

Evaluation of the Burn Healing Properties of *Arnebia Euchroma* Rolye (Johnst) in Diabetic Rats

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Abstract— the ethanol extract of *Arnebia euchroma* roots were used to evaluate the burn healing activity at 200 mg/kg/day dose in alloxan-induced diabetic rats. Burns were induced in Wistar rats divided into four groups as following; Group-1, normal rats were treated with simple ointment base. Group-2, diabetic rats were treated with simple ointment base (control). Group-3 diabetic rats were treated with simple ointment base containing of extract (diabetic animals), Group-4 diabetic rats received the standard drug (Silver sulphadiazine). The efficacy of treatment was evaluated based on burn wound area relative and histopathological characteristics. The extract-treated diabetic animals showed significant reduction in the wound area when compared with control. Also, histopathological studies of the tissue obtained on days 9th and 16th from the extract-treated by extracts showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells. So we concluded that *Arnebia euchroma* in the form of ointment had a good potential for acceleration of burn wound healing in rats.

Keywords- Burn, diabetic, *Arnebia euchroma*, folk medicine

I. 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 [9]. Many of the synthetic drugs pose problems such as allergy, drug resistance, etc., forcing scientists to seek alternative drugs [13].

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, 6]. 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 [8].

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 habiting the rural areas for wounds healing purpose [4, 5, 18]. For example, nomadic tribal (Bakhtiari) in southwest Iran, the roots of *Arnebia euchroma* used as a remedy for burn wounds [4].

Arnebia euchroma Rolye. (Johnst.) (Boraginaceae), a well-known traditional herb used in tribal medicine of Iran, is locally known as "Sorkh Giyah or Heveh Choaeh". Shikonin derivatives isolated from the roots of *Arnebia euchroma* have been reported to have antimicrobial, anti-inflammatory and anti-tumor activities and thus to be considered as important compounds for potentially medicinal use [7].

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

II. MATERIALS AND METHODS

A. Plant materials

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.

B. Preparation of the extract

About 100 g of powdered roots of *Arnebia euchroma* was extracted with absolute 75% ethanol (Merck®) using Soxhlet

apparatus for 12 h. The extracts filtered on Whatman paper and lyophilized a residue (yield: 10% w/w).

C. 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*.

D. Burn wound creation

Burn wounds were created on dorsal part of shaved rats using a metal rod (1.5 cm diameter) heated to $80\text{--}85^\circ\text{C}$ and exposed for 20s [16]. After 24 h, dead tissues were excised using sterile surgical blade (Fig 1). Control rats were dressed with cold cream alone, while experimental rats were dressed with the 10% (w/w) ointment formulated.

E. Experimental design

The animals were divided randomly into four groups of nine each. Group-1, normal rats were treated with simple ointment base. Group-2, diabetic rats were treated with simple ointment base (control). Group-3 diabetic rats were treated with simple ointment base containing of extract (diabetic animals), Group-4 diabetic rats received the standard drug (Silver sulphadiazine).

F. 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 histological examination. The excision skin biopsies were fixed in 4% formaldehyde solution 48 h during the experimentation period.

III. RESULTS AND DISCUSSION

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 burn wound area when compared with other groups. The animals treated with *Arnebia euchroma* extract (according ethnobotany survey) showed a significant reduction in the burn wound area when compared with other groups. The animals treated with *Arnebia euchroma* extract showed faster epithelialization than those treated with the standard drug and other extracts.

The best results of histopathological evaluation were obtained with *Arnebia euchroma* extract and standard drug (Silver sulphadiazine), when compared to the other groups as well as to the control. These results offer pharmacological evidence on the folkloric use of *Arnebia euchroma* for burn healing. The study of the histological structure showed the tissue regeneration was greater in the skin wound treated with *Arnebia euchroma* extract.

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 extract of *Arnebia euchroma* roots. Finally, the epithelialization time was also found to be shorter in animals treated with *Arnebia euchroma*. In our study extracts significantly increased the rate of wound contraction and collagen turnover. Collagen, the major component which strengthens and supports extracellular tissue [15].

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 [12]. 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 [10, 11]. 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 [17] related that naphthoquinone derivative, arnebin-1 (b,b-dimethylacrylalkannin), significantly accelerated wound healing with or without hydrocortisone treatment [17]. 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-epithelization of the wounds [1]. Shikonin derivatives isolated from the roots of *Arnebia euchroma* have been reported by Kim et al. (2001) reduced to shikonin semiquinone radical by O_2 and these scavenging activities may play an important role in the wound healing enhancement of the plant extract [7].

IV. CONCLUSION

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

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TABLE I. EFFECT OF THE TREATMENTS ON BURN WOUND HEALING IN DIABETIC RATS

Treatments	Wound area relative (cm ²)					
	3 th	6 th	9 th	12 th	15 th	18 th
Arnebia euchroma + Simple ointment base (diabetic rats)	1.381	1.08	0.855	0.641	0.442	0.261
Standard drug (diabetic rats)	1.401	1.345	1.242	1.131	0.882	0.729
Simple ointment (diabetic)	1.5	1.5	1.5	1.49	1.48	1.48
Simple ointment (non diabetic)	1.485	1.423	1.323	1.153	0.996	0.708

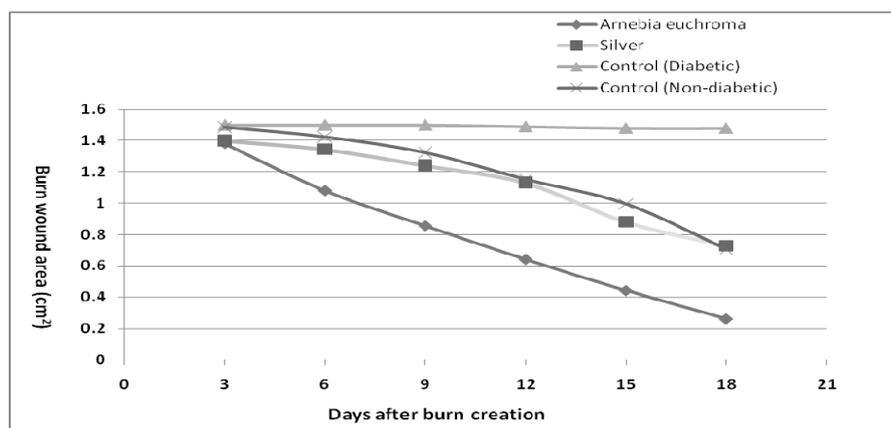


Figure 1. effect of the treatments on burn wound healing in rats