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Original article

In vivo wound healing activity of a herbal ointment in rat

M. Karami[1,2], D. Yazdani[3*], M.R. Jalali Nadoushan[4], S. Geravand[2]

[1]*Neurophysiology Research Center, Shahed University, Tehran, Iran.*
[2]*Department of Biology, Faculty of Basic Sciences, Shahed University, Tehran, Iran.*
[3]*Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.*
[4]*Department of Pathology, School of Medicine, Shahed University, Tehran, Iran.*

Abstract

Background and objectives: The wounds are still the health tribulations at the present time. In the present research the effect of a new herbal ointment (Oppyheal) in treatment of rat's wound has been investigated. The effectiveness of the ointment was compared with the Fibrinolysin/DNAse. **Methods**: Fifty four male Wistar rats were used. The wound was created on the back cervical skin of the animals under anesthesia in three different sizes. The animals in each size group were randomly divided into three groups. The control group did not receive the ointments. The reference group was given the Fibrinolysin/DNAse and the third group was treated with the Oppyheal. The products were topically used once per day until the wounds of one group were completely healed. The size of the wound area was measured in days 0-20 by a standard reference ruler. The reduction in size of the wound was calculated and analyzed. The recovered skin of all animals were examined histologically, $p<0.05$ was considered as significant. **Results**: The results showed a significant difference in wound contraction between the treated groups and the control group ($p<0.0001$). The new skin of ointment-treated rats showed healing features in comparison to the control group. **Conclusion**: This study may introduce a suitable topical ointment, Oppyheal, for wound care.

Keywords: Fibrinolysin/DNAse, herbal ointment, rat, skin, wound healing

Introduction

Skin wounds are produced as a result of hurt in healthy skin. Injuries are induced by damaging stimuli, pathogens or other factors. Along with the depth and degree of the wound, healing process is also dependent on several factors such as cell inflammation mediators, causal diseases, type of injury, etc. It deals with a series of cellular and molecular events for example inflammation, angiogenesis, fibroplasia, epithelialization, and wound contraction as well as remodeling of matrix [1]. If treatment is not effective, then the skin is not healed and chronic wound is formed.

Chronic dermal injuries affect a person's life, and require more attention, and they also need much cost. To restore the health and physiology of the affected part, use of proper remedies are recommended. The main result of optimal wound healing is to lessen the tissue damage and provide adequate tissue healing [2].

Available at: http://rjpharmacognosy.ir
*Corresponding author: dayazdani@yahoo.com, Tel: +9821-66465036; Fax: +9821-66497572

Wound healing treatment is commonly obtained with the Fibrinolysin/DNAse, as an available proteolytic agent [3]. An ointment containing collagenase or papain has degrading effect on wound components, such as collagen, fibrin, and elastin both *in vitro* and in patients [4]. There are now more trends to herbal remedies for open skin injuries. The natural ingredients with antibacterial action seem to help combat infection and inflammation. Several plants have been used as wound healing agents since ancient times. Some of them have been examined scientifically for the evaluation of their wound-healing activity in different pharmacological models and patients [5-7], but the potentiality of most plants remains unknown.

The genus *Aloe* with at least four species, has been traditionally applied for the medicinal practice over thousands of years in many cultures for its curative purposes. *Aloe vera* L. (Xanthorrhoeaceae) is the most widely studied species so far for its clinical effectiveness against a variety of skin disorders including burns and wounds [8,9].

The positive influence of *A. vera* on skin wound repair, for example, anti-inflammation, antimicrobial, immunomodulation and hematopoiesis stimulation and absorbent quality has been reported. This effectiveness has been attributed to its diverse constituents, in particular, the polysaccharides. Apart from polysaccharides, miscellaneous bioactive constituents have been identified from *A. vera*. These compounds belong to different bioactive compounds as alkaloids, anthraquinones, saccharides, enzymes, amino acids, inorganic minerals, etc [8].

Hypericum perforatum L. (Hypericaceae) (St John's Wort) has long been used topically for healing of wounds and burns in folk medicine of various countries. In a clinical study, it has been demonstrated that oily extract of St John's Wort promotes healing of surgical wounds from childbirth with caesarean section as a result of the increase in epithelial reconstruction [10]. St John's Wort is a well-known plant in herbal medicine for its therapeutic effects. Mukherjee *et al.* have reported that the tribal people of Nilgiris, Tamilnadu, India use the leaves of *H. perforatum* for its wound healing potential [11]. Based on the traditional Turkish folk medicine, *H. perforatum* has potent wound healing activity [12].

The official 2009 Herbal Medicinal Products (HMPC) monograph of the European Medicines Agency accepted the use of topical St John's Wort preparations for "symptomatic treatment of minor wounds" in the context of traditional medicine [13].

The wide range of indigenous uses of *Papaver somniferum* L. (Papaveraceae) in traditional medicine have been reported. The seeds can be used in remedies for constipation, intestinal inflammatory pain and dysentery [14].

With reference to the traditional use, it should be noted that *P. somniferum* was recommended for pain relief by Avicenna [15]; also in Iranian Traditional Medicine (ITM), opium was used for pain relief [16] while it is still used in modern medicinal products [17]. Thus in the present study, the wound healing potential of the mixture containing the extracts of the mentioned plants were considered.

The present experiment aimed to examine the wound healing efficacy of the ointment, Oppyheal (St John's Wort, *Aloe* and poppy seed oil), in the experimental model of skin wound.

Experimental

Plant material

Flowering shoots of *H. perforatum* L. were collected from Alamut region in Qazvin province, Iran (June 2014). They were identified at the Herbarium of Institute of Medicinal Plants, Karaj, Iran. The leaves of *A. vera* were collected from the *A.vera* plants grown in a greenhouse located in Institute of Medicinal Plants, Karaj, Iran. A voucher specimen of each species was deposited at the Herbarium of Institute of Medicinal Plants, Karaj, Iran for future reference (519 MPIH and 4528 MPIH, respectively).

Opium Poppy (*Papaver somniferum* L.) seeds

were supplied from Sam Arian Exir Company, Tehran, Iran, an importer of poppy seed oils from Afghanistan for medical purposes. A voucher specimen (MPISB 1338) has been deposited in gene bank of Institute of Medicinal Plants, Karaj, Iran for future references.

Extraction

The flowering shoots of St John's Wort were dried in shade at room temperature, powdered by a grinder, and stored in a well closed vessel before use. The powdered material (500 g) of *H. perforatum* was extracted with methanol using maceration method for 72 h (solvent was changed every 24 h with fresh methanol). The methanol extract was concentrated using rotary evaporator below 50 °C under reduced pressure to get the crude extract. The obtained semi-solid mass was used for the ointment formulation.

Aloe vera gel powder was prepared from *A. vera* L. leaf gel according to Rajasekaran *et al*. [18].

The leaves of *A. vera* were cut transversely and epidermis was selectively removed. The solid gel in the center of the leaf was homogenized. The homogenate gel was lyophilized and the lyophilized sample was extracted using 95% ethanol by solid- liquid extraction technique (perculation). The filtrate was evaporated to dryness under reduced pressure in a rotary evaporator. The residue was stored at 4 °C.

Opium poppy seed oil was extracted using a cold press technique from seeds of *P. somniferum.*

Ointment

Each 100 g of the formulation contained 20 g St John's Wort extract (equivalent to 28 mg hypericine), 0.5 g *A.vera* dried gel and 10 mL poppy seed oil incorporated in simple ointment base. Fibrinolysin/DNAse was used as the standard drug for comparing the wound healing potential of the ointment in the animal model.

Animals

Male adult Wistar rats (250-300 g) were obtained from Pasteur Institute of Iran, Tehran, Iran and housed in standard cages as groups of 2 at animal care center under 22±3 °C and 12:12-hour dark/light cycle. The experiments were performed during the light phase (from 09.00 a.m. to 11.00 a.m.) of the cycle. Each animal received treatment once per day in the experimental period (20 days). At the end of experiments the rat was anaesthetized by ketamine (100 mg/kg) and xylazine (20 mg/kg) purchased from Iran's Veterinary Organization. The rats' skin samples were collected in 10% formalin for histological verification. All experiments were carried out in accordance with the National Institutes of Health Guide for care and use of laboratory animals (NIH publications) and approved by the local committee of ethics at Shahed University.

Wound induction

The back neck hairs of rats were clipped off in day 0 [19,20]. Excision wounds were made over 25 mm×25 mm or 15 mm×10 mm or 10 mm×10 mm areas of intact skin of back neck of animals under anesthesia. Animals were housed individually in autoclavable animal cages at animal care center of Shahed University, Tehran, Iran.

Animals were grouped based on the size of wound and the type of the treatments. For each wound size, three groups of animals (n = 6) were categorized as follows:

Group I – The control group: They passed surgery, but, they did not receive any cream. They were simply treated by saline solution.

Group II – The animals treated by Fibrinolysin/DNAse: they were taken surgery and treated with Fibrinolysin/DNAse (positive control).

Group III – The rats treated with Oppyheal ointment: they received Oppyheal after surgery.

(The drugs were gently applied onto the wounds with swap in the size of the swap head.)

Wound contraction rate

The wound areas were defined as large (25 mm×25 mm), moderate (15 mm×10 mm), and

small (10 mm×10 mm). They were measured using millimeter standard reference ruler at days 0, 1, 6, 9, 13, and 20.

Histopathology

The samples of the newly formed skins were isolated from all animals by ending the experiments to evaluate the histopathological alterations. The samples were fixed in 10% formalin at least for 10 days before the commencement of tissue processing. The samples were then processed and blocked with paraffin. They were finally sectioned at 3-5 µm thickness and stained with hematoxylin and eosin (H&E). The skin specimen of each rat was studied.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). The Tukey's HSD *post hoc* analysis was applied for between group comparisons. The *p* value less than 0.05 was considered as significant.

Results and Discussion

The results of the wound size measurements for all animals have been shown in figures 1-3. The wound area (mm^2) in the animals (the control, the reference and the test groups) per wound size was measured in days 0-20. The size of the wounds resulted in a significance decrease in the herbal Oppyheal as well as Fibrinolysin/DNAse in contrast to the control group. Thus, the healing time shortened significantly based on the wound facet measurement between the drugs-treated animals and the control group. Referring to the further analysis by Tukey's HSD post hoc though Oppyheal showed even earlier improvement for the large wounds ($p< 0.001$), in medium and small wounds acted in accordance with the standard drug ($p< 0.01$).

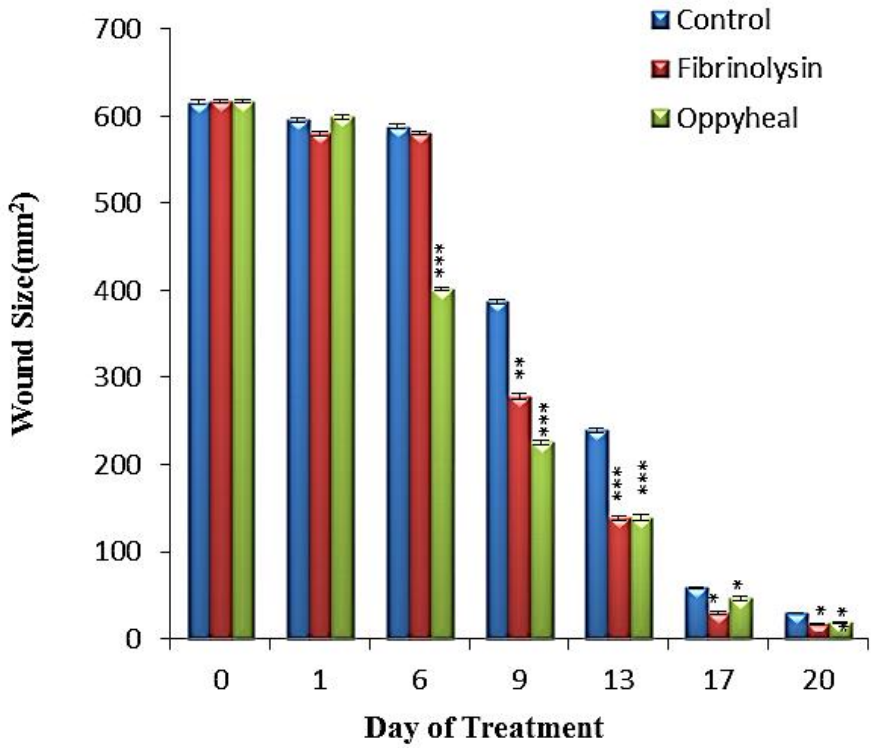

Figure 1. Change in size of large (25 mm×25 mm) wound throughout the treatments. The data have been expressed as mean ± SEM from 6 rats *p<0.05, **p<0.01, ***p<0.001 to the control saline group according to the Tukey's *post hoc*.

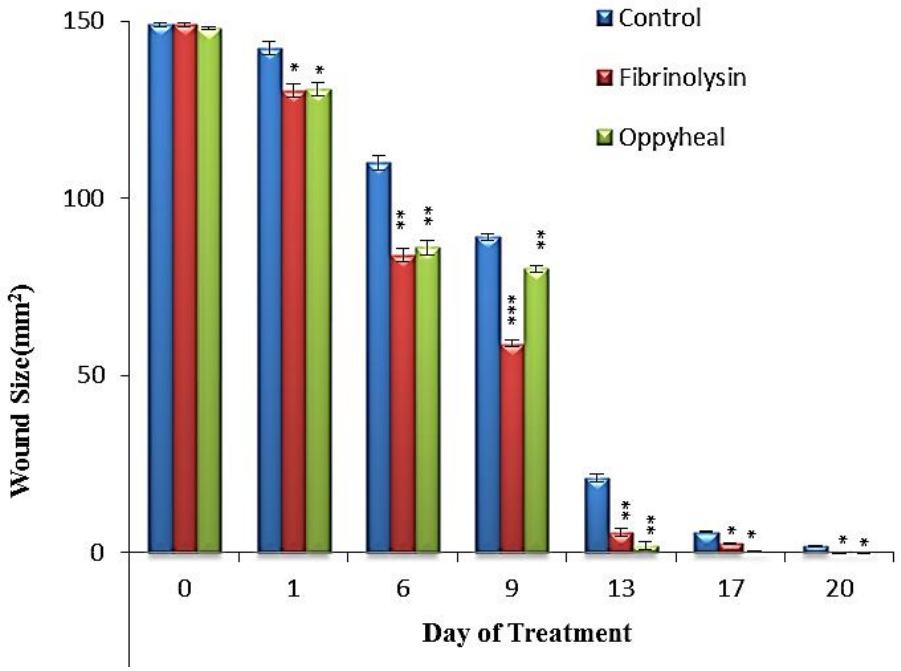

Figure 2. Change in size of moderate (15 mm×10 mm) wound throughout the experimental procedure. The data are expressed as mean ± SEM from 6 rats *p<0.05, **p<0.01, ***p<0.001 based on the *post hoc* in a comparison with the control saline group.

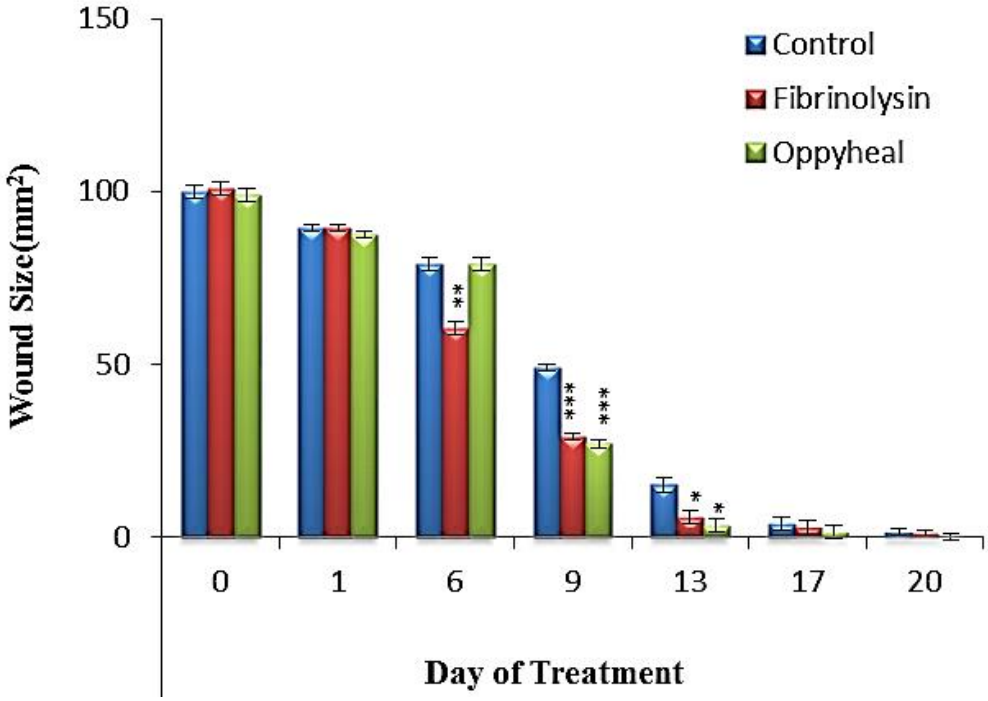

Figure 3. Change in size of small (10 mm×10 mm) wound throughout the experiment. The data are expressed as mean ± SEM from 6 rats *p<0.05, **p<0.01, ***p<0.001 in comparison with the control saline group.

Data show no significant change among the groups (p>0.05) proposing a non-infectious feature. The tissue characteristics of the skins of drug-treated animals including the epithelial layers, the adipose glands, the hair follicles, the muscle layer, and the loose connective tissue after the cut off healing time (20th day) were in appropriate renewal states. The finding demonstrated the protective role and healing value of the herbal ointment and the reference drug (figure 4).

Wounds may cause problems. Although, they are less likely to cause death than other skin problems (*e.g.* burn), they might induce pain and secondary infection.

The present study evaluated the healing effect of a new herbal ointment (Oppyheal) on experimentally induced deep wounds in back cervical skin of Wistar rats in comparison to reference ointment, Fibrinolysin/DNAse.

The results showed significant differences in contraction of the wounds and the healing time (p<0.0001) between the reference treatment Fibrinolysin/DNAse- and the Oppyheal ointment-treated groups versus the negative control group mostly in days 6,9 and 13.

Additionally, the regenerated skin in the ointment treated rats showed the best healing features compared to the control groups.

Many previous studies have investigated the effects of herbal ointments on healing time of wounds in laboratory animals [21-23]. They have shown that the herbal materials have healing effect on the skin after multiple applications. *Aloe* and its products have been used as cosmetic ingredients, including *Aloe andongensis* extract and leaf juice, *A. arborescens* leaf extract, leaf juice, and leaf protoplasts, *A. barbadensis* flower extract, leaf, leaf extract, *A. ferox* leaf extract, leaf juice, and leaf juice extract [24].

The previous studies have demonstrated that the polysaccharide components of *Aloe* species have a dehydrating effect on the skin after multiple applications [23]. The study about the streptozotocin-induced diabetes in rat, indicated

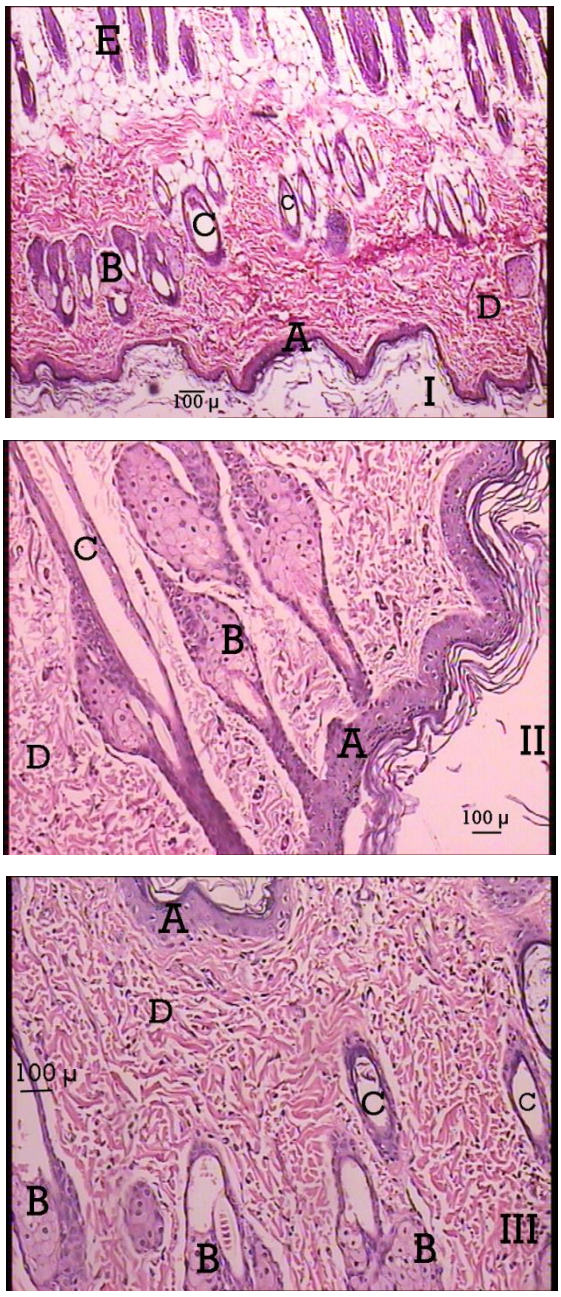

Figure 4. Photomicrographs of skin sections of Wistar rats stained with hematoxylin and eosin (H & E). (I): the saline control; group (II): the Fibrinolysin/DNAse-treated; group (III): the herbal Oppyheal ointment-treated animals. A: Epithelial Layers; B: Adipose Gland; C: Hair Follicle; D: Loose Connective Tissue; E: Muscle Layer

an antioxidant effect of *A. vera* gel extract in the animal model [18]. Other researchers have also evaluated the potential anticancer properties and modulatory effect of selected *A. vera* active principles on antioxidant enzyme activities [25]. *Aloe*-derived ingredients are used in a wide variety of cosmetic products at concentrations of 0.1-20% [24].

The concentrated material derived from *Aloe* in the present study has been prepared according to a previously published work [18]. It contained the *Hypericum perforatum* L. extract, freeze dried powder of *Aloe* leaf juice and *Papaver somniferum* seed oil. The results of the present study indicated that the new herbal Oppyheal ointment was as effective as the known Fibrinolysin/DNAse ointment in reducing the healing time and covering the injured tissue when given in animals with wounds on their back cervical skin. It has been previously demonstrated that aerial parts of *H. perforatum* possess remarkable wound healing and anti-inflammatory activities [20].

It seems that the present new herbal ointment which contained *H. perforatum* extract, freeze dried powder of *A. vera* leaf juice and *P. somniferum* seed oil could be useful to patients suffering from deep skin wounds.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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