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Research Article

EFFECT OF *AMYGDALUS EBURNEA* ON THIRD-DEGREE BURNS IN RATS AND COMPARISON WITH SILVER SULFADIAZINE OINTMENT

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ABSTRACT

Objective*: Amygdalus eburnea* is a perennial herb that has been valued for its important biological perspectives and it has been used to treat the burn. This study aims to evaluate wound healing activity of *A. eburnea* extract compared with silver sulfadiazine (a synthetic burn ointment) for treating the induced third-degree burn in rat.

Methods: In this study, a deep, third-degree burn wound was induced by a hot plate warmed 5 minutes within boiling water and placed for 3 seconds on the skin. The shell root of *A. eburnea* an Iranian medicinal herb is investigated on its wound healing activity in comparison with silver sulfadiazine ointment as the standard treatment for burn wound in rats.

Results: Our results demonstrated that *A. eburnea* can be an effective treatment for third-degree burns. Advanced clinical and pharmaceutical studies are recommended to the production of novel natural drugs for burn wound treatment.

Conclusion: *A. eburnea* can be an effective treatment for third-degree burns. Advanced clinical and pharmaceutical studies are recommended to the production of novel natural drugs for burn wound treatment.

Keywords: *Amygdalus eburnea*, Rat, Wound healing, Silver sulfadiazine, Burns.

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INTRODUCTION

Burn injury is a global public health issue especially for the developing [1] and undeveloped countries, which suffer from lack of adequate medical facilities. Burns are one of the most common forms of injury with devastating concerns [2]. Burn is tissue injury caused by heat, electricity, chemicals, radiation, etc. According to the depth, burn wounds are categorized as first degree (superficial), second degree (partial thickness), and third degree (full thickness) [3]. Dressing is required to protect against the environmental flora and evaporative heat loss and accentuate the healing period because thermal injury disrupts the protective barrier function of skin. The ultimate burn dressing wound is cheap and comfortable; it cause the burn to heal rapidly and it cleans the wound and devitalized tissue and debride fragments of separated eschar and have an antibacterial activity [4,5]. Wound healing is a complex process including many physiological actions and responses, requiring the collaborative effects of different cells and tissues [6]. Severe burn wounds must be treated rapidly since any delay may cause infection or postpone the healing process [3]. Most of the early treatments include topical application of medicaments are necessary to prevent infections [7]. In severe burns, septicemia and infection are the main factors of mortality among these patients [8]. Burn wound healing is still a challenge to modern medicine despite the discovery of several antiseptics [2]. Although many improvements have been made to treat burn injuries, the best treatment to hasten healing has not been reached yet [9].

Many of the synthetic drugs cause problems such as drug resistance and allergy. Hence, many scientists are trying to seek alternative drugs [10]. More than 80% of the world's population utilizes traditional medicines for various skin diseases [11]. Recently, the traditional usage of plants has received considerable attention for wound healing by the scientific community [11,12]. Approximately, one-third of all using traditional medicines are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs [13,14]. Herbal products have moderate efficacy with no or less toxicity and are cheaper than synthetic drugs [15]. Medicinal plants can act as wound healing agents because of their vast variety of different constituents such as alkaloids, tannins, terpenoids, essential oils, saponins, flavonoids, fatty acids, and phenolic compounds [16], which have the capability to improve healing process of burn wound. Low cost, availability and fewer side effects such as allergy and drug resistance are other advantages of herbal remedies [3,17,18].

Amygdalus eburnea Spach. is a plant from *Rosaceae* family and it is a type of almond which is naturally grown and distributed in Iran. This plant is called "Ghosk" in Persian, which has been used in semi-desert areas to control soil erosion and stabilized watersheds [19]. Moreover, it has been used as wound healing of burn since ancient time by people of southeast of Iran. In traditional Iranian medicine, *A. eburnea* has also been utilized successfully as laxative and anti-worm. Furthermore, brew of dermal tissue is used for cough, respiratory distress, and paregoric. Moreover, in modern medicine, various pharmacological properties such as antioxidant, antifungal, antibacterial, and antidermatophyte have been related to this plant [20-24]. This study aims to evaluate wound healing activity of *A. eburnea* extract compared with silver sulfadiazine (a synthetic burn ointment) for treating the induced third-degree burn in rat.

METHODS

Collection of plant materials

The shell root of *A. eburnea* was collected from rural regions of from Baft district, the Southeast of Iran, in April 2013. They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Iran (KF 1136).

Preparing of extracts
About 100 g of powdered plant material was separately extracted by percolation method with methanol (80%) and water successively for 72 hrs in room temperature. The extracts were passed through filter paper (Whatman No. 3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at −20°C, until testing [25-27].

Thermal injury model

Ethical statement

This study was conducted in strict accordance with the recommendations in the guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Kerman University of Medical Science (Permit Number: 1508). Moreover, all efforts were made to minimize suffering.

Animals

Mal Wistar rats weighing from 200 to 250 g were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12 hrs light/dark cycle at 21±2°C and were handled according to standard protocols for the use of laboratory animals. During the tests, the temperature of the room and humidity were controlled. For the experiments, the animals were selected randomly into three groups: Topical *A. eburnea* treated group, topical silver sulfadiazine treated group, and untreated or control group.

Establishment of third-degree burn wound
A deep, third-degree burn wound was induced by a hot plate warmed 5 minutes within boiling water and placed for 3 seconds on the skin. Immediately after burn, animals received an intraperitoneal injection of 1 ml normal saline to prevent shock caused by burns. Burns were dressed daily with *A. eburnea* extract in Group 1 and silver sulfadiazine in Group 2. Responses to the treatment were assessed by digital photography during the treatment until day 15.

Wound analysis
The wound closure rate was assessed by tracing the wound on day 3, 6, 12 and 15 then percentage of recovery of all groups was compared. Time to wound closure was defined when the wound bed was completely re-epithelialized and filled with new tissue. The wound healing rate was calculated with a formula as following:

$$\text{Percent of wound} = \text{Area of remaining wound} / \text{Area of original wound} \times 100 \quad (1)$$

Percent of recovery=100−Percent of wound.

Pathological study
Pathology examination was carried out on the skin biopsies on the 6 and 12 days after injury. Skin biopsies were examined after blocking and preparing cuts of 3 μm by microtome (Leitz 1512) and stained with hematoxylin-eosin.

RESULTS AND DISCUSSION

Wound healing rates were measured at day 3, 6, 12, and 15 after the wounding. The effect of the extract on wound healing activity in rats compared with the control rats and silver sulfadiazine treated group values of $p<0.05$ were considered as statistically significant. The average percent of wound healing rates on the 3rd day for extract, silver and control group was 9.51%, 7.17% and 7.88%, respectively. The overall average wound healing rates on the 3rd day were 8.19% that the highest (25.66%) and lowest (−1.48%) wound healing rates on the 3rd day were belonging to extract and silver group, respectively. However, the silver group shows highest and lowest 47.54% and 4.07% wound healing rates on the 6th day. Nevertheless, in the 12th and 15th day, the procedure was same that the maximum wound healing rates that are belong to extract group were 79.74% and 90.32%, respectively. In all groups, wound healing rates increased with time. A significant increase in the wound-healing activity was observed in the animals treated with the extract, compared with those received the control treatments. This investigate showed that healing percentage in *A. eburnea* extract was 85.91%, while in silver groups was 81.50% and in control groups was 68.78% ($p<0.05$). Therefore, healing percent progress on *A. eburnean* extract was more than silver sulfadiazine and control groups in all days, and healing percent progress on control group was loss than another group. Healing time in treatment with *A. eburnean* was significantly shorter than silver sulfadiazine.

According to the homogeneity of variances tests at day 3, 6, 12, and 15 confidence level are 0.338, 0.502, 0.115, and 0.074 (>0.05), respectively, and conclude that the variance is constant.

The one-way ANOVA is used to test for differences among control, extract and silver groups at day 3, 6, 12, and 15. Since the confidence interval on the 3rd day and 6th is 0.89 and 0.849 respectively ($p>0.05$) can be said healing rate percentage are same for all three groups in these 2 days. Differences of wound healing rate percentage in extract and silver groups are not significant at 12th day, but according to descriptive statistics, it can be concluded wound healing rate in control group is less than them. As a result, statistically, the percentage of wound contraction showed that there is high significant difference between the different groups in the 15th day. Moreover, Tukey test demonstrates the difference of wound healing between silver and extract groups was not significant, but their percentage of healing rate was better than the control group. Investigate of wound healing rate in all days for three groups indicate that differences of wound healing surface area for all three groups were not significant, although the best results of wound healing in all days were obtained in extract group. The difference between silver and extract group was not significant, but in extract group, healing rate was better 7th, 8th and near the end of the study. However, healing rate in control group was similar to that of extract and silver group in 1st, 2nd, 6th and 9th days, the speed of healing was lower in the other [28].

Wound healing process consists of inflammation, re-epithelialization, granulation and neovascularization, which result in wound contraction. In histopathological study regarding the inflammation, in 6th day re-epithelialization was not significant in control group, and a little granulation tissue formation was observed. Furthermore, necrotic tissue with severe edema and infiltration of inflammatory cells with dominance neutrophil is visible. In the extract group, there was less inflammatory reaction of neutrophils and edema. Inflammatory infiltration was observed. Furthermore, in addition to neutrophils, a large number of macrophages infiltrated. In wound margins, epithelial hyperplasia occurred. Granulation tissue formation was with angiogenesis. The healing process in silver group was similar to the extract group.

In the 12th day of experiment, incomplete monolayer of epidermal cells (in most of cases, about 30% of the damaged area was still without re-epithelialization) with evidence of crusting in control group was also observed. Furthermore, granulation tissue with good thickness in the wound edges observed. In some cases, immature granulation tissue with high levels of inflammatory cells in the dermis was seen. In extract group, epidermis exhibited well-structured layers without any crusting. Extract was effective on burn wound with increase in thickness of granulation tissue and re-epithelialization. It also decreased the inflammatory reactions. Although up to 80% burns in silver were covered by the new re-epithelialization, thickness of re-epithelialization was not significant. Granulation tissue with minimum inflammatory cells in the dermis was made. Re-epithelialization was prominent in extract group.

A. eburnea has been used as a medicinal herb since ancient times in Iran. The present study has shown that the extract from the *A. eburnean* as an available and inexpensive herb possesses wound healing activity,

and thus provided the evidence for its traditional use value and it is suitable substitute in healing of burn wounds.

A. eburnea extract to restore burned tissue and facilitate skin regeneration. Moreover, *A. eburnea* inhibit the growth of microorganisms, therefore, this finding support the use of *A. eburnea* in the management of burn wound infection. In concluded, *A. eburnea* can be an effective treatment for third-degree burns. Advanced clinical and pharmaceutical studies are recommended to the production of novel natural drugs for burn wound treatment.

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