ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 01 Issue - 03 MAY-JUN 2013

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Review Article

WOUND HEALING ACTIVITY OF PREPARED POLYHERBAL FORMULATION

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Received: 06 May 2013, *Revised and Accepted: 15May 2013*

ABSTRACT

In this study, the effect of prepared polyherbal formulation for effective healing of wound was evaluated by applying excision and incision wound models in healthy albino rats. In the excision wound model, rats were categorized into 3 groups of five rats each. Groups 1 served as controls, Group 2, received standard treatment with Nitrofurazone, while Groups 3 were treated with prepared Polyherbal ointment (study group). The wound-healing activity was assessed by estimating reduction in wound surface area, the time required for complete epithelialization, and skin-breaking strength. Histological study of the granulation tissue was carried out to know the extent of collagen formation in the wound tissue. In the Excision wound model, Polyherbal-treated animals showed significant (P<0.001) reduction in the wound surface area and faster rate of epithelialization as compared to controls (23.17±0.54 days vs 26.26±0.40 days, respectively). Again, rats in three groups of 05 animals each, were studied using incision wound model, where, polyherbal-formulation treated animals demonstrated significantly (P<0.001) higher skin-breaking strength (420.33±5.92 g vs 277.86±0.3.19 g). Furthermore, histological studies showed a significantly higher activity in the formulation-treated group. Our present study revealed that the prepared polyherbal formulation possesses a potent wound healing activity, and though less potent than standard Nitrofurazone, could be a good choice of remedy for wound healing.

Key Words: *Excision wound model, Incision wound model, Polyherbal ointment, Histopathological studies.*

INTRODUCTION

The concept of developing herbal drugs from medicinal plants used in indigenous medicine is followed from time immemorial. In some cases direct link between a local person and a medical practitioner exists. In other cases the relationship is much more diverse and complex. Wounds and especially chronic wounds are major concerns for the patient as well as the clinician. Chronic wounds affect a large number of patients and seriously reduce their normal life and life span.

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Worldwide the herbal industry is picking up at a fast pace. Herbs and botanicals now appear in more products and have more medicinal applications than ever before. India is blessed with diverse agro-climatic conditions and is a major source for a wide variety of medicinal plants. But the production potential is largely underexploited [1]. The therapeutic efficacies of many indigenous plants for various diseases have been described by traditional herbal medicine practitioners [2]. Natural products are a source of synthetic and traditional herbal medicine [3]. The Indian traditional system of medicine described several drugs of plant, mineral, and animal origin are in the Ayurveda for their wound healing properties, Scientists who are trying to develop newer drugs from natural resources are looking toward the Ayurveda [4]. The presence of various life sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties. Researchers who have explored the complex dynamics of tissue repair have identified several nutritional cofactors involved in tissue regeneration, including vitamins A, C, and E, zinc, arginine, glutamine, and glucosaminev [5].Cutaneous wounds are the result of disrupted skin integrity. The healing process depends on local wound factors, systemic mediators, the underlying disease, and the type of injury. These factors combine to determine if physiologic or acute wound healing occurs, or if there is an abnormal healing process, also called chronic wound healing. Chronic wounds are the result of an inadequate repair process that is unable to restore anatomic and functional integrity in an appropriate length of time. Chronic wounds affect about 1% of the European population and are frequently a management challenge, even with an interdisciplinary approach. In addition to having an adverse effect on the quality of life of the affected individuals, chronic wounds also create a significant economic burden: nearly 2% of health budgets are devoted to the care of chronic wounds [6].Tissue injury initiates a response that first clears the wound of devitalized tissue and foreign material, setting the stage for subsequent tissue healing and regeneration. The second phase of wound healing, the inflammatory phase, presents itself as erythema, swelling, and warmth, and is often associated with pain [7]. In the late inflammatory phase, monocytes converted in the tissue to macrophages, which digest and kill bacterial pathogens, scavenge tissue debris and destroy remaining neutrophils. The subsequent proliferative phase is dominated by the formation of granulation tissue and epithelialization. Its duration is dependent on the size of the wound. Collagen levels rise continually for approximately three weeks. The amount of collagen secreted during this period determines the tensile strength of the wound. The final phase of wound healing is wound remodeling, including a reorganization of new collagen fibers, to form more organized lattice structure that progressively continues to increase wound tensile strength [8].

The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient. At the site of wound closure a flexible and fine scar with high tensile strength is desired. Understanding the healing process and nutritional influences on wound outcome is critical to successful management of wound patients. Therefore the present research is towards exploring scar and wound healing properties of a polyherbal formulation using excision and incision wound models.

MATERIALS & METHODS:

Materials:

Neem (*Azadirichta indica*) extract was prepared by maceration in the laboratory which was further purified and dried, Haldi (*Curcuma longa*) powder (edible) was purchased from local market, Ghee (edible and prepared from cow milk) was purchased from local dairy and Ras kapoor was procured as gift sample from Pratibha herbs (Indore, M.P.), all other chemicles used were of AR grade and were purchased from local vendor (s).Healthy Albino rats weighing 150-200g were used for the study. They were individually housed and maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after the experiment. All animals used in this study received human care as defined by the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publications no. 80-23, revised 1978). The Ethics Committee for animal experimentation approved the study protocol.

Experimental methodology:

Preparation of polyherbal ointment:

The polyherbal ointment was prepared by using Neem Extract (20.00%), Haldi powder (7.00%), Ghee (2.00%), Ras kapoor (0.10%), and Base (q.s.), on lab-scale ointment mill, which was further characterized for viscosity and stability at various temperatures [9,10].

Excision wound model (Experiment 1):

In this experiment, the wound site was prepared as pre-established method (s) [9]. Animals were anesthetized by diethyl ether and the stage was maintained during infliction of the experimental wound. Then, under sterile conditions, a wound was made on dorsal thoracic region (500 sq. mm) and animals were allowed to recover spontaneously. The rats so prepared were assigned to one of three different experimental groups of five animals each. Group 1 animals received simple ointment and served as controls; Group 2 animals received standard treatment with 0.2% w/w Nitrofurazone; while Group 3 animals were treated with the polyherbal ointment (Study Group). Animals which showed signs of postoperative infection were separated, excluded from the study and replaced with new animals. Now, all animals were observed at regular intervals for wound healing and time of complete epithelialization at days 5, 10 & 15 post-operatively. Incision wound model (Experiment 2): Similar to Experiment 1, Albino rats (150-200 g) were categorized in three different experimental groups of five rats each. Under inhalational ether anesthesia, the dorsal fur of the animals was shaved with an electric clipper. A 6-cm longitudinal paravertebral incision was made through the skin and cutaneous tissue on the back [10]. After the incision, the parted skin was sutured, 1 cm apart, using a surgical thread and curved needle. The wounds were left undressed [11]. Formulation was topically applied to the wound once a day. The sutures were removed on the 8th day (post wound) and application of the formulation was continued thereafter. The skin-breaking strength was measured by the method of Lee [12] on the 10th day evening after the last application.

Table 1: Excision wound model

Group	***Wound area (mm^2) post wounding days***				***Period of epithelialization***
	0	***5***	***10***	***15***	
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*
Polyherbal formulation	513.3±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 =5 animals in each group; no in parenthesis indicates percentage of wound contraction;* p≤0.001 when compared to control group using one way ANOVA.

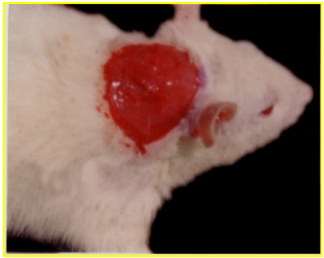
Table 2 -Effect of Polyherbal Formulation on Tensile Strength of Resutured Incision Wound on 10th Post Wounding Day

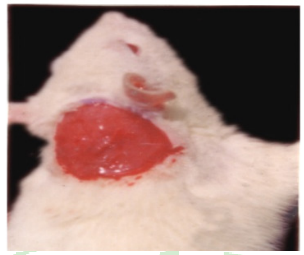
Group	***Tensile strength (g)***
Control	277.86±03.19
Standard	497.13±06.06
Polyherbal formulation	420.33±5.92***

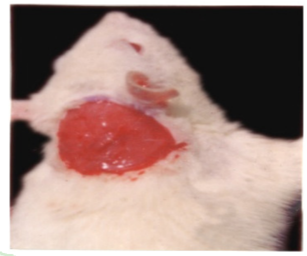
Values are expressed as mean±SEM; n =5 animals in each group; *** p≤0.001 when compared to control group using one way ANOVA.

Figure I. Comparison of wound site by excision wound model in control, Standard and Study groups at days 0 and 15 post-operatively.

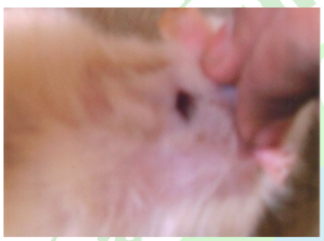
Control (Simple Ointment) ***Standard (0.2% w/w nitro furazone)*** ***Test (Poly-I)***







Day 0 **Day 0** **Day 0**



Day 15 postoperatively **Day 15 postoperatively** **Day 15 postoperatively**

Histopathological study:

The healing tissues (granulation tissue) obtained on the 10^{th} day from all three groups of animals of the incision wound model were processed for histological study. The amount of collagen was quantified using Vangeison stain.

Statistical analysis:

All the data were collected and expressed as mean values ± SEM, which were further subjected to compared using ANOVA with correction for the small numbers in each group (Values of $P<0.05$ were considered to be statistically significant).

Figure II. Histological study of granulation tissue obtained from the control, standard and study groups using Vangeison stain

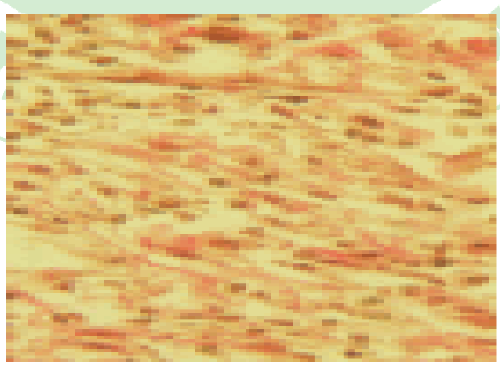


Figure II-a: Granulation tissue of group 1 animal (control) showing with less collagen and more macrophages (Vangeison stain).

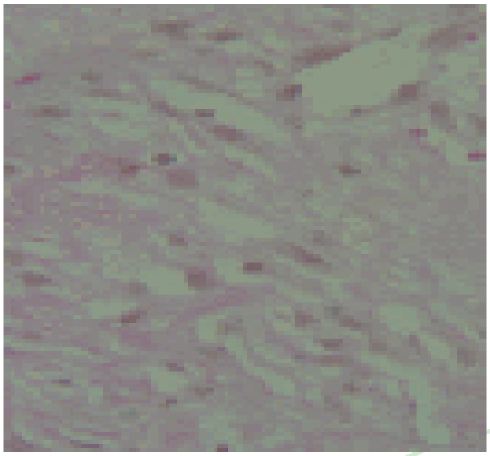


Figure II-b: Granulation tissue of group 2 (standard) animal showing moderate deposition collagen (Vangeison stain).

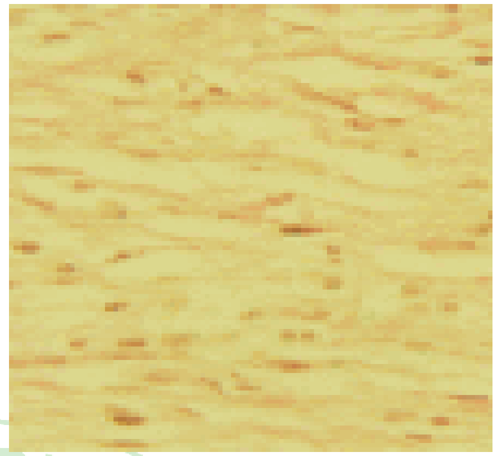


Figure II-c: Granulation tissue of group 3 animal showing more collagen and less macrophages (Vangeison stain).

RESULTS

In both excision and incision wound models, significantly improved wound-healing activity has been observed with the prepared polyherbal ointment, compared to that of the reference standard and control group of animals. In the excision wound model (Experiment 1), marketed formulation treated animals showed significant reduction in the wound area (P< 0.001) (Figure I), faster rate of epithelialization (23.17±0.54 days *vs* 26.26±0.40 days, respectively) (Table 1), increased dry weight of the tissue (P<0.001) and increased hydroxyproline content (P<0.001) (compared with the control group of animals). While, in incision wound model (Table 2), the treated animals in the study group demonstrated significant skin-breaking strength when compared to control animals (420.33±5.92g *vs* 277.86±0.3.19 g, respectively), along with significant increase in the weight of the granulation tissue (P<0.001) and hydroxyproline (P<0.001) content were also observed in animals treated with the polyherbal formulation when compared to the control group of animals. The histological studies of the tissue obtained from the treated group showed significant increase in collagen deposition, with very little macrophages, less tissue edema and more fibroblasts (Figures II-a and Figure II-c, respectively), as compared to control. It was more or less equal to Group 2 animals treated with standard 0.2% w/w Nitro-furazone (Figure II-b).

DISCUSSION

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 mainly depends on the repairing ability of the tissue, type and extent of 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 composed of amino acid (hydroxyproline) is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides; measurement of the hydroxyproline could be used as an index for collagen turnover. Based on the data presented, it may be concluded that prepared polyherbal ointment promotes wound-healing activity. It may therefore be suggested for treating various types of wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity.

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