) and silver sulfadiazine (p<0.05) was significantly reduced compared to the sham group. The mean number of fibroblasts in both SP and silver sulfadiazine groups was significantly increased compared to the sham group (P<0.001). The number of fibroblast

Table 1. Comparison of different groups for the number of inflammatory cells on different days.

Parameter	Days of the study	Groups				
		Salvadora persica 5%	*Salvadora persica* 10%	Silver sulfadiazine	Vaseline	Sham
Neutrophils	4	3.1±1.44	2.2±1.88	1.9±1.66*	4.2±1.47	5.1±1.66
	7	37.7±7.86	31.3±5.75*	32.5±5.75*	42.8±6.35	44.7±4.54
	10	46.6±2.17	43.5±2.36*-**	43.0±2.26*-**	43.0±2.26	51.4±1.34
	14	43.4±6.41	38.7±4.21*	39.0±7.83*	51.9±7.72	53.0±8.78
Lymphocytes	7	3.4±2.11	2.3±1.25	2.5±1.77	4.3±1.88	5.3±1.94
	10	5.9±0.99*	5.3±1.15*	*5.3±1.15	8.4±0.84	8.9±1.19
	14	4.6±1.17*	4.00±1.05*	*3.8±1.31	6.7±0.94	7.7±1.25

* Significant difference with the vaseline and sham group (P<0.05).
**Significant difference with the *Salvadora persica* 5% group (P<0.05).

Table 2. Comparison of different groups for burn wound healing parameters on different days.

Parameter	Days of the study	Groups				
		Salvadora persica 5%	*Salvadora persica* 10%	Silver sulfadiazine	Vaseline	Sham
Fibroblasts	7	4.7±0.94*	6.2±1.47*-***	5.6±1.17*	2.5±0.84	2.2±1.03
	10	22.8±3.99*	29.7±3.83*-***	25.6±3.13*	17.2±2.93	16.5±4.88
	14	43.3±7.70*	52.1±4.81*-**-***	44.9±5.50*	32.3±4.21	31.2±4.10
Epithelialization in the margins	10	56.5±8.06	60.17±6.28	54.98±9.11	51.67±5.16	50.36±7.01
	14	86.96±6.89*	88.01±10.00*	78.88±5.76*	71.0±3.13	65.36±4.21
Density of collagen fibers	10	0.284±0.007*	0.299±0.003*-**-***	0.285±0.005*	0.265±0.003	0.261±0.009
	14	0.361±0.008*	0.373±0.009*-**-***	0.359±0.005*	0.339±0.007	0.337±0.009
Number of hair follicles in the margins	10	3.3±1.76	4.1±0.99*	3.6±1.26	2.2±1.22	2.1±1.28
	14	4.4±1.07*	5.1±1.10*	5.0±0.94*	2.7±1.33	2.2±1.39

*Significant differences with vaseline and sham groups (P<0.05).
**Significant difference with silver sulfadiazine group (P<0.05).
***Significant difference between *Salvadora persica* group 10% to 5% (P<0.05).

cells in the group treated with 10% SP extract showed a significant increase compared to the 5% SP extract group (p<0.05). On day 7, no evidence of collagen synthesis, hair follicles and the onset of epithelialization was observed.

Evaluation of the parameters on day 10

The number of neutrophils and lymphocytes in both groups treated with 10% and 5% SP extracts and silver sulfadiazine group were significantly decreased compared to the sham group (P<0.001). The number of neutrophil cells in the groups treated with 10% extract (P<0.05) and reduction compared to the 5% extract group. The number of fibroblasts in both groups treated with 5% (P<0.01) and 10% SP (P<0.001) extracts and silver sulfadiazine (P<0.001) was significantly increased compared to the sham group. The number of fibroblasts in the group treated with 10% extract had a significant increase compared to 5% (P<0.01). Collagen fibers density was significantly increased in both treatment and silver sulfadiazine groups compared to the sham group (P<0.001). The 10% SP extract group showed a significant increase compared to the silver sulfadiazine group (P <0.001) and the 5% extract group (P<0.01). On day 10, epithelialization and hair follicles were not observed at the center of the wound, so the height of the epithelium and the number of newly formed hair follicles in the wound margins were examined. The height of epithelium and the number of newly formed hair follicles in the wound margins were significantly higher only in the 10% SP group compared to the sham group (P <0.05) (Figure 1).

Evaluation of the parameters on day 14

The number of neutrophils and lymphocytes in both groups of 5% (P<0.05) and 10% SP (P<0.001) and silver

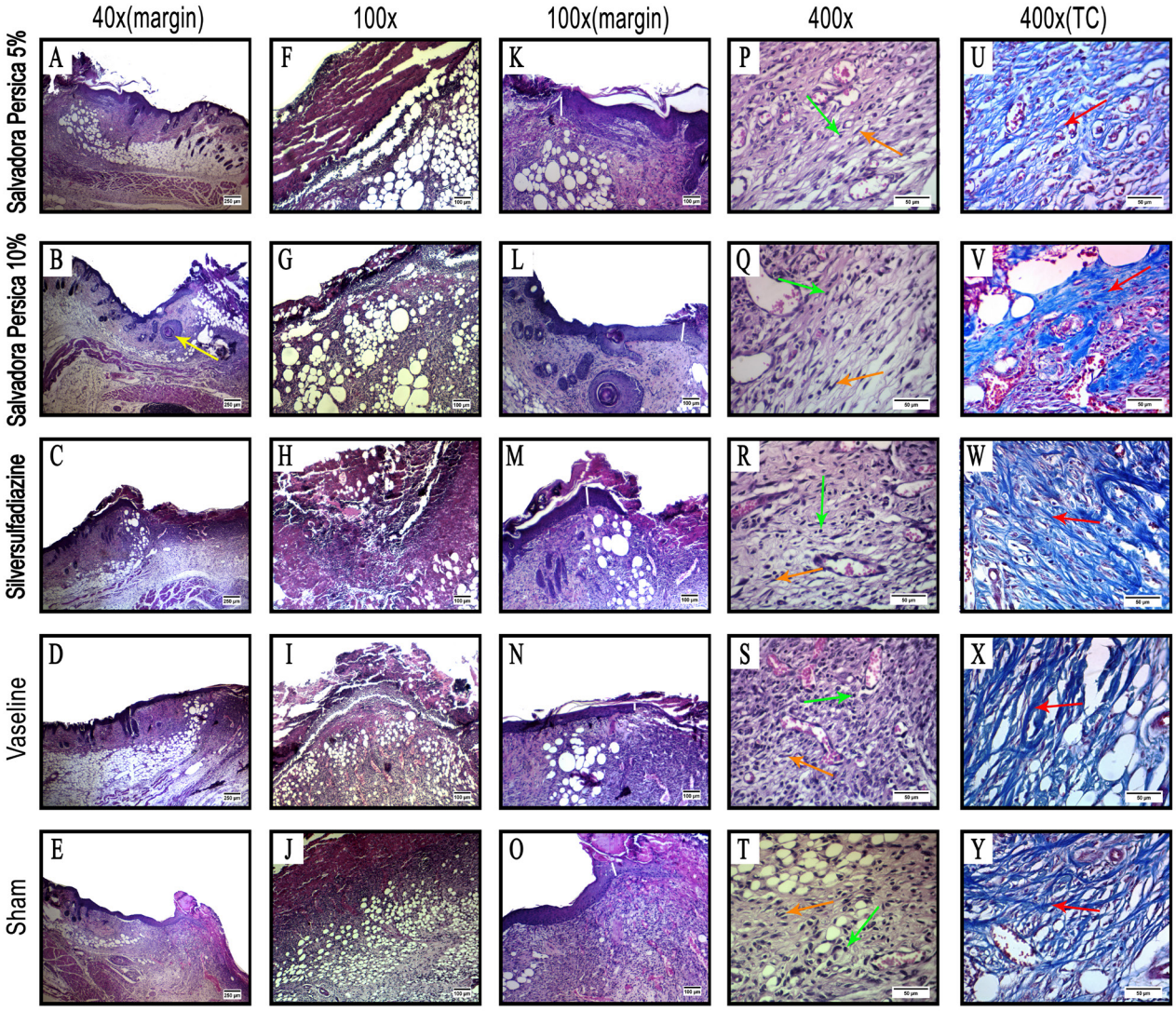

Figure 1. Representative photomicrographs of histopathological evaluation of wound healing processes in the groups administered *Salvadora persica* 5% (A,F,K,P,U), *Salvadora persica* 10% (B, G, L, Q, V), silver sulfadiazine (C, H, M, R, W),Vaseline (D, I, N, S, X) and sham (E, J, O, T, Y) on day 10; A, B, C, D and E: Newly formed hair follicle (yellow arrow) in the margin of the wound on H&E staining, 40x; F, G, H, I and J: Wound area on H&E staining,100x; K, L, M, N and O: Epithelialization in the margin of the wound (white lines) on H&E staining, 100x; P, Q, R, S and T: Neutrophils (green arrows) and fibroblasts (orange arrows) on H&E staining, 400x; U, V, W, X and Y: Collagen fibers (red arrows) on Masson's trichrome staining, 400x.

sulfadiazine (P<0.01) group showed a significant decrease compared to the sham group. The number of fibroblasts and collagen fiber density were significantly increased in both the treatment groups and the silver sulfadiazine group compared to the sham group (P<0.001); besides, in the group treated with 10% SP extract, a significant increase was observed in both fibroblast cells number and collagen fibers density compared to the silver sulfadiazine group (P<0.05) and the 5% extract group (P<0.01). On the 14th day of repairing, epithelization and hair follicles formation in the center of the wound were not observed yet; so these two parameters were examined in the margin of the wound. Epithelium height and the number of newly formed hair follicles at the margin of wounds in both groups of 5% (P<0.01) and 10% SP (P<0.001) extracts and silver sulfadiazine (P<0.001) showed a significant increase compared to the sham group (Figure 2).

Discussion

Burn wound is a common injury all over the world.[29] The efficacy of many herbal products on wound healing has been shown in several studies.[12-13,15] Herbal products are more affordable and less toxic than synthetic medications.[30] In our study, *Salvadora persica* extract was compared with silver sulfadiazine for burn wounds in mice. The results of this study showed that topical application of *Salvadora persica* aqueous-alcoholic extract could have positive effects on the healing of second-degree skin burn wounds. The wound healing process is a set of sequential reactions that being the result of interaction between

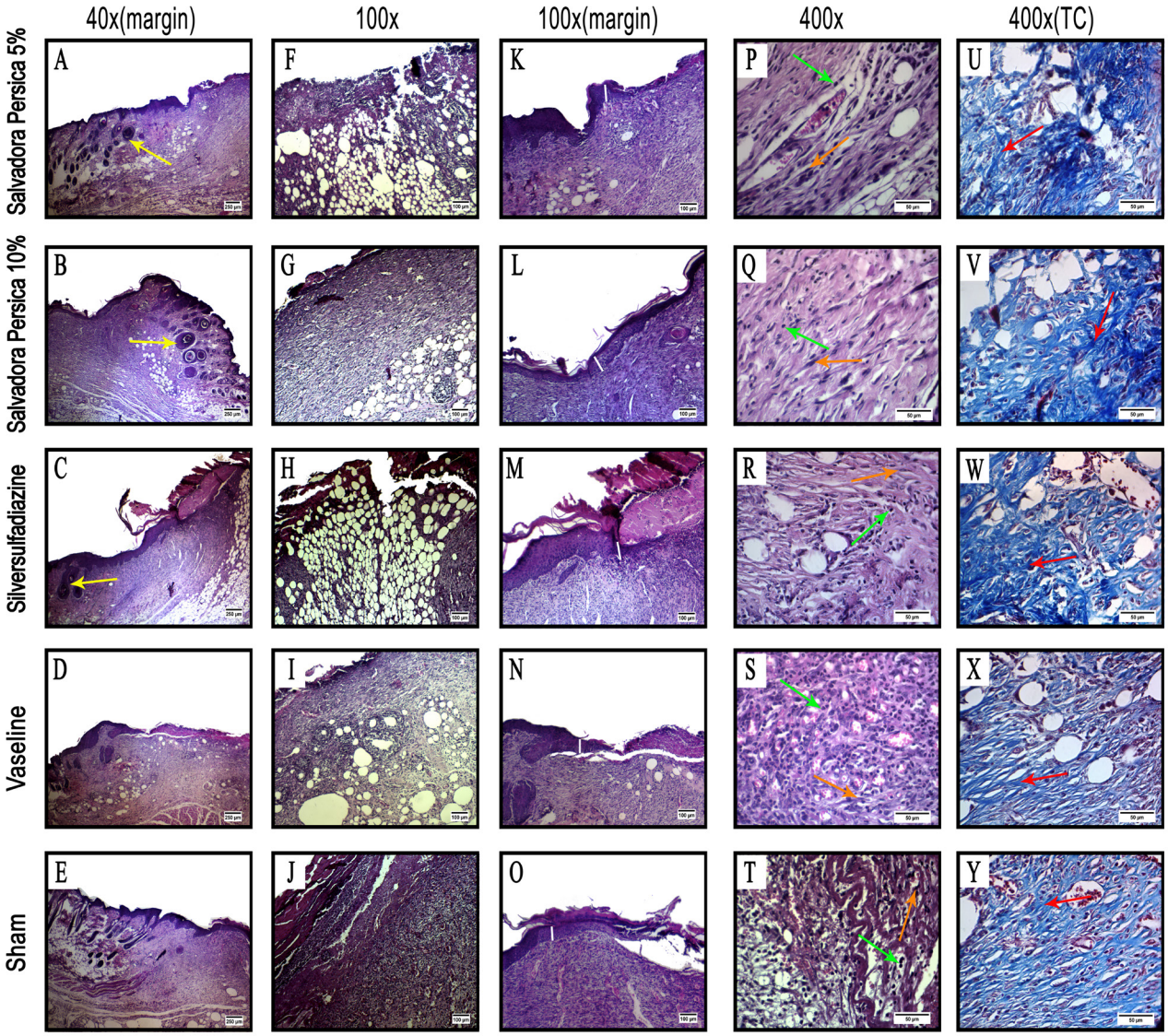

Figure 2. Representative photomicrographs of histopathological evaluation of wound healing processes in the groups administered Salvadora persica 5% (A, F, K, P, U), Salvadora persica 10% (B, G, L, Q, V), silver sulfadiazine (C, H, M, R, W),Vaseline (D, I, N, S, X) and sham (E, J, O, T, Y) on day 14; A, B, C, D and E: Newly formed hair follicles(yellow arrows) in the margin of the wound on H&E staining,40x; F, G, H, I and J:Wound area on H&E staining, 100x; K, L, M, N and O: Epithelialization in the margin of the wound (white lines) on H&E staining,100x; P, Q, R, S and T: Neutrophils (green arrows) and fibroblasts (orange arrows) on H&E staining, 400x; U, V, W, X and Y: Collagen fibers(red arrows) on Masson's trichrome staining, 400x.

different types of cells, molecules and extracellular matrix, begin immediately after skin is damaged, and may continue for a long time.[31] After the first phase of homeostasis and coagulation, an important second phase called inflammation is formed at the wound site. In the inflammatory phase, white blood cells, including neutrophils and lymphocytes, and macrophages migrate to the wound site, and their main activity there would be phagocytosis and infection prevention.[6] There is much evidence that pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α secreted by macrophages, increase the inflammatory response and if continued, it might pospone wound healing and even might cause chronic wounds. Thus, in case the wound healing process is accompanied by proper and temporary regulation of pro-inflammatory cytokine levels, it would accelerate wound healing.[32] The results of this study showed that inflammation and infiltration of neutrophils and lymphocytes in the group treated with 10% *Salvadora persica* extract was lower compared to the group treated with 5% SP extract, and it has almost had similar anti-inflammatory effects to the silver sulfadiazine group. The anti-inflammatory effects of this plant have been confirmed by a number of recent studies. A study done by Lebda *et al.*,[21] investigating the effect of SP aqueous extract on pro-inflammatory cytokines, nitric oxide synthesis, apoptosis pathways and oxidation and antioxidants pathways involved in gastric ulcer healing, were performed on rats. It was shown that by inhibiting the NF-κB signaling pathway, SP extract owing to its anti-inflammatory properties, reduced the production of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α. Also, due to its antioxidant compounds such as furan, vitamin C, tannin, saponin, flavonoid and lycopene, α-linolenic acid, oleic acid, lycoxanthin and retinoic acid can also produce additional anti-inflammatory effects by reducing pro-apoptotic protein expression Bax, lipid peroxidation and TNF-α secretion. Another beneficial effect of this plant is the increase in NO production by up-regulating endothelial nitric oxide synthase (eNOS), which leads to increased mucosal blood flow and mucus production and secretion, and thus the gastric mucosa is enhanced; therefore, all these factors cause this plant to have positive effects in the prevention and treatment of gastric ulcers. Another study done by Ibrahim *et al.*,[18] investigated the anti-inflammatory effects of ethyl acetate SP extract on paw oedema induced by carrageenan in rats, It was found that the ethyl acetate extract of this plant reduced inflammation and inflammatory mediators such as IL-1β. IL-6, TNF-α, and TGF-β and had similar anti-inflammatory effects to indomethacin as a reference treatment. The anti-inflammatory effects of SP extract is probably due to the flavonoids in that.

On the other hand, SP is a rich source of sulfur.[20] Sulfur compounds have strong antioxidant, anti-inflammatory and anti-cancer effects. Studies have shown that sulfur-containing compounds inhibit the production of nitric-oxide, prostaglandin E2, and reduce the expression of proinflammatory cytokines including TNF-α, IL-1β, IL-6 by inhibiting the NF-κB pathway.[33]

Eleven flavonoid compounds have been known, all of which are flavonol glycerides, including apigenin rutin and quercetin extracted from *Salvadora persica*.[18,20,34] Flavonoid compounds prevent the inflammatory and destructive effects on tissues by inhibiting the degranulation of mast cells, inhibiting the release of inflammatory and pro-inflammatory mediators such as prostaglandins, histamine, IL-1β, TNF-α and TGF-β, and reduction of inflammatory cells.[35-37] In our previous studies, we found the beneficial effects of quercetin in preventing the inflammatory process, ageing and the harmful effects of hyperglycemia on kidney tissue.[38]

Recent studies have shown that apigenin has significant anti-inflammatory, antioxidant and anti-cancer effects. It is also effective in treating the symptoms of gastritis, gastric ulcers and other mucosal inflammations; besides, some studies have shown that apigenin can effectively treat skin inflammation arising from free radicals such as UV, X and γ and chemicals.[39-40] A study done by Shukla *et al.*,[41] evaluated the effect of apigenin on the healing of diabetic wounds in rats, and it was shown that the rate of wound closure and collagen production in the wounds of diabetic rats treated with apigenin were similar to healthy rats having never received any treatment.

In a study done by Chen *et al.*,[42] to evaluate the effectiveness of intraperitoneal injection of rutin in wound healing in hyperglycemic rats, it was found that rutin accelerates wound healing by having effects on controlling blood sugar, reducing the synthesis of inflammatory cytokines and growth factors, and promoting enzyme production with antioxidant properties.

SP extract contains fatty acids such as linoleic acid (the main fatty acid of the epidermis), oleic acid and stearic acid.[43] Unsaturated fatty acid mitigates the inflammatory response.[44] The results of a study showed that consumption of both linoleic acid and oleic acid, compared to control groups, shortened the inflammatory phase, reduced the number of inflammatory cells and inflammatory mediators such as IL-6 and IL-1 in the first 24 hours and also shortened the closure of wound within 7 days after wound creation.[45] It was also found that the inflammatory response in wounds treated with oleic acid was modulated, and the wound size got smaller than the control group after 5 days.[44]

Oxidants and oxygen free radicals pospone tissue regeneration and wound healing, so wounds treatment with antioxidants reduces tissue damages resulting from oxygen free radicals, and improves skin regeneration.[46] Furan compounds derived from methanolic extract of SP have strong antioxidant activity. In addition, the antioxidant effect of this plant is closely related to the presence of compounds such as phenolic compounds, anthocyanin, carotenoids, Tocopherols, vitamin C and flavonoids. The peroxidase enzymes, catalase, superoxide dismutase and polyphenol oxidase also exist in the SP extract, which have

antioxidant effects and have a synergistic effect with the antioxidant compounds of this plant.[19,36] In the previous studies done on the healing effects of *Malva sylvestris* extract, due to the antioxidant compounds abundance in this plant, we achieved similar results in incisional wound healing.[26]

On the other hand, the repair of skin lesions is mostly related to the major role of fibroblast cells in the regeneration of the extracellular environment by producing large amounts of collagen.[47] In the present study, it was indicated that *Salvadora persica* extract, by increasing the number of fibroblast cells, the collagen fibers density, the epithelium regeneration and the formation of new hair follicles in the wound margins, accelerates the burn wounds healing. Numerous studies have shown that the use of this plant can increase the growth of fibroblasts and collagen production and also can help the granulation tissue regeneration in ulcers treated with SP extract.[19,48]

Quantitative and qualitative analysis of *Salvadora persica* plant extract has shown that this plant is a rich source of vitamin C and other chemicals, including silica, resin, sodium chloride and potassium chloride.[49] There have been several reports based on the positive effects of vitamin C on the increase and proliferation of fibroblasts as well as increased expression of collagen genes type 1 and type 4 in these cells. Vitamin C is also a co-factor of lysyl and prolyl hydroxylase, which is required to stabilise the third structure of collagen.[50]

Another study performed by Hina Imran *et al.*[23] examined the effect of *Salvadora persica* ointment on the excisional wound healing process in rats. He found that being compared to other groups, in the ointment-treated group, wound enclosure time was 10% shorter, and epithelial regeneration was greater, which is consistent with the results of our study.

According to the compounds in *Salvadora persica* and the presented report, it might be concluded that the aqueous-alcoholic extract of this plant has anti-inflammatory, antioxidant and antimicrobial effects and also increases the number of fibroblasts, collagen production and regeneration of the epidermis and hair follicle formation; Therefore, it positively influences on the phase of inflammation, proliferation and remodelling in wound healing.

In this experiment, small population size, the anatomical and physiological variations, and the difference in the rate of wound healing between mice and human beings shall be considered as limitations. So, for clinical use of this natural product, future clinical trials with large sample sizes and longer follow-up periods are recommended. Additionally, due to the positive effects of this plant in the burn wound healing process, it is suggested that a similar study be carried out to evaluate its effect on diabetic wounds.

Conclusion

The results of the present study showed that *Salvadora persica* extract accelerated burn wound healing by reducing inflammation. On the other hand, the extract of this plant had significant effects on increasing the number of fibroblast cells and collagen production, as well as increasing the epithelialization and the new hair follicles formation; Therefore, the mentioned extract can be used as a suitable candidate for healing process of burn wounds.

Ethical Issues

This study was approved by the research ethics committee at Birjand University of Medical Sciences with the approved code of 455460 (ethics code ir.bums.REC.1397.89).

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Author Contributions

MA conceived and designed the study and also supervised the manuscript. HL carried out all the experimental work, analyzed the data, performed statistical analysis and prepared the manuscript. MHT collaborated in interpretation of the data and took part in manuscript preparation too. MZ supervised the pathological part. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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