

Evaluation of the healing effects of *Onosma bolbotrichum* root extract on second degree burn wound in rabbit

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ABSTRACT

Objective: Burn injury is one of the main causes of death by injury worldwide and healing of burn is still a challenge in modern medicine. In present study we investigated the healing effects of n-hexane-dichloromethane (1:1) extract of *Onosma bolbotrichum* roots on second degree burn wound in rabbit.

Methods: 36 Iranian rabbits were used in this study. The rabbits were randomly divided into 6 groups. After creating of standard second degree burn wound on the back of rabbits, different treatment modalities were investigated in six groups. Group 1 received no treatment, group 2 was treated with cold cream (vehicle), group 3, 4 and 5 were treated with creams containing 0.5%, 1% and 2% (W/W) of plant extract, respectively. The 6th group served as standard group and was treated with Silver Sulfadiazine. Healing was assessed by the rate of wound healing, and total collagen content of treated skin samples. Histopathological evaluation of wound samples was also performed.

Results: The cream containing 0.5% (W/W) of *Onosma bolbotrichum* root extract significantly ($P < 0.01$) increased the rate of wound contraction and tissue collagen and non-collagenous proteins content when compared to vehicle and no-treatment groups. Additionally, histopathological evaluation confirmed these results.

Conclusion: The results of this study indicated that *Onosma bolbotrichum* root extract has the potential for treatment of burn wound and it can be used to treat different types of wounds in human beings also.

INTRODUCTION

A burn is a type of injury to skin, or other tissues, caused by heat, cold, electricity, chemicals, friction, or radiation (Shlash *et al.*, 2016). Skin is one of the most important organs of human body since it performs an array of various vital functions. Protection from harmful materials in the external environment, defense against microorganisms, fluid, protein and electrolyte homeostasis, thermoregulation, neurosensory and

metabolism are some of the most important functions which are performed by the skin (Tortora, 1993). A burn injury results in either the loss or disruption of some or all of these functions. The burn injuries are among the leading causes of disability and mortality resulting large costs in health care system all over the world (Rowan *et al.*, 2015). The healing of burn wounds is a complex process including: inflammation, neo-vascularization, granulation tissue formation, re-epithelialization and wound contraction (Hettiaratchy and Dziewulski, 2004).

Although various treatment options are available for burn wounds management, but further research is required for identifying novel targets and treatment paradigms to further improve burn wound care. Medicinal plants have been used in the treatment of burns for centuries almost in all cultures worldwide and play an

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important role in managing skin disorder (Bahramsoltani *et al.*, 2014).

Iranians have long used plant remedies in the treatment of many diseases. Thousands of years of history and hundreds of books have placed Iranian traditional medicine among the oldest and richest alternative medicines (Sahranavard *et al.*, 2014).

Onosma bolbutrichum (Boraginaceae) is a biennial herbaceous plant widely found in western and northern parts of Iran. In traditional medicine genus *Onosma* L plants are used in the treatment of variety of diseases such as bronchitis, hemorrhoids, wounds, peptic ulcer and hypertension. The plants of genus *Onosma* L. contains alkannin and shikonin, flavonoids, ferulic and vanillic acids, which may responsible for anti-inflammatory, wound healing, analgesic and its antibacterial actions (Kumar *et al.*, 2013).

In Iranian traditional medicine different extracts of root of *O. bolbutrichum* are used topically for the treatment of burns and wounds. Use of these extracts is only based on folklore without any scientific evidence of effectiveness. To determine whether its traditional use are supported by biological effects, the healing effects of n-hexane- dichloromethane (1:1) extract of *Onosma bolbutrichum* roots on second degree burns was investigated by assessment of some healing parameters and comparing its healing effects to those of Silver sulfadiazine.

MATERIALS AND METHODS

Plant material

The plant of *Onosma bolbutrichum* was collected from the west of Iran (Lorestan province) and identified by the Department of Botany, Faculty of Sciences, Shahid Chamran University of Ahvaz. The roots were separated, washed and dried at room temperature.

Extract preparation

The dried roots were placed in to blender to be grounded into powder. 300g of powdered root was extracted with n-hexane dichloromethane (1:1) (250 ml) in soxhlet apparatus. The extract was concentrated by rotary vacuum evaporator (Yamato RE300, Japan) and then air dried.

Topical Preparation of root extract of *O. bolbutrichum*

The creams containing 0.5%, 1% and 2% weight of extract per weight of cold cream were prepared. Briefly, after weighing, the plant extract was solubilized in a small amount of ethanol 70%, the extract was emulsified in the cream base, by continuous mixing, at room temperature.

Experimental animals

36 healthy Iranian rabbits (weighing between 2000-2200g) of either sex were used in this study. The animals were housed under standard conditions of temperature (22± 3°C), relatively humidity (55% ± 5) and light (12h light/ dark cycles)

with adequate supply of food and water *ad libitum*. All experimental methods were approved by the laboratory animal's ethical committee of the Ahvaz Jundishapur University of Medical Sciences in Ahvaz, Iran, and followed national guidelines for the care and use of laboratory animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). Rabbits were divided into six equal groups. The control group did not receive any treatment, vehicle group treated with cold cream, treatment groups received different doses of *O. bolbutrichum* root extract (OBRE) (0.5%, 1 % and 2% in cold cream, W/W), respectively, the sixth group was treated with Silver sulfadiazine (SSD) cream 1% (Sobhan Darou Co, Iran) as standard treatment.

Thermal injury model

Two identical wounds were created on the back of each rabbit, at a distance of more than 2 cm from each other. The dorsal hairs of rabbits were shaved and the shaved area was disinfected with ethanol 70 % and anesthetized with Lidocaine 2%. Animals were subjected to full-thickness second-degree skin burns with 2.5cm surface area diameter by hot plate. The plate was immersed in boiling (100 °C) water until thermal equilibrium was reached. The plate was then placed on the back of the rabbits for 20seconds without applying pressure (Abdullahi *et al.*, 2014). Extracts were topically applied to the wound twice a day.

Wound healing rate

The wound healing rate was measured as reduction in the wound size. The wounds were photographed every day and the wound area was calculated using Image-J software. The healing percentage of each wound was calculated as below:

$$\text{wound healing \%} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Histopathological evaluation

Samples were taken for histopathological study with a small excision containing part of the wound area from skin after 10 days of treatment. Samples were fixed in 10% buffered formalin, processed, blocked with paraffin, then sectioned in to 5 µm sections, and stained with hematoxylin and eosin.

Collagen and non-collagenous proteins content assay

After complete healing of the wounds, total collagen and non-collagenous proteins content of skin samples obtained from experimental groups were determined using Sirius Red/Fast Green Collagen Staining Kit (Chondrex), according to manufacturer's protocol. The tissue sections were 15µm thick, and collagen and non- collagenous proteins were determined in tissue sections.

Statistical analysis

Results are expressed as mean ± SD. Data were evaluated using the two-way ANOVA followed by Bonferroni posthoc test. Values of $P < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Wound healing rate

The percentage of wound closure in experimental groups on days 7, 14 and 16 after burn injury are shown in Figure 1. The significant reduction in wound area was observed in the animals treated with SSD and OBRE 0.5% compared with those who received vehicle or no treatment ($P<0.01$) [Figure 2]. Although the wounds treated with SSD healed more quickly (16days) than those which treated with OBRE0.5% (17 days) but there were no statistically significant differences between two groups. The degree of wound healing did not differ significantly among OBRE 1%, OBRE5%, vehicle and no treatment groups.

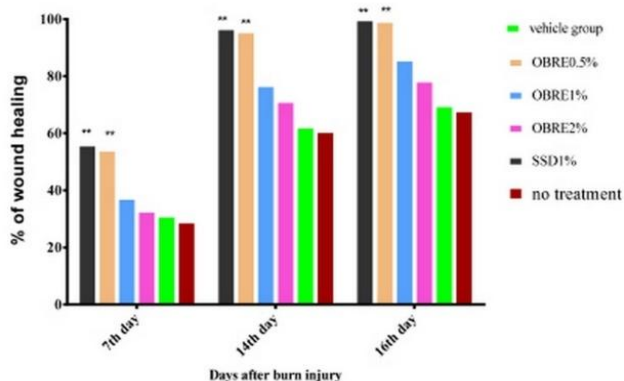


Fig. 1: Percentages of wound healing in experimental groups in day 7, 14 and 16 after burn injury. Data are expressed as Mean \pm SD, $**P<0.01$ when compare to no treatment and vehicle groups. SSD: Silver sulfadiazine, OBRE: Onosma bolbutric).

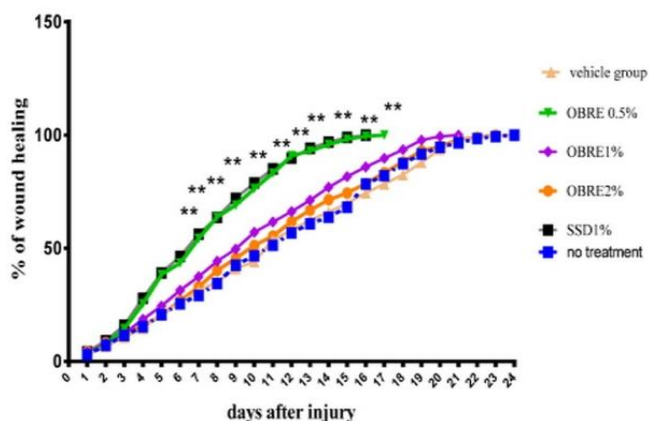


Fig. 2: Effect of Onosma bolbutricum root extract on wound healing rate in second degree burn wound. Data are expressed as Mean \pm SD, $**P<0.01$ when compare to no treatment and vehicle groups. SSD: Silver sulfadiazine.

Histopathological studies

Histopathological features of skin samples obtained from experimental groups on day 10 after burn injury are illustrated in Figure 3. In untreated and vehicle treated wounds, the surface of wounds was covered with exudate, the re-epithelialization was incomplete and the granulation tissue was observed [Figure 3. A & B], whereas, in SSD and OBRE 0.5% treated wounds more complete re-epithelialization, little exudate and thick granulation

tissue were observed [Figure3.A & B]. In OBRE1% and 2% treated wounds the migrating epithelial cells and granulation tissue were observed. There were inflammatory cells and red blood cells in wound site [Figure3 C & D].

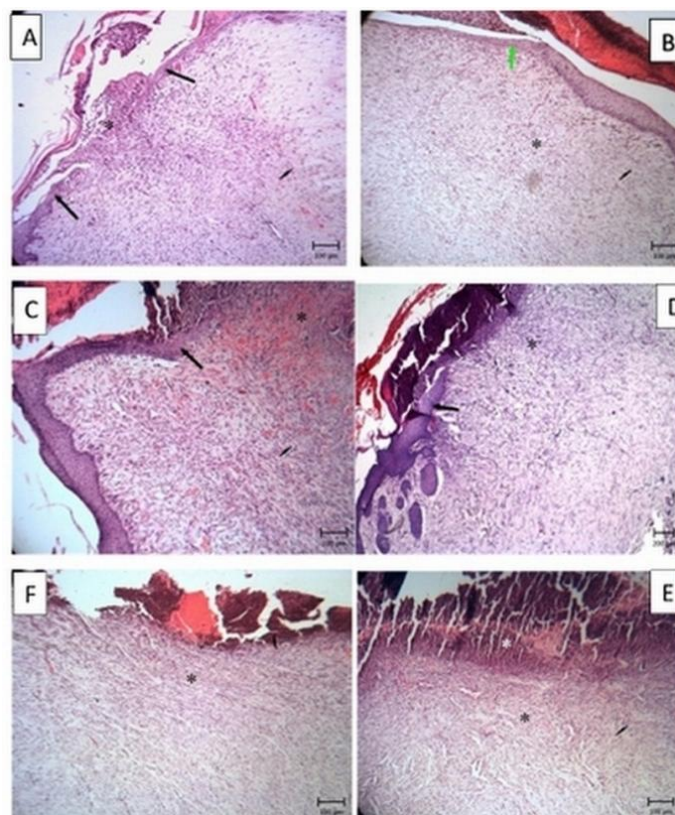


Fig. 3: Histopathological features of skin tissue samples obtained from experimental groups at day 10 after burn injury. A) SSD treated group, B) OBRE 0.5% treated group, C) OBRE1% treated group, D) OBRE 2% treated group, E) Vehicle treated).

Collagen and non-collagenous proteins in tissue samples

Wounds collagen content significantly ($P<0.01$) increased in SSD and OBRE0.5% treated groups after complete healing, when compared to the vehicle treated and no treatment group [Table 1]. The non-collagenous proteins content in SSD, OBRE 0.5%, OBRE 1% and OBRE2% treated animals was significantly ($P<0.01$) higher than that in vehicle treated and untreated animals [Table 2].

Table 1: The collagen content of skin tissue samples of experimental groups after complete wound healing.

Experimental groups	Collagen content ($\mu\text{g/g}$ tissue)
SSD 1%	17.917 \pm 0.176**
OBRE 0.5%	19.150 \pm 0.155**
OBRE 1%	16.529 \pm 0.183
OBRE 2%	16.197 \pm 0.131
Cold cream	9.390 \pm 0.137
No treatment	8.641 \pm 0.171

Data are expressed as Mean \pm SD, $**P<0.01$ when compare to cold cream and no treatment groups. OBRE: Onosma bolbutricum root extract, SSD: Silver Sulfadiazine.

Table 2: The non-collagenous proteins content of skin tissue sample of experimental groups after complete wound healing.

Experimental groups	Non-collagenous proteins ($\mu\text{g/g tissue}$)
SSD 1%	295.92 \pm 4.600**
OBRE 0.5%	285.80 \pm 7.581**
OBRE 1%	278.41 \pm 5.927**
OBRE 2%	250.28 \pm 9.00**
Cold cream	115.18 \pm 4.820
No treatment	92.67 \pm 3.080

Data are expressed as Mean \pm SD, **P<0.01 when compare to cold cream and no treatment groups. OBRE: *Onosma bolbutrichum* root extract, SSD: Silver Sulfadiazine.

Effective treatment of burn wounds remains an important challenge for clinicians and researchers. Plants and their derivatives have the potential for the treatment of burn wounds and evaluation of their efficacy in the treatment of wound injuries is necessary (Mohammadi-Barzelighi *et al.*, 2011). The results of present study demonstrated the wound healing activity of *Onosma bolbutrichum* root extract, but the involved mechanism is unclear. Many species of *Onosma* have been studied with respect to their bioactive chemical compounds, because the plants of this genus have been used in traditional medicine to treat variety of diseases (Shahina Naz *et al.*, 2006). Alkannins and shikonins have been found in the external layer of the roots of many species of the Boraginaceae family (Mehrabian *et al.*, 2012, Shahina Naz *et al.*, 2006). Some studies have shown the antioxidant, antibacterial and anti-inflammatory activity of shikonins (Lee *et al.*, 2015, Chen *et al.*, 2001). The inflammatory response is an important part of the wound healing process, but long-term persistence of inflammation is a reason for impaired wound healing (Singer and Clark, 1999). Infection is one of the leading causes of morbidity and mortality in burn patient (Williams *et al.*, 2009). Topical antibacterial agents decrease morbidity and mortality in patient with major burn injuries (Church *et al.*, 2006). Free radicals are necessary for proper healing of skin wounds, but over production of free radicals causes oxidative stress, a condition that weakens immune system, accelerates aging and increases the risk of chronic diseases. Free radicals damage collagen, protein and lipid which are key components of wound healing (Wortsman, 2013). Therefore reducing production of free radicals is essential for proper wound healing. Thus, the healing activity of *O. bolbutrichum* could be partly due to its antioxidant, antibacterial and anti-inflammatory activity.

CONCLUSION

This study concluded that OBRE extract promote burn wound healing in second degree burns in rabbit and it may be suggested to use for treating burn wounds in human being. However further studies are required to identify and isolation of its active constituents responsible for this pharmacologic effect.

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Conflict of Interests: There are no conflicts of interest.

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