Original Article

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Induced third-degree Burn in Rat: Healing by *Melilotus officinalis* Extract as Medicinal Plant

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Abstract

Introduction: burn wounds are common happenings which cause many people have irreversible defects and to be dead around the world every year. In this study, we evaluated the effects of *Melilotus officinalis* (*M. officinalis*) and Alpha ointment in the healing of burn wounds and compare their findings with silver sulfadiazine (SSD). Seventy-five male Sprague-Dawley rats were randomly divided into five groups, and similar burn ulcers were produced on the back of rats. The first group of rats received no treatment, the base gel was used topically to group II, in groups III-V, Alpha, SSD and *M. officinalis* preparations were applied, respectively. Wound healing, contraction and histopathological examination were evaluated at the end of 7, 14, and 21 days. Alpha ointment was equally effective as *M. officinalis* gel, while its efficacy was better compared to SSD for all parameters of wound healing on days 7, 14 and 21. Alpha and *M. officinalis* preparations as less expensive medications which significantly improve the quality of wound healing and scar formation and also are more appropriate treatment choices than SSD. Alpha and *M. officinalis* are recommended as the alternative to SSD, especially in patients with low economic backgrounds or in those who show adverse reactions to SSD.

Keywords: Melilotus officinalis, Rat, Alpha, Silver sulfadiazine, Wound healing.

1. Introduction

Burn injuries especially third-degree burn cause the most damage, extending through all layers of skin as a major public health issue, which there is not much that can be done to relieve its symptoms (1, 2). Third-degree burns carry the most risk for complications, such as infections,

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blood loss, and shock. At the same time, all burns carry the risk of infections because bacteria can enter broken skin (3, 4). Many of the popular drugs pose complications such as; adverse reaction and drug resistance, that has forced researchers to look for alternative remedies (5). The effect of herbal medications includes herbs, herbal materials, herbal preparations and finished herbal products that contain an active ingredient, parts of plants, or other plant materials, or combinations in the healing process of burn injuries have been documented (6, 7). Herbal byproducts possess efficacy with no or fewer complications and are mostly inexpensive compared with synthetic drugs (8). Many plants extract have shown to hold potent wound-healing properties (9).

Alpha is a topical ointment with ingredients extracted from Lawsone (*Lythraceae intermis*) (10). Several studies have demonstrated its healing properties in burn wounds (11-13).

MO is annual or biennial herbs of the legume family (Fabaceae), sweet-scented. It shows good properties and characters of the following field: treatments of wounds, inflammation, swollen joints, stomach ulcers and Swollen, and acts as an antioxidant and antibacterial due to dicumarol, which is a broad spectrum bactericide (14, 15). It has been reported that *Melilotus officinalis* (*M. officinalis*) stimulates wound healing and might be effective in reducing the duration of wound healing (16).

The active substances of *M. officinalis* reduce inflammation and increase the amount of blood flow (17).

The main chemical components of *M. officinalis* include; Coumarin, the crystalline, saponin and flavonoids (quercetin, rutin, narcissin, isorhamnetin, kaempferol), and other active ingredients (18).

It seems that *M. officinalis* can be effective for managing second and third-degree burns (19). Thus, based on individual conditions the recommended doses can vary. For the first time, we applied a higher does (40%) of a *M. officinalis* topical gel to assess its wound healing effect on third-degree burn injuries in experimental rat model compared to Alpha and Silver sulfadiazine (SSD).

2. Materials and methods

2.1 Materials

Ethanol, Carboxy Methyl Cellulose Sodium, Methyl Paraben Sodium and Propyl Paraben Sodium, Sodium metabisulphite, Triethanolamine, and EDTA were purchased from Merck (Germany). Carbopol 934 as a gelling agent was purchased from Sigma-Aldrich, USA.

2.2. Plant material, preparation of extract and formulation

Flowering aerial parts of *M. officinalis* were collected during April 2014 from rural areas around Shiraz, Fars, south of Iran, and it was authenticated by Prof. Khosravi at Botany department of Shiraz University. The plant was dried at the room temperature. Fifty gram of dried powder plant were macerated in 80% aqueous EtOH (100 ml) at room temperature for 48 hours. The extract was filtered and concentrated under reduced pressure and low temperature (40 °C) on a rotary evaporator to dryness. The extract yield was 36 mg/g of dried plant.

To prepare *M. officinalis* gel Sodium metabisulphite, Methylparaben sodium and Propylparaben sodium were dissolved in water. Gelling agent was added to it and stirred continuously till it got swollen completely. Triethanolamine was slowly added to the dispersion with continuous stirring which resulted in a stiff gel. *M. officinalis* extract was added to it and stirred for 15 min. Volume was made with water and stirred continuously till a uniform gel was formed (40%).

2.3. Animal study

Seventy-five male Sprague-Dawley rats (180-250 g) were selected and housed in separate cages having a temperature-regulated conventional animal room at 22±3 °C, 40-60% relative humidity with an artificial light cycle of 12-12 hours light/dark. The research protocol has complied with the guidelines for animal care of Shiraz University of Medical Sciences, Shiraz, Iran. All experiments were carried out under aseptic conditions in Laboratory Animal Center of Shiraz University of Medical Sciences. All efforts were made to minimize suffering during the exposure period. Rats were randomly allocated into five indepen-

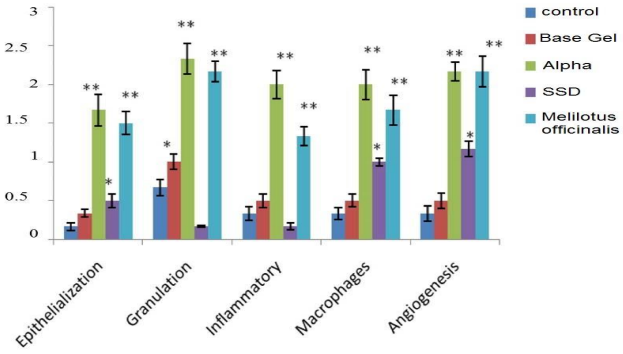

Figure 1. Histological scoring indices of wound healing activity in experimental groups on the 7th day.
0: absent; 1: mild; 2: moderate; 3: severe. Data are presented as Mean±SD (n=5).
SSD: Silver Sulfadiazine.
*Significantly different as compared with control group (P<0.05).
**Significantly different as compared with control group (P<0.01).

dent equal groups after wound induction.

2.4. Experimental protocol

The rats were under general anesthetize 90 mg/kg/IP ketamine (GmbH, Germany), and 10 mg/kg/i.p. xylazine (Alfasan, Netherlands). Back of the neck was shaved, and their skin was disinfected with povidone-iodine and sterile water. The third-degree burn was inflicted based on previous studies (20). Then, the rats were placed in an individual cage in order to recover. Two hours after inducing the injury, animals were randomly divided into five equal groups of 15. From each group, five animals were selected in three different time periods (7, 14, and 21 days). Each group had subgroups including; without treatment (negative control), gel base-treated, Alpha, SSD (positive control) and *M. officinalis* treatments.

2.5. Histopathological evaluation

Tissue samples were provided after 7, 14, and 21 days therapies for further histological assessment. All of the subjects were sacrificed under deep anesthesia, and the burn areas were removed and transferred to 10% neutral-buffered formalin until tissue processing for evaluation as described by Hosseini *et al.* with some minor modifications (12). The minimum and maximum scores for each criterion were minus and 3+ or (3.5), respectively.

2.6. Statistical analysis

All data were shown as the mean and standard deviation (SD), and statistical analysis was performed using Mann–Whitney test (SPSS version 11.5, SPSS Inc., Chicago, IL, USA). P<0.05 was considered as significant.

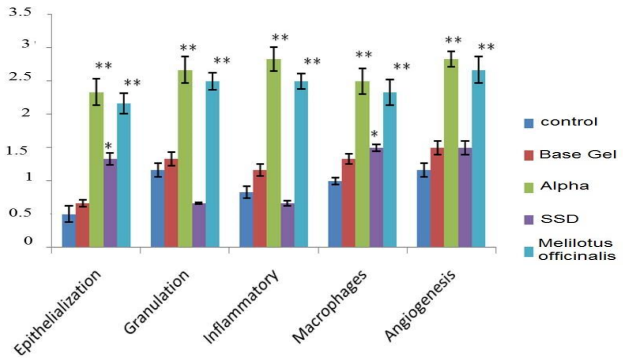

Figure 2. Histological scoring indices of wound healing activity in experimental groups on the 14th day.
0: absent; 1: mild; 2: moderate; 3: severe. Data are presented as Mean±SD (n=5).
SSD: Silver Sulfadiazine.
*Significantly different as compared with control group (P<0.05).
**Significantly different as compared with control group (P<0.01).

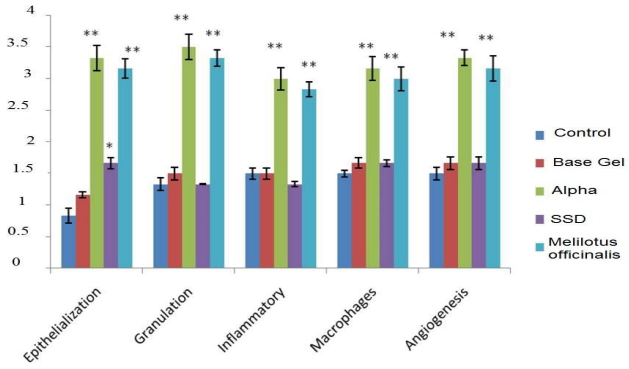

Figure 3. Histological scoring indices of wound healing activity in experimental groups on the 21th day.
0: absent; 1: mild; 2: moderate; 3: severe. Data are presented as Mean±SD (n=5).
SSD: Silver Sulfadiazine.
*Significantly different as compared with control group (P<0.05).
**Significantly different as compared with control group (P<0.01).

3. Results

None of the animals expired during the study. Figure 1, 2, 3 show histological scores of wounds. Histopathological evaluations on days 7, 14, and 21 showed that burn healing was better in the Alpha and *M. officinalis* groups in comparison with the control group (P<0.01) (Figure 4, 5, 6). There was a marked infiltration of inflammatory cells, improved angiogenesis, and boosted proliferation of cells or epithelialization due to treatment with Alpha and *M. officinalis*.

On the other hand, on days 7, 14, and 21 histological findings demonstrated that there were no significant differences between the Alpha and *M. officinalis* groups (P>0.05). While the best result for re-epithelialization was observed on day 21 for the Alpha and *M. officinalis* groups (scores 3.5).

In fact, wound healing effects of the Alpha and *M. officinalis* groups were much better in comparison with SSD group (P<0.05).

4. Discussion

Wound healing is a process described by homeostasis, re-epithelialization, and granulation of tissues. Even though healing process occurs by itself without any facilitation, numerous risk factors such as; infection and delay in healing process have attracted the attention of many researchers in this domain (21).

SSD topical ointment at 1% is the most widely used topical treatment for burn injury (22). Due to its anti-microbial properties, it is widely used. However, its side effects have been report-

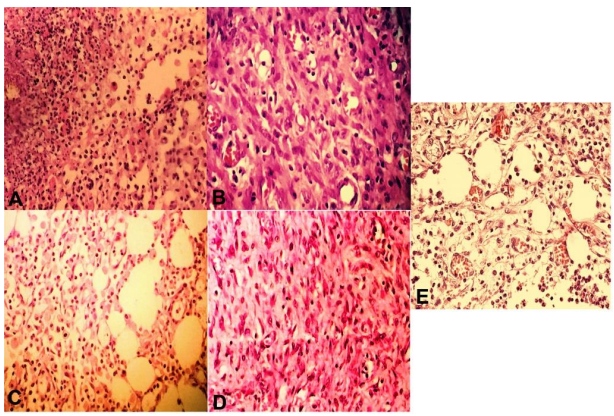

Figure 4. Microscopic appearance of burned skin on the 7th day.
Photographs of rats show infilteration of inflamatory cells in different groups.
A=Control; B=Base gel; C=Alpha; D=SSD; E=MO (H&E, 400×).

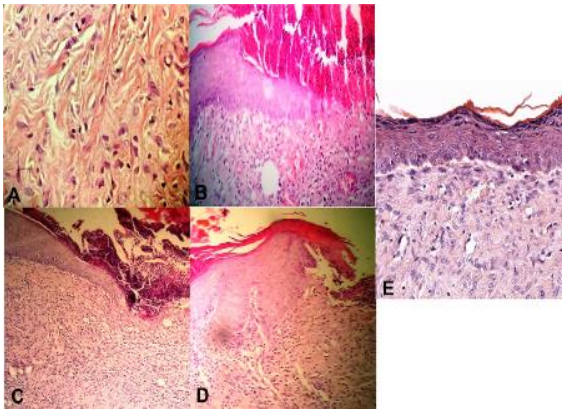

Figure 5. Microscopic appearance of burned skin on the 14th day.
Photographs of rats show proliferation of epithelial cells with Partial epithelialization in different groups.
A=Control; B=Base gel; C=Alpha; D=SSD; E=MO (H&E, 400×).

ed in several reports such as; renal toxicity, and leukopenia. Therefore, it is not recommended that this topical ointment is used for a long duration of deep wounds (23).

L. inermis is commonly known as Hanna in Iran which is called Mehndi in India is a very popular natural dye for coloring hands and hair. L. inermis has been known for its medicinal values in traditional medicine (24). In Iran, Alpha is a new topical ointment which is used in medicine for sores, scars, blisters, and burns (25).

Hosseini *et al*. evaluated the efficacy of Alpha ointment in the treatment of *Pseudomonas infections* burn and compared its results with silver sulfadiazine. They showed that infected wounds were significantly less common in Alpha ointment group compared to SSD (12).

Also, the effect of *M. officinalis* extract was examined in experimentally induced burn in rats, which the result indicated the efficiency of *M. officinalis* extract on increasing the antioxidant defense mechanism in order to decrease burn injury (26). Farzamfar *et al*. (2008) applied *M. officinalis* extract on dermal wound healing in diabetic mice and reported that it provides full wound healing with accelerated wound closure, and improves the quality of the tissue in the healing wound with very efficient hair growth on the scars which confirmed our findings.

M. officinalis has been found to have strong antioxidant components such as oleanene glucuronide, flavonoids, and coumarins and also due to beneficial effects such as improvement of venous blood circulation, reduction of inflammation, and improvement of the immune system can be effective in wound healing (27, 28).

In this study, the healing effects of Alpha and *M. officinalis* as natural products were evalu-

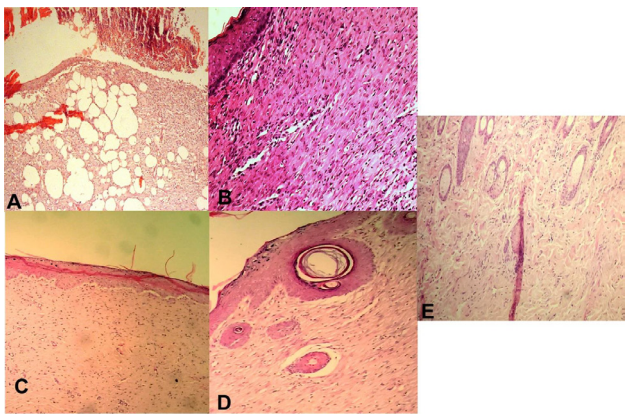

Figure 6. Microscopic appearance of burned skin on the 21th day.
Photographs of rats show completed ephithelialization with dermal mature collagenization in different groups.
A=Control; B=Base gel; C=Alpha; D=SSD; E=MO (H&E, 400×).

ated and compared with SSD. Our findings confirmed that both Alpha and *M. officinalis* have beneficial effects on healing process after burn injuries in all evaluated parameters including gross and histopathological features. Histological findings demonstrated no significant difference between the Alpha and *M. officinalis*.

5. Conclusion

To sum up, Alpha and *M. officinalis* ointments can be suitable alternatives for SSD. In addition, these topical formulations are more cost-effective.

It is suggested that further studies should be conducted on larger sample sizes or on human cases for a longer period to thoroughly evaluate the efficacy of Alpha and *M. officinalis* formulations on burn wounds in human.

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Conflict of Interest

None declared.

6. References

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