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BORAGO OFFICINALIS HYDROETHANOLIC EXTRACT IMPROVED FULL THICKNESS WOUND HEALING PROCESS IN EXPERIMENTAL ANIMALS

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ABSTRACT

Borago officinalis has a wide for rheumatoid arthritis, cardiovascular disorder, and some type of gastrointestinal disorder, and we want to identify its effect on wound healing. For this goal we used forty-four inbred Wistar rats. Two circular surgical wounds were performed with a 7mm biopsy punch on the back of each animal. In the control group we did not use any ointment on wound area but in the second group as a placebo, we just used (60% Vaseline+ 40% Eucerin). The animals in our treatment groups were treated respectively, with 2% and 4% of Borago officinalis ointment during 21 days. The base of ointment is placebo in all groups. The test of wound closer were carried out on each Second, 4th, 6th, 8th, 10th, 12th and 14th days; and also histopathology evaluation were carried out on each 3rd, 7th, and 14th days. The result showed that two treatment groups, especially high concentration, have the most effect on wound healing process (granulation tissue formation and re-epithelialization) and these groups were more effective on the number of mononuclear cells, fibroblast and new vessel formation. With these effects and we concluded that the Borago officinalis extract, especially in higher doses (4%) could be useful for healing the excisional wounds.

Keywords: *Borago officinalis*, Hydroalcoholic Extract, Wound Healing, Full-Thickness Wounds, Rat

INTRODUCTION

One complication may happen time and again, during the life of a human being or animal, is skin laceration. Following rupture and break the protective barrier of the skin, immediately being complex natural process of wound healing, with the initial contraction of the torn blood vessels, and fallow it, migration of immune and fibroblast cells into the wound site. Typically, these events in the classic model of wound healing may be categorized into three or four sequential including the inflammatory, proliferates, and remodeling phases, that overlapping in time **[1]**.

Borago officinalis a member of the Boraginaceae family is an annual herb **[2]**. This plant is one of the best sources of gamma-linolenic acid (GLA), oleic acid, linoleic acid [3,4], phenolic compounds **[3,5]** and also and Rosmarinic acid **[6]**. There are many reports that derivatives in different parts of this plant, including leaves, flowers and or seeds, are used in cooking and medicine. They can be useful for many diseases such as rheumatoid arthritis and multiple sclerosis **[7]**, gastrointestinal, respiratory and cardiovascular disorders **[8]**.

The aim of the present study is to investigate of enhancing wound healing activity and wound contraction in topical application of hydroethanolic extract of Borago officinalis aerial part, which is used in the traditional form in some parts of Iran.

MATERIAL AND METHODS

Plant and Hydroalcoholic extract preparation

Fresh aerial part (leaves and flowers) of Borago officinalis was collected locally during June 2012 in the region of Hamadan city (latitude: 36 46', longitude: 48 34'); and identified by the Department of Botany Sciences, Herb Garden Avicenna in Hamadan Province, Hamadan, Iran. The plant material was cleaned and dried naturally on laboratory benches at room temperature (23-24ºC) for 10 days until crisp, and powdered in an electric blender. Then 350 g of the powder of plant material was suspended in 500 mL of an aqueous ethanol solution (7:3, v/v) for 7^{th} day at room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No 1). After filtration, the solvent was completely evaporated under vacuum at 40˚C in a rotary evaporator, and the hydroethanolic Borago officinalis was obtained after lyophilization.

Preliminary phytochemical assessment

Aerial part (flower and leaves) of Borago officinalis was undergone preliminary phytochemical evaluation in order to elevate major phytoconstituents in the extract formulation. The presence of phytochemicals, alkaloids, flavonoids, phenol and terpenoid were performed based on previous reports **[9]**.

Experimental animals

Forty-four healthy inbred male Wistar rats weighing (180-190 g) approximately nine weeks of age were obtained from the experimental animal house; Veterinary Colleges of Urmia University. The animals were left in separate cages for five days at room conditions for acclimatization. Animal houses were in standard environmental conditions of temperature (22 ±3°C), humidity (60 ± 5%), and a 12h light/dark cycle. The animals were maintained on standard pellet diet and tap water. The study was approved by the ethics committee for animal experimentation by the Faculty of Veterinary Medicine, Islamic Azad University-Urmia Branch.

Formulation of topical wound application forms

We prepared three variants of the topical application, form comprising of Commercial ointment base formulation (PARS DARUO. Ltd., Tehran. Iran) for this study. After creating of the surgical wound, all rats randomly were labeled by none toxic color and divided into four groups. Two groups served as controls: Group 1, didn't receive any administration and Group 2, placebo, animals were applied with base formulation. Groups 3 and 4, applied respectively, with the 2g and 4g of Borago officinalis hydroethanolic extract mixed with base formulation (BOO). The ointments were topically applied once a day, starting from the day of operation, on the wound area until the wound healed completely.

Circular excision wound model

This model of wound was used to monitor wound closure time and wound contraction. After induction of anesthesia with Xylazine HCL 2% (5 mg/kg/IP/Alfasan- Netherlands) and ketamine HCL 5% (60 mg/kg/IP/ Alfasan- Netherlands) rats were fixed in a ventral posture on a surgery table. The skin of the dorsal area from the scapula to the ileum was shaved with an electric clipper. After wound area preparation and scrubbed with 70% alcohol, two circular, full thickness surgical wounds with diameters of 7mm, 1 cm away from both sides of the backbone, were made with a 7mm biopsy punch. Post-surgery all animals housed and randomly divided into four groups of six each **[10,11]**.

Wound area measurement

A graph paper and a permanent marker were used in order to measure and record the wound size. The wound size was computed at the second, 4th, 6th, 8th, 10th, 12th and 14th days after surgery **[11,12]**.

Histologic evaluation

In order to, specimens from skin were taken in the 3th, 7th and 14th days after surgery, with the rats under general anesthesia. Sample tissues, excised along with 1 to 2mm surrounding normal skin and in a depth of approximately 3mm, were pinned on a flat cork surface and fixed in neutral-buffered formalin 10%. Then the sample tissues were routinely processed, paraffin wax embedded, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E) stain. Stained sections were then microscopically (by light microscope, Olympus CX31RBSF attached cameraman) evaluated to assess the predominant stage of wound healing **[11,12,13]**.

Statistical Analysis

All values are presented as mean ± S.E.M. Analysis were done by Variance method (ANOVA) considering P value < 0.05 significant. The statistical differences among groups were compared by Duncan multiple range test. Statistical analysis was performed using SPSS 18 software.

RESULTS

Preliminary phytochemical assessment

The phytochemical analysis of Borago officinalis hydroethanolic extract is presented in **Table 1**. According to the data, Borago officinalis hydroethanolic extract contains alkaloids, flavonoids, phenols and terpenoid **(Table 1)**.

Wound enclosure

The extract showed a significant decrease in the wound size beginning the third day (P<0.05). The sizes were reduced more quickly in the treatment groups compared to the control group. At the last day of measurement the wound in the treatment groups were approximately enclosed, while the control and placebo had rather large wounds **(Figure 1)**.

Wound epithelialization

When the wound is completely covered with epithelial tissue, named epithelialization time. As shown in Figure 2, wound surface were covered faster with by epithelial tissue in high percentage of BOO (4%) at 11.6 days after wound creation, while this time (average of the epithelialization time), in the group treated with the extract 2% BOO, equal to 13.2; and in groups placebo and control equal to 14.5 and 15.8, respectively. According to this finding, there were significant differences (p<0.05) in the mean epithelialization time between to treatment groups (specially BOO 4%), compared with control group, that this indicated the covered wound surface with epithelial tissue was dose dependent and high concentration of Borago officinalis hydroalcoholic extract, was effective.

Histologic evaluation

Observations demonstrated that, animals in BOO-treated groups exhibited well-formed granulation tissue on day 7 after wound creation. However, this situation was more considerable in 4% BOO-treated animals compared 2% BOO-treated and control groups. In three and seven days after wound creation, in treated groups, specially BOO 4%, significantly (P<0.05) lower poly morph nuclear (PMN) infiltration into the wound site compared to control group (P<0.05) **(Figure 4)**. Moreover, in same days, significantly (P<0.05) higher mononuclear cell infiltration observed in the treated groups, specially BOO 4%, comparing to control group **(Figure 5)**. Depending on administrated concentration of BOO, the new vessel formation, significantly (P<0.05) were increased by the time, specially BOO 4%, comparing to control group (Figure 6). Depending on administrated concentration of BOO, the fibroblasts distribution, significantly (P<0.05) were increased by the time, specially BOO 4%, comparing to control group **(Figure 7)**.

Table 1: Phytochemical Screening of Secondary Metabolites of hydroethanol aerial part extract of Borago officinalis aerial part	
Secondary metabolites	**hydroethanol extract**
Alkaloids	+
Flavonoids	+
Steroids	-
Terpenoid	+
Phenol	+
Note: + = present, - = absent	

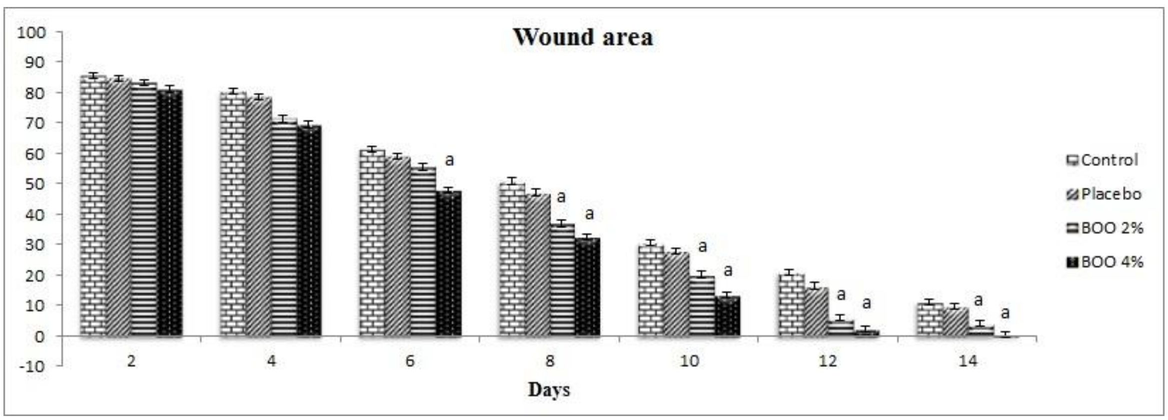

Figure 1: Effect of Borago officinalis hydroethanolic extracts on wound area contraction
Note: n= 6 rats in each group. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group.

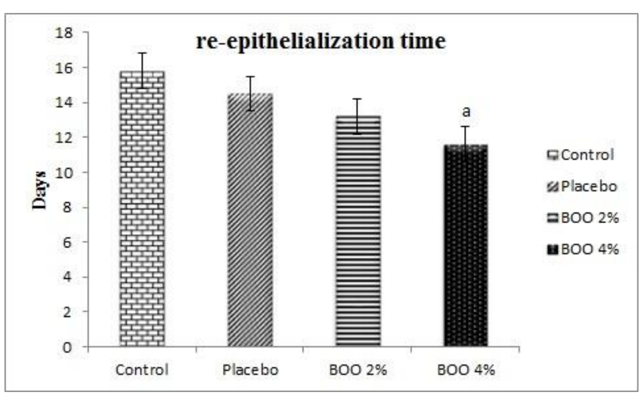

Figure 2: Effect of Borago officinalis extracts on days of re-epithelialization.
NOTE: n= 6 rats in each group. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group.

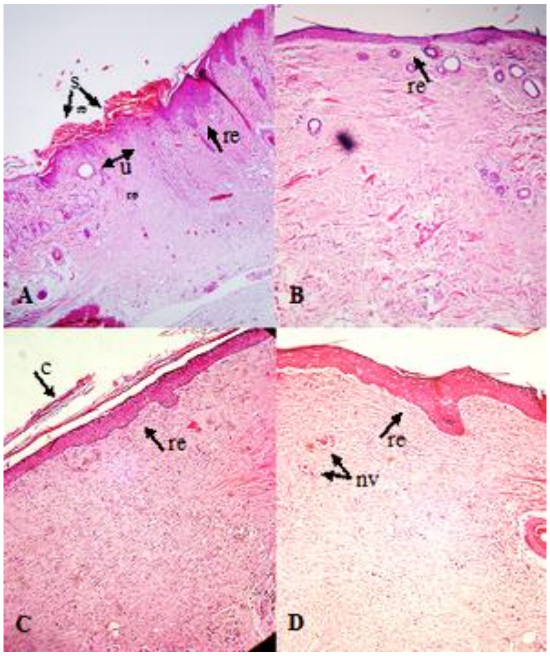

Figure 3: Histopathological view of skin wound healing in the 14th day after surgery
NOTE: A: control, B: placebo, C: Borago officinalis hydroethanolic extract ointment 2% and D: Borago officinalis hydroethanolic extract ointment 4%, administered animals. Skin sections show the hematoxylin and eosin (HE) stained in original magnification 100×. c: corneous tissue layer; nv: new vessels; re: re-epithelialization; s: scab and u: ulcer.

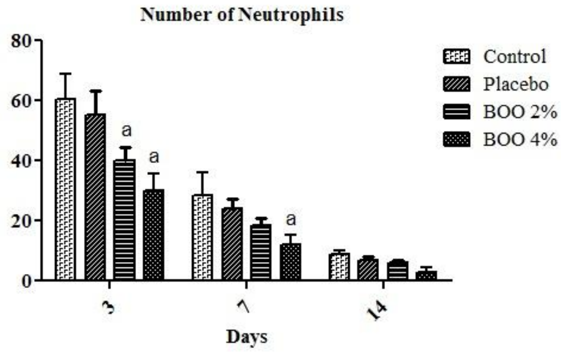

Figure 4: Effect of Borago officinalis hydroethanolic extract on poly morph nuclear cells (PMN) count.
n= 3 rats in each group and at every day. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group.

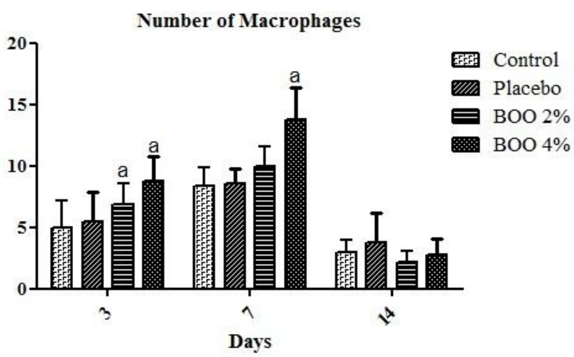

Figure 5: Effect of Borago officinalis hydroethanolic extract on mononuclear cells (MNC) count.
n= 3 rats in each group and at every day. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group

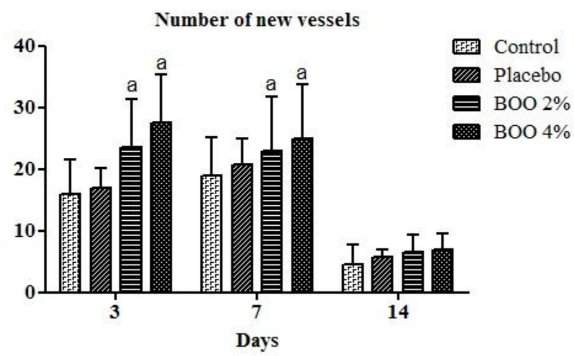

Figure 6: Effect of Borago officinalis extract on new vessels formation count
NOTE: n= 3 rats in each group and at every day. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group

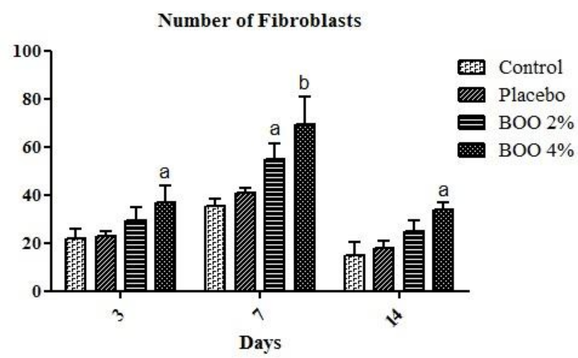

Figure 7: Effect of Borago officinalis extract on fibroblast cell disturbation.
n= 3 rats in each group and at every day. Values are mean ± S.D. [a] $p < 0.05$ compared to the control group

DISCUSSION

In the present study, we assessed the accelerating effect of Borago officinalis hydroethanolic xtract on full thickness wound healing process. Our phytochemical analyses showed that Borago officinalis hydroethanolic extract contained phenol and flavonoid. These components are known for antioxidant and anti-inflammatory properties **[14, 15]**.

There are many reports that Borago officinalis have antioxidant properties **[16, 17]**. On the other side many antioxidant components such as phenolic compounds and rosmarinic acid, which synthesized by many plants, as free radical scavengers **[18]**, have a multitude of biological activities and therapeutic effect such as antioxidant and anti-inflammatory activity **[19, 20]**, wound healing **[14, 21]**, antibacterial and antiviral **[22]**.

Immune cells, such as poly morph nuclear and Mononuclear cells infiltrate to the wound site during the inflammatory phase **[1]**. Neutrophils are the most important cells in the first phase of the healing process, which are indicative of tissue inflammation **[1, 23]**. Based on our histopathology findings, topical use of BOO, especially in higher doses, causes a significant decrease of poly morph nuclear cells (neutrophils) at the wound site, comparison control group at seven days after wound creation. Almost at the same time, BOO causes the significant increase of the mononuclear cells at the wound site; These effects may be due to the presence of phenolic compounds in Borago officinalis hydroethanol extract **[17]**. These cellular changes, indicating the shortening of the inflammatory phase, and began the proliferative phase immediately, in the treated animals.

By reducing inflammation in the wound site, granulation tissue increased. New vascular formation is needed on the wound site to feed the proliferation phase cells **[1]**. Our results showed that BOO, especially in higher doses (BOO 4%), causes the significant increase of new vascular formation in treated animals compared a control group at day 3 after wound creation.

Mononuclear cells, macrophages, as principal cells in the inflammatory phase of wound healing **[1, 23]**, in addition to phagocytosis, by secreting growth factors and cytokines, cause the fibroblasts migration into the wound site **[1, 23]**. Fibroblasts are needed to promote and facilitate collagen synthesis **[1]**. Our microscopic results and analyses showed that BOO, especially at higher doses (BOO 4%), caused a significant increase of the fibroblasts migration into the wound site in treated animals compared control group at day 7 after wound creation.

Follow collagen synthesis and secreting at wound site, wound contraction occurs, that with increasing the amount of secreted collagen, wound more contracts **[1]**. Our results showed that the BOO cause the significant decrease of wound area, especially at higher doses (BOO 4%). Moreover, our results showed that the BOO cause the significant increase of re-epithelialization, especially at higher doses (BOO 4%), comparison control group.

CONCLUSION

Based on the results obtained in this experimental research study, we suggest that topical administration of the Borago officinalis hydroethanol extract in differential concentration, especially ointment 4%, have beneficial effects on full thickness wound healing, as an in vivo experimental wound models in rats, and it may be suggested for treating various types of wounds in animals and human beings.

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