

## Wound Healing Effect of *Arnebia euchroma* gel on Excisional Wounds in Rats

### Abstract

**Background:** Wound healing is a complex process leading to regeneration of damaged skin tissue. *Arnebia euchroma* (AE) have many effective activities such as anti-inflammatory, antimicrobial, antioxidative, and anti-tumoral effects. The extract of AE has positive effects on burn wounds. This study is designed to investigate the healing effects of AE on excisional wounds in rats. **Materials and Methods:** Thirty six rats with the age of 8 weeks divided into three groups. One group (E1) was treated with AE gel at a concentration of 10%. Control group (C1) received normal saline and the vehicle group (C2) was treated with carboxymethyl cellulose gel as the vehicle for 14 days. Stereological analysis was done to investigate the collagen bundle and hair follicle synthesis, vascularization, fibroblast proliferation. Pathological evaluation was also conducted. **Results:** In this study, pathological evaluation showed severe acute inflammation in C2 group, chronic and acute inflammation in C1 and also more wound contraction in E1 in comparison with other groups. There was a meaningful difference between E1 and C1 regarding fibroblast proliferation ( $P < 0.05$ ). **Conclusion:** Results of this study revealed the healing effect of AE on excisional wounds and recommend its administration after further clinical investigations.

**Keywords:** *Arnebia euchroma*, excisional wound, healing, skin

### Introduction

Wound healing is a natural response to tissue injuries. It consists of complex cellular processes leading to damaged tissue repair. According to previous studies, different methods has been used to accelerate the healing process including dressing, different systemic and topical medications, low potent laser, ultrasound, hyperbaric oxygen, growth factors, electrical stimulation and also gene therapy.<sup>[1,2]</sup>

Wound healing includes three phases; inflammation, proliferation and remodeling. Inflammatory phase is characterized with hemostasis and inflammation. This phase starts with clotting cascade (both internal and external ways) which is activated by collagens exposed after injury.<sup>[3,4]</sup> Growth and remodeling usually happens during eight days to even one year after the injury. Maturation and remodeling are the most important phases. The impairment of matrix deposits due to disease or diet can severely weaken the wound healing. The collagen of the granulation tissue has biochemical differences with normal skin. It has

more hydroxylation and glycosylation of lysine as amino acid; moreover, increased glycosylation can cause thinner fibers of collagen. The collagen of scar tissue never will arrange as same as the normal skin and also the solidarity and the power of the injured skin never become 100% again. During the first week after damage its power is about 3% of final power and along 3 weeks and 3months is 30% and 80% of it, respectively.<sup>[5,6]</sup>

Recently, herbal therapies are widespread among patients and clinicians. Thousands of years, herbal treatments are used in Europe and Asia for skin lesions.<sup>[7]</sup> The *Arnebia* genus is from the family of *Boraginacea* which has various species growing in Asia and drier regions of North Africa. *Arnebia euchroma* (AE) has pharmacological properties including anti-inflammatory, anti-microbial, anti-inflammatory, and anti-tumoral activities. Different studies demonstrated the effect of AE in comparison with other medications in burn wounds healing.<sup>[8,9]</sup>

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The aim of this study is to determine the effect of AE on the wound healing process and to more evaluate whether it is possible to recommend the herb as an alternative treatment for excisional wounds.

## Materials and Methods

This study was done in Islamic Azad University, Tehran medical branch. 36 rats with the age of 8 weeks kept at temperature of  $25 \pm 5^\circ$  centigrade and 50% air humidity were randomly divided into three groups for a treatment course of 14 days. Root and stalk of AE were used for extracting. Then the dried material was ground into powder and extracted with a mixture of water and ethanol, to change the shape of extract to gel, it was mixed with a vehicle made of Carboxy-Methyl-Cellulose (CMC).

A 1 cm<sup>2</sup> standard circular wound was created by excising the skin of the posterior surface of animals back by a surgical knife under general anesthesia with injecting Xylazine 10 mg/kg intraperitoneal and 5 mg/kg Ketamine.

C1 group had daily cleaning of the wound by normal saline, C2 group received CMC gel without the AE extract and experimental group (E1) received AE extract at concentration of 10% in CMC gel.

At the end, all rats were euthanized with ether and specimens from the wound site were taken. To determine the wound area, digital photographs were captured every other day from the wound area and the surfaces were measured by using stereological methods.<sup>[10]</sup>

A full thickness skin sample was provided from the wound site and was embedded in a cylindrical paraffin block. Five  $\mu$ m and 15  $\mu$ m sections were provided by microtone and stained with both Heidenhain's Azan and Hematoxylin and Eosin. Then it was inspected by stereological analysis with the point of collagen synthesis, fibroblast proliferation, vascularization and pathological evaluation of inflammation, fibrosis and scar.<sup>[10]</sup> The duration of the study was 15 days started at the day the wounds were created.

Data were expressed as Mean  $\pm$  standard deviation (SD). Kruskal Wallis and Man-Whitney U-tests were used to compare the groups. All data were analyzed with SPSS software version 14.0.

## Results

Pathological evaluations showed that in group E1 there was mainly a mild inflammation, in group C2 severe inflammation and in C1 acute and chronic inflammation. Mean re-epithelization was more than 50% in E1; however, less than 50% re-epithelization was visible in C1 and C2 wounds. The contraction of the wounds in C1 and C2 was significantly less than E1 ( $P < 0.05$ ).

Mean wound closure rate was 15.52 mm<sup>2</sup>/day in E1 and 8.51 mm<sup>2</sup>/day in C2 and 5.01 mm<sup>2</sup>/day in C1. The mean reduction of wound surface was significantly meaningful in E1 group in comparison with other groups. As stereological evaluations showed [Table 1], fibroblast production in E1 was  $\sim$  61% higher than C1 which showed a considerable difference between E1 and C1 ( $P < 0.05$ ); however, no difference was found between C1 and C2 ( $P < 0.05$ ). The density of collagen bundles in E1 was  $\sim$  41% more than C1 and  $\sim$  34% more than C2 ( $P = 0.031$  and  $P = 0.043$ , respectively). Length density of the vessels in E1 was  $\sim$ 6% more than that of group C1. The differences between the other groups were not meaningful. With regard to mean diameter of vessels and their length density there were no statistically significant differences.

## Discussion

Large group of world population use traditional drugs in developing countries. Herbal drugs are extremely used to treat wounds and burn wounds. Wound healing is a dynamic response consisting of inflammation, formation of granulation tissue, and remodeling.<sup>[11]</sup>

Many studies on plants of *Boraginacea* family were done all over the world. In a study by Papageorgiou *et al.* showed that Alkanins and Shikonins have positive impacts on wound healing by their anti-inflammatory, antimicrobial and anti-oxidative functions.<sup>[12]</sup> Our findings are agreed with their results.

Previous studies have showed the role of Alkanins and Shikonins in both accelerating formation of granulation tissue and wound healing in inflammation phase.<sup>[13]</sup> This study demonstrated that AE enhances fibroblast proliferation, collagen bundle synthesis,

**Table 1: Mean (SD) of the numerical density of the fibroblasts ( $\times 10^3$  per mm<sup>3</sup>), volume densities of the collagen bundles ( $V_v$  collagen/dermis; %), vessels ( $V_v$  vessel/dermis; %), length density (mm/mm<sup>3</sup>) and mean diameter ( $\mu$ m) of vessels in the dermis of the wounded rats treated with *Arnebia euchroma* gel, normal saline treated group (C1) and gel base (C2)**

Groups	Fibroblast proliferation ( $10^3$ per mm <sup>3</sup> )	Vv of collagen bundles	Vv of vessels	Length density of vessels (mm/mm <sup>3</sup> )	Mean diameter of vessels ( $\mu$ m)
C1	207.5 (11.6)	56.3% (6.1%)	3.7% (1.1%)	14.31 (4.1)	1.12 (0.29)
C2	216.31 (11.2)	59.6% (8.2%)	3.4% (0.7%)	13.18 (1.9)	1.13 (0.30)
E1	333.81 (9.1)*	79.6% (6.1%)*	4.1% (0.8%)	15.21 (1.7)	1.11 (0.21)
P	0.021	0.032	0.68	0.47	0.076

\* $P < 0.05$  in comparison with C1 and C2 groups. SD: Standard deviation

and vascularization and it showed anti-inflammatory impacts in the healing process of excisional wounds. Pirbalouti *et al.* in 2009 reported that both AE and *Malva sylvestris* causes more wound contraction and our results are agreed with their findings.<sup>[14]</sup> In two studies by Ashkani-Esfahani *et al.*, it was shown that AE cream in concentrations of 10% and 20% affects the burn wounds, second and third degree ones, and improves the parameters such as fibroblast proliferation rate, collagen and vascular regeneration, and enhances wound closure rate.<sup>[10,15]</sup> They found was no significant difference between the two AE treated groups. Our findings are agreed with their report. According to our result and previous studies AE shows promising potentials for repairing different kinds of skin wounds including burn-caused or excisional ones. However, more clinical studies are required to evaluate the effects of this plant and also its side effects for wound treatment.

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### Conflicts of interest

There are no conflicts of interest.

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