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Wound healing effects of *Artemisia sieberi* extract on the second degree burn in mice skin

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ABSTRACT

Introduction: Previous studies have shown anti-ulcerogenic effects of *Artemisia sieberi (A. sieberi)* on gastric lesions and experimental skin wound, so this study was conducted to evaluate healing effects of *A. sieberi* on the experimental second degree burn in mice skin.
Methods: Ninety adult male mice in 3 groups were used. Second degree burn was made in the dorsum and then silver sulfadiazine (SSD) 1% ointment and *A. sieberi* extract were applied in the positive control and treatment groups respectively, while in the negative control no medication was done. Digital photographs were taken daily for determination of healing percentage. For histopathological assessment, 5 mice of each group on the days 1, 3, 7, 14 & 21 post burn, were chosen and after euthanasia, a full thickness skin flap of the burn was taken and after tissue processing, specimens were stained with Hematoxylin-Eosin (H&E) and examined for granulation tissue, inflammation, re-epithelialization and collagen sediment, also hydroxyproline content of burn was measured. Data were presented as mean ± SE and analyzed by using Kruskal-Wallis and Dunn post huc tests ($P \leq 0.05$).
Results: *A. sieberi* enhanced wound healing via significantly decreased inflammation, increased granulation tissue, hydroxyproline content and healing percentage in comparison to negative control in such a manner which was comparable to standard SSD.
Conclusion: It seems that *A. sieberi* can promote burn healing due to anti-inflammatory, antioxidant, anti-microbial and mitogenic properties of the plant.

Implication for health policy/practice/research/medical education
A. sieberi increased granulation tissue, hydroxyproline content and healing percentage parallel to decreased inflammation which leading to promote burn healing due to anti-inflammatory, antioxidant, anti-microbial and mitogenic properties
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Introduction

Burn is a tissue injury due to extreme heat, electrical, radiation, corrosive chemical and friction sources. It is one of the major reasons of death and inability and accounts the fourth cause of injuries worldwide (1). Burns are classified most commonly based on the depth of injury into first degree, second degree, third degree and forth degree. In the second degree burn, injury extends to the superficial/deep dermis. Many of the burn injuries are due to disturbance in the normal protective and physiological functions of skin (2). Cave portraits indicated that burn and its management came back to more than 3500 years ago (3). Various folk remedies particularly herbal medications had been indicated in different civilizations, for example Avicenna recommitted medicinal plant for sore dressing (4). Today there are topical treatments for burn wounds e.g. silver sulfadiazine (SSD), silver impregnated dressings, silver nitrate, mafenide acetate, neomycin, polymyxin B, mupirocin. Although SSD 1% ointment is considered as the gold standard for topical burn treatment, nephrotoxicity, leukopenia, risk of antibiotic resistant and delayed wound healing are considered as its adverse effect (5). So, finding new, efficient therapeutic agents with more safety has always been a great concern (6). Medicinal plants lived long before human being and nowadays, they are prominent source of indigenous products for human health, because of suitable efficacy, less toxicity and side
effects than chemical drugs (4,7,8). *Artemisia* species are medicinal plants with a long history of different indications

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in the traditional and modern medicine as anti-malaria, anti-pyretic, analgesic, anti-oxidant, anti-inflammatory, anti-hepatitis, anti-ulcerogenic, anti-helminthic, anti-amoebic and anti-hemorrhagic (9-13). Anti-ulcerogenic action of some sesquiterpene lactones of *A. annua* and ethanolic extract of its aerial parts had been shown (14,15). Histopathological and biochemical analysis of *A. Anuua* extract showed that it can hasten healing of experimental dermal injuries (16). Later accelerating effects of ethanolic extract of *A. aucheri* on wound healing and reducing the healing period was reported (17). Also the ability of *A. sieberi* extract to increase thickness of skin layers and collagen fibers have been reported (8). So due to anti-microbial, anti-inflammatory, anti-oxidant and anti-ulcerogenic activity of *Artemisia* species and its suitable effects on the histopathological, histomorphometric and biochemical parameters of the skin and wound healing, this study was conducted to evaluate the effects of *A. sieberi* extract on the healing of second degree burn.

Materials and methods

Plant extraction

Aerial parts of *A. sieberi* were collected from Yazd province in the center of Iran, dried at room temperature, grounded and sieved and then Artemisinin extraction was done by using petroleum ether (8,11).

Animals

Ninety adult male mice (20-25 g) in three experiments were used. Animals were housed under standard laboratory conditions in accordance with the European community guidelines for laboratory animals (12 hours light/dark cycle, ambient temperature of 22 ± 1°C, 60% humidity) and under supervision of the Ethical Committee of the University, while chow pellet and water were freely available. After 1 week of acclimatization, they were divided accidentally into three groups (n = 30). Mice were anesthetized with an intra-peritoneal (i.p) injection of 40 mg/kg sodium thiopental (UVB Pharma, Czech Republic). The skin of dorsum was shaved and thoroughly depilated and thereinafter washed with povidone iodine. Burn was made by contact of a round 1 cm diameter metal plate (105°C, 5 seconds, without pressure) and then for prevention of shock, 1 ml of normal saline was injected i.p and each animal was kept in a separate cage. Group one was treated with SSD 1% ointment (King Pharmaceuticals, USA), group two was treated with *A. sieberi* extract but group three was selected as negative control and received no medication. Treatments were done every 12 hours for 21 days.

Histopathological evaluation

The burns were monitored for sign of infection and digital photographs were taken daily and after analysis by using ImageJ software, they were used for measurement of wound area and calculating healing percentage (18):
[(area of the first day- area of the second day)/ (area of the first day)]×100.

For histopathological study, 5 mice of each group on the days 1, 3, 7, 14 and 21 post-treatments were chosen randomly and after euthanasia, a full thickness skin flap of the burn was taken and fixed. Tissue processing was done (Autotechnicon tissue processor Citadel 1000, Thermo Shandon, UK), specimens were stained with H&E and examined for tissue granulation, inflammation, re-epithelialization and collagen sediment (19,20). Hydroxyproline content of burn as the index of tissue collagen was measured (21).

Statistical analysis

Data were presented as mean±SD and analyzed by using SPSS version 16.0 (Chicago, IL, USA). Kruskal-Wallis and Dunn post hoc tests were used for data analysis ($P \leq 0.05$).

Results

Healing percentage

Healing percentage of *A. sieberi* and SSD was significantly higher than control on days 7&14 ($P \leq 0.05$), while on day 7, the difference between *A. sieberi* and SSD was not significant ($P > 0.05$; Figure 1).

Inflammation

From day 1 to 21 inflammation score was decreasing, but only on days 3 & 7 in the *A. sieberi* and SSD groups it was significantly lower than control ($P \leq 0.05$), while on the rest of days such a significant difference was not seen ($P > 0.05$; Figure 2).

Granulation tissue

On day 3 granulation tissue of *A. sieberi* and SSD was significantly higher than control ($P \leq 0.05$), while there was no significant difference neither between *A. sieberi* and SSD on day 3, and nor between *A. sieberi* and SSD with control on other days ($P > 0.05$; Figure 3).

Hydroxyproline

Hydroxyproline content of burn as the index of collagen synthesis was measured on day 14. It was significantly

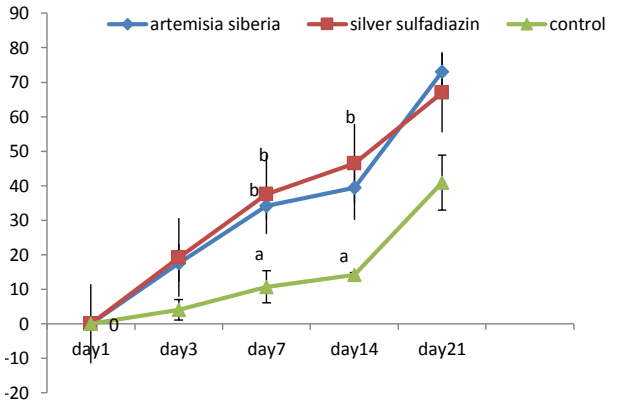

Figure 1. Healing percentage of burn in the silver sulfadiazine (SSD), *Artemisia sieberi* and control groups on sampling days. Different letters indicate significant difference ($P > 0.05$; Dunn test).

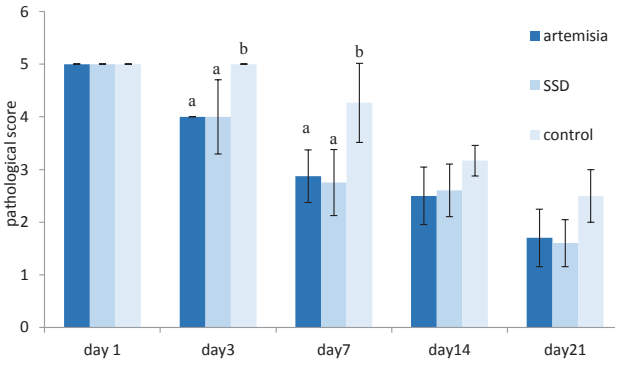

Figure 2. Pathological score of the inflammation of burn in the silver sulfadiazine (SSD), *Artemisia sieberi* and control groups on sampling days. Different letters indicate significant difference ($P > 0.05$; Dunn test).

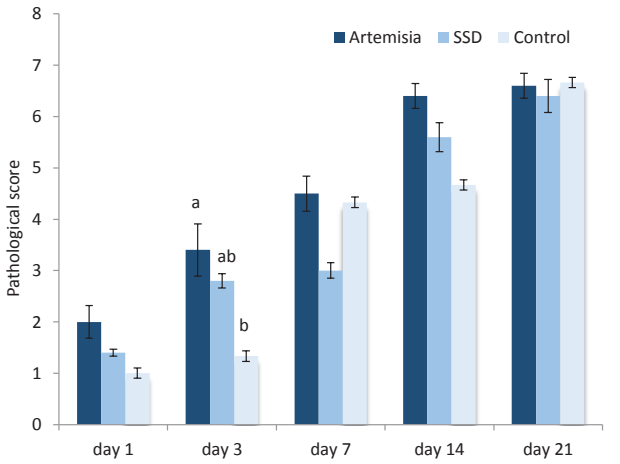

Figure 3. Pathologic score of granulation tissue of burn in the silver sulfadiazine (SSD), *Artemisia sieberi* and control groups on sampling days. Different letters indicate significant difference ($P > 0.05$; Dunn test).

higher in SSD and *A. sieberi* groups than control ($P \leq 0.05$), but the difference between SSD and *A. sieberi*, was not significant ($P > 0.05$; Figure 4).

Histopathological results

Histopathological evaluation of the burn is illustrated in Figure 5. On the day 3,in the *A. sieberi* group, tissue necrosis was seen on the surface of the burn, inflammation was evident and many inflammatory cells especially neutrophils were presented, and despite that regeneration of the epithelium has been stared but it was not complete and number of blood vessels was few (Figure 5A). On the day 7, healing proceed in the *A. sieberi* group, so that epithelial tissue has been regenerated but surface keratinization has not been done and also collagen fibers have been formed (Figure 5B). In the day 21 in the both SSD and *A. sieberi* groups healing has been completed, epithelial and connective tissues were completely regenerated and their structures were seen normally, so that in the SSD group hair follicles were clearly visible. Collagen fibers in the dense bundles and horizontal orientation were seen, numbers of fibrocytes increased

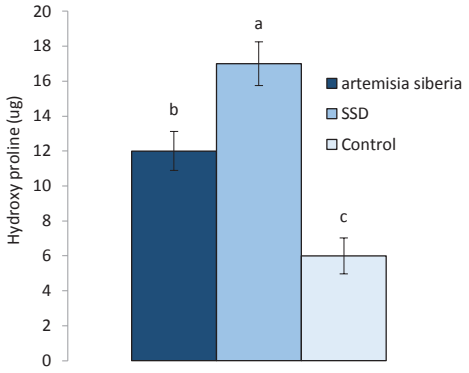

Figure 4. Comparison of hydroxyproline content of burn in the silver sulfadiazine (SSD), *Artemisia sieberi* and control groups on day 14. Different letters indicate significant difference ($P > 0.05$; Dunn test).

and were more than fibroblasts and new vascularization has been decreased (Figure 5C and 5D).

Discussion

Wound healing in the least possible time and decreasing functional and emotional consequences of the burn injuries is the ultimate purpose of burn treatment (5). Form past times abundant plants and derivative products have been used in traditional and modern medicine for wound healing (4,8,22). Burn healing is a complex, time consuming process, which inflammation is the first stage of this process, but overtime it may offend wound area and surrounding tissue and thus hinder healing, so, modulation of inflammation can promote wound healing (6,23). In this study *Artemisia* extract significantly decreased inflammation score on the days 3 & 7. Previously it was shown that almost *Artemisia* genus possess immunosuppressive and anti-inflammatory potential (8,12,14,16). Sesquiterpene lactones including Artemisinin, dehydroleucodine, and flavones e.g. apigenin, crisilineol and 6-methoxytricin are active ingredients of *Artemisia* which seems to exert anti-inflammatory and immunosuppressive activity via interaction with nuclear factor κB (NF-κB) and inhibiting liberation of inflammatory mediators (8,12,14,16,24). Scoparone is another constituent of the plant that inhibits iNOS and COX-2 enzymes which decline release of inflammatory mediators (8,12,16). Additionally plant extract contains phenolic components such as tannin, xanthines, cumarine and flavonoids and micronutrients e.g. copper, zinc & manganese which are potent anti-oxidant and anti-free radicals (8,16). *Artemisia* extract increases superoxide dismutase, catalase and glutathione S-transferase which potentiate the antioxidant capacity against free radicals (8,12,16). Modulation of inflammation and application of antioxidants in the initial days of healing and in the proliferative stage accelerate healing process, so it seems that *Artemisia* plant via impeding lipid peroxidation, lessening the inflammation and collecting and counteracting free radicals help and enhance healing. Burn infection is one of the major complications of the burn which hinders healing and is the principle reason

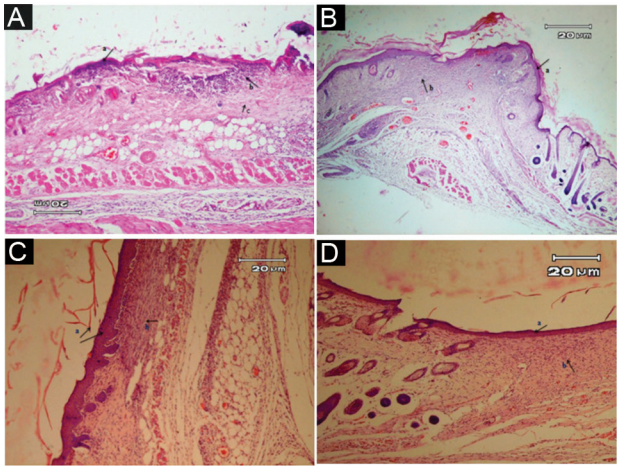

Figure 5. A) Light microscope structure of the burn in the *A. sieberi* group, day 3. Necrotic tissue on the surface and starting of epithelialization (a), tissue inflammation with plenty of neutrophil cells (b), little number of blood vessels (c). **B)** Light microscope structure of the burn in the *A. sieberi* group, day 7. Regeneration of epithelium, without keratinization (a), collagen fiber formation (b). **C)** Light microscope structure of the burn in the *A. sieberi* group day 21. Complete regeneration of epithelium and keratinization (a), suitable arrangement of collagen fibers (b). **D)** Light microscope structure of the burn in the SSD group, day 21. Complete regeneration of epithelium and keratinization, skin structures and hair follicles are seen (a), complete connective tissue and dense collagen fibers on the horizontal surface of the epithelium, vascularization decreased, fibrocytes are more than fibroblasts (b). H&E staining, magnification ×20.

of death in severe burns, therefore decreasing the hazard of burn infection can improve healing (4,6,18). Prior studies have shown that *Artemisia* possesses antibacterial property against bacteria including *Staphylococcus aureus, Streptococcus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and dermatophytic infections, which are the most important infectious agents of the burn. These anti-bacterial effects is attributed to the constituents such as verbenol, camphor, cineole, linalool, borneol, cymene, sabinene, α & β thujone and 1,8- cineole in the *Artemisia* plant that may be another mechanism responsible for promotion of wound healing (8,13,25-29). This study indicated that *Artemisia* during days 7&14 significantly improved healing, so that in the day 14 it was significantly better that SSD. Some sesquiterpene lactones of *Artemisia* exhibit antiulcerogenic effects by augmentation of gastric glycoprotein, mucus and prostaglandin synthesis against gastric ulcers (14,15). Later, increasing number of fibroblast, capillary buds, epithelial gap and improving the tensile stability of the wound following application of *A. sieberi* extract was reported (16). Also *A. sieberi* extract significantly increased epidermis, dermis and hypodermis thickness and percentage of collagen fiber in normal skin (8). Intensification of collagen formation and epithelialization can promote healing. During remodeling stage of wound healing, collagen fibers play a vital role to supply strength of the extracellular matrix and support wound and eventually intensifying tensile of the skin (16,18,23). The effects of *Artemisia* on the increment of the hydroxyproline content of burn can be due to the mitogenic characteristic of the plant leading to more collagen synthesis and durability of the healing tissue, improvement of re-epithelialization and establishment of granulation tissue which finally leads to enhancement of healing (8,16).

Conclusion

Artemisia extract promotes burn healing which can be due to antioxidant, anti-inflammatory, anti-microbial and mitogenic properties of the plant leading to modulation of inflammation, prevention of oxidative stress and free radical collecting, prevention of wound infection, amplification of collagen synthesis, re-epithelialization and granulation tissue.

Authors' contributions

JK: research design & manuscript preparation; JK and BK: performing animal experiments; BK, HN and MR: histopathological examinations & statistical analysis

Conflict of interests

The authors of the manuscript declare that they have no conflict of interest.

Ethical considerations

The authors have been entirely regarded ethical issues such as plagiarisms, data assembly, fraud, double publication or submission. All animal experiments had been approved by the ethical committee of the University and carried out according to the International Guide for the Care and Use of Laboratory Animals.

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