

Evaluation of Wound Healing Activity of Herbal Formulation

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The wound healing activity of one herbal formulation (Zakhme heal ointment) containing Rai, Suhaga, Fitkari, Swarnageru, Singdaraj in paraffin base was evaluated for its wound healing potential in two types of wound models in rats (i) excision wound model and (ii) incision wound model. The formulations responded significantly in both the wound models tested. The results were also comparable to that of the standard drug nitrofurazone used as a standard drug for comparison in this present investigation. The results were also comparable in terms of wound contracting ability, epithelialization period, tensile strength and regeneration of tissues at the wound area. Thus, this investigation confirms the use of the Zakhme heal ointment as wound-healing formulation.

Key Words: Zakhme heal ointment, Nitrofurazone, Excision wound, Incision wound, Wound healing.

INTRODUCTION

The wounds are trauma, which are defined as disruption of cellular and anatomic or functional continuity of living tissues. Wounds may occur due to physical, chemical or radiological insult to the living tissue¹. The wounds exposed to the external environment are likely to infect by microorganisms. Moreover wounds are associated with pain, making the patient feel uncomfortable and unable to work. There is a considerable economic loss to the nation by loss man hours due to wounds. Hence the wound healing is an important subject in therapeutics. The wound healing is a complex phenomenon involving self generated autocooids and hormones working in a systematic synchrony leading to wound healing. The plants are rich source of phytochemicals, which are having wound healing properties^{2,3}. These plants are used in different formulation as wound healing agents. Here in this project one such formulation was taken up to ascertain its activity as wound healing agent.

EXPERIMENTAL

For the present work a marketed herbal formulation was taken up for screening of wound healing activity. Zakhme heal ointment was purchased from local market of Mandsaur (Madhaya Pradesh), India. Content of the ointment are Rai (450 mg), Suhaga (375 mg), Fitkari (375 mg), Swarnageru (450 mg), Singdaraj (300 mg) in paraffin base (q.s.).

Wound-healing activity: Excision and incision wound models were used to evaluate the wound-healing activity. Albino rats of either sex weighing 150-200 g were taken for study and maintained at standard housing conditions. The animals were fed with a commercial diet and water *ad libitum* during the experiment. The institutional animal ethics committee, B.R. Nahata College of Pharmacy, BRNSS-CRC, Mandsaur, permitted the study.

Excision wound: The rats were inflicted with excision wounds as described by Morton and Malone⁴ under light ether anesthesia. A circular wound of about 250 sq. mm was made on depilated ethanol sterilized dorsal thoracic region of the rats. The animals were divided into 3 groups of six each. The animals of group I were treated with simple ointment and considered as the control, group II served as reference standard and treated with 0.2% w/w nitrofurazone ointment (NO), animals of group III were treated with 50 mg of the tested ointment. The ointment was topically applied once a day, starting from the day of the operation, till complete epithelialization. The parameters studied were wound closure and epithelialization time. The wounds were traced on mm² graph paper on 6th, 12th and 18th days and thereafter on alternate days until healing was complete. The percentage of wound closure was calculated. The 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.

Incision wound: In incision wound model, 6 cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat as described by Ehrlich and Hunt⁵. The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into 3 groups of six animals each. The animals of group I were treated and considered as the control, the group II served as reference standard and received 0.2% w/w nitrofurazone (NO) ointment, animals in groups III were treated with 50 mg of the tested ointment. The ointment was topically applied once in a day. The sutures were removed on the 8th post wound day. The skin tensile strength of the wounds was measured on the 10th d as described in the method of Lee *et al.*⁶

RESULTS AND DISCUSSION

Significant promotion of wound-healing activity was observed in the Zakhme heal ointment in the both of the wound models such as excision and incision wound. In excision wound model, the mean percentage closure of wound area was calculated on the 6, 12 and 18 post wounding days (Table-1). The period of epithelialization was 20.03 ± 0.39 in the case of standard drug 0.2 % w/w nitrofurazone (NO) ointment. In incision wound model, the Zakhme heal ointment treated animals showed increase in tensile strength (420.33 ± 5.92), when compared to the control (277.86 ± 03.19). The mean tensile strength in animals treated with standard drug was 497.13 ± 06.06 (Table-2). Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen is the major component of extra cellular tissue, which gives support and strength and is composed of amino acid (hydroxyproline). Increase in tensile strength of granulation tissue of Zakhme heal ointment treated animals indicated the enhanced collagen maturation by increased crosslinking⁷. Similar types of wound-healing activity were reported on two herbal formulations Himax ointment and lotion⁸. In conclusion the marketed herbal formulations Zakhme heal ointment was found to contain significant wound healing activity.

TABLE-1
EFFECT OF ORAL ADMINISTRATION OF HERBAL FORMULATION ON
HEALING OF EXCISION WOUND MODEL

Group	Wound area (mm ²) Post wounding (d)				Period of epithelialization
	0	6	12	18	
Control	502.6 ± 5.37 (0.0)	412.83 ± 15.05 (17.86)	304.66 ± 10.82 (39.38)	191.83 ± 9.803 (61.83)	26.26 ± 0.40*
Standard	506.5 ± 5.51 (0.0)	294.5 ± 17.30 (41.85)	304.66 ± 10.82 (39.38)	1.003 ± 0.82 (99.80)	20.03 ± 0.39*
Herbal formulation	51.33 ± 4.6* (0.0)	399.66 ± 6.86* (21.68)	194.33 ± 7.75* (61.92)	49.66 ± 4.41* (90.269)	23.17 ± 0.54*

Values are expressed as mean ± SEM; n = animals in each group-6; no. in parenthesis indicates percentage of wound contraction; *p ≤ 0.001 when compared to control group.

TABLE-2
EFFECT OF ORAL ADMINISTRATION OF HERBAL FORMULATION
ON TENSILE STRENGTH (gms) OF RESUTURED INCISION WOUND
ON 10th POST WOUNDING (d)

Group	Tensile strength (gms)
Control	277.86 ± 3.19
Standard	497.13 ± 06.06
Herbal formulations	420.33 ± 5.92*

Values are expressed as mean ± SEM; n = animals in each group-6;

*p ≤ 0.001 when compared to control group.

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