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The effects of *Arnebia euchroma* ointment on second-degree burn wounds: a randomized clinical trial

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ABSTRACT

Ethnobotanical relevance: Burn injuries can cause detrimental long-term consequences and call for immediate management. Avicenna's Canon of Medicine, describing the use of Abu-Khalsa (*Arnebia euchroma)* (AE) as being effective for burn healing. The purpose of this study was to evaluate the healing effects of AE ointment (AEO) on patients with a second-degree burn wound and compare its results with silver sulfadiazine cream (SSD).

Materials and methods: In this prospective, single-blind clinical trial, 45 patients with similar types of second-degree burns at two different sites of the body were randomly assigned to the two treatment groups. One burn wound site of the patient was treated with SSD and another similar burn wound site with AEO once a day until complete healing was achieved. Wound size and percentage of wound healing were evaluated at 15 days. Satisfaction, clinical adverse events such as pain, burning, warming, erythema, edema, infection, inflammation, and general wound area were assessed on a visual analogue scales, and 6-point scales.

Results: The healing time was significantly shorter in the site treated with AEO than SSD (13.9 ± 5.3 vs. 17.5 ± 6.9 days, respectively). The severity of pain and burning were reduced in the AEO site compared with SSD site at the time of dressing change, while the warming score was significantly higher in the AEO wound area. Side-effects were lower in the site treated with AEO.

Conclusion: In this clinical study, we demonstrated that AEO has benefits over SSD in the treatment of second-degree burn wounds and wound healing and is a viable medication for the management of second-degree burns.

1. Introduction

Burn injuries are among the most distressing of all injuries, with many physical, mental, and serious complications if not treated at the right time (Forjuoh, 2006; Kaushik et al., 2013). Burns can be life threatening and have varying morbidity and mortality across countries. In developing nations, burn injuries constitute the top ten causes of mortality and a main cause of disability. In the East Mediterranean Region (EMR) countries, burn injuries cause a significant health problem especially in the low-income and middle-income countries of the EMR. Data from the World Health Organization reveal fire-related unintentional burns to be highest in South-East Asia followed by the EMR countries (Fazeli et al., 2014; Othman and Kendrick, 2010; Seo et al., 2015). Over 90% of fatal fire-related burns occur in developing or low and middle income countries such as Iran (Atiyeh et al., 2009; Othman and Kendrick, 2010; Sadeghi-Bazargani and Mohammadi, 2012). Most minor burns can be managed on an outpatient emergency basis at the nearest medical center where they are first evaluated, however major burns require careful evaluation including assessment of other associated injuries (Genuino et al., 2014; Taghavi et al., 2010). The healing of a burn wound is a multistage process primarily involving three phases: inflammatory, proliferative, and remodeling. Initial vasodilation and edema is followed by collagen and fibroblast proliferation and neovascularization. The final remodeling phase includes re-epithelization, wound contraction, and scar formation (Hemmati et al., 2015; Kim et al., 2009; Rowan

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et al., 2015). Burns are classified according to their degree to estimate treatment and prognosis. First-degree burns do not produce blisters, whereas second-degree burns cause pain, bleeding, and blisters. Second-degree burns can be superficial or deep and take more than 2 weeks to heal (Alharbi et al., 2012; Rowan et al., 2015).

Burn wounds are increasingly susceptible to bacterial infections. As a consequence, *anti*biotics and topical antimicrobial agents including silver sulfadiazine (SSD), silver nitrate, mafenide acetate, bacitracin, and mupirocin are employed to prevent burn wound infections and promote healing (Hosseinimehr et al., 2010; Kaushik et al., 2013). SSD is widely used topically and many burn dressings contain SSD. It has been used for more than fifty years but its use requires frequent dressing changes and can cause unwanted side-effects: delay in re-epithelialization, allergic reactions, transient leukopenia, neutropenia, erythema multiforme, crystalluria, and methemoglobinemia (Ahuja and Chatterjee, 2014; Fuller, 2009; Kaushik et al., 2013). In addition, mafenide acetate cream, the other common topical agent, presents with disadvantages including painful application, metabolic acidosis, and delay in re-epithelialization (Kaushik et al., 2013).

Some herbal plants having burn wound healing properties have been described in Chinese, Indian, and Iranian traditional medicine and have been scientifically evaluated (Aliasl and Khoshzaban, 2013; Avicenna, 2011; Nasiri et al., 2014; Pirbalouti et al., 2012; Rezaeizadeh et al., 2009). This has led to various products that promote wound healing and are being used worldwide (Ashkani-Esfahani et al., 2012). Recently, *Arnebia euchroma* (Royle) I. M. Johnst. from the Boraginaceae family, a traditionally used herbal plant in Iran and other countries for various skin disorders has been assessed by scientists in burn wound healing in experimental animal models (Aliasl and Khoshzaban, 2013; Arora et al., 2012; Ashkani-Esfahani et al., 2012; Kaith et al., 1996; Nasiri et al., 2015; Pirbalouti et al., 2009; Singh et al., 2003). These studies have reported that application of *A. euchroma* extracts has significant positive outcomes in the healing of second-degree burn wounds than SSD in animal models. Additionally, most of these studies note that there are no side-effects with *A. euchroma* treatment. *A. euchroma* grows in several alpine regions of the world including North Africa, Himalaya, and several other parts of Asia. The antibacterial, anti-inflammatory and wound healing properties are due to naphthoquinone derivatives (Aliasl and Khoshzaban, 2013; Arora et al., 2012; Ashkani-Esfahani et al., 2012; Kaith et al., 1996; Nasiri et al., 2015; Pirbalouti et al., 2009; Singh et al., 2003). These recent findings are in agreement with Avicenna's Canon of Medicine, written over six centuries ago, describing the use of Abu-Khalsa (*Arnebia euchroma)* as being effective for burn healing (Aghili, 2008; Avicenna, 2011; Momen-Tonkaboni, 2008). However, clinical evidence is needed to establish the effective use of this agent as a therapeutic option to promote wound healing in patients with burn injuries.

The purpose of this study was to determine the healing effects of *A. euchroma* ointment (AEO) on second-degree burn wounds in patients and compare the results with silver sulfadiazine cream (SSD).

2. Material and methods

2.1. Preparation of Arnebia euchroma ointment (AEO)

Arnebia euchroma herb and roots were bought from the local herbal market in Sari, north Iran, during September 2014. This whole herb was identified by Professor Mohammad Azadbakht and Dr. Masoud Azadbakht, and was kept at the herbarium of Faculty of Pharmacy, Mazandaran University of Medical Sciences, under the herbarium number: 1001. The whole roots of *A. euchroma* were washed and dried in an air oven at 40 °C for 8 h. The AEO was made according to an Iranian traditional medicine formula used in previous studies. We used following formula with slightly modification (Aghili, 2008; Aliasl et al., 2014; Avicenna, 2011; Momen-Tonkaboni, 2008; Nasiri et al., 2015). The components of AEO 10% were made from *A. euchroma roots* 10 g, goat fat 20 g, cow butter 15 g glycerin 20 g, and eucerin 35 g. Weight ratio of the *Arnebia euchroma* ointment was 10% of primary materials. Within the container, the dried roots of *A. euchroma* were chopped and heated in goat fat, cow butter, and glycerin at 95–100 °C for 30 min Then the container of samples was autoclaved for 18 min at 121 °C. The composition sample was filtered and mixed with eucerin, methyl paraben 0.025 g, and propylene paraben 0.015 g and levigated for 5 min to form a homogenous ointment. The product was filled in the sterile tubes 50 g and placed in a clean environment. In order to evaluate the consistency and stability of the ointment formulation, they were stored in 4, 25 and 40 °C for 2 weeks. This formulation with the weight ratio of AEO (1:2:1.5: 2: 3.5) was prepared as described before (Aliasl et al., 2014; Nasiri et al., 2015). To check the microbial quality of this product, examination of microbial contamination level of AEO was performed according to the United States Pharmacopeia *(USP)* Monograph using Soybean-Casein Digest Medium (SCDM) culture environment.

2.2. Patients

This was a prospective, randomized, single-blind, clinical trial performed after obtaining approval from the Ethical committee at Mazandaran University of Medical Sciences, (protocol number: 118–92, 11–03-2013). The study was registered at the site of Iranian Registry of Clinical Trials (IRCT), http://www.irct.ir/user.php (IRCT ID: IRCT201412154492N2). This study was carried out at the Burn division of Zare' s hospital, which is referral center of burn injuries in the Mazandaran province and north of Iran. In addition, before starting the study, patients signed a written informed consent form according to the Helsinki Declaration and Iranian research ethical code.

All potentially eligible burn patients were invited to participate of the study. Patients were eligible for entry into the study if they were the ages of 16–65 years, admitted to the Burn emergency ward of Zare hospital, diagnosed by the same expert emergency burn physician based on the presentation of two same sites of second-degree burns. The other including criteria were as follows: the burn should have occurred within 24 h before the beginning of treatment, second-degree burn on two sides of the same patient's body, and with a less than 15% total burn surface area. Patients with epilepsy, diabetes, immunodeficiency disease, electrical and chemical burns, known allergy and sensitivity to either AEO or SSD, or pregnant women were excluded from the study. The site of burn for each participant was randomly assigned to either *A. euchroma* treatment or SSD control group. The label “A” denoted right-sided site of the body and “B” represented left-sided site of body. These areas were randomly assigned to AEO treatment and the opposite site was treated with conventional treatment with SSD cream. A simple coin-based randomization was performed for each patient after enrollment by the blinded staff nurse. This allocation continued until complete wound healing.

2.3. Burn assessments and care

After admission and primary preparation, the wounds were washed with normal saline or sterile water and dried with sterile gases. The general condition of the wound areas were first observed and evaluated by the expert emergency burn physician and the Burn unit special nurse prior to utilization of topical agents.

Thereafter, before each dressing, the wounds were assessed by same team who were unaware of the assigned treatment to each side and the ointment applied on the wounds for treatment.

General Wound Appearance (GWA) was determined by the following 4-point scale: 0=poor, 1=fair, 2=good, 3=very good (32). Every day, instructions were given to the patients regarding the wound protection, dressing, bandage, and nutrition. Once the study patients were discharged from the hospital, they came back only for dressing and inspection of their wounds. The study drugs, AEO 10% and SSD 1%, were used to opposite sides of the body until complete wound healing had taken place as decided by the special nurse who had no role in the evaluation of the treatment process. The participant's wounds were evaluated on day 1, 3, 5, 7, 10, 13, 15, 20, 25, and 30 days of the burn injury and to take wound area measurements and digital photographs. The evaluator was blinded to the kind of treatment. The surfaces of the wound areas were calculated in square centimeters and compared in the two groups. This process was followed until re-epithelialization of wounds occurred for the two sides in each patient. The percentage of wound contraction and healing times were recorded. The following formula was used for wound closure:

Wound closure (%)=([initial area of wound – Nth day (day of evaluation) area of wound]/initial area of wound) × 100(1).

All patients received daily dressing and application of topical agents on a once daily basis. All study patients were similarly supplied with routine drugs such as oral antibiotics and analgesic drugs. Epithelialization period was defined as the time in days required for falling of eschar without any residual raw wound, and wound healing was defined as complete epithelial covering as observed by an expert emergency burn physician.

2.4. Complications assessment

The treatment complications of erythema, edema, infection, inflammation, and general wound appearance were evaluated on day 3, 5, 7, 10, 13, 15, 20, 25, and 30 days after treatment. These were assessed using the following 4-point scales: 0=absent, 1=mild, 2=moderate, and 3=severe. Signs of clinical infection were specifically evaluated further with a 6-point scale (0=no sign, 1–5=(warmth and redness, tenderness after sensation, swelling and erythema or inflammation, fever, and pus (1 point for each component). Increasing points related to increasing severity. Risk of clinical infection or inflammation was based on the Infection scale: (0= no sign, 1–5=(warmth, tenderness, swelling, fever, and pus). This risk was assessed by the blind expert nurse above the components during dressing.

Itching was recorded by the patient being queried in the two sites of wound treatment with a 0–10 Visual Analogue Scale Itching (VAS-I) as itching (0=none, 10=worst possible condition) after one and 2 weeks of procedure. The itch severity was analyzed by using a 6-points grading scales: 0=none, 1–2=mild =1, 3–4=moderate=2, 5–6=severe=3, 7–8=very severe=4, 9–10=worst condition=5 (Aliasl et al., 2014; Sarnoff, 2001).

The VAS was employed to measure the pain, burning, and warmth experienced during the first 15 min of topical application and dressing changes. These scores were defined from 0 (no problem) to 10 (extreme problem). These components were recorded by the patient being questioned in the two sites of the treated wound in 1, 5, and 15 min after the use of topical agents on the both wounds. In addition, these scores were analyzed by using the 6-points grading scales: 0=none, 1–2=mild=1, 3–4=moderate=2, 5–6=severe=3, 7–8=very severe=4, 9–10=worst possible condition=5.

The objective dermatological abnormal reactions of application agents on the site of the wounds such as purulent exudates, inflammation, indurations, infection, and other events were evaluated by the blinded emergency burn physician, expert nurse, and patients and recorded in data forms provided by the study investigators.

Post-procedure patient satisfaction was evaluated by using the visual analogue satisfaction scale (VAS-S), which was scored as 0 (not satisfied), to 10 (very satisfied). Satisfaction was defined as pain-free dressing removal, pain and burning during night, sleeping, and general condition process about wounds and trend of treatment for each site. Further, the quality of satisfaction was evaluated with a 6-point scale (0=not satisfied, 1=slightly satisfied, 2=moderately satisfied, 3=satisfied or good, 4=very satisfied or very good and, 5=excellent) (Aliasl et al., 2014; Brown et al., 2014; Sarnoff, 2001; Singh et al., 2003).

Two months after wound repair, participants were contacted by telephone and asked about the wound healing progress and their overall satisfaction.

2.5. HPLC analysis

A 35 g of dried and powdered root of *A. euchroma* was extracted by refluxing in 350 mL of chloroform for 2 h. The chloroform solution was filtered and evaporated under reduced pressure at 40 °C to give a dry residue. Identification and quantification of juglone as a naphthoquinone derivative in *A. euchroma* was carried out by a Knauer Smartline HPLC consisting of a pump 1000 and solvent delivery system equipped with a sampler injector and a photodiode array detector model SmartLine DAD 2800 (all from Knauer Assoc., Germany) with ChromGate software (Version 3.1.7). Analysis was performed using an ODS-C18 column (250 × 4.6 mm i.d., 5 μm particle size, MZ-analysentechnik GmbH, Germany), and the corresponding guard column (5 × 4.6 mm i.d., 5 μm particle size). The mobile phase was phosphate buffer-acetonitrile (50:50 v/v) at pH 4. The mobile phase flow rate was 1.0 mL/min, and all the measurements were done at ambient temperature. Quantification of juglone in *A. euchroma* was done using an external standard method. Different concentrations of standard juglone (Merck, Germany) were prepared to plot the calibration curve.

2.6. Statistical analysis

Calculation of the sample size was based on the decrease of healing days according to previous same studies. In a previous experimental study, the estimated decrease of healing time of a second-degree burn from natural products such as AEO compared to SSD was 2–8 days (Khorasani et al., 2009; Nasiri et al., 2015). Assuming that the *A. euchroma* topical application reduces the wound healing time by 3 days with an (Alfa) of 0.05, 44 patients would be required in each treatment group (assuming a power of study 0.80). Anticipating a study drop-out rate of 15%, we included 51 patients per group. All analysis were performed with SPSS for windows (ver. 15.0 SPSS, Chicago. IL., USA). All values were expressed as number of patients (%), mean and standard deviation. Data were analyzed for normally distributed continuous variables using a 2-tailed paired *t* test and for non-normally distributed continuous variables using the Wilcoxon rank test. P-values < 0.05 were considered statistically significant. The base of the analysis was performed on the “intention-to-treat” principle, where included participants were randomized according to the treatment they were allocated.

3. Results

3.1. Characteristics of participants

Between November 22, 2014 and March 10, 2015, a total of 51

eligible patients were registered. Forty-nine of them signed the consent form and were randomly allocated sequentially to the two sides and two treatment groups. Four patients were lost to follow-up. Therefore, 45 patient's results were eligible for data analysis (Fig. 1). In addition, 1 patient in both groups needed bilateral skin graft on the day of 11th according to the plastic surgeon's decision. Furthermore, 2 patients in the SSD group needed skin graft from days 11–14, but their treatment area on the opposite area with AEO healed after 5 and 7 days, respectively.

The average age of the patients were 39.9 ± 15.6 years, of which mostly were women. Most of the burns in the participants were caused by hot liquids such as boiling water; 30 (66.7%), direct flame; 14 (31.1%), and contact burn; 1 (2.2%), respectively. The percentage of the surface wound area was 3.7 ± 2.4, (range: 1–13%). The mean weight of participants was 70.5 ± 11.4 kg (range: 55–105 kg). More than 44% of the burns involved the lower limbs (Table 1).

Table 1.
Comparison of burned on part of body between *Arnebia euchroma* ointment (AEO) and SSD.

Site of body/Groups	SSD Number (%)	AEO Number (%)
Upper limbs	15 (33.3%)	17 (37.8%)
Lower limbs	21(46.7%)	20 (44.4%)
Anterior(abdomen)	5 (11.1%)	4 (8.9%)
Posterior (back part)	4 (8.9%)	4 (8.9%)

3.2. Condition of wound size and healing time

The results showed no significant differences in the wound area between SSD and AEO groups during the 1, 3, and 5 days after treatment (Table 2).

Time to re-epithelialization (healing time) ranged from 7 to 29 days in the AEO group and 8–36 days in the SSD group. The average time to re-epithelialization was shorter in the herbal AEO group by more than 3.6 days compared to the conventional SSD group (Table 3).

After 13 days of treatment, approximately 24 (53%) of participants were healed in the AEO group, while 13 (28.9%) of SSD group were cured. Table 4 shows the difference rate of re-epithelialization time through each day in this study.

Table 2.
Mean burn wound area (cm^2) of the 45 patients treated with *Arnebia euchroma* ointments (AEO) and silver sulfadiazine (SSD) during study.

Day/mean ± SD	AEO (Cm^2)	SSD (cm^2)	P value
1	55.7 ± 35.7	54 ± 31.7	0.408
3	50.8 ± 32.8	50.9 ± 31.4	0934
5	38.5 ± 28.3	41.2 ± 27.9	0.086
7	24.5 ± 22.7	30.4 ± 23.9	0.002
10	13.7 ± 18	19.4 ± 20.5	0.001
13	7.5 ± 10	14.3 ± 17	0.001
15	4 ± 7	9.9 ± 15.7	0.012
20[a]	1.3 ± 3.6	6.9 ± 16.2	0.011
25[a]	0.7 ± 2	6.2 ± 19.2	0.043
30[a]	0 ± 0	0.71 ± 1.8	0.157

[a] Kolmogorov-simirnov Z was shown that wound area had not normal distribution within 20, 25, and 30 days after treatment. Therefore, The Wilcoxon rank test was done. AEO=Arnebia euchroma ointment, SSD=Silver sulfadiazine.

3.3. Quality of wound healing

The experienced emergency physician before redressing, without knowing how the bilateral wounds were treated,

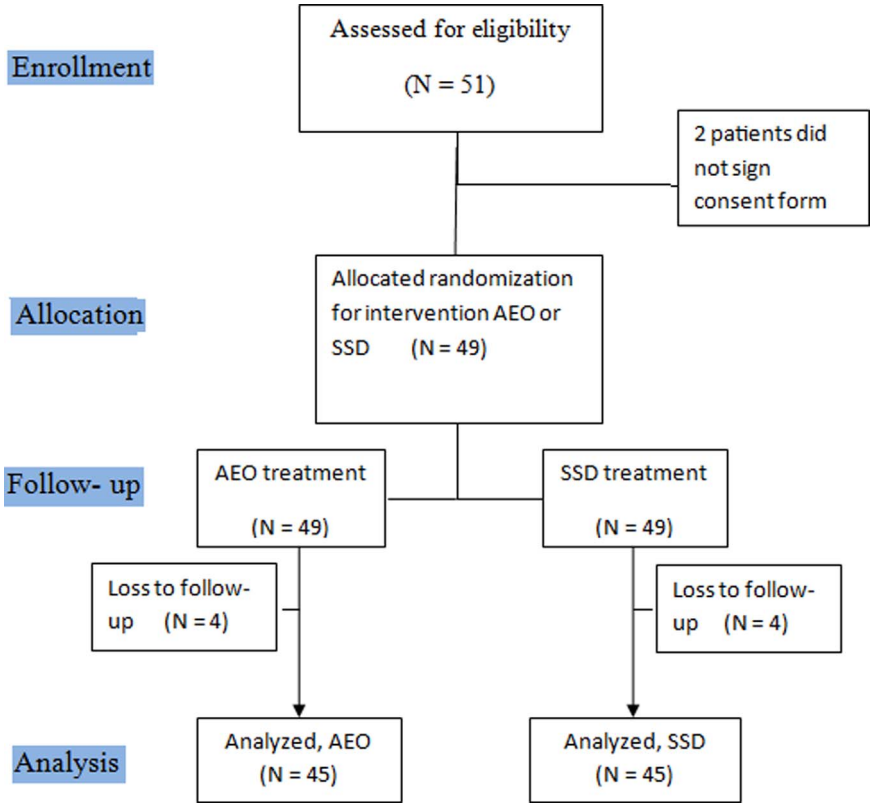

Fig. 1. CONSORT diagram, including the number of patients who started and continued trial treatment, and stopped. (AEO=*Arnebia euchroma* ointment, SSD=Silver sulfadiazine).

Table 3.
Comparison of the mean healing time between AEO and SSD groups.

Healing time (day)	Mean ± SD	95% Confidence Interval (CI)	Range (day)
AEO (N=45)	13.9 ± 5.3	12.3–15.5	7–29
SSD (N=45)	17.5 ± 6.9	15.4–19.6	8–36
P value	0.001		

Table 4.
Difference rate of re-epithelialization and healing time in the *Arnebia euchroma* ointment (AEO) and silver sulfadiazine (SSD) groups in 45 participants.

Healing time (day)	Number of patients healing (%) in AEO group	Number of patients healing (%) in SSD group	Difference rate (%)
7th day	3(6.7%)	0	3(6.7%)
10th day	14(31.1%)	8(17.8%)	6(13.3%)
13th day	24(53.4%)	13(28.9%)	11(24.5%)
15th day	29(64.4%)	24(53.3%)	5(11.1%)
20th day	41(91.1%)	35(77.8%)	6(13.3%)
25th day	42(95.5%)	38(84.4%)	4(11.1%)
30th day	45(100%)	43(95.6%)	2(4.4%)

evaluated the treatment progress. General wound healing during 5, 10, 15, 20, and 25 days after treatment according to the physician opinion is illustrated in Fig. 2.

In addition, AEO treatment resulted in better GWA scores than SSD treatment on days 7, 10, and the following days by Wilcoxon Signed Ranks Test ($p < 0.001$), while, this was not significantly different between the AEO site and the SSD treatment sites on day1, 3, and 5 days after treatment (Fig. 3).

3.4. Complications

The study findings show that complications such as pain, and burning were different between the two treatment sites with AEO and SSD. Burning score was assessed in the early first 15 min after dressing change and three nights after the burn injury. Burning scores of the wound area related to AEO treatment was better than SSD wound treatment at home during 1, 3, and 5 days after injury during nights (Fig. 4).

The itching scores (VAS-I) of burn wound environments show no significant difference at the end of first and second weeks between two treatments. More patients in the AEO treatment site 40 (89%) had no itching during the first week period compared to the SSD site treatment 38 (84.5%). In the second AEO treatment week,

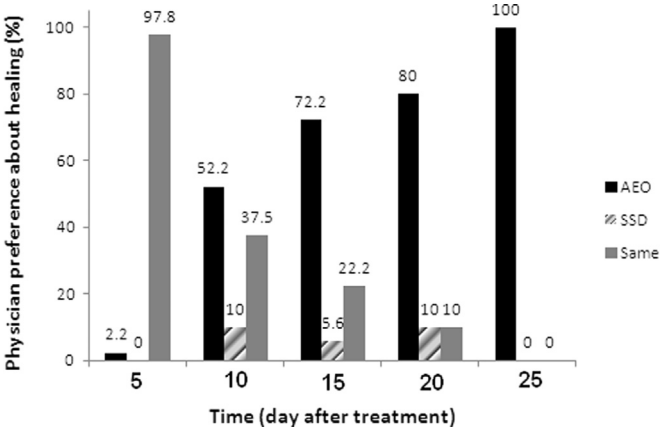

Fig. 2. Physician preference about healing condition sites on 5, 10, 15, 20, and 25 days after treatment. AEO=*Arnebia euchroma* ointment, SSD=silver sulfadiazine, Same=the wound sites condition were equal according to expert blinded burn physician.

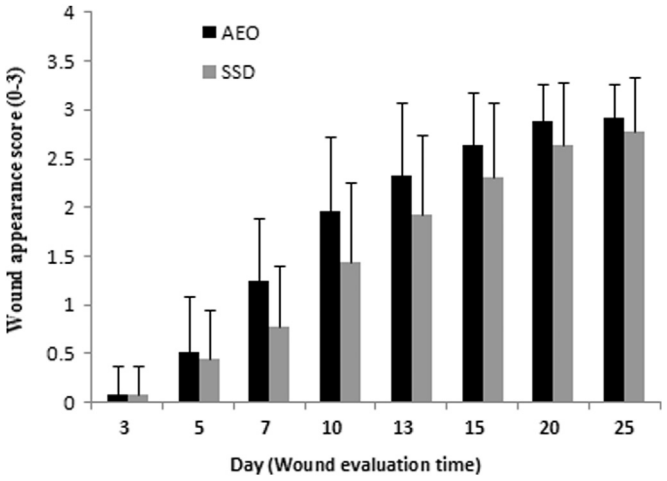

Fig. 3. Mean general wound appearance score according to experience nurse evaluated: 0=poor, 1=fair, 2=good, 3=very good. AEO=Arnebia euchroma ointment, SSD=Silver sulfadiazine on 3, 5, 7, 10, 13, 15, 20, and 25th day after treatment.

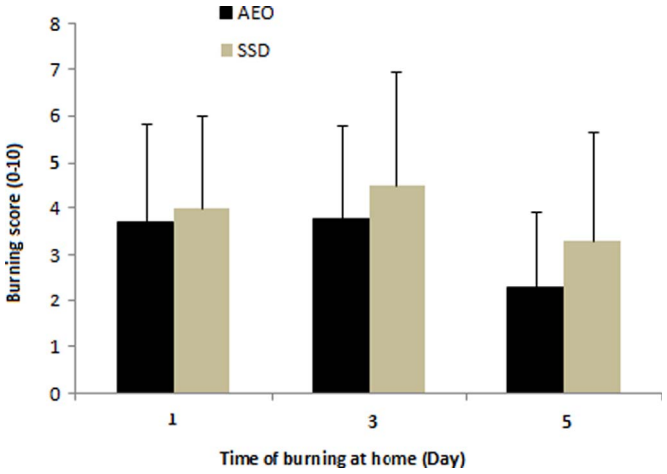

Fig. 4. Comparison of burning score between AEO and SSD sites at home on the1, 3, and 5 days after burn injury during night.

15 (33.3%) patients had mild itching, 8 (17.8%) patients had moderate itching, 1 (2.2%) patient had very severe itching, and 1 (2.2%) patient had the worst possible itching. On the other hand, patients treated with SSD in the second week, 14 (31.3%) patients had mild itching, 7 (15.6%) patients had moderate itching, 1 (2.2%) patient had severe itching, 1 (2.2%) patient had very severe itching, and 2 (4.4%) patients had worst possible itching. Burning pain and warming after application of AEO and SSD on burn wounds were different between the two sides at 1, 3, 5, and 10 days post-burn injury during 1, 5, and 15 min after application of the agents and dressing (Fig. 5).

The warming scores of the wound area treatment with AEO were significantly higher than the wound treated with the SSD cream (Fig. 5(C)). Severity of clinical infection signs was higher in the SSD sites as compared with AEO-treated wound areas (Table 5).

No local allergic reaction was observed on both wound areas related to herbal AEO and SSD treatments. The risk of skin graft according to esthetic and plastic surgeon examination after 6–10 days topical treatment for AEO site was 2.2%, 95% CI (2.2–6.7) compared to the SSD site of 6.7%, 95% CI (0.9–14.3). Risk difference of need to skin graft was 4.5% and relative risk for AEO was 0.33.

Risk of clinical infection sings or inflammation based on Infection scale: (0=no any sign, 1–5=(warmth, tenderness, swelling,

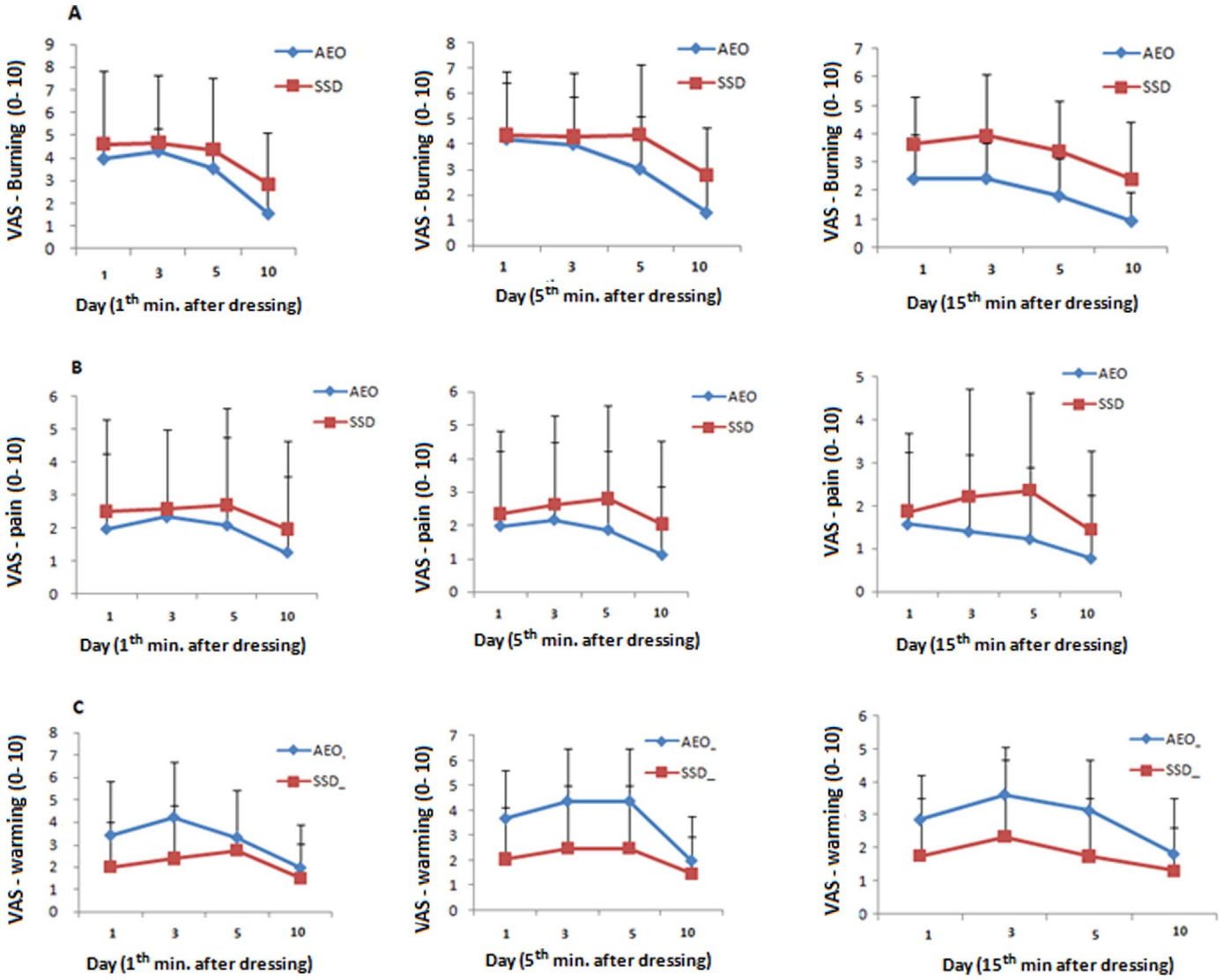

Fig. 5. Burning (A), pain(B), and warming (C) after and application AEO and SSD on wound 1, 5, 15 min after dressing change on 1, 3, 5 and 10 days post burn treatment. (AEO=*Arnebia euchroma* ointment, SSD= Silver sulfadiazine). VAS = Visual analogue scale (0–10), warming, pain and burning were different between groups, $p < 0.05$).

Table 5.
Comparison of infection score [(0=no infection sign, 5= worst infection) between AEO and SSD treatment on burn wound.

Score (-05)	Infection sign AEO; No (%)	Infection sign SSD; No (%)
0	37 (82.2%)	31 (69%)
1	7 (15.6%)	11 (24.4%)
2	1 (2.2%)	2 (4.4%)
3	0	1 (2.2%)

Table 6.
Comparison of the quality patient satisfaction (0=no satisfy, 5=excellent satisfy) between AEO and SSD treatment on burn wound process.

Satisfaction scale (0-5)	(AEO) No (%)	(SSD) No (%)
2 (moderate)	1 (2.2%)	4 (8.9%)
3 (Good)	14 (31.1%)	32 (71.1%)
4 (Very good)	23 (51.1%)	8 (17.8%)
5 (Excellent)	7 (15.6%)	1 (2.2%)

fever, and pus) were evaluated (Table 5).

The average score patient satisfaction related to the site of AEO treatment was 7.2 ± 1.8, while for the SSD site treatment, it was 5.3 ± 1.7 ($p < 0.001$).

The Quality of Satisfaction grades according to Satisfaction scale (VAS-S) for many patients was very satisfied or excellent in the AEO site, while for SSD site was satisfied or moderate satisfied (Table 6).

A typical example of a second-degree burn healed with topical AEO and SSD sites is shown in Fig. 6. This wound corresponds to a 65 year old female patient with a flame-related burn injury admitted to the Burn ward less than 2 h after her injury. The comparative process of healing on the two treatment sites is also depicted in the figure.

In this study, an isocratic elution of phosphate buffer-acetonitrile (50:50 v/v) at pH 4 was used to achieve complete separation of juglone in the extract. This naphthoquinone derivative had a typical retention time of 12.5–13 min Purity of juglone peak in HPLC chromatogram was confirmed with photodiode array detector. Juglone was identified in the chromatogram of *A. euchroma* by comparing the retention time and UV spectra with this of the standard (Fig. 7). The extract was standardized based on juglone by HPLC method. The calibration curve of juglone was linear over the range 0.01–0.5 mg/mL with a correlation coefficient of 0.994. The juglone content of *A. euchroma* was 2.8 ± 0.05 μg/ mg of extract powder.

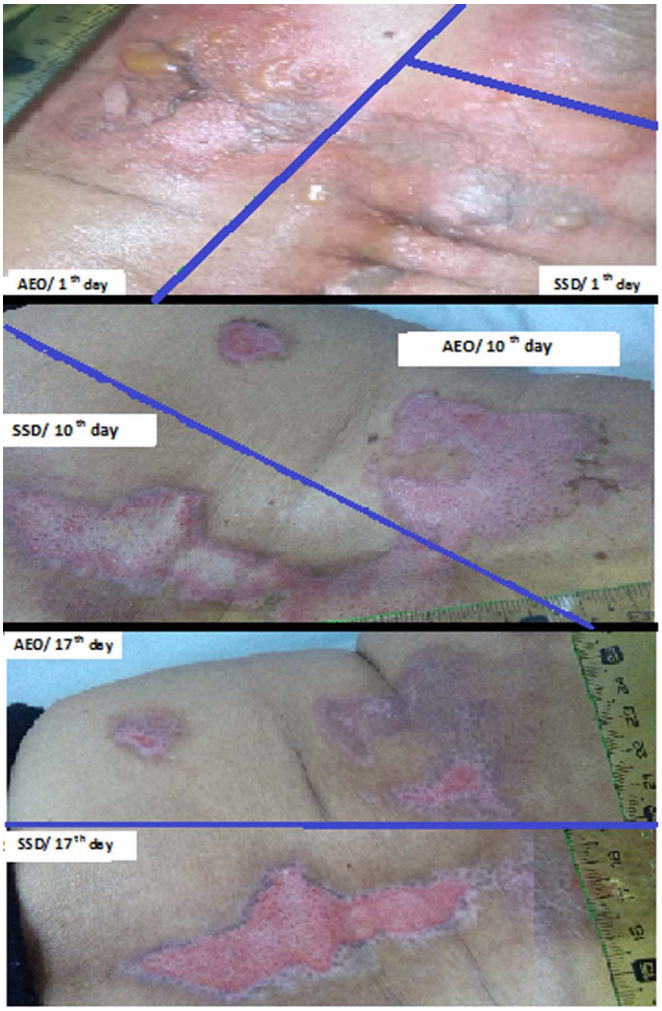

Fig. 6. Comparison of the wound healing condition between two sites treatment with *Arnebia euchroma* ointment (AEO) and silver sulfadiazine (SSD), female 65 years old that her back burned, the process of healing on 1, 10, and 17 days after treatment.

4. Discussion

This prospective, randomized, single-blind study investigated the wound healing effects of AEO on second-degree burns as compared with SSD cream treatment. AEO significantly decreased the healing time of burn wound as compared SSD (13.9 ± 5.3 days vs 17.5 ± 6.9 days, respectively) and increased the patient satisfaction scores in AEO as compared with the SSD cream (mean score: 7.2 ± 1.8 vs 5.3 ± 1.7, respectively). Compared with sites receiving SSD, wound sites which received the AEO had better wound healing, shorter healing time, and a clinically significant decrease in pain scores as assessed using visual analogue scales, with lower reported adverse effects as assessed by an expert Burns nurse and physician in the Burn emergency department as well as in the self-reported forms by the patients during management of wound care until complete re-epithelialization. Treatment interventions for faster healing times require shorter hospital stays. In our study, the average time to re-epithelialization was shorter in the herbal AEO group by more than 3.6 days compared to the SSD-treated group. According to Brown et al., treatment intervention is clinically important even if healing can take place two days earlier (Brown et al., 2014). Hence, it may be concluded that the more than three days difference between the treatment groups for second-degree burns becomes highly clinically important, when infection, scarring, and cost may be eliminated. This finding in the current human clinical trial is in line with previous animal model studies that have demonstrated *A. euchroma* to be effective for burn wound healing in rats (Aliasl et al., 2014; Ashkani-Esfahani et al., 2012; Nasiri et al., 2015).

Active constituents in *A. euchroma* roots have been determined in previous studies. Naphthoquinone derivaties such as alkannin, alkannan, shikonins, and arnebin-2 are the components of the *Arnebia* roots from Boraginaceae family. Previous studies revealed that *A. euchroma* was rich in naphthoquinones, hydroxynaphthoquinone, phenolic acids and alkaloids (Ashkani-Esfahani et al., 2012; Papageorgiou et al., 2008). Pharmaceutical formulations with wound healing properties based on naphthoquinone derivaties have been reported for many years. Naphthoquinine derivatives from *A. euchroma* had anti-inflammatory and wound healing properties(Papageorgiou et al., 2008; Pirbalouti et al., 2012; Xiao et al., 2011). Shikonin, as a 1,4-naphthoquinone derivative, of *Arnebia euchroma* was found to be active against methicillin resistant *Staphylococcus aureus* and Enterococci (Shen et al., 2002). It has been used for ulcer and skin diseases in traditional medicine for centuries (Aliasl et al., 2014). In this study we showed *A. euchroma* extract is containing juglone as a 5-hydroxy-1,4-naphthoquinone derivative that was confirmed with HPLC analysis. There are several animal reports providing pharmacological and histopathological evidence of AEO benefits with varying mechanisms that contribute to wound healing, anti-inflammatory and antibacterial effects such as diminishing tissue edema, secretion, erythema, improved matrix of collagen formation, extent of granulation, neovascularization, fibroblast proliferation, and degree of inflammation(Ashkani-Esfahani et al., 2012; Henry and Garner, 2003; Kaith et al., 1996; Kaushik et al., 2013; Kosger et al., 2009; Nasiri et al., 2015). *A. euchroma* preparations have many favorable and biological effects including antitumor, antidiabetes, antivirus(Shen et al., 2002; Xiao et al., 2011). anti-inflammation, antibacterial, wound healing(Ahmadian-Attari et al., 2009; Kosger et al., 2009; Pirbalouti et al., 2009) and pain relief in osteoarthritis (Soltanian et al., 2010). The antimicrobial effect is related to its constituents including naphthoquinone derivatives. This antimicrobial effect could possibly contribute to the reduction of pain and promotion of wound healing by *A. euchroma* (Ashkani-Esfahani et al., 2012; Damianakos et al., 2012; Kaith et al., 1996; Singh et al., 2003). Recently, a study reported that AEO was not effective on post-laser resurfacing wound healing in patients with atrophic facial acne scars(Aliasl et al., 2014). The authors concluded that post-laser wounds differed from other wounds and the components of AEO could create dryness impairing wound healing. However, the application of AEO resulted in increased dermal fibroblast formation and re-epithelialization during the early stages of post-laser wound repair. Furthermore, rats with experimental second-degree burns demonstrated that *A. euchroma* increased the collagen content of the granulation tissue promoting collagen synthesis or increased the proliferation of the fibroblast synthesis of collagen, or both, aiding wound healing (Nasiri et al., 2015).

In this study, we showed that AEO significantly increased the warming in the wound area as compared with SSD, suggesting increased angiogenesis thereby enhancing cell proliferation and migration for efficient wound repair (Cho et al., 2006). Wound healing involves biological processes such as inflammation and granulation tissue formation, a process that normally heals within 2 months (Papageorgiou et al., 2008). This process is performed through different stages, including inflammatory, proliferative, and a remodeling phase. During the inflammatory phase, homeostasis takes place and immune cells, neutrophils, macrophages, and platelets are activated with the release of inflammatory mediators, cytokines, and growth factors. The protective layer created by the fibroblasts, secrete growth factors throughout the

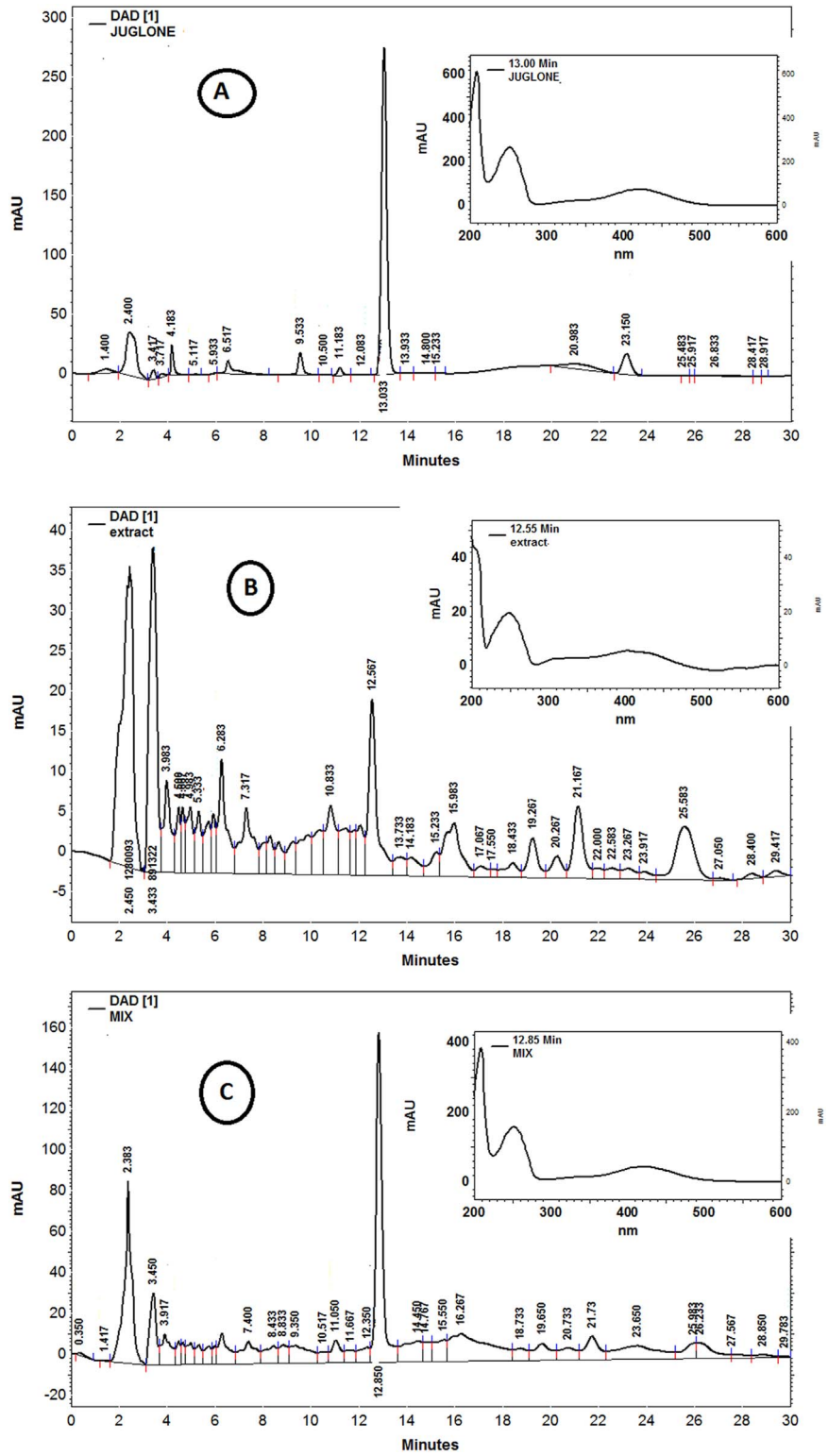

Fig. 7. HPLC profiles of juglone and the chloroform extract of *A. euchroma*. A: HPLC chromatogram of standard juglone (retention time: 13 min), and its UV spectrum, B: HPLC chromatogram of *A. euchroma* extract (retention time: 12.6 min), and UV spectrum of peak with retention time at 12.6 min, C: a mixture of *A. euchroma* extract and standard juglone, and UV spectrum of a single peak retention time at 12.8 min.

proliferative phase. Finally, cell proliferation and scar degradation by proteases is continued in the remodeling phase (Khorasani et al., 2009; Kim et al., 2009; Nasiri et al., 2015).

The need for a skin graft after 6–10 days of topical treatment was lower in the AEO-treated sites than SSD, thus minimizing the need for a surgical procedure and the associated risk for anesthetic complications, and costs.

Our data suggest that AEO application improves repair of second-degree wound areas and may protect against infection, cell damage, and increase wound area circulation. SSD cream as an anti-bacterial agent is still one of the most commonly prescribed topical agent for burn injuries, and liable for many side-effects

mentioned earlier (Khorasani et al., 2009; Nasiri et al., 2015). The opinion of the blinded burn expert physician and staff nurse demonstrated that the AEO-treated site healed better and faster than the SSD-treated areas. These findings may be attributed to the various effects of AEO on second-degree burn wound healing. Angiogenesis and optimal circulation in the first few days are essential for wound healing in the proliferative phase. Naphthoquinone derivatives are able to directly and significantly induce regenerative effects on tissues early where the homeostasis has been destabilized (Papageorgiou et al., 2008). AEO application promotes angiogenesis through vasodilatation or neovascularization with increased expression of matrix mucopolysaccharide deposition, collagen synthesis, fibroblast proliferation, and vascular endothelial growth factor. According to previous studies, collagen plays an important role in wound healing, and as a principal component of connective tissue provides a structural framework for tissue regeneration. Increased cell density and deposition of connective tissue at the wound area improves wound healing and also, release of growth factors cause wound contraction which is an important part of the wound healing process (Ashkani-Esfahani et al., 2012; Hemmati et al., 2015; Kosger et al., 2009; Nasiri et al., 2015).

In this work, we observed that topical AEO application significantly induces warming wound area, and reduces pain and burning for brief times in adults with second-degree burn injuries. In addition, there were no systemic or local adverse reactions observed related to AEO application. Furthermore, the patients were more satisfied with AEO treatment as compared to SSD application.

The AEO was almost red in color, while SSD cream was white in color. Therefore, the patients were informed the kind of treatment agent used for each site; however, the patients were presented to the blinded expert physician and nurse after a thorough cleaning of wound before reapplication. This process made our study a single-blind clinical trial.

5. Conclusion

In conclusion, AEO significantly improved wound healing, decreased healing time, and increased satisfaction score as compared SSD cream in patients with second-degree burns. The efficacy of *A. euchroma* in the process of healing burn wounds may be explained by its ability to enhance tissue regeneration by promoting fibroblast proliferation, re-vascularization, and collagen formation combined with associated anti-bacterial and anti-inflammatory properties. Therefore, AEO has an undoubted place in the treatment of second-degree burn patients.

Conflict of interest statement

The author declared no potential conflict of interest with respect to the research, authorship, and/or publication of this review.

Authors contribution

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