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WOUND HEALING ACTIVITY OF *SAMBUCUS EBULUS*

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ABSTRACT: This study aimed to evaluation of healing potential of *Sambucus ebulus* in full-thickness wound model in rats. A dorsal skin wound was created in rats. The 5% ointment applied to the wound area once a day for 12 days. Healing process was compared histologically between the groups. Parameters of wound healing were evaluated in the wound area. Extract improved wound contraction and closure. Histopathology study certified an organization of wound tissues. Extract reduced the number of inflammatory cells, increased wound-healing rate, epithelialization and significantly improved collagen formation. Granulation tissue formation was more organized when compared with control groups. *S. ebulus* showed positive effects on wound healing process and improved healing wounds.

INTRODUCTION: Wound healing is a physiologic process including several phases as inflammation, proliferation, granular tissue formation, re-epithelialization, matrix deposition and remodeling. Loss of skin integrity and wounds is leading to homeostatic imbalance¹. Wound accompanies with major concern for medical staff and seriously reduces the patient's life quality. Therefore, efforts to accelerate skin lesions along with ideal performance are clinical goals². Natural products are safe and have good physiological properties, as great sources for treating skin damage.

Sambucus ebulus (Caprifoliaceae) is widely grows in Iran³. Iranian herbal medicine uses it to treat some cases of inflammation like arthritis³. The effect of *S. ebulus* fruit on *Paederus* dermatitis has been reported via controlling the burning, pain, inflammation and infection⁴. Its antibacterial, antioxidant effects and its effect on treating burn, wounds, eczema, rash, inflammation and rheumatism have been studied^{3, 5}. It seems *S. ebulus* plays role in treating chronic inflammatory processes by expression inhibition of TNF α ⁶. The aim of the present study was to evaluate the *in vivo* wound healing effect of the *S. ebulus* fruit extract in rats.

MATERIALS AND METHODS:

Animals: Male Wistar albino rats (250–300 g) were housed in 3 groups at room temperature (22 \pm 2°C) and were maintained on a 12-h light/dark cycle with free access to standard diet and tap

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water. Animals were handled daily for at least three days before experiments. All experiments approved by the Research and Ethics Committee of Mazandaran University of Medical Science. Each animal was used only once.

Plant material and extraction: *S. ebulus* fruit were collected from Sari, Iran in Aug 2015 and a voucher specimen (No. 135) was deposited in Faculty of Pharmacy herbarium. Fruit were dried at room temperature. 500 gram of sample was fractionated by successive solvent extraction by percolation with hexane (2 L \times 3) then ethyl acetate (2 L \times 3) and finally methanol (2 L \times 3). We have recently improved toxicity of ethyl acetate fraction and safety of methanol fraction³. The resulting methanol extract was concentrated over a rotary vacuum evaporator until a solid extract sample was obtained which was then freeze-dried. A 5% ointment was prepared.

Wound creation and treatments: Rats were anaesthetized prior to creation of wounds, with intraperitoneal injection of ketamine (50 mg/kg) and xylezine (5 mg/kg). The dorsum of neck area was shaved and disinfected. A full-thickness wound of size 4 cm² was made by cutting out a 2 \times 2 cm piece of skin from the shaven area. After wound creation, animal were divided into three groups: I: control (C); II: negative control (ointment base treated, NC) and III: topically treatment 5% ointment (SE); the wounds were of full-thickness type extending up to the subcutaneous tissue. Materials were applied to the wound surface topically once daily. Wound healing rate determination was traced on 2 mm graph paper on days 3, 6, 9 and 12. Animal were sacrificed on last day.

The percentage of wound closure was calculated. Period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound. The areas of the wound were measured by tracing the wound boundaries using millimeter-scale transparent graph paper with a permanent marker. The degree of wound healing was calculated using the following formula: percentage of wound healing = $1 - [\text{wound area on the corresponding day (cm}^2\text{) / wound area on day zero (cm}^2\text{)}] \times 100$. WHR (%) = $[(W_i - W_s) / W_i] \times 100$.

Where W_i is initial wound area and W_s is specific day wound area⁷.

Histopathological studies: Skin specimens of healthy and healed area were cut out and immediately fixed in formalin for 24 h. After embedding in paraffin and processing, the specimens were sliced 4 μ m sections and stained with hematoxylin and eosin (HE) staining. Sections were qualitatively assessed under the light microscope with the Bio report OLYSIA soft Imaging System GmbH, version 3.2 (Build 670) and observed in respect of epithelialization, granulation tissue formation and severity of inflammation in healed area. For assessment the collagen deposition stained with trichrome masson.

Statistical Analysis: The results are expressed as Means \pm SEM. Differences among experimental groups were compared by one-way analysis of variance (ANOVA). All Statistical analyses were performed using SPSS statistical version 16.0 software package. P values <0.05 were considered statistically significant.

RESULTS: Skin damage has been evaluated macroscopically in all groups within 12 days. Wound healing process has been obvious in all groups but its speed and wound area contraction in extract treated group has been more than that of other groups. In microscopic evaluation, the mean epidermis thickness and collagen level have been studied. In extract treated group, the epidermal layer thickness (105.12 \pm 4.38 μ m) compared with that of C and NC groups (78.09 \pm 3.8 and 81.53 \pm 4.1 μ m, respectively) and had significantly increased (P<0.001) (**Fig.1**).

At 12th day, more intense and extensively spread inflammation with further inflammatory cells and severe necrosis have been observed in the control groups. Also the presence of neo-vascularization and the high counts of fibroblast cells and further collagen fibers have been spotted in experimental group in comparison with the control one. In experimental group, wound healing process proceed through reduced inflammation and fewer inflammatory cells, no necrosis, re-epithelialization, collagen deposit, and more developed differentiated fibers (**Fig.2**). Trichrome masson staining has led to meaningful increased

collagen level compared to control groups ($P < 0.001$) (**Fig.2**). The wounds treated extract in microscopic analysis with further fibroblasts and collagen fibers have been more organized than those of control groups (**Fig.2**). In order to analyze the inflammation, the neutrophil and lymphocyte cell counts in histological slides in groups have

been studied. A significant decrease has been observed in extract-treated group ($P < 0.001$). On 12th day, extract treated group showed about 95% wound healing, whereas it was 87% and 84% for rats treated with base and control groups, respectively.

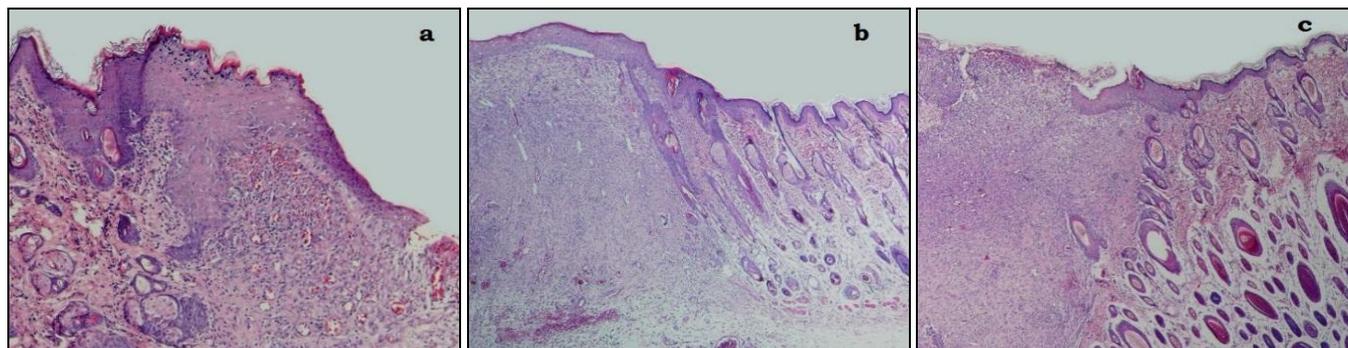


FIG. 1: PHOTOMICROGRAPH OF HISTOPATHOLOGICAL SECTION OF WOUND TISSUE OF RATS (STAINED WITH H&E, 40 X MAGNIFICATIONS). (a) CONTROL; (b) OINTMENT BASE TREATED AND (c) EXTRACT TREATED GROUP.

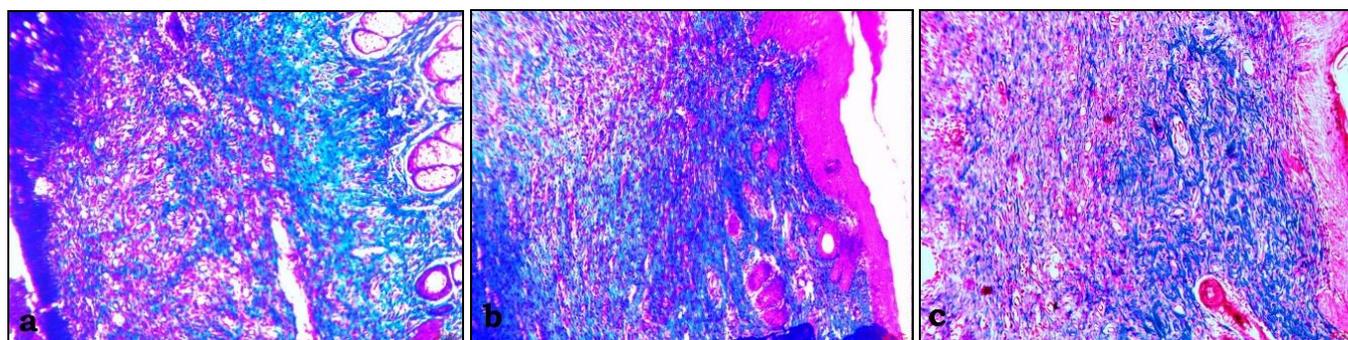


FIG. 2: PHOTOMICROGRAPH OF HISTOPATHOLOGICAL SECTION OF WOUND TISSUE OF RATS (STAINED WITH TRICHOME MASON, 40 X MAGNIFICATIONS). (a) CONTROL; (b) OINTMENT BASE TREATED AND (c) EXTRACT TREATED GROUP.

DISCUSSION: Results revealed a good efficacy of extract on wound healing progress by accelerating healing process. Fibroblasts count goes up and collagen forms and the inflammatory cells count drops. They required for the wound healing acceleration and the granular tissue formation. Histological evaluation of the wound area showed a more organized tissue in TS groups. Wound healing is a spontaneous process and encompasses homeostasis, inflammation, reproduction and regeneration phases. When wound is created tissue is at risk of infection. Acceleration wound healing, minimizing pain and scar formation and prevention of infection is a goal ⁸.

The wound model has been employed for evaluating the wound surface retraction and the plant extract's re-epithelialization capacity on the wound. The wound surface retraction signifies the

wound getting healed faster. In histopathological evaluation, the stages of wound healing processes (inflammation, proliferation and remodeling) have been observed. **Fig. 1** depicts the wound healing process delay in groups. In the extract treatment group, re-epithelialization and proliferation has been seen more rapidly.

The wound surface contraction speed in groups has had clearer effect in extract treated group from 3rd day compared to other groups. Anti-inflammatory activity of extract may have a major role in its wound healing ⁴. By reducing the inflammation intensity, extract can move the wound to healing process remodeling stage. At 12th day in experimental group, the inflammatory cells infiltration in the wound location has got lower. The new capillaries and fibroblast in the experimental group have been significantly clear

and observable. Collagen is produced by fibroblast cells in the proliferation phase of wound healing process⁹. Trichrome masson staining improved increasing collagen density in the wound location in extract treated group. The process of wound healing depends on biosynthesis and collagen deposit and its maturation. Critically increase in granular tissue formation was observed in extract treated group.

Many *S. ebulus* photochemicals such as flavonoid glycosides have been reported¹⁰. They are anti-ulcer property¹¹. The anti-inflammatory effect of *S. ebulus* may be due to its flavonoid or steroid substances. Flavonoids inhibit lipid peroxidation, and cell damage prevention result in collagen fibers increase¹². Base on anti-microbial properties, flavonoids accelerate wound healing process.

CONCLUSIONS: These findings show extract influences the wound healing proliferation and regeneration through revascularization pathway, collagen deposit, granular tissue formation, re-epithelialization and wound surface contraction. These result support positive effects of extract on wound healing. These results introduced *S. ebulus* as easily accessible source of natural products.

CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest.

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