

## *Althaea officinalis* improves wound healing in rats: a stereological study

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**SUMMARY** *Althaea officinalis* (AO) is reported to have the ability to activate fibroblasts as well as anti-inflammatory and antioxidative properties. Herein, we investigated the effects of this herbal medicine on wound healing in rat models by using stereological methods. In this experiment, 48 male Wistar rats were divided into four groups randomly ( $n = 12$ ): the control group with no treatment, the gel-base treated group, 5% and 10% AO-gel treated groups. The treatments were administered every 24 hours. Wound closure rate, volume densities of collagen bundles, hair follicles, and vessels, vessel's length density and mean diameter, and fibroblast populations were estimated. Fibroblast populations, hair follicles, and mean diameter of vessels in the dermis of AO-treated groups were noticeably higher than those of control and base groups. Also, collagen bundles synthesis was significantly higher in the AO10%-treated group compared to the control and base groups. According to our research and previous studies, AO has the potential to be considered as an alternative medicine in wound healing treatment; however, further clinical investigations are suggested.

**Keywords** *Althaea officinalis*, wound healing, stereology, rat

### 1. Introduction

The skin is a bi-layer tissue that has the three roles, protection, sensation, and regulation, which could be compromised by injuries (1). The process of wound healing contains 5 stages of re-epithelialization, angiogenesis, activation and migration of fibroblasts, and endothelial cell proliferation (2). All of these stages are associated with inflammation and oxidation in the injured tissue (1). The release of growth factors from platelets and inflammatory cells like neutrophils leads to the initiation of the repair process immediately after the injury (3,4). Empowering the mechanisms involved in the process of wound closure and reducing scar formation are the main goals of wound treatment, specifically for skin wounds (5).

Nowadays, herbal medicines are used all around the world because of being efficient, safe, and also having fewer side effects and better compatibility with

the human body (6). *Althaea officinalis* (AO), marsh-mallow, is a perennial plant from the Malvaceae family (7). This agent has been traditionally consumed in different cultures both as food and medicine. Recently, several studies have mentioned that the alcoholic, hydroalcoholic, and methanol extracts of this herb have considerable anti-inflammatory, antioxidative, and antibacterial effects (7-11). Besides, the aqueous extract of AO has great amounts of mucilage polysaccharides that stimulate the proliferative activity of fibroblasts and keratinocytes (12). AO extract has the potential to increase the release of cytokines that are responsible for apoptosis, and rise the gene expressions of molecules involved in creating the extracellular matrix and the bio-adhesive proteins (13).

In this study, we aimed to investigate the effects of hydroalcoholic extract of AO on wound healing in rat models by using histomorphometric and stereological evaluations.

## 2. Materials and Methods

### 2.1. Preparation of AO gel

AO was received from SIMR Co., Shiraz, Iran (Code: 3104). All plant organs were occupied as the herbal material for making the extract. We dried the material at room temperature for 5-6 days. Then, it was ground into powder. Thereafter, the powder was extracted with a mixture of ethanol: water (1:1, v/v) for 72 hours. The obtained product was then filtered and then evaporated to make a hydro-alcoholic extract (yield: 16.23%). We dissolved 5 mg of AO extract in 2 mL of ethanol (70%) in order to prepare 5% gel and then mixed the solution with 2% carboxymethylcellulose (CMC) (2 g/dL). The 10% gel was prepared with the same method by using 10 mg of AO. We also similarly used the vehicle for the control group without the AO component.

### 2.2. Animal models and procedures

In this experimental study, we used 48 male Wistar rats (200 ± 20 g) aged 2.5 months on average, which were kept in standard cages with sufficient food and water *ad libitum*. Animals were divided into 4 groups randomly ( $n = 12$ ): the control group with no treatment, the gel-base treated group, 5%, and 10% AO gel treated groups. The study protocol was approved by the animal ethics committee of Shiraz University of Medical Sciences. In the beginning, on day 0, a 1 cm<sup>2</sup> circular full-thickness wound was created *via* excision of the skin. Under general anesthesia, wounds were created on the dorsal surface of each rat's neck. Every day, before applying the treatments, we irrigated the entire wound with normal saline. The control group received no treatment and the vehicle group received vehicle gel while the other groups were treated with 5% and 10% AO gels. According to a prior pilot study the end of the study was set as day 15. On the last day, we euthanized all the animals with a high dose of ether inhalation and took the skin samples from the wound site and transferred them into buffered formaldehyde (pH = 7.2) for making microscopic slides for stereological studies.

### 2.3. Stereological study

Every 4 days, we took a digital picture from the wound surface with a digital camera to measure the wound closure rate. A ruler was put next to the wound in each picture to measure the magnification on the monitor of computers. The "point grid" method was used to estimate the closure rate (14) by using the following formula: Wound closure rate (%) = ((area at visit 1 – area at each visit)/area at visit 1) × 100.

In a systematic random sampling manner, 8-13 pieces (each piece 1 mm<sup>2</sup>) of each skin sample were obtained and were put in a cylindrical paraffin block.

After Isotropic Uniformly Random (IUR) sectioning of the blocks with thicknesses of 4 μm and 20 μm (14), the slides were made and stained with hematoxylin and eosin (H&E; Figure 1).

Stereological parameters including volume densities of the collagen bundles and vessels, vessel's length density and mean diameter, and fibroblast populations were measured according to the study conducted by Ashkani-Esfahani *et al.* (14).

### 2.4. Statistical data analysis

Results were reported as mean and standard deviation (mean ± SD). SPSS statistical software (ver.19.0, IBM™, USA) was used to do statistical comparisons between the groups. The statistical analyses were carried out by employing Kruskal Wallis and Mann Whitney U tests. Furthermore,  $p \leq 0.05$  was considered as statistically considerable.

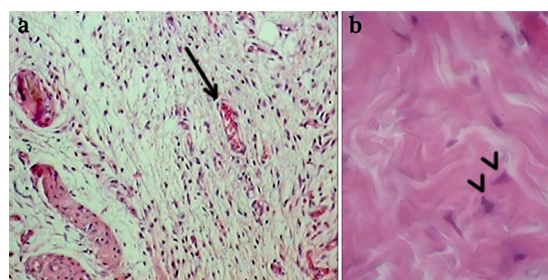
## 3. Results and Discussion

### 3.1. Wound closure

The mean initial wound area in all four groups was 104.22 ± 7.26 mm<sup>2</sup> with no considerable contrast among the groups. Wound closure rates of 5%-AO (6.03%/day) and 10%-AO (5.94%/day) groups were noticeably higher than the control (3.14%/day) and gel-base treated (3.44%/day) groups ( $p < 0.05$ ; Figure 2).

### 3.2. Fibroblast population

The numerical densities of the fibroblasts in the dermis of the AO-treated groups were noticeably higher than those of control and gel base groups. The numerical densities of the fibroblasts in 5% and 10% AO-treated groups were 112.8% ( $p = 0.021$ ) and 45.3% ( $p = 0.049$ ) higher than the controls, respectively, and 117.8% ( $p = 0.018$ ) and 49.18% ( $p = 0.027$ ) higher than the gel base group, respectively (Table 1).

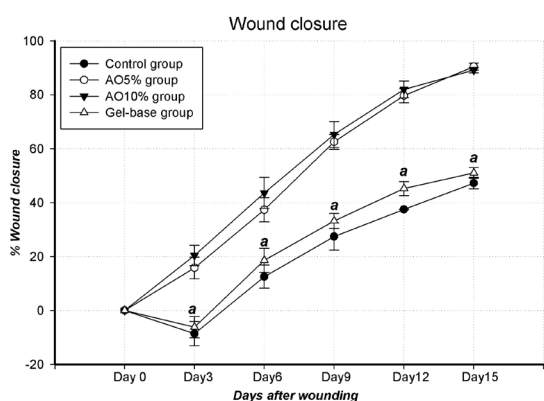


**Figure 1.** The effect of *Althaea officinalis* on the wound closure rate in rat models. The vessels (arrow) and collagen bundles were appreciated using 4 μm slices (a) while the fibroblasts (arrowheads) were counted using 20 μm slides (b). Stereological methods were used to evaluate the skin tissues.

**Table 1.** Mean (SD) of the numerical density of the fibroblasts ( $\times 10^3$  per  $\text{mm}^3$ ), volume densities of the collagen bundles ( $V_{\text{collagen/dermis}}$ ; %) and vessels ( $V_{\text{vessel/dermis}}$ ; %), length density ( $\text{mm}/\text{mm}^3$ ) and mean diameter ( $\mu\text{m}$ ) of vessels in the dermis of the wounded rats treated with 5% AO and 10% AO gels, gel-base and untreated wounded group (Control).

Groups	Fibroblasts	Collagen bundles		Vessels		Hair Follicles
	Numerical density	Volume density	Volume density	Length density	Mean diameter	Volume density
Control	225.4 (21.1)	51.2% (4.8%)	0.8% (0.5%)	27.5 (8.9)	11.8 (1.8)	3.2% (1.4%)
5% AO	477.7 (70.1)*	78.3% (5.6%)	1.4% (0.8%)	27.6 (9.8)	24.3 (10.3)*	9.1% (4.1%)*
10% AO	328.5 (63.3)*	85.1% (5.3%)*	1.3% (0.4%)	28.2 (10.2)	21.3 (4.9)*	9.9% (2.7%)*
Gel-base	220.2 (53.1)	54.2% (5.2%)	0.9% (0.7%)	17.16 (7.3)	11.5 (2.1)	2.8% (1.1%)

\*  $p < 0.05$  in comparison with control and gel-base groups.



**Figure 2.** The effect of *Althaea officinalis* (AO) on the wound closure rate in rats of the control group, gel-base treated, 5%, and 10% AO-treated groups. Each point exhibits the mean  $\pm$  SD of the twelve wounds. The "a" letter demonstrates considerable difference for the 5% AO and 10% AO treated rats compared to the control group and the base group ( $p < 0.05$ ).

### 3.3. Volume densities of the collagen bundles and hair follicles

The volume densities of the collagen bundles were significantly higher by 66.2% ( $p < 0.001$ ) and 57.1% ( $p < 0.001$ ) in 10% AO-treated group in comparison to the control and base groups, respectively (Table 1). However, the collagen bundles' volume density in the 5% AO-treated group was not significantly higher in comparison to the control and base groups. The volume densities of the hair follicles in the 5% and 10% AO-treated groups were significantly higher in comparison to the base group ( $p = 0.038$  and  $p = 0.008$ , respectively) and the control group ( $p = 0.037$  and  $p = 0.009$ , respectively) (Table 1).

### 3.4. Volume density, length density, and diameter of the vessels

The length and volume densities of the vessels in the 5% and 10% AO-treated groups were not significantly higher in comparison to the gel base and control groups. However, there are considerable differences regarding the mean diameters of the vessels between the AO-

treated groups, and the control and the gel base groups (Table 1).

Based on previous studies, we hypothesized that AO extract can be used as a treatment to accelerate the wound healing process. The results of this study revealed that hydroalcoholic gel-based AO extract has the potential to be an alternative treatment for wound healing. Our investigation demonstrated that AO extract increases the volume density of collagen fibers and the population of fibroblasts, besides it improves the process of vascularization. These results are consistent with previous reports which have mentioned various effects of the agent such as anti-inflammatory, antioxidant, and anti-microbial properties, as well as fibroblast proliferation-inducing effect (7-11). Böker *et al.* in their study showed that aqueous extract of AO has N-phenylpropenoyl-L-amino acids (NPA) which can stimulate keratinocytes and increases cellular activity of fibroblasts (12). Moreover, Benbassat *et al.* indicated that the ethanolic extract of AO has antioxidant activity that improves vascular endothelial function (10). Another study also mentioned the same property for aqueous extracts of AO, as it can stimulate tissue regeneration of epithelial cells (13). Our results highlighted that topical administration of AO extract improved fibroblast proliferation, collagen bundle synthesis, and re-vascularization in skin injuries. Based on previous studies and according to the present evaluations, it is revealed that AO improves dermal tissue reconstruction.

Our research shows that AO positively affects the processes of wound healing including angiogenesis, the proliferation of fibroblasts, and the accumulation of collagens. However, we suggest further experimental and clinical studies to evaluate the therapeutic properties of this herb and compare it with current treatments.

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**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this paper.

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