To Develop and Evaluate Polyherbal formulation for wound healing activity

Pragya Balodiya, Dr. R.B. Goswami, Mr. Manoj Sahu.

Sagar Institute of Research and Technology-Pharmacy, Bhopal

Objective- To develop and evaluate polyherbal formulation for wound healing activity.

Materials and Methods-Wistar albino rats(130-150gm)were divided into four groups (n=6). Group I(control) animals received no treatment, group II animals were applied standard preparation(Betadine), group III animals were applied 2% herbal ointment formulation and group IV were applied 5% herbal ointment formulation topically. The treatment continued till the complete healing of the wound. The Rate of wound contraction and the period of epithelization was evaluated thereafter. The data were analyzed by one- way anova. $P<0.05$ is considered significant. In incision wound model, the treatment was given for 10 days only from day of wound, skin breaking strength were estimated.

Keywords-Azadirachta indica, Hibiscus rosa-sinesis, wound healing, Polyherbal ointment, excision wound model, incision wound model, Betadine

INTRODUCTION

Wound is defined as the cellular and anatomic disruption of tissue that may be caused by chemical, physical, microbial, thermal, or immunological damage to the tissue. Wound healing is the restoration of the structure and function of injured tissue to approximate pre wound characteristics.In other words, the wound is damage or disruption to the normal anatomical structure and function. This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs, and even bone. (**Ikobi et al., 2012)[1].**

Wound healing is a complex process in which the skin, and the tissues under it, repair themselves after injury. In this article, wound healing is depicted in a discrete timeline of physical attributes (phases) constituting the post-trauma repairing process. In undamaged skin,the epidermis (surfaceand dermis (deeper layer) form a protective barrier against the external environment. When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage. This process is divided into predictable phases: blood clotting (hemostasis), inflammation, tissue growth (proliferation), and tissue remodeling (maturation). Blood clotting may be considered to be part of the inflammation stage instead of a separate stage. **(Ngugen D.T, Orgill DP)[2].**

Wound healing begins at the moment of injury and involves both resident and migratory cell populations, extracellular matrix and the action of soluble mediators. The mechanisms underlying the processes described

above involve: (i) inflammatory mediators and growth factors; (ii) cell-cell and cell-extracellular matrix interactions that govern cell proliferation, migration, and differentiation; (iii) events involved with epithelialization, fibroplasia and angiogenesis; (iv) wound contraction; and (v) remodeling.These mechanisms are initiated at the time of physical injury and proceed continuously throughout the repair process. **(Labler et al., 2006)[3]**. Even though the processes of repair begin immediately after an injury in all tissues and that all wounds go through similar phases of healing, specialized tissues such as liver, skeletal tissue, and the eye have distinctive forms of regeneration and repair and follow separate pathways.

MATERIAL AND METHODS

Plant collection and Authentication

The bark of medicinal plant Azadirachta indica and flowers of Hibiscus rosa- sinesis was collected locally from Bhopal, M.P. and were authenticated by Dr. Saba naaz, Department of Botany, Safia Science College, Bhopal, M.P. A voucher specimen has been deposited in the department for further reference.

Drying and Size Reduction of Plants

The bark and flowers of medicinal plant were sorted, cleaned and shade dried at room temperature for 3 weeks and then coarse powdered to prepare the extract.

Extraction of Plant

The air dried medicinal plant of Azadirachta indica (Bark) and hibiscus rosa- sinesis (flowers) was coarsely powdered and extracted by cold maceration process, using ethanol (75% v/v) and kept for 4 days. Extract after filtration was dried by using rotary vaccum evaporator under low pressure at 40 -50 ℃ and finally air dried. The percentage yield was calculated using the following formula: **(PatelA. 2013)[4].**

Percentage Yield = Practical yield / Theoretical yield

Formulation of ointment

a) **Preparation of 100gm water soluble ointment:**

An ointment with water soluble base was of first choice due to their ease of preparation and also eases of cleaning after application. PEG 4000 (40%) and PEG 600 (60%) ointment base are mainly prepared by fusion method. In fusion method, the component are melted in decreasing order of their melting point, i.e, PEG 4000 are melted first on water bath at 65-70℃ and then lowest melting point PEG 600 is added to it and stirred it continuously till homogenous mixture is formed.

a) 2gm of ethanolic extract was added separately into 100gm of ointment base (2% herbal ointment).

b) 5gm of ethanolic extract was added separately into 100 gm of ointment base (5% herbal ointment).**(M. Ismail shareef)[5].**

Composition of water solubleointment (100 gm)

S.no.	Ingredients	Quantity taken (2%herbal ointment)	Quantity taken (5%herbal ointment)
1.	PEG 4000	49 gm	47.5 gm
2.	PEG 600	49 gm	47.5 gm
3.	Propyl paraben	Q.S.	Q.S.

Evaluation of the Polyherbal Formulation

The polyherbal formulation was evaluated by the following physiochemical parameters:

- **Colour and odour**

  Colour and odour was examined by visual examination.

- **Loss on drying**

Loss on drying was determined by placing the ointment in a petridish on a water bath and dried until constant weight was obtained.

- **pH**

The pH of the formulation was recordrd using digital pH meter. The weight, quantity of the sample was dissolved in distilled water and stored for 2 hours and pH value is measured.

- **Spreadability**

The spreadability was expressed in terms of time in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of load. Spreadability was calculated by using the following formula:

S = (M.L/T)

Where, S= spreadability, M = Weight tied to upper slide, L = Length of glass slides,

T = time taken to separate the slides**(Kavitha AN)[6].**

- **Extrudability**

The formulation was filled in the standard collapsible aluminium tubes which were sealed at the end. The weight of each tube was determined and recorded. Then the tubes were placed in between two glass slides which were clamped by having standard weight of 0.5 kg over the glass plate. Then the cap made to remove and weigh the extruded ointment from the tube. The percentage of extruded ointment was calculated.

In - vivo wound healing activity

1. Protocol
2. Skin irritation study
3. Wound Healing Models

Protocol

Albino Wistar Rats, weighing 130-150 gm was used in the study and fed with standard laboratory pellet diet provided water ad libitum and were maintained at 22-25℃,35 to 60% humidity. The experimental protocol duly approved by institutional animal ethics committee(IAEC)(**Ansar .M)**[7].

Grouping of Animals

Animals were divided into four groups, each group consisting of six rats.

Group I : Normal control group

Group II: Standard (Betadine ointment 5%)

Group III: TestgroupI - Treated with 2% herbal ointment formulation

Group IV: Test group II – Treated with 5% herbal ointment formulation**(Gauravdubey)[8].**

Skin Irritation Studies

To test skin irritation studies, 2% and 5% ointment formulation were applied individually to an area of approximately 5cm^2 of skin and covered with gauze pauch. After 1hr, ointment was removed. The gel was applied to the skin once in a day for 7 days and was observed for any sensitivity and reactions.(**Esfahani S.A.)[9].**

Excision Wound Model

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 100 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 and applied twice a day. The wounds were traced on transparent tracing paper by permanent marker on theday of wounding and subsequently on alternate days until healing were complete. Wound areas were measured on days 4, 8 ,12, 16 and 20 for all groups.

The % of wound contraction was calculated by following formula:

% wound contraction = Wound area on initial day – Wound area on day Nth/ Wound area on initial day ×100

Where n= no. Of days (4, 8,12,16,20 days) **(DashG.K.)[10].**

Incision Wound model

All rats were anesthetized with diethyl ether. Paravertebral incision of 6cm length was made through the entire thickness of the shaved skin, on either side of the vertebral column of the rats with the help of a sharp scalpel. After complete hemostasis, the wound was stitched by means of interrupted sutures placed approximately 1cm apart using black silk surgical thread (no.000) and a curved needle no. 11. After stitching, the wound was left undressed and animals were treated daily for 10 days. On the 10 day, all rats were anesthetized and sutures were removed and tensile strength of cured wound skin was measured using tensiometer.**(GuptaN**).

StatisticalAnalysis

The results were then analyzed by using one- way analysis of variance (ANOVA) followed by Tukey's comparison test with equal sample size. The difference was considered as significant when P-values <0.05.All the values were expressed as mean±SEM.

Results and Discussion

Percentageyield

The percentage yield of the ethanolic extract of bark and leaves of Azadirachta Indica and Hibiscus rosa- sinesis is:

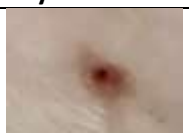
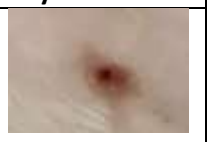
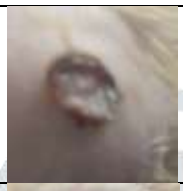
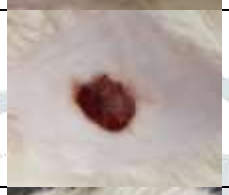
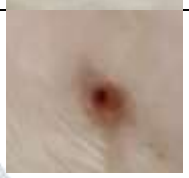
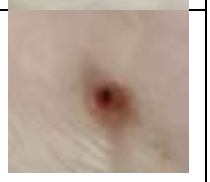
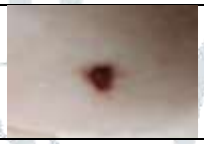
Practical Yield = 50

Theoretical yield = 12.1

Percentage yield= Practical yield/ Theoretical yield× 100

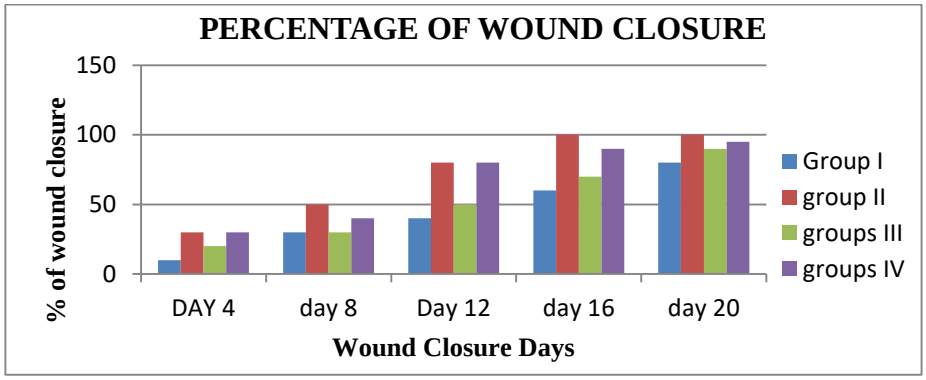
% yield= 12.1/50 ×100 = 24.2 gm

Excision wound model

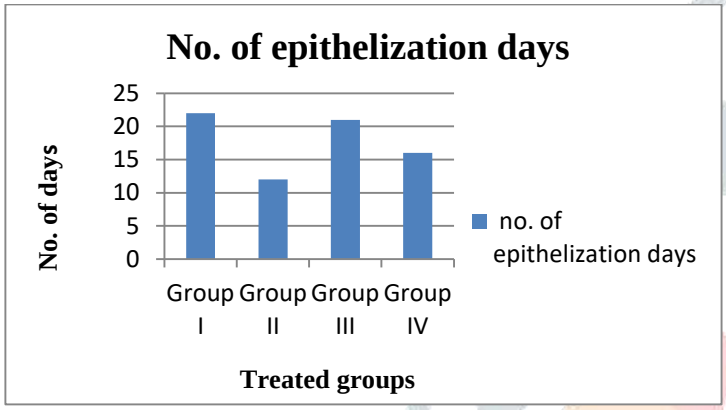
Groups	Day 0th	Day 4th	Day 8th	Day 12th	Day 16th	Day 21th
Group-I (Control)						
Group- II (Standard)						
Group-III (2% herbal ointment)						
Group-IV (5% herbal ointment)						

Effect of Polyherbal formulation on wound area and percentage of wound contraction in exicision wound model

Groups	Area of wound closure (sq mm± S.E.M)					
	4th8th	12th16th21th				Epithelization in days
Group I (Control)	90±23.68 (10%)	70±22.60 (30%)	60±26.69 (40%)	40±24.50 (60%)	20±25.62 (80%)	22±27.50
Group II (Standard)	70±28.54 (30%)	50±27.53 (50%)	20±28.54 (80%)	00	00	13±27.53
Group III (2% oint.)	80±22.34 (20%)	70±23.30 (30%)	50±20.35 (50%)	30±22.34 (70%)	20±24.33 (90%)	22±24.50
Group IV (5% oint.)	70±28.86 (30%)	60±26.86 (40%)	20±29.84 (80%)	10±27.53 (95%)	00	17±22.34



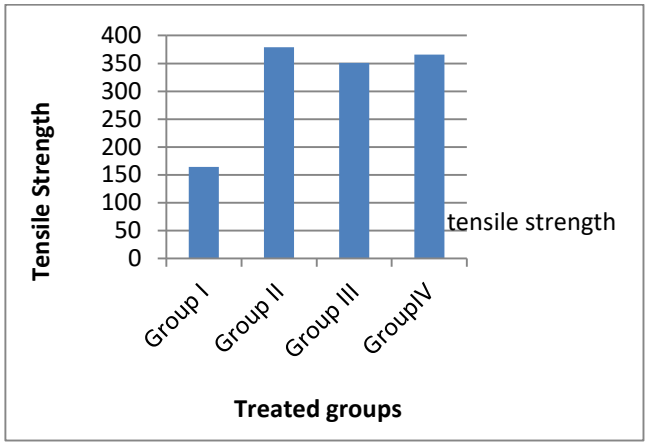
Effect of polyherbal formulation on percentage of wound closure in excision wound model



Effects of polyherbal formulation on epithelisation period in excision wound model

Effect of polyherbal formulation in incision wound model

S.no.	Groups	Tensile strength of skin(g)
1.	Group- I (Control)	164.55±3.11
2.	Group- II(Standard)	379.98±2.94
3.	Group-III (2% herbal ointment formulation)	351.35±3.27
4.	Group-IV (5% herbal ointment formulation)	366.08±2.31



Result

The polyherbal formulation (Azadirachta indica and Hibiscus rosa sinesis)show presence of phytochemical constituents flavanoids, tannins,carbohydrates, glycosides.

The studies on excision wound healing reveals that all the four groups showed decreased wound area from day to day. However, on 21th post wounding days, Group-I animals showed 80% of healing ,whereas Group-II and Group-III showed 100% and 90% healing and Group-IV showed 95% healing.

In Incision wound model, a significant tensile strength was observed in the skin breaking strength. The breaking strength of group-IV(5% herbal ointment) formulation showed significantincrease in breaking strength as compared to group-I(control) and Group-III(2% herbal ointment) but less than the Group-II(Standard) treated group.

Thus, Group-IV(5% herbal ointment) formulation as well as Group-II(Standard) show a significant increase in breaking strength in the 10 days old wound.

References

1. E. U. Ikobi, C. I. Igwilo, O. Awodele, and P. C. Azubuike, "Antibacterial and Wound Healing Properties of Methanolic extract of dried fresh Gossypium barbadense Leaves," Asian Journal of Biomedical and Pharmaceutical Sciences, vol. 2, no. 13, p. 32, 2012.
2. Nguyen DT, Orgill DP, "The pathophysiologic basis for wound healing properties of Indian medicinal plant'',pp.25-57.
3. Labler L, Mica L, Härter L, et al: Influence of V.A.C.-therapy on cytokines and growth factors in traumatic wounds. Zentralbl Chir 2006
4. Nikunjana A. Patel*, Megha Patel, Rakesh P. Patel Formulation and Evaluation of Polyherbal Gel for Wound Healing N. A. Patel et al / Intl. R. J. of Pharmaceuticals (2011), Vol. 01, Issue 01, pp. 15-20
5. M. Ismail Shareef1, P. Jagan Mohan Reddy1 *, S. M. Gopinath1 **, K. S. Dayananda1, Ajay Mandal2, Purushotham K.M Evaluation of wound healing activity of polyherbal formulation of roots of TerminaliaArjuna International Journal of Innovative Research in Science, Engineering and Technology 2014

6. Kavitha A. Et al, Design, formulation and evaluation of a polyherbal ointment for its wound healing activity, International research journal, 2013,4(5), 175-180.

7. Ansar M. Patel*1, S.M. Kurbetti2, R.V.Savadi3, V.A. Thorat 4, V. V. Takale 5, S.V. Horkeri Preparation and Evaluation of Wound Healing Activity of New Polyherbal Formulations in Rats AJPCT[1][6][2013]498-506.

8. Dubey G.et al, Evaluation of wound healing activity of some flavanoidal drugs and their polyherbal formulation on wound healing models, IOSR journal of pharmacy, 2017, 12(5), 52-63.

9. Esfahani S.A. et al, The healing effect of Plantago major and Aloe vera mixture in excisional full thickness skin wounds, World journal of pharmaceutical sciences, 2018, 8, 51-57.

10. Dash G.K.et al, The wound healing effects of a new polyherbal formulation, Der Pharmacia , 2011, 3(1), 342-349.

11. Gupta N.et al, Comparative wound healing activity of two polyherbal formulation on experimental in rats, Indian journal of Pharmaceutical education and research, 2014, 48(2), 77-86.

12. Robson MC, Steed DL, Franz MG: Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg 2001; 38: 72 – 140

13. Degreef H: How to heal a wound fast. Dermatol Clin 1998; 16: 365 – 375.

14. Bischoff M, Kinzl L, Schmelz A: The complicated wound. Unfallchirurg 1999; 102: 797 – 804.

15. Attinger CE, Janis JE, Steinberg J, et al: Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. Plast Reconstr Surg 2006: 117(7 suppl): 72S – 109S.