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Formulation and Evaluation of New Polyherbal Formulations for Their Wound Healing Activity in Rat

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Abstract

The newly prepared polyherbal formulations (Ointment and Gel) were evaluated for their wound healing activity in Excision and Incision wound models in rats. The wound-healing activity was assessed by the rate of wound contraction, period of epithelialization and skin-breaking strength. In Excision wound model the polyherbal Ointment treated group exhibit 100% reduction in wound area on 20th day when compared to standard Betadine Ointment and controls which was 98.55±0.40 % and 90.10±0.75% and faster rate of epithelization (16.55 ± 0.54). In the incision wound model, there was a significant increase in tensile strength (538.1± 2.6) was observed in ointment treated group. In all cases, there was a progressive decrease in wound area with time, indicating an efficacy of the formulations in healing the induced wounds. Our present study reveals that the new polyherbal formulations posses potent wound healing activity, which could be a good choice of remedy for wound healing.

KEYWORDS: *Excision wound model, Incision wound model, Polyherbal Formulations, Betadine Ointment.*

Introduction

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[1]. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue [2] . Wound healing is the process of repair that follows injury to the skin and other soft tissues. Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation (0–3days), cellular proliferation (3–12 days) and remodeling (3–6 months) (Glynn, 1981; Clark, 1996; Martin, 1996) [1]. Healing of wounds is one of the important areas of clinical medicines explained in many Ayurvedic texts under the heading "Vranaropaka". According to the Ayurveda, Vrana (wounds or ulcers) is the discontinuation of lining membrane that after healing leaves a scar for life closely resembling the modern definition [3]. Similarly, inflammation is considered to be an early phase in the pathogenesis of wounds termed Vranashotha. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient [4] .

Plants and their extracts have immense potential for the management and treatment of wounds. The phyto-medicines for wound healing are not only cheap and affordable but are also safe as hyper sensitive reactions are rarely encountered with the use of these agents [2]. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds.

Materials and Methods

Materials:

All **crude drugs** (*Erythrina indica, Bergenia ciliata , Cissampelos pareira)* were provided by **SG. Phyto Pharma Pvt. Ltd. Kolhapur, Maharashtra.** which were authenticated as per Ayurvedic standards

as well as our Pharmacognostic authentification is also included to establish proper selection. And also all the **Chemicals** like Liquid paraffin, Hard paraffin, Glycerin, Triethanolamine, Petroleum jelly, Cetostearyl alcohol, PEG 6000,Methylparaben, Carbomer934P were supplied by the **SG. Phyto Pharma Pvt. Ltd. Kolhapur, Maharashtra.**

Methods:

Preparation of extracts:

Air dried coarsely powdered plant materials of (*Erythrina indica, Bergenia ciliata & Cissampelos pareira*) were extracted with ethanol (95%) using soxhlet apparatus for 4-5 hrs. All the extracts were concentrated at low pressure by rotary flash evaporator and finally air-dried.

Formulation:

The dried ethanolic extracts of *Erythrina indica, Bergenia ciliata & Cissampelos pareira (5 gm each)* were taken for the preparation of 100gm Ointment and 100gm Gel formulations. The Ointment and Gel formulations were prepared by using an Ointment base and Gel base. Standard method of fusion were used, where the solid fats were melted and mixed by continuous trituration [4]. The required quantity of the ointment base and gel base was weighed and melted at a temperature of about 70^{O}C in a hot water bath. The designated quantity of the extract (s) were respectively added to the melted base at 40^{O}C and the mix, stirred gently and continuously until a homogenous dispersion is obtained [5].

Animals:

Healthy wistar rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard diet and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions .

Grouping of animals

Animals were divided in to six groups, each group consisting of 6 rats.

Group I : Treated with plain ointment base and served as control-I.

Group II : Treated with reference standard ointment i.e. Betadine ointment.

Group III : Treated with new polyherbal ointment dosage form.

Group IV : Treated with plain Gel base and served as control-II.

Group V : Treated with reference standard drug i.e. Framycetin sulphate cream.

Group VI : Treated with new polyherbal Gel dosage form.

***Excision wound model*: [6]**

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circular wound of about 300 mm^2 area and 2 mm depth was excised on depilated dorsal thoracic region of excised rats, 5 cm away from ear . The entire wound was left open. The treatment was done topically in all the cases. The wounds were traced on transparent tracing paper by permanent marker on the day of wounding and subsequently on alternate days until healing was complete. Wound areas were measured on days 1, 4, 8 ,16 and 20 for all groups.

***Incision wound model:* [7]**

Two 6-cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat. Wounds were closed with interrupted sutures, 1 cm apart with the help of black silk surgical thread and a curved needle(no.11). The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding by continuous constant water supply technique.[17]

Statistical Analysis:

The data was statistically analyzed by one-way ANOVA followed by Dunnett multiple comparison test with equal sample size. The difference was considered significant when $p<0.001$. All the values were expressed as mean ± standard deviation (S.D.).

Table 1: Effect of New Polyherbal Formulations on Healing of Excision Wound Model

Group	Percentage wound contraction on post wounding days					Epithelialization in days
	4th	8th	12th	16th	20th	
Group I	17.32±1.05	34.41±0.90	58.66±0.59	81.03±1.25	90.10±0.75	22.5 ± 0.4
Group II	32.22±0.55	63.70±0.50	78.05±0.60	89.12±0.25	98.55±0.40	17.80 ± 0.35
Group III	33.52±0.23	67.81±0.10	82.55±0.31	93.40.±0.34	100±0.0	16.55 ± 0.54
Group IV	18.20±0.34	35.97±0.40	61.50±0.67	82.25±0.35	91.02±0.76	21.52 ± 0.45
Group V	31.85±0.10	64.05±0.12	83.44±0.44	91.80±0.77	98.20±0.55	17.50 ± 0.40
Group VI	30.36±0.05	63.91±0.78	81.90±0.30	90.30±1.55	97.65±0.75	18.20 ± 0.30

Table 2: Effect of New Polyherbal Formulations On Healing Of Incision Wound Model

Sr. No	Groups	Tensile strength of skin (g)
1	Group -I	359.60 ± 4.4*
2	Group -II	493.06 ± 3.3*
3	Group- III	538.10± 2.6*
4	Group- IV	348.30 ± 3.7
5	Group -V	491.06 ± 3.6*
6	Group -VI	484.60 ± 2.4*

Values are Mean ± S.D. of six animals in each group. *p<0.001 as compared to control

Results

The studies on excision wound healing model reveals that all the six groups showed decreased wound area from day to day. However on 20th post wounding day, Group-I animals showed 90.10±0.75 % of healing , where as Group-II and Group-III animals showed 98.55±0.40 % and 100.00 % healing. On the other hand, Group IV animals showed 91.02±0.76 % of wound healing , while Group V and Group VI animals showed 98.20±0.55% and 97.65±0.75% of wound healing (table 1). All readings are found to be statistically significant and comparable with control. The epithelization time i.e. time at which complete scar formation occurs, also suggest that both polyherbal ointments and polyherbal gel treated group were found to be significant and comparable with control (table 2). On the basis of the results obtained in the present investigation, it is concluded that the new polyherbal ointment and polyherbal Gel has significant wound healing activity. Wound healing activity of new polyherbal ointment formulation was found to be better than the standard Betadine ointment group.

Discussion

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation which are concurrent but independent to each other. Hence in the present study two different wound models were used. In the excision wound model, animals treated with the polyherbal ointment showed better and fast healing compared to the standard and control groups. Also there was significant decrease in the epithelization period. In the incision wound model, there was significant increase in the tensile strength of the 10 days old wound due to treatment with polyherbal ointment .

Conclusion

Literature survey revealed that the above plants were used traditionally for various ailments, especially for its wound healing property. Extensive scientific studies were not performed on these plants. Its wound healing property was not under taken for any scientific study. Both formulations i,e Polyherbal ointment and polyherbal Gel prepared from the ethanolic extracts of *Erythrina indica, Bergenia ciliata & Cissampelos pareira* showed the better wound healing activity in rats. Further experiments have to be conducted in other animal models for wound healing activity. Based on above tests, trails on human being can be performed.

"Cite this article"

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