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Comparing Effects of *Arnebia euchroma* and Alpha Ointment on Wound Healing Process

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Background: Wound healing is a dynamic process with inflammatory response and oxidative reaction in the damaged area. Alpha ointment (AO) and *Arnebia euchroma* (Arnebia) are herbal medicines with antioxidant and anti-inflammatory effects that can be used as wound healing agents. AO is a commonly used ointment while Arnebia is a newly introduced one.

Objectives: We aimed to compare the effects of Arnebia with those of AO on the skin wound healing process in rats by using stereological methods.

Materials and Methods: A total of 48 female Wistar rats were randomly allocated to four groups of 12. One group was treated with AO (E1), one was treated with Arnebia gel with a concentration of 10% of herbal extract (E2), the first control group (C1) received no treatment, and the other group (C2) was treated with the vehicle gel. Treatments were performed every 24 hours for 14 days. The volume densities of the collagen bundles and vessels, vessel's length density and diameter, and fibroblast populations were estimated by using stereological methods. Mann-Whitney U test was used for statistical analysis.

Results: According to our results, the average of reduction in wound areas, volume density of collagen bundles, fibroblast populations, and length density of vessels in E1 and E2 groups were significantly higher than C1 and C2 groups. The differences between E1 and E2 were not statistically significant regarding the stereological parameters.

Conclusions: According to our results, Arnebia and AO showed similar efficacies in improving the wound healing process and tissue regeneration.

Keywords: Wound Healing; Rats; *Arnebia euchroma*; Alpha Ointment; Stereology; Plants, Medicinal

1. Background

Wound healing is a dynamic and normal biologic process involving fibroblast activation and migration, re-epithelization, proliferation of endothelial cells, and angiogenesis, which are accompanied by inflammatory response and oxidative reactions in the damaged area (1, 2). Re-epithelialization of wounds and tissue regeneration begins immediately after the injury by the release of different factors and activation of different mechanisms, which will finally lead to tissue regeneration and sometimes, scar formation (2, 3). Enhancing the involved mechanisms in the process of wound closure and reducing scar formation are the main goals of wound treatment, specifically skin wounds.

Alpha ointment (AO) is a combination of Lawson (natural Henna) and unsaturated fatty acids (4). Unsaturated fatty acids and Henna derivatives in AO have anti-inflammatory and antioxidant properties. This ointment is commonly used as a wound healing herbal medicine (5, 6). *Arnebia euchroma*, from the family of Boraginaceae, distributed in Asia and the drier regions of northern Africa (7, 8), has chemical components such as naphthoquinones, alkannins, shikonins, and their derivatives, which have widespread biologic properties including wound healing, antibacterial, antifungal, antiviral, antiamoebic, anti-inflammatory, antitumor, and anticancer effects (9-12).

2. Objectives

In this study, we aimed to compare the effects of *A. euchroma*, a newly introduced herbal agent that was seen to be effective in the process of wound healing, and those of AO on wound healing in rats as animal models by employing stereological analysis for estimating the wound closure rate as well as quantitative estimation of vascularization, collagen bundle synthesis, hair follicle production, and fibroblast proliferation.

3. Materials and Methods

3.1. Plant Material

Arnebia euchroma herb was collected (June 2011) from Sisakht City at altitude of 880 m. A voucher specimen of the plant (by Jafari MD, 151) was deposited at the herbarium of Research Center of Agriculture and Natural Sources, Yasuj University of Medical Sciences, Yasuj, Iran. Hydroalcoholic extract of leaves and roots of the plant was prepared using a method introduced by Maddocks-Jennings et al. (5). To facilitate the application of the extract, a vehicle gel was produced according to the method reported by You et al. (10). The concentrated plant extract was introduced to the gel in 10% v/v.

3.2. Animals

A total of 48 female Wistar rats (220 ± 20 g, non-fasted) were randomly allocated to four groups of 12. Under general anesthesia, we induced a 1×1 cm^2 standard full-thickness skin wound on the dorsum of each animal's neck. One group was treated with AO (E1), one group with 10% *A. euchroma* extract (E2), the control group (C1) received no treatment, and one group received carboxymethyl-cellulose (CMC) gel (vehicle) (C2). The wounds were treated every 24 hours for 14 days. All animal experiments were approved by the Animal Ethics Committee of Shiraz University of Medical Sciences and care was in accordance with guidelines of Ethics Committee of Shiraz University of Medical Sciences.

3.3. Stereological Evaluation of Healing

To determine the rate of reduction in wound area, digital photographs were captured from the wound surfaces every other day. A standard ruler was laid at the wound level to find the magnification on the computer monitor. The wound area was calculated by using a method introduced by Ashkani-Esfahani et al. (13). A full-thickness circle of 10 mm was removed from the skin of the wounds and embedded in a cylindrical paraffin block. Then 5-μm and 15-μm sections were prepared and stained with both Heidenhain's azan (for estimating collagen synthesis and vascularization) and Hematoxylin and Eosin (H and E; for estimating fibroblast populations). Microscopic analyses were done using a video-microscopy system made up of a microscope (E-200, Nikon™, Japan) connected to a video camera.

The volume densities (Vv [structure/dermis]) of collagen bundles were estimated at the final magnification of ×180 by using the reported method by Ashkani-Esfahani et al. (14). The length density of the vessels (Lv) and mean diameter of the vessels were estimated at the final magnification of ×180 by occupying a method introduced by Ashkani-Esfahani et al. (13). For these purposes, 5-μm sections were employed. The numerical densities (Nv; number of cell per volume unit) of fibroblasts were estimated by employing 15-μm sections at the magnification of × 450 on the monitor and the "optical disector" method.

3.4. Statistical Analyses

Data were collected, analyzed, and reported as mean and standard deviation (mean ± S.D). Statistical comparisons between groups were performed by using SPSS 14.0 (SPSS Inc., Chicago. IL). Kruskal-Wallis test was employed for an overall comparison between results and mean ranks of all groups and Mann-Whitney U test was used for one-on-one comparison between every two groups. $P \leq 0.05$ was considered statistically significant.

4. Results

C1 and C2 groups showed slower wound contracture in comparison with E1 and E2 ($P < 0.05$) groups. The mean of reduction in wound areas was 15.52 mm^2/d in E1, 14.07 mm^2/d in E2, and 8.51 mm^2/d in C2 groups, which were significant in comparison to the mean of 5.01 mm^2/d in C1 group ($P < 0.05$). Fibroblast proliferation rates (Nv) were higher than C1 by approximately 61% in E1 ($P < 0.05$) and by 54% in E2 groups ($P < 0.05$). There were no significant differences between E1 and E2 as well as C1 and C2 groups regarding Nv.

Vv of collagen bundles in E1 and E2 groups were similar but were significantly different from those of C1 and C2 groups ($P < 0.03$; Table 1). Vv of collagens in E1 and E2 groups were respectively 41% and 27% higher than those in C1 group were ($P < 0.05$). In addition, in comparison to C2 group, Vv of collagens in E1 and E2 groups were respectively 34% and 20% higher ($P < 0.05$). Lv in E1 was 6% higher than C, which was not significantly different ($P > 0.1$); there were no significant differences regarding the Lv, mean diameter of the vessels, and Vv among all study groups.

Table 1. Comparing Collagen Bundles in Different Groups [a,b]

Groups	Parameters				
	Nv, $\times 10^3$/mm^3	Vv_1, %	Vv_2, mm/mm^3	Lv, $\times 1000$/mm^3	Mean Diameter of Vessels, μm
C1	207.51 ± 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 [c]	79.6 ± 6.1 [c]	4.1 ± 0.6	15.21 ± 1.7	1.11 ± 0.21
E2	319.84 ± 9.7 [c]	71.8 ± 5.1 [c]	3.9 ± 0.7	14.11 ± 1.8	1.14 ± 0.31

[a] Data are presented as mean ± SD.
[b] Abbreviations: C1, untreated wounded rats group; C2, wounded rats treated with 10% vehicle gel; E1, wounded rats treated with 10% *A. euchroma* gel; E2, wounded rats treated with 10% Alpha ointment; Lv, length density; Nv, numerical densities of the fibroblasts; Vv_1, volume densities of the collagen bundles; Vv_2, volume densities of vessels.
[c] $P < 0.05$ *vs.* C1 and C2.

5. Discussion

Finding more beneficial agents to enhance and improve the wound healing process has always been a concern for the researchers. *Arnebia euchroma* has chemical components such as naphthoquinones, alkannins, shikonins, and their derivatives, which have widespread biologic properties such as wound healing, antibacterial, antifungal, antiviral, antiamoebic, anti-inflammatory, anti-tumor, and anti-cancer effects (9-12). Previous studies have reported anti-inflammatory, fibroblast proliferation, and inducing collagen synthesis as the effects of alkannins and shikonins in *A. euchroma* (15-17). Based on the study conducted by Sidhu et al., naphthoquinone derivative in *A. euchroma* could enhance wound healing (18). Moreover, the results of a study by Ashkani-Esfahani et al. showed enhancement of fibroblast Proliferation, vascularization, and collagen synthesis in the healing process of burn wounds by administration of topical *A. euchroma* in both second- and third-degree wounds (13, 14).

In an animal study conducted by Nayak et al., wound healing activity of natural henna were demonstrated on excision, incision, and dead space wound models (19). One study demonstrated the effectiveness of AO in comparison with topical silver sulfadiazine in third-degree burn wounds through evaluating wound healing, contraction, culture, and scar formation regarding pathologic parameters (20). In addition, it was reported that topical AO was more effective on the dermatitis healing induced by radiation than topical hydrocortisone cream (1%) in the second week of intervention (4). Overall, AO, as a commonly prescribed agent has been proven to have considerable positive effects on the process of wound healing. The results of the present study showed that both AO and *A. euchroma* had hastened the wound healing process by quicker reduction in wound area and enhancing the tissue regeneration by improving fibroblast proliferation, collagen bundles synthesis, and revascularization.

In conclusion, this study showed that *A. euchroma* gel has the potential to be introduced as a new herbal treatment or an alternative for today's commonly used agents such as AO; however, further studies, specifically clinical trials, are needed for evaluating and comparison of this agent with other herbal and chemical medicines.

Authors' Contributions

Study concept and design: Maryam Mohsenikia. Acquisition of data: Neda Jamalnia and Hasti Nuraei. Analysis and interpretation of data: Alireza Moradi and Fatemeh Karimi. Drafting the manuscript: Soheil Ashkani Esfahani. Critical revision of the manuscript for important intellectual content: Soheil Ashkani Esfahani and Maryam Mohsenikia. Statistical analysis: Shima Rafiee. Administrative, technical, and material support: Hasti Nuraei. Study supervision: Zahra Azizian.

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