Journal of Medicinal Plants and By-products (2025) 06: 632– 638

Burn Wound Healing Effect of Traditionally Used Persian Medicine Ointment Containing Herbal Extracts and Yogurt Butter

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Article Info

Article Type
Original Article

Article History
Received: 01 March 2025
Accepted: 28 April 2025

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ABSTRACT

This study aimed to assess the burn healing efficacy of this traditionally used formulation (herbal ointment) in deep second-degree burns in a Florida white rabbit model. On the dorsal surface of the two rabbits, six burn wounds of the same size were inflicted. Herbal ointment was applied to the wounds on the right side, and silver sulfadiazine was applied to the wounds on the left side. On the 5th and 18th day of the treatment, the burn wound site was excised in a square shape with a narrow surrounding healthy skin for histopathological examination. At the end of day 18 of the treatment period, all the ulcers in the traditional herbal ointment group were covered entirely with epithelial tissue, while in the silver sulfadiazine group, the mean and SD of the ulcer diameter were (1.4 ± 0.1). The duration of pain, blister, and open wound in the silver sulfadiazine group was 6, 8, and 18 days; however, in the traditional herbal ointment group, on the second day, none of these complications were observed. Regarding the pathological findings, epithelial tissue renewal on day 18th was 6.63 ± 0.74 in the traditional herbal ointment group compared to 4.93 ± 0.48 in the silver sulfadiazine ($P< 0.05$). The findings indicate that the traditional herbal ointment can reduce the duration of the wound healing process as well as the complications, including pain and blister formation. The pathological findings also suggest better epithelial tissue renewal in the traditional herbal ointment group.

Keywords: Burn wound, Persian medicine; Herbal ointment, Yogurt butter, Silver sulfadiazine

How to cite this paper
Ansari, F., Pourjafar, H., Alian Samakkhah, Sh., Kazemikhoo, N., Lotfi, R., Armand, N., Atarod, A., Neshat Gharamaleki, M. Burn Wound Healing Effect of Traditionally Used Persian Medicine Ointment Containing Herbal Extracts and Yogurt Butter. Journal of Medicinal Plants and By-products, 2025;14(6):632-638. doi: 10.22034/jmpb.2025.368757.1912

INTRODUCTION

Burn injuries are the most widespread injuries with significant morbidity and mortality. Burns, especially severe burns, are associated with immune and inflammatory responses, metabolic alterations, and distributive shock, which are difficult to manage and can lead to multiple organ failures. Injuries affect not only the patient's physical health but also their mental health and quality of life [1, 2]. A patient with burn ulcers is therefore not considered to have recovered once the wound has healed, as burns result in significant long-term changes that must be addressed to optimize quality of life [3, 4]. Many topical treatments for burns are already available worldwide, including 1% silver sulfadiazine, moist ointment for exposed burns (MEBO), 10% mafenide acetate cream, and povidone-iodine [1, 5].

Silver sulfadiazine has been used since 1968 to reduce the risk of infection in burn wounds and has a broad spectrum of antimicrobial activity. This ointment has an antibacterial effect, is easy to use, and doesn't cause pain or a burning sensation during application. However, dressings made with silver sulfadiazine often produce scars due to their adhesion to the wound surface and cause a delay in the wound healing process by disrupting keratinocyte regeneration. Other side effects include leukopenia, silver poisoning, increased pigmentation, skin discoloration, drug resistance, epidermal necrolysis, neutropenia, failure of the skin to return to its original state, delay in the separation of scar tissue from the skin, and the possibility of fungal infection has been reported in some patients [6-8].

Several natural products such as honey, beeswax, olive oil, and butter [9, 10], and herbal medicine such as *Aloe vera* [11], green tea [12], *Teucrium polium* L.[13, 14] and curcumin [15] have been reported to treat burns in laboratory and clinical studies.

The application of Persian medicine formulations for the treatment of burn wounds in Iran has a long history. One such formulation, which is prescribed for treating burn wounds, is a combination of

natural fatty substances such as yogurt butter, olive oil, turmeric, and beeswax. All of these components have been shown to have positive effects on the wound healing process due to their antioxidant, anti-inflammatory, and anti-microbial effects [10, 16-19]. The mixture of olive oil and beeswax has been traditionally used in various countries, such as Egypt [20] and Turkey [9] to treat burn wounds. Olive oil is rich in fatty acids, vitamin E, and phenol compounds with antimicrobial, antioxidant, and anti-inflammatory activities [21]. Beeswax also contains flavonoids, with antioxidant and antibacterial properties, and can regulate cytokine production [10, 20]. The fatty acids can play a significant role in the acceleration of the wound healing process [10, 20]. Turmeric and its active component have been reported to have therapeutic effects on burn pain and wound healing [15, 22]. Since burn wound treatment has always been problematic and effective drugs that simultaneously control infection, reduce pain, accelerate wound healing, and prevent hypertrophic scars are still lacking, the introduction of new materials for burns remains vital [23, 24]. The present study aimed to investigate the effects of herbal ointment on burn wounds compared with silver sulfadiazine in an animal model.

MATERIALS AND METHODS

Ointment Components

The traditional method of preparation was used to prepare the ointment. In this method, the yogurt butter (40%), olive oil (*Olea europaea*) (40%), turmeric powder (*Curcuma longa*) (5%), and beeswax (*Cera alba*) (5%) were mixed at a temperature of 60 degrees Celsius. Then the ointment was poured into sterilized tubes and stored at room temperature until use.

Animal Studies

Two healthy white Florida rabbits with an average weight of 2 kg were purchased from the animal house of Alborz University of Medical Sciences and placed in separate cages one week before the start of the experiment to acclimate them to the environmental and dietary conditions of the laboratory.

24 hours before burn wound induction, the dorsal surface of the animals was shaved with an electric shaving machine, followed by the application of a depilatory cream for complete hair removal. To induce burn wounds, the animals were anesthetized with intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg).

On the dorsal surface of each of the two rabbits, six standardized second-degree burn wounds of equal size (three wounds on the right side and three wounds on the left side) were created using the method described by Kaufmann *et al.* [25].

At that time, square-shaped instruments with a diameter of 1.5 cm were immersed in 75 ˚C water to preheat and then placed in full contact with the rabbits' shaved skin for exactly 5 s, the pressure exerted being solely due to the weight of the rod itself.

Treatment Groups

Burn wounds on the right side of the dorsal surface were randomly assigned to herbal ointment and the left side to silver sulfadiazine. Sufficient amounts of herbal ointment were applied to the right burn wound and silver sulfadiazine to the left wound.

Wounds were dressed using band-aid, and dressings were changed every day. The rabbits were kept in two separate cages and had free access to food and water. Each wound is considered an experimental unit. In this study, one animal was selected to observe the early effects (after 5 days of burn ulcer) and one animal to examine the late effects of the ointment (after 18 days of burn ulcer).

Assessing the Size of the Wounds

The initial wounds were created by a square-shaped instrument, and the wounds were supposed to have four dimensions of the same size; however, during the treatment process, the shape of some of the wounds changed. The size of the wounds was measured each day by measuring the greatest diameter of the wound with a standard ruler (Fig. 1).

Fig. 1 Standard ruler used for daily measurement of the wound size (18th day).

Assessing the Pain

Surgical forceps were used to mechanically stimulate the surrounding skin of the burn wound, and the behavior of the animals was analyzed to assess if they were feeling pain. We observed pain-like behaviors, including agitation, increased grooming in the affected area, or the withdrawal of a body part [26]. The findings were recorded systematically as either yes or no regarding the presence of pain, with assessments conducted daily.

Assessing the Complications

The general condition of the wounds was observed daily, and any alterations, including the presence of infection, blister, scab, or callus formation, were recorded.

Histopathology

One rabbit was euthanized in a state of unconsciousness on the 5th day, and another on the 18th day of the treatment period. The burn wound repair site was cut and separated with a narrow surrounding healthy skin.

The removed tissue samples were fixed in 10% buffered formalin and sent to the pathology laboratory for histological studies. The criteria for evaluation of homeostasis, grading of epithelial tissue regeneration, degree of inflammation, and the process of fibroplasia in the repair site are described in Table 1.

Ethical Issues

Ethical approval was obtained from the Alborz University of Medical Sciences, Karaj, Iran (Ethical Code: IR.ABZUMS.AEC.1401.003). All the experimental procedures were conducted by the internationally accepted principles for laboratory animal use and care as found in the European Community Guidelines/ECG (EEC Directive of 1986; 86/609/EEC).

Table 1 Histopathological grading of hemostasis, regeneration of epithelial tissue, reduction of the severity of edema and fibroplasia at the site of burn repair.

Evaluation of homeostasis
1. Bleeding in the wound more than half of the microscopic field with low magnification (×10)
2. Bleeding in the wound between one quarter and one half of the microscopic field with low magnification (×10)
3. Bleeding in the wound is less than a quarter of the microscopic field with low magnification (×10)
4. Bleeding is limited to the edges of the surgical incision
5. Partial bleeding only in a part of the cut
6. Absence of bleeding
Grading of epithelial tissue regeneration
1. Absence of epithelial tissue regeneration
2. Observing the beginning of regeneration of the epithelial tissue from the edges of the wound
3. Regeneration of the epithelial tissue so that it covers about a quarter of the wound surface.
4. Regeneration of the epithelial tissue so that it covers about half of the wound surface.
5. Regeneration of the epithelial tissue so that it covers about three-quarters of the wound surface.
6. Regeneration of the epithelial tissue so that it covers the entire surface of the wound with irregular thickness and inappropriate quality and organization.
7. Regeneration of the epithelial tissue so that it covers the entire wound properly and with good quality and organization.
Degree of reduction of inflammation
1. Perivascular infiltration and observation of edematous cells in more than half of the microscopic field of view with low magnification (×10)
2. Perivascular infiltration and observation of edematous cells in the range between a quarter and a half of the microscopic field of view with low magnification (×10)
3. Perivascular infiltration and observation of edematous cells less than a quarter of the microscopic field of view with low magnification (×10)
4. Partial and scattered infiltration of tumor cells inside the connective tissue
5. Absence of inflammation
The process of fibroplasia in the repair site
1. Absence of fleshy bud tissue
2. The initial appearance of fleshy bud tissue at the wound site
3. Expansion of fleshy bud tissue full of vessels and cells
4. The initial appearance of collagen fibers
5. Expansion of collagen fibers
6. Accumulation, density, and creating order in collagen fibers (scars)
7. Appearance of skin appendages in scar tissues

Statistical Analysis

Data are expressed as mean ± SD. The normality of the data was examined by the Kolmogorov-Smirnov statistic test, and the parametric test was selected for normally distributed data and the non-parametric test for data with non-normal distribution. The statistical significance between the proportion of wound closure on the 18th day and histopathological scores in two treatment groups was analyzed by independent samples T-test and Mann-Whitney U Test, respectively, using SPSS version 26 software (SPSS Inc., Chicago, IL, USA). A P< 0.05 was considered statistically significant. The work has been reported by the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments) [27].

RESULTS

Wound Size

As shown in Figure 1, the appearance of the wounds in the two groups was different from the first day after treatment. This difference was obvious on the 5th day when the right side wounds (herbal ointment group) were closed and the left side wounds (Silver sulfadiazine group) were open with blisters and significant tenderness. On day 5, the bed of ulcers in the herbal ointment group was completely covered by granulation tissue, and the squamous cells were expanding from the margins of the ulcers to the center. While in the silver sulfadiazine group, the bed of the ulcers was partially covered with granulation tissue and a scab on top of them. These findings, along with data from clinical assessments including wound size and complications of the wounds, suggest a faster and better healing process in the herbal ointment group.

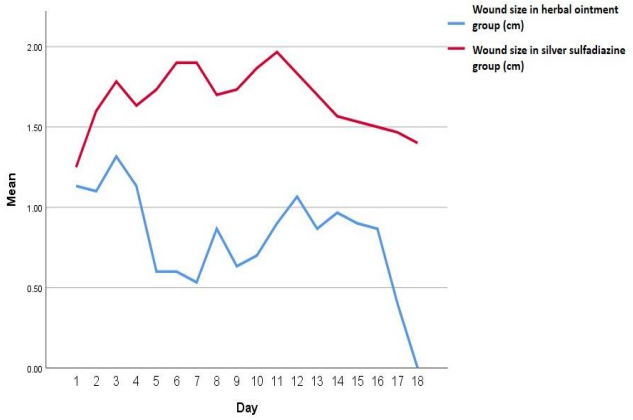

Fig. 2 Comparison of the average wound size of herbal ointment (blue line) and silver sulfadiazine (red line), in each of the two groups of silver sulfadiazine and herbal ointment. At first, we had two rabbits with 6 wounds in each treatment group. On the fifth day, we continued the study with one rabbit, i.e., with 6 burn wounds, three in each treatment group.

Complications

The initial diameter of the wounds was 1.5 cm. On the first day, the mean and SD of the wound size in the herbal ointment and silver sulfadiazine groups were 1.13 ± 0.12 and 1.25 ± 0.08, respectively. On the 18th day, the wounds in the herbal ointment group were completely closed, while the mean and SD in the silver sulfadiazine group were 1.4 ± 0.10. Based on the results, on the 18th day, the severity of swelling and bleeding in the healing site of the burn wound was reduced in both groups and was limited just to the surface and edges of the wounds. Although this reduction was significant in the herbal ointment group. The difference between wound closure in the two studied groups was significant (P< 0.001).

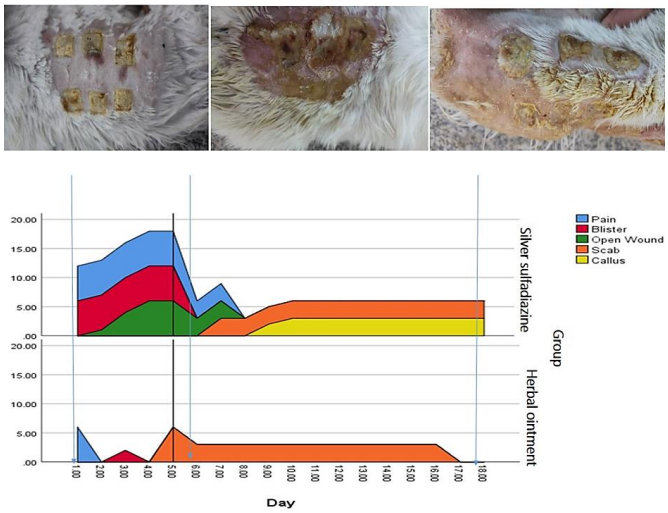

Fig. 3 Timeline of the frequency of presence of pain, blister, open wound, scab, and callus in each of the two groups of silver sulfadiazine and herbal ointment (The upper side shows the wounds treated with silver sulfadiazine and the lower side includes the wounds treated with the herbal ointment) - At first we had two rabbits with 6 wounds in each treatment group, on the fifth day we continued the study with one rabbit, i.e. with 6 burn wounds, three in each treatment group.

The wound size difference between herbal ointment and silver sulfadiazine during the study period is presented in Figure 2.

None of the wounds were infected during the study period in both groups. The frequency of other assessed complications, including pain, blister, open wound, scab, and callus, is presented in Figure 3. The expansion of collagen fibers and the maturation of connective tissue in the wounds treated with the herbal ointment showed more progress than the wounds treated with silver sulfadiazine, and in this way, the mature granular tissue was organized with thick, dense, regular collagen fibers filling the lower parts of the wound. Above all, in the herbal ointment group, the wounds were fully covered by an epithelial layer, and hair follicles appeared in the vicinity of the scar tissue.

Histopathological Findings

On the fifth day of the experimental period, no significant difference was observed in terms of wound healing between the silver sulfadiazine and herbal ointment. In both groups, the bed of the ulcers was covered by blood clots containing fibrin and blood cells, and the surface of the wounds was covered by scabs, made of accumulated and dense corneal material and necrosed tissue deposits. Hyperemia and severe edema with the presence of neutrophils indicated the formation of a fibrinous and purulent discharge in the wound healing site. In the treatment group with herbal ointment, the beginning of epithelial tissue regeneration was visible from the edges of the wound. However, at this stage, no effect of epithelial tissue regeneration was observed in the wounds treated with silver sulfadiazine (Fig. 4). Regarding these results on the 18th day, the advantages of the herbal ointment were clear in all the pathological indexes, including homeostasis, reduction of inflammation, fibroplasia, and re-epithelialization. Scab and callus were also apparent on the wounds of the silver sulphadiazine group, while the skin appearance was partially normal in the herbal ointment group. According to the obtained results, herbal ointment showed more efficiency in inducing the healing process in burn ulcers and resulted in better scar formation than silver sulfadiazine. The comparison of the healing degree of skin on the 5th day of the treatment period based on histopathological parameters is shown in Table 2. It indicates an overall augmented improvement in the herbal ointment group. A significant enhanced epithelial tissue renewal was seen in the herbal ointment compared with the silver sulfadiazine group (P< 0.001). The difference of homeostasis (P= 0.448), reduction of Inflammation (P= 0.194), and fibroplasia (P= 0.056) was not significant between silver sulfadiazine and herbal ointment; however, all of these factors were shown to be slightly better in the herbal ointment group.

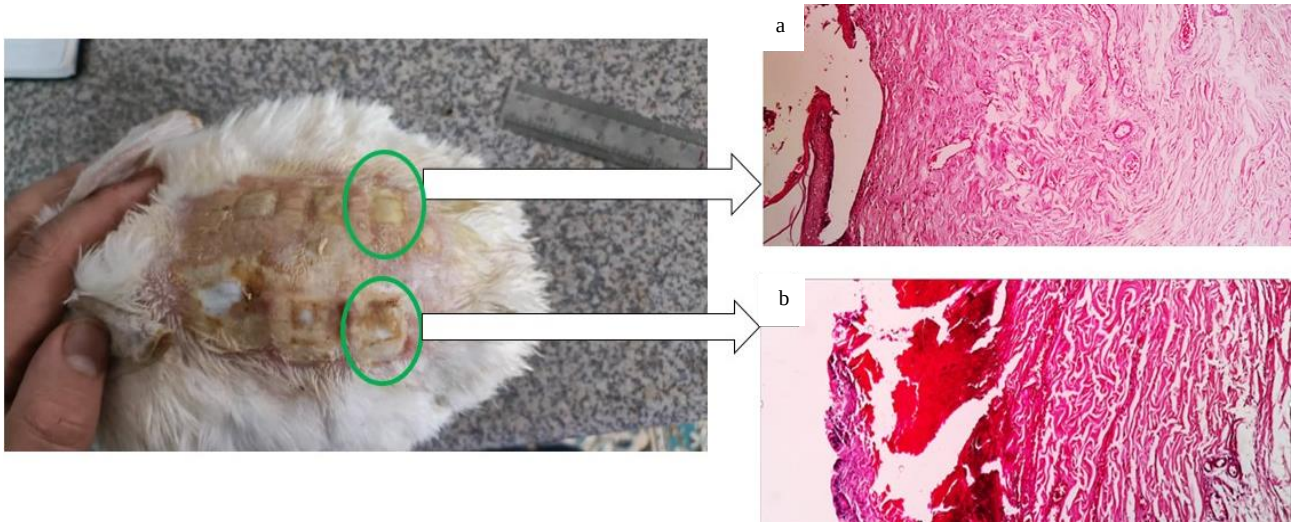

Fig. 4 a) Microscopic view of the burn wound site, treated with the herbal ointment on day 5. The bed of the ulcer is covered by granulation tissue and epithelialization expanding from the borders to the center of the wound. Certain hyperemia is also observed in the deeper tissues of this area (Hematoxylin-eosin, × 120 magnification). b) Microscopic view of the burn wound site treated with silver sulfadiazine on day 5. The surface of the wound is covered by a scab consisting of a blood clot containing coagulated fibrin, inflammatory cells, and the remains of necrotic tissues. A specific hematoma is observed in the superficial parts of this area. The bed of the ulcer is covered by granulation tissue (Hematoxylin-eosin, ×120 magnification).

In the wounds treated with silver sulfadiazine, the continuation of epithelial tissue regeneration was visible from the edges of the wound, and the wound surface was partially covered by new epithelial tissue. Granular connective tissue had completely occupied the space inside the wound, and the appearance of very fine collagen fibers could also be seen from the side of the tissue adjacent to the repair tissue. The expansion of collagen fibers and the maturation of connective tissue in wounds treated with herbal ointment showed more progress than in wounds treated with silver sulfadiazine, and mature granular tissue organized with thick, dense, regular collagen fibers filled the deeper space of the wound. A marked finding in the wounds treated with herbal ointment was the coverage of the entire surface of the wound by new epithelial tissue; however, this epithelial tissue, especially in the middle part, was disorganized and lacked corneous substance. On the sides of the wounds treated with herbal ointment, skin appendages, including hair follicles, appeared in the vicinity of the scar tissue (Fig. 5).

Table 2 Histopathological parameters on day 5 of the treatment (mean ± SD).

Repair index / Group	Homeostasis	Reduction of inflammation	Fibroplasia	Epithelial tissue renewal
Silver sulfadiazine (n=3)	2.85 ± 0.44 a	1.89 ± 0.21 a	2.98 ± 0.19 a	1.10 ± 0.06 a
Herbal ointment (n=3)	3.10 ± 0.27 a	2.17 ± 0.23 a	3.66 ± 0.40 a	2.13 ± 0.14 b
P value	0.448	0.194	0.056	<0.001

Different letters in each column indicate significant difference (P< 0.05).

On the 18th day of the treatment period, the severity of swelling and bleeding in the healing site of the burn wound was reduced in both types of treatment and was limited only to the surface and edges of the wound, but this reduction was higher in the treatment with herbal ointment (Table 3). All the assessed factors, including homeostasis, reduction of inflammation, fibroplasia, and epithelial tissue renewal, were significantly better in the herbal ointment group.

Table 3 Burn wound recovery on the day 18th of the treatment period based on histopathological parameters (mean ± SD).

Repair index / Group	Homeostasis	Reduction of inflammation	Fibroplasia	Epithelial tissue renewal
Silver sulfadiazine (n=3)	3.15 ± 0.37 a	2.92 ± 0.27 a	3.84 ± 0.16 a	4.93 ± 0.48a
Herbal ointment (n=3)	5.10 ± 0.24 b	4.46 ± 0.24 b	5.74 ± 0.49 b	6.63 ± 0.74 b
P value	<0.001	<0.001	0.003	0.029

Different letters in each column indicate a significant difference (P< 0.05).

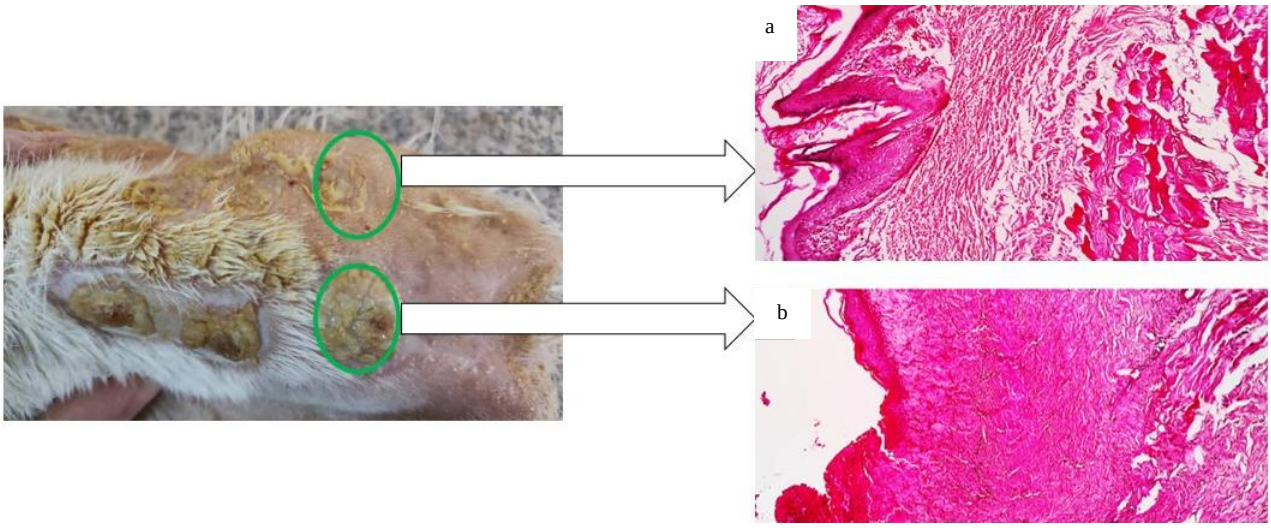

Fig. 5 a) Microscopic view of the burn wound site, treated with the herbal ointment on the 18th day. The relatively organized squamous epithelial tissue of the new foundation has completely covered the surface of the wound. The wound space is also occupied by relatively mature granulation tissue. Inflammatory cells are visible to some extent, and mild to moderate hyperemia is observed in the superficial parts of the healing site (Hematoxylin-eosin, ×120 magnification). b) Microscopic view of the burn wound site, treated with silver sulfadiazine on the 18th day. The squamous epithelial tissue of the new foundation has covered most of the wound surface, and only a small part of the wound surface lacks new epithelial tissue and is covered by a scab consisting of a blood clot containing coagulated fibrin, edematous cells, and the remains of necrotic tissues. The wound space is completely occupied by relatively mature granulation tissue and fibrous strands. Inflammatory cells are visible, and moderate hyperemia is also observed (Hematoxylin-eosin, ×120 magnification).

DISCUSSION

Wound healing is an important physiological process for maintaining skin integrity after accidents and deliberate interventions. Normal wound healing involves three sequential but overlapping phases, including the hemostasis/inflammatory, the proliferative, and the remodeling phase. Wound-healing disorders, such as excessive wound healing (hypertrophic scars and keloids) or chronic wounds, will cause unsatisfactory consequences for the patients [28]. Several experimental studies have provided insights into wound healing [3, 29]. The present study was conducted to investigate the effects of a traditional herbal ointment containing a mixture of yogurt, olive oil, turmeric powder, and beeswax on the healing process of burn wounds. This ointment has been traditionally used in the treatment of burns since ancient times. In this study, the histopathological changes of the skin after wound induction were examined in two stages on the fifth and eighteenth days after treatment. This study demonstrates that the traditional herbal ointment containing yogurt butter and medicinal extracts significantly outperforms silver sulfadiazine in promoting burn wound healing, as evidenced by accelerated wound closure, enhanced epithelial regeneration, and superior histopathological recovery.

By day 18, wounds treated with the herbal ointment achieved complete closure, whereas silver sulfadiazine-treated wounds remained unhealed (1.4 ± 0.10 cm; P< 0.001). Early differences were notable by Day 5, with the herbal group showing initiation of epithelial regeneration, which was absent in the silver sulfadiazine-treated group (Fig. 4, Table 2). Also, by day 18, the herbal ointment group exhibited complete epithelial coverage, organized collagen fibers, and reappearance of skin appendages (e.g., hair follicles; Fig.5). In contrast, silver sulfadiazine-treated wounds still had partial epithelial gaps and immature collagen. The herbal ointment showed significantly higher reduction in inflammation (4.46 vs. 2.92; Table 3), likely due to phytochemicals modulating neutrophil infiltration (Fig. 4). Neither group experienced infections, but the herbal ointment reduced blistering and scabbing (Fig. 3), suggesting better preservation of wound bed integrity.

As proposed mechanisms for yogurt butter, its lactic acid and lipid content (as part of the postbiotic content in yogurt, originating from native probiotics) likely maintains a moist wound environment, facilitating cell migration, reducing scab formation, and eliminating infection [30-37]. In case of herbal extract, bioactive compounds of olive (e.g. phenolic compounds) [31, 38], turmeric [39, 40], and also beeswax [41, 42] may have stimulated fibroblast proliferation and collagen synthesis, as reflected in the higher fibroplasia score (5.74 vs. 3.84; P< 0.05; Table 3). In a similar study, Bayir *et al.* (2019) studied the effects of beeswax, olive oil, and butter impregnated bandage on burn wound healing. The results showed that the Beeswax–olive oil–butter (BOB) treatment increased TGF-β1 and VEGF-α expressions compared to the burn

group. The histopathological analyses indicated that the epidermis and dermis layers were injured due to the burn. BOB treatment improved the regeneration of these layers and increased fibroblast activity and keratinization, which play an important role in the new blood vessels [10]. The results of this study are largely consistent with the present study.

In an experimental study on rats, using crocodile oil burn ointment, accelerated wound closure, and it had anti-inflammatory and analgesic effects compared to silver sulfadiazine [3]. In another study, the effect of *Aloe vera*, milk, and honey mixture was investigated on second-degree burns. The result showed that the ointment accelerated wound closure rate, cell proliferation, revascularization, and collagen fiber density in treated animals. It also reduced wound discharge, inflammation, and scarring [43].

CONCLUSION

In conclusion, the results of this study suggest that the herbal ointment may improve wound healing and reduce pain and other complications of burn wounds. The authors claim that this herbal ointment may be an effective alternative to silver sulfadiazine for the treatment of burn wounds, provided that further experimental and clinical trials are conducted to assess stronger evidence of its efficacy and safety.

Acknowledgments

We thank the Vice Chancellor for Research and Technology of Alborz University of Medical Sciences for the scientific support of this research project.

Medical Ethics

Ethical approval was obtained from the Alborz University of Medical Sciences, Karaj, Iran (Ethical Code: IR.ABZUMS.AEC.1401.003).

Author Contributions

Abbreviations List

MEBO: moist ointment for exposed burns; ECG: European Community Guidelines

Conflict of Interest

The authors declare no conflict of interest.

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