ORIGINAL RESEARCH REPORTS

Effect of *Arnebia euchroma* ointment on post-laser wound healing in rats

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Abstract

Introduction: *Arnebia euchroma* ointment has been used in Iranian Traditional Medicine for burn wound healing. The aim of this study is to evaluate wound healing efficacy of *A. euchroma* ointment on wounds induced after fractional CO2 laser in rats.

Material and methods: In this study, after anesthetizing two bilateral burn wounds were induced on dorsal skin of the rat using fractional ablative CO2 laser. After applying laser, *A. euchroma* ointment, petrolatum, and silver sulfadiazine cream were used topically on wounds twice daily for 10 days. Digital photographs were captured from the wound surfaces every day. At the end of the study, two blinded dermatologists observed the photograph of 3rd, 5th, 7th, and 9th days after laser injury and assessed erythema, crusting/scabbing, epithelial confluence, and general wound appearance to determine the efficacy of wound healing. These wound-healing parameters were assessed using the 5-point scales.

Results: This study showed significantly less erythema and crusting (P<0.024 and P<0.004, respectively) on 9th day and higher epithelial confluence and general wound appearance scores on 7th (P<0.037 and P<0.016, respectively) and 9th days (P=0.008 and P=0.016, respectively) in *A. euchroma* ointment compared with other groups.

Conclusion: This study showed *A. euchroma* ointment has good healing effects on post-laser wounds in rats.

Key Words: *Arnebia euchroma*, Iranian Traditional Medicine, laser, wound healing

Introduction

Laser skin resurfacing (LSR) for the rejuvenation of facial skin has remained a popular cosmetic procedure. Wound care after LSR is critical for achieving a successful result (1). Nowadays, there is no standard of care for post-laser resurfacing treatment of the face. Ideally, treatment should speed re-epithelialization and reduce downtime, with minimal irritation (2). It has been reported that the post-laser resurfacing wound care is important in promoting wound healing and preventing early and late complications. In fact, the final outcome of laser resurfacing depends on a great amount of the efficiency of wound care (3). Since ancient time, plants have been rich sources of effective and safe medicines. Herbal medicines have been the main source of primary health care in many nations. About 80% of world populations are still dependent on traditional medicines (4). The *Arnebia* genus from the family of Boraginaceae has various species growing in Asia and the drier regions of northern Africa (5). Shikonin and
ALKANIN are very prominent chemical components of *A. euchroma*, which have widespread pharmacological properties including anti-inflammatory, antimicrobial, and anti-tumoral activities (6–9). The root of *A. euchroma* is effective in treating skin rash, eczema, ulcers, and burns (8). *A. euchroma* in Iranian Traditional Medicine (ITM) is named Havachoobe or Abokhalsa (6). Roots of Havachoobe were used topically for wound healing in ITM (10). The purpose of this study is to determine the healing effect of *A. euchroma* on wound caused by fractional ablative CO₂ laser in rats.

**Materials and methods**

**Plant material**

The dried roots of *A. euchroma* were purchased from local market in Tehran bazar and identified by Professor Gholamreza Amin, and were kept at the herbarium of Faculty of Pharmacy, Tehran University of Medicinal Sciences, under the voucher number PMP-216.

**Preparation of *A. euchroma* ointment and vehicle**

*A. euchroma* ointment was made using *A. euchroma* roots (10 g), sesame oil (100 g), and wax (10 g) in the weight ratio of 1:10:1, respectively (11). The dried roots of *A. euchroma* were chopped and heated in sesame oil at 40–60°C for 12 h. Then, the samples were filtered and mixed with melted wax. Vehicle was a mixture of sesame oil and wax.

**Animals**

In this study, 12 male Wistar rats (200–250 g) with age of 2–3 months were used. These animals were born, reared, and housed in the Faculty of Pharmacy, Tehran University of Medicinal Sciences. The animals were kept in standard environmental conditions of temperature, humidity, and a 12-h light/dark cycle. During experimental time, rats were given free access to standard diet and water. The Ethics Committee of Shahed University approved the protocol (approval number: 148489). The animals were kept in separate cages to prevent licking or biting of wound areas by other animals.

**Wound creation**

In this experiment, wounds were caused by anesthetizing rats with intra-peritoneal injection of ketamin and xylazine (1.5 mg/kg), shaving the dorsal skin of the Wistar rats, and inducing two bilateral burn wounds (20 × 20 mm) on the dorsal skin of the rat (Figure 1) using fractional ablative
CO\textsubscript{2} laser at the Laser Research Center of Shahid Beheshti University of Medical Sciences. Fractional CO\textsubscript{2} laser resurfacing was performed using a Mixel CO\textsubscript{2} Fractional Laser System (Hironic Co, Ltd. [Korea]) with the following setting parameters: laser power = 50mj, distance = 0.3–0.4 mm, overlap = 3 steps, and repeat = 0.5 s.

Experimental design

After applying laser, each animal had two wounds on its back. Right and left sides of each animal were different groups; on the other hand, six animals received two types of drugs—on right side, \textit{A. euchroma} ointment (group 1) and on left side, vehicle (sesame oil + wax) (group 2). Also, the other six animals received petrolatum (group 3) and silver sulfadiazine cream (group 4) on right and left sides, respectively. Treatments were carried out twice daily for 10 days. To determine the rate of wound healing, digital photographs were captured from the wound surfaces every day. On the 10th day, the experiment was terminated and the wound area was removed from the expired animals for histological examination. All the specimens were formalin-fixed and stained with hematoxylin–eosin. At the end of the study, two blinded dermatologists observed the photographs and a blinded pathologist assessed microscopic specimens.

Wound healing assessment

Two blinded dermatologists observed photographs of 3rd, 5th, 7th, and 9th days after laser injury and assessed erythema, crusting/scabbing, epithelial confluence, and general wound appearance to determine the efficacy of wound healing. These wound-healing parameters were assessed using the 5-point scales (Table I).

Histopathological examination of laser wounds

A specimen of the skin (0.5 × 0.5 cm) was taken from the middle of the burnt area 10 days after applying laser. These tissues were preserved in a 10% fresh, neutral buffered solution of formaldehyde for at least 24 h. Sections were stained with hematoxylin and eosin dyes and examined using a light microscope (Figures 3).

Analysis of data

Data were collected, analyzed, and reported as mean and standard deviation (Mean ± S.D.). Statistical comparisons between groups were carried out using SPSS software (Version 16.0, Chicago, IL, USA). One-way ANOVA followed by Duncan’s post hoc test were used to analyze the data. \( P \leq 0.05 \) was considered statistically significant.

Figure 3. Photomicrograph of wound healing after 10 days of laser in four groups (H&E ×100).
Table I. Grading score.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Scale</th>
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<tbody>
<tr>
<td>Erythema</td>
<td>0 = none or absent, 1 = mild, 2 = moderate, 3 = marked, 4 = severe</td>
</tr>
<tr>
<td>Epithelial confluence</td>
<td>0 = none, 1 = slight (up to 30%), 2 = moderate (31–60%), 3 = extensive (61–90%), 4 = almost complete (91–100%)</td>
</tr>
<tr>
<td>Crusting/scabbing</td>
<td>0 = none, 1 = slight (up to 29%), 2 = moderate (30–59%), 3 = extensive (60–90%), 4 = almost complete (91–100%)</td>
</tr>
<tr>
<td>General wound appearance</td>
<td>0 = poor, 1 = fair, 2 = good, 3 = very good, 4 = excellent</td>
</tr>
</tbody>
</table>

Results

This study showed significantly less erythema and crusting (P = 0.024 and P = 0.004, respectively) on 9th day and higher epithelial confluence and general wound appearance scores on 7th (P = 0.037 and P = 0.016, respectively) and 9th days (P = 0.008 and P = 0.016, respectively) in group 1 compared with other groups (Figures 4).

Discussion

In this study, *Arnebia* ointment has good healing effect on post-laser wound in rats. *A. euchroma* ointment is traditionally used for wound healing (10,11). It has anti-inflammatory (12) and antimicrobial properties (13,14), and improves re-epithelialization, fibroblast proliferation, collagen bundle
synthesis, and vascularity in the injured tissues (15). It also has burn wound healing effect (7,9).

There are no similar studies about A. euchroma on post-laser wounds and our study is the first research in this field. However, there are many studies about A. euchroma on burn wound healing.

There are some studies for evaluation of A. euchroma on burn wound healing. In one experimental study, the healing effect of A. euchroma extract was compared with that of silver sulfadiazine in second-degree burn wounds on rats. This study revealed that A. euchroma and silver sulfadiazine significantly improved re-epithelization, fibroblast proliferation, and collagen bundle synthesis, and had a remarkable anti-inflammatory effect. This study showed that A. euchroma herbal extract was an effective treatment for second-degree burn wounds when compared with silver sulfadiazine (7). In another experimental study, efficacy of A. euchroma on third-degree burn wound was assessed in 48 female Sprague-Dawley rats, which showed positive effects (16). On the other hand, studies showed that alkannin and shikonin, which are the main components of A. euchroma, are active against methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci (17). Shikonin is famous because of its natural red-purple color and medicinal properties. It was used for curing burnt skin and ulcers in traditional medicine. Modern medicinal studies also support its old-known uses and suggest additional applications against cancers and HIV (14). Another in vivo study in mouse skin tissues suggested that topical treatment with shikonin can confer a potent stimulatory effect on epithelial–mesenchymal transition and suppress the expression of the associated microRNAs in skin wound healing. Altogether, these cellular and molecular data provide further evidence in support of previous findings on the specific pharmacological effects of shikonin in wound healing and immune modulation (12).

Conclusion
This study showed that A. euchroma ointment, an effective drug for burn wound healing which has been used by people for many years in many places (Iran and East of Asia), has good healing effects on post-laser wounds in comparison with petrolatum and silver sulfadiazine in rats. We suggested clinical study for assessment of effect A. euchroma ointment on post-laser wound healing.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References