Macroscopic evaluation of wound healing activity of the Persian shallot, *Allium hirtifolium* in rat

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**Abstract**

Wound is a break in the outer layer of skin. There are several different methods to provoke wound healing process, consisting topically or orally administration of medicinal drugs or herbal remedies. Persian shallot, *Allium hirtifolium* which belongs to the same genus of garlic, naturally grows in different parts of Iran. In this study, we have evaluated the wound healing activity of hydro-alcoholic extract of *A. hirtifolium* Boiss. Four bilateral full-thickness wounds (2 on each side) were made on the dorsal area of four adult albino rats weighing 165±35 gr under general anesthesia. Right side wounds were treated in experimental groups, while left side wounds were considered as control littermates. One day after surgery, a gel-like 1:1 mixture of *A. hirtifolium* extract and methylcellulose were topically applied (100 mg/kg/day) to the experimental wounds while the wounds in control groups were treated with the extract-free gel for 12 days. At days 1, 3, 6, 9 and 12, digital photos of wounds were taken using of Scion image software, for analyzing the percentage of wound contraction, epithelialisation and speed of healing. The results revealed that *A. hirtifolium* can accelerate wound healing by increasing the rate of epithelialisation. We concluded that *A. hirtifolium* extract may be clinically useful in management of open wounds treatment procedures.

**Keywords:** *Allium hirtifolium*, epithelialisation, extract, wound healing

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Introduction

The repair or recovery process of wound, after a trauma or a surgical incision is called wound healing. The wound healing process has multiple complicated stages of cellular and biochemical events in response to any disruption of normal anatomy, leading to structural and functional restoration of damaged tissue (Martin, 1996; Tanya and Martin, 2009). There are several reports indicating that herbal medicines possess pro-healing effects. They may accelerate or promote wound healing by their different properties such as encouraging blood clotting, promoting the expression and release of growth factors and cytokines, or even via antibacterial effects.

Pharmacological effects of many herbal remedies had been observed on humans long before their mechanism of action being discovered. Based upon thousands of years of experience, herbal drugs and their derivatives are the main sources of many conventional drugs and provide potential alternative to modern medicine. Therefore their effectiveness, structure, properties and their proper doses should be identified and formulated for different purposes such as management or treatment of wounds (Polasek et al., 2007).

Materials and methods

Herbal extract preparation

Persian shallot (A. hirtifolium) bulbs were collected from Khansar area in Isfahan province in early summer 2008. The specimen was identified and approved by Department of Biology, Tehran University, Iran. The bulbs were washed in tap water and, cut into small slices, air dried and ground into powder using a blender. The extract was made using 100 gr percolated powder in 250 ml distilled water mixed with 250 ml Alcohol 96% (1:1) and kept at 4 ºC for 24 hours. The solution filtered through cotton cloth for three times, left under the hood until dryness, powdered and stored at 4 ºC for later use (Polasek et al., 2007).
Preparation of treatment and control gels

For preparation of treatment gel (80mg/ml), 8gr of dry extract along with 3gr Methyl cellulose dissolved in 100 ml distilled water to form a gel-like mixture. The similar gel was made of Methyl cellulose alone for use as control agent.

Animals and experimental set-up:

Four adult albino rats weighing 165±35g were purchased from Razi Vaccine and Serum Research Institute, Mashhad, Iran. They were kept in standard conditions, in a temperature and light-controlled environment with free access to standard laboratory rodent food and fresh drinking water.

Anesthesia was induced by intramuscular (IM) injection of a combination of ketamine (40 mg/kg) and xylazin (10 mg/kg). Four full-thickness circular wounds (8.75 mm diameter) were made by biopsy punch on the dorsal area of each animal. The right side wounds were treated as experimental groups, while left side wounds were treated as control groups. One day after wounding, treatment gel was applied topically to the experimental wounds (100 mg/kg), while control groups were treated with the extract-free gel. The treatment was repeated once a day for 12 days. At days 1, 3, 6, 9 and 12, digital photos were taken from wounds. Scion image software was used to measure the percentage of wound contraction, epithelialisation and speed of wound healing. The following formulate were used:

Wound contraction:
1. Wound size at day \((x)\) mm\(^2\)/ wound size at day \((0)\) mm\(^2\) x 100 = percentage wound size at day \((x)\) compared to wound size at day \((0)\)
2. Percentage wound size at day \((x)\) compared to day \((0)\) -100 = percentage wound contraction

Wound epithelialisation:
Size of epithelialisation area at day \((x)\) mm\(^2\)/Size of the wound at day \((x)\) mm\(^2\)/t x 100 = percentage epithelialisation

Wound healing:
1. Granulation tissue at day \((x)\) mm\(^2\)/ wound size at day \((0)\) mm\(^2\) x 100 = percentage non-healed area compared to the wound size at day \((0)\)
2. Percentage non-healed area compared to the wound size at day \((0)\) -100 = percentage healing

Statistical analysis

Effects of time on wound healing, epithelialization and contraction was examined using repeated measurement ANOVA, including time as fixed factor and rats as random factor. Statistical analysis was performed using the SPSS 9 program for Windows (SPSS Inc. Chicago IL, USA). A value of \(p<0.05\) was considered significant.

Results

The wounds treated with \(A.\ hirtifolium\) extract exhibit noticeable healing improvement from day 6, healed faster and closed in shorter time than wounds in control group. All wounds in treatment group were completely healed within 11 to 12 days while it took about 13 to 14 days in control group (Figure 1).

A statistically significant difference was seen only in wound epithelialization between treatment and control group during this study \(\(p<0.05\)\) (Figure 2), while there was no significant difference in wound contraction and wound healing between these two groups (Figures 3 and 4).

Discussion

The wound healing process is to reduce the pain and closing the wound as soon as possible to accelerate recovery of injured tissue with minimum cosmetic defects. In brief, it has three phases including inflammation, cellular proliferation and remodeling (Reddy et al., 2002).

The potential of traditional medicine to manage wound healing has been considered in some communities for thousands of years (Krishnan, 2006). \(A.\ hirtifolium\) is used as a herbal medicine in folklore. According to author’s knowledge, no information is available about the wound healing activity of...
Figure 1: Wound healing in animals treated with solution contain *A. hirtifolium* extract (right) and treated with Methyl cellulose alone (left), in day 6 (up), day 9 (middle) and day 12 (bottom).

Figure 2: Percentage of wound epithelization in treatment and control wounds.
Figure 3: Percentage of wound healing in treatment and control wounds

Figure 4: Percentage of wound contraction in treatment and control wounds
this plant. Therefore, the present study may be the first to evaluate the effects of *A. hirtifolium* on wound healing.

The results of this study revealed that *A. hirtifolium* significantly affects wound epithelialisation, as the first step of wound healing by which proliferation and migration of epidermal cells take place (Shukla and Mossman, 2008). The observed improvement in rate of wound closure, healing and reduction in healing time might be due to enhanced epithelialisation.

*A. hirtifolium* belongs to the same genus as *A. sativum* (garlic). Several reports have been shown that garlic is rich of allicin (diallyl thiosulfinate) (Panus, 2008), allicin has also been in *A. hirtifolium*, quantified in the amount of 3.4± 0.1 gr⁻¹ (Ghodrati et al., 2008; Ghodrati et al., 2009). Sardari and colleagues have reported that topical application of allicin has no significant effect on wound contraction, epithelialization and healing in full-thickness wound in dogs (Sardari et al., 2006). Therefore, it seems that allicin is not responsible for wound epithelialization. However, non-significant effect of *A. hirtifolium* on wound contraction and healing in the present study is similar to what sardari and colleagues have shown about allicin. In addition to allicin, *A. hirtifolium* contains several secondary metabolites such as alliin, alliinase, S-allyl-cysteine (SAC), diallyldisulphide (DADS), diallyltrisulphide (DATS), and methylallyltrisuphide (Block et al., 1992), which may be responsible for its effect on epithelialisation lonely or in combination. Although it is unlikely that experimental animals (rats and dogs) make differences in results but we should consider that, different animals may respond to plant extract in a different way.

The results of this study raise the possibility that topical application of *A. hirtifolium* can accelerates or improves the healing of small granulating wounds by encourage the epitheliazation and may be clinically useful in management of open wounds.

Further studies should perform with a variety of doses of *A. hirtifolium* extract to establish the appropriate dosage. Also use of the fractionation of active compounds may lead to better result in the process of wound healing activity of this herbal medicine.

**References**


Jafarian, A., Ghannadi, A. and Elyasi, A.


ارزیابی ماکروسکوپی تأثیر عصاره موسر
(Allium hirtifolium)
بر ترمیم زخم در رتبه‌های آزادی ۱، بهره‌وری فتحی ۱، هنیه شاطر زاده، مرجع اشیبی، حسن کاظمی مهرچری، محسن ملکی

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چکیده

زخم نوعی تغییر در بدنتان است که منجر به تغییر در شکل و حجم جسمان شده است. اگر زخم بتواند به صورت صحیح درمان شود و بتواند درمان شود، می‌تواند به صورت صحیح درمان شود. اگر زخم بتواند به صورت صحیح درمان شود و بتواند درمان شود، می‌تواند به صورت صحیح درمان شود. اگر زخم بتواند به صورت صحیح درمان شود و بتواند درمان شود، می‌تواند به صورت صحیح درمان شود. اگر زخم بتواند به صورت صحیح درمان شود و بتواند درمان شود، می‌تواند به صورت صحیح درمان شود.

واژگان کلیدی: موسر، ایپیتریپاسیون، عصاره، بهبود زخم
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Abstract

Wound is a break in the outer layer of skin. There are several different methods to provoke wound healing process, consisting topically or orally administration of medicinal drugs or herbal remedies. Persian shallot, Allium hirtifolium which belongs to the same genus of garlic, naturally grows in different parts of Iran. In this study, we have evaluated the wound healing activity of hydro-alcoholic extract of A. hirtifolium Boiss. Four bilateral full-thickness wounds (2 on each side) were made on the dorsal area of four adult albino rats weighing 165±35 gr under general anesthesia. Right side wounds were treated in experimental groups, while left side wounds were considered as control littermates. One day after surgery, a gel-like 1:1 mixture of A. hirtifolium extract and methylcellulose were topically applied (100 mg/kg/day) to the experimental wounds while the wounds in control groups were treated with the extract-free gel for 12 days. At days 1, 3, 6, 9 and 12, digital photos of wounds were taken using of Scion image software, for analyzing the percentage of wound contraction, epithelialisation and speed of healing. The results revealed that A. hirtifolium can accelerate wound healing by increasing the rate of epithelialisation. We concluded that A. hirtifolium extract may be clinically useful in management of open wounds treatment procedures.