

Evaluation of Burn Healing Properties of *Arnebia euchroma* and *Malva sylvestris*

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Abstract

Arnebia euchroma Rolye (Johnst) (Boraginaceae) and *Malva sylvestris* L. (Malvaceae) are traditionally used to treat various skin disorders, and for antimicrobial, and anti-inflammatory activities. The goat lipid containing roots of *Arnebia euchroma* is widely used as a remedy for burn wounds in nomadic tribal (Bakhtyari) southwest Iran. We examined the effectiveness of diethyl ether extracts of *Arnebia euchroma* roots and *Malva sylvestris* flowers at 200 mg/kg/day dose. Burns were induced in Wistar rats divided into six groups as following; Group-I was treated with cold cream. Groups-II and -III were treated with cold cream containing of two extracts, Group-IV was treated with goat lipid containing of *Arnebia euchroma* extract (according ethnobotany survey). Group-V was treated with goat lipid. Group-VI received the standard drug (silver sulphadiazine cream). The efficacy of treatment was evaluated based on reduction of burn wound area and histopathological characteristics. The extracts-treated animals showed significant reduction ($P \leq 0.05$) in the wound area when compared with other groups. Also, histological studies of the tissue obtained on days 6, 9 and 16 from the extract-treated by goat lipid containing of *Arnebia euchroma* and *Malva sylvestris* showed increased well organized bands of collagen, more fibroblasts, and few inflammatory cells. So we concluded that *Arnebia euchroma* and *Malva sylvestris* in the form of ointment had a good potential for acceleration of burn wound healing in rats.

Keywords: Burn; medicinal plants; *Arnebia euchroma*; *Malva sylvestris*.

1. Introduction

Burn wounds are very common in both developed and developing countries, however, in developing countries burns constitute a major health problem

because the incidence of severe complications is high, and financial resources are limited. Burn wound healing is a complex process that does not require much help, but still causes discomfort, and wounds are prone to infection and other complications. Infection is a major complication of burn injury and is responsible for 50-75% of hospital deaths [11]. Many of the synthetic drugs pose problems such as allergy, drug resistance, etc., forcing scientists to seek alternative drugs [15]. More than 80% of the world's population depends upon traditional medicines for various skin diseases [2]. Recently, the traditional use of plants for wound healing has received attention by the scientific community [2, 8]. Approximately one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs [10].

Several plants used as traditional healing remedies have been reported to treat skin disorders, including burn and cut wounds. In Iran, a survey of the ethnobotanical studies indicated the use of several of plant species by the inhabitants of the area, especially by those inhabiting the rural areas for wounds healing purpose [6, 7, 22]. For example, nomadic tribal (Bakhtyari) in southwest Iran, the roots of *Arnebia euchroma* with goat lipid used as a remedy for burn wounds [6].

Arnebia euchroma Rolye. (Johnst.) (Boraginaceae), a well-known traditional herb used in tribal medicine of Iran, is locally known as "Sorkh Giyah or Heveh Choae". Shikonin derivatives isolated from the roots of *Arnebia euchroma* have been reported to have antimicrobial, anti-inflammatory and anti-tumour activities and thus to be considered as important compounds for potentially medicinal use [9].

Malva sylvestris Linn. (Malvaceae), known locally as "Panirak", is an important medicinal plants in Iran whose flowers are used as a remedy for cut wound, eczema, dermal infected wounds, bronchitis, digestive problems and inflammatory in *Unani*

medicinal (Iranian Traditional Medicine) literatures [7,22]. A new anthocyanin, malvidin 3-(6'-malonylglucoside)-5-glucoside has been characterized in both wild and cultivated forms of *Malva sylvestris* [20]. The malvone A (2-methyl-3-methoxy-5,6-dihydroxy-1,4-naphthoquinone) is reported [4, 21].

No systematic studies have yet been carried out on the clinical evaluation of the burn wound healing potency of *Malva sylvestris* and *Arnebia euchroma*, so these effects were investigated using excision, histopathological characteristics and dead space wound repair models in rats.

2. Materials and Methods

Plant materials

The flowers of *Malva sylvestris* were collected on the slopes of the Zagross Mountains (1700–1800 m), District of Chaharmahal and Bakhtiari, Iran, during May 2007. The roots of *Arnebia euchroma* collected from the Jahanbin Mountain, Shahrekord, Iran in August 2007. Dr Valiollah Mozaffarian, Researches Institute of Forests and Rangeland, Tehran, Iran, authenticated the plants.

Preparation of the extract

About 100 g of powdered roots of *Arnebia euchroma* and flowers of *Malva sylvestris* were extracted with absolute 75% diethyl ether (Merck®) using Soxhlet apparatus for 12 h. The extracts filtered on Whatman paper and lyophilized a residue (yield: 10% w/w).

Animals

Male Wistar rats (200-250 g) of 2-3 months were used. The animals were housed in standard environmental conditions of temperature ($22 \pm 3^\circ\text{C}$), humidity ($60 \pm 5\%$) and a 12 h light/dark cycle. During experimental time rats were given standard pellet diet (Pastor Institute, Iran) and water *ad libitum*.

Burn wound creation

Burn wounds were created on dorsal part of shaved rats using a metal rod (1.5 cm diameter) heated to 80-85°C and exposed for 20s [18]. After 24 h, dead tissues were excised using sterile surgical blade (Figure 1). Control rats were dressed with cold cream alone, while experimental rats were dressed with the 10% (w/w) ointment formulated.

Experimental design

The animals were divided randomly into six groups of nine each. Group-I was treated with cold cream. Groups-II and -III were treated with cold cream containing of two extracts (200 mg/kg/day), Group-IV was treated with goat lipid containing of *Arnebia euchroma* extract (200 mg/kg/day) (according ethnobotany survey). Group-V was treated with goat

lipid. Group-VI received the standard drug (silver sulphadiazine cream) (200 mg/kg/day).

Burn healing

During the burn wound healing period and at the present time intervals, the burn wound area was traced manually and photographed. The burn wound area was calculated using Auto CAD RL 14 (Autodesk Company) software. At days 6th, 9th and 16th the experiment was terminated and the wound area was removed from the surviving animals for histologicsal examination. The excision skin biopsies were fixed in 4% formaldehyde solution 48 h during the experimentation period.

Analysis of data

The relative burn wound area was statistically analyzed as mean \pm S.D and statistically significance between treated and control groups were analyzed by means of Student's *t*-test. Data are significant; *P*-values ≤ 0.05 compared with control by the program "SAS ver 6.12 full".

3. Results

The efficacy of treatment was evaluated based on reduction of burn wound area and histopathological characteristics. The extracts-treated animals showed significant reduction ($p \leq 0.05$) in the wound area when compared with other groups. The animals treated with goat lipid containing of *Arnebia euchroma* extract (according ethnobotany survey) showed a significant reduction in the wound area when compared with other groups (Table 1). The animals treated with goat lipid containing of *Arnebia euchroma* extract showed faster epithelialization than those treated with the standard drug and other extracts (Table 2). Sorry, four animals treated with goat lipid deceased by high infection in wound.

Table1. Effect of the treatments on wound healing in rats.

Treatments	Wound area relative (cm ²)		
	6 th	9 th	16 th
<i>Arnebia euchroma</i> + Goat lipid	0.85 \pm 0.01 ***	0.66 \pm 0.18 *	0.08 \pm 0.06 ***
<i>Arnebia euchroma</i> + Cold cream	1.02 \pm 0.03 *	0.75 \pm 0.37 **	0.41 \pm 0.02 *
<i>Malva sylvestris</i> + Cold cream	0.87 \pm 0.05 **	0.84 \pm 0.05 *	0.23 \pm 0.09 **
Silver sulphadiazine	1.17 \pm 0.05 *	1.08 \pm 0.12 *	0.52 \pm 0.05 *
Control (Cold cream)	1.42	1.32	0.99

Note: Each value represents mean \pm S.D. *N*= 9 animals.

**: *P* ≤ 0.01 , *: *P* ≤ 0.05 levels of significance.

Table2. Effect of the treatments on the evolution of wounds in rats after 6, 9 and 16 days of topical application.

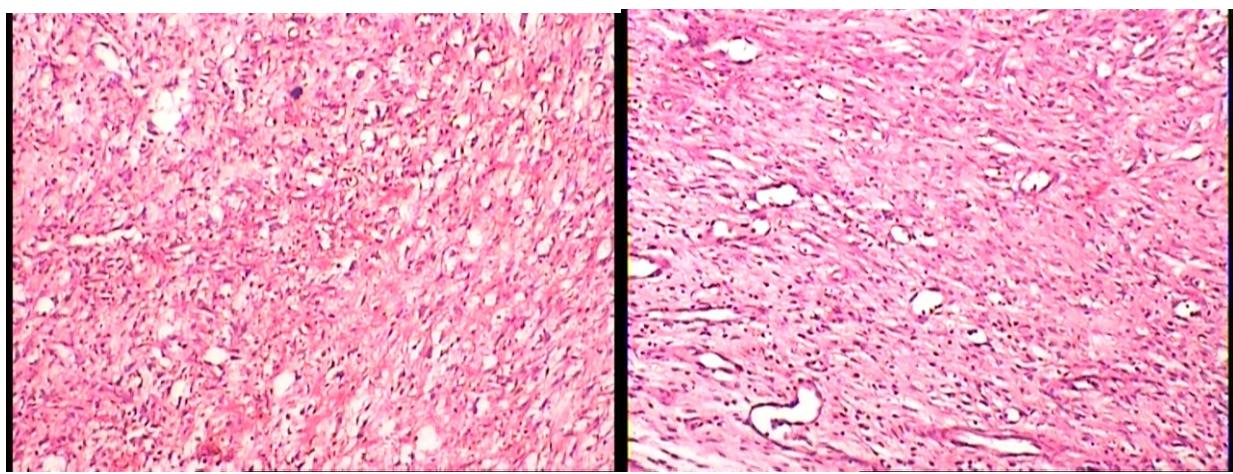
Treatments	Inflammatory cells			Collagen fibers			Re-epithelialization			Organization of the collagen			Necrosis			Fibrin		
	6	9	16	6	9	16	6	9	16	6	9	16	6	9	16	6	9	16
Arnebia euchroma+ Goat lipid	+	+	+	+	++	+++	+	++	+++	++	++	+++	-	-	-	+	+	-
Arnebia euchroma+ Cold cream	++	++	-	+	+	++	-	+	++	+	+	++	+	-	-	+	+	-
Malva sylvestris+ Cold cream	+	+	+	+	+	++	-	++	+++	+	+	++	-	-	-	+	+	-
Silver sulphadiazine	++	++	++	+	+	++	-	+	++	+	+	++	+	-	-	+	+	+
Control (Cold cream)	+++	+++	+++	-	-	+	-	-	+	-	-	+	+++	++	+	+++	++	+

Note: +: slight, ++: moderate, +++: extensive, -: absent.

The best results of histopathological evaluation were obtained with goat lipid containing of *Arnebia euchroma* extract and following *Malva sylvestris* extract, when compared to the other groups as well as to the control and the standard drug (Table 2 & Figure 2). These results offer pharmacological evidence on the folkloric use of *Arnebia euchroma* and *Malva sylvestris* for burn healing. The study of the histological structure showed the tissue regeneration was greater in the skin wound treated with goat lipid containing of *Arnebia euchroma* extract (Table 2 & Figure 2). The animal treated with cold cream (control) presented edema, monocyte cells and area with cellular necrosis that were not observed in the treated with herbal ointments and standard drug (Table 2 & Figure 2).



Figure 1. Wound burn induction in rats.



Arnebia euchroma+ Goat lipid (a)

Arnebia euchroma+ cold cream (b)

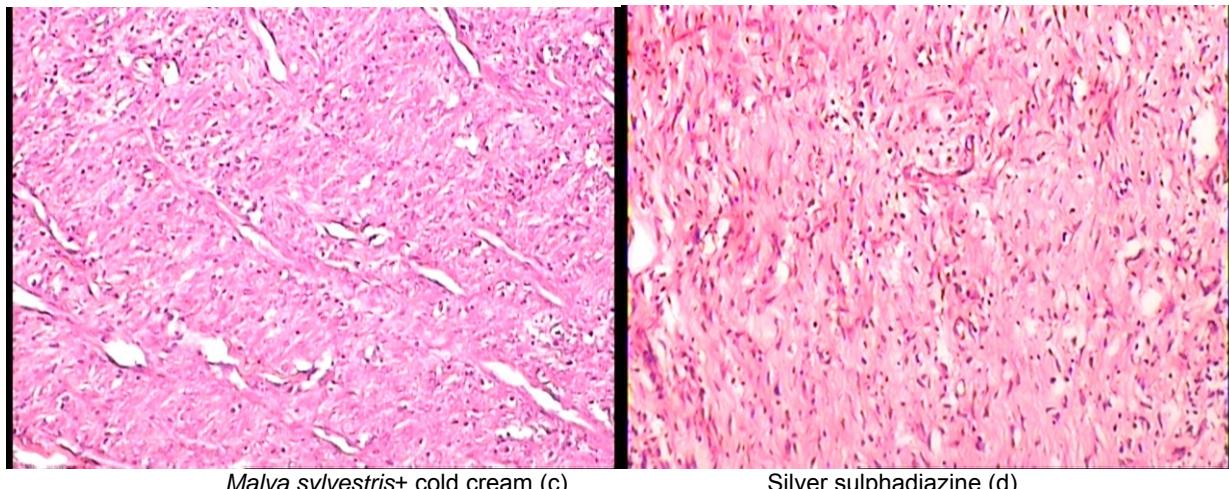


Figure 2. Histological evaluation after 16 days of burn creation in four groups (a, b, c & d).

4. Discussions and Conclusions

Burn and wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of the area of the wound. It is mainly dependent upon the type and extent of damage, the general state of health and the ability of the tissue to repair [3]. The wound contraction was significantly faster and higher in percentage in animals treated with goat lipid containing extracts of *Arnebia euchroma* roots and cold cream containing extracts of *Malva sylvestris* (Table 1). Finally, the epithelialization time was also found to be shorter in animals treated with goat lipid containing *Arnebia euchroma* (Table 1). In our study extracts significantly increased the rate of wound contraction and collagen turnover. Collagen, the major component which strengthens and supports extracellular tissue [17].

The roots of some genera of the Boraginaceae family such as *Arnebia*, *Alkanna*, *Onosma*, *Lithospermum* and *Echium* species are rich in naphthoquinones. alkannin, shikonin and their derivatives. Alkannin esters showed excellent healing properties in a clinical study conducted on 72 patients suffering from indolent ulcer on the lower part of the leg, due to varicose veins [14]. Accelerative effect of an ether extract of two Boraginaceae species *Lithospermum erythrorhizon* roots and *Macrotomia euchroma* roots on the proliferation of granuloma tissue in rats has been demonstrated [12,13]. They suggested that the accelerative effect of the roots on the proliferation of granuloma tissue depended mostly on the total content of naphthoquinone derivatives and the accelerative effect induced by ether extract might be an additive effect of these naphthoquinone derivatives. According previous studied [19] related that naphthoquinone derivative, arnebin-1 (b,b-dimethylacrylalkannin), significantly accelerated wound healing with or without hydrocortisone treatment [19]. Akkol et al. (2009) showed that, the

treatment with arnebin-1 showed reduction in the wound width and gap length compared with controls, moreover, promoted cell proliferation, migration and vessel formation to form a thick granulation tissue and re-epithelialization of the wounds. Shikonin derivatives isolated from the roots of *Arnebia euchroma* have been reported by Kim et al. (2001) reduced to shikonin semiquinone radical by O₂ and these scavenging activities may play an important role in the wound healing enhancement of the plant extract [16].

Any one of the phytochemical constituents (malvone A: 2-methyl-3-methoxy-5, 6-dihydroxy-1,4-naphthoquinone) present in *Malva sylvestris* may be responsible for antimicrobial activity [5]. It may be either due to the individual or additive effect of the phyto-constituents that hastens the process of wound healing.

The result of the present study offers pharmacological evidence on the folkloric use of *Arnebia euchroma* roots with goat lipid for healing wounds. Hence, the results support the traditional use of *Arnebia euchroma* roots to treat skin disorders including burns.

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