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PHARMACOLOGICAL EVALUATION OF POLYHERBAL FORMULATION FOR WOUND HEALING ACTIVITY IN RATS

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ABSTRACT

The aim of the present study to examine wound healing activity of polyherbal formulation using ***Martynia annua***, ***Aloe vera*** and ***Calendula officinalis*** extract. The parameters of studied included rate of wound contraction, measurement of wound index, wound area, and the period of epithelialization in excision wound model. The extracts were topically applied in the form ointment (using 6% hydroalcoholic extracts as a bioactive ingredient and was formulated in the form of simple ointment base BP) daily once times, starting from the day 1 on the excision of the rats, till complete epithelialization. In the excision wound model, the polyherbal ointment group exhibit 97.696±0.83% reduction in wound area on the 20th day when compared to control treated group which was 92.928±1.40% and faster rate of epithelialization is 21.66±0.33 days. Polyherbal ointment significantly reduced the period of epithelialization and increased wound contraction rate compared to the control group ($P < 0.05$). In present study reveals that the polyherbal formulation having potent wound activity which could be a good choice of remedy for wound healing.

KEYWORDS: Martynia annua, Aloe vera and Calendula officinalis, period of epithelialization, rate of wound contraction, polyherbal ointment.

1. INTRODUCTION

A wound is a cellular and anatomic injury which is caused by an external force and it can involve any tissue or organ. Cutaneous Healing of a wound is a complex and protracted process of tissue repair and remodeling in response to injury.[1] Healing is a complex process that consists of four main stages: hemostasis, inflammation, proliferation, and remodeling of the tissue.[2] *In-vivo* small animals provide a myriad of model choices for various human

wound conditions. *In-vivo* models include excision models, incision models, dead space models, burn models. Wound healing property measurement can be categorized into physical attributes like wound contraction, epithelization and scar remodelling which can be monitored by measuring the total wound area, open wound area and noting the physical changes in scar e.g. size, shape and colour etc, mechanical attributes like tensile strength, biochemical attributes like estimation of hydroxyproline, hexosamine and hexuronic acid and histopathological attributes. Excision wound model was used for the study of the rate of contraction of wound and epithelization. India has a rich tradition of plant-based knowledge on the healthcare system.[3] Several herbs and medicinal plants proved to be wound healers were identified and formulated for the treatment and management of wounds. Many herbal products have been used in the management and treatment of wounds over the years. In *Ayurveda*, multiple herbs (polyherbal) or single are used for the treatment. The *Ayurvedic* literature *Sarangdhar Samhita*' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desired therapeutic effects. When combining the various herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity.[4] *Martynia annua*, *Aloe vera*, and *Calendula officinalis* are one of the medicinal plants used traditionally for the treatment of wounds. However, there were no scientific reports documented of polyherbal preparation so far on the wound healing activities of these plants.

1.1. Agent use for wound healing activity

Wound healing agent is defined as the drug or any agent which heals the wound.

Martynia annua belongs to family Martyniaceae (or Pedaliaceae), is a well-known small herbaceous annual plant, distributed throughout India. It is commonly known as the Cat's claw or Devil's claw refers to the inner woody capsule which splits open at one end into two curved horns or claws. They produce strange seed pods that attach to the feet and legs of large animals, and include some of the largest hitchhiker fruits in the world.[5] In Ayurveda, the plant is known as kakanasika, which is being used in Indian traditional medicines for wound healing.[6]

Aloe vera is a plant that belongs to Liliaceae family that grows easily in hot and arid regions. The existing mucilage tissue at the center of leaves in this plant that is also so-called aloe gel is used for various cosmetics and medical applications. The peripheral leaf cells in this plant

produce bitter and yellowcolor latex that is called aloes. Aloe Vera promoted complete healing of wounds.[7]

Calendula officinalis is a common garden plant belonging to the Compositae family. It is one of the commonly used medicinal plantsin India, China, Europe and US.[8] *Calendula officinalis*, commonly known as marigold flower has wound healing property.[9]

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of ***Martynia annua***, flowers of ***Calendula officinalis*** *and* leaves of ***Aloe vera*** were collected from local areas of Bareilly during the August-September. The plant was authenticated and taxonomically identified by Dr. Alok Srivastava, associate professor in the department of Plant science M.J.P. Rohilkhand University, Bareilly. The plant materials were dried in shade, powdered and stored in well-closed containers.

2.2. Extraction

Powder of ***Martynia annua*** leaves, ***Calendula officinalis*** flower, *and* ***Aloe vera*** leaves in the ratio of 2:1:1 was subjected to extraction with 60% hydroalcohol by maceration for seven days at room temperature. The extract was filtered and concentrated at room temperature, after extraction.[10]

2.3. Development of polyherbal formulation

The polyherbal ointment was prepared by using 6.0% hydroalcoholic extracts as a bioactive ingredient and was formulated in the form of simple ointment base BP. [10]Dissolve bellow specified quantity of extract in 9.3 ml of water and added remaining ingredients in water and heat the solution to 70^0C in a beaker. Melt the white petrolatum and stearyl alcohol on a hot plate. Heat this mixture to 70^0C. Add the oleaginous phase slowly to the aqueous phase with constant stirring. Remove from the heat and stir the mixture until it congeals. The ingredients and their quantity were used to prepare formulation or ointment specified in **Table 3.**

2.4. Stability study of formulation

The stability study was performed as per The ***International Conference on Harmonisation*** of Technical Requirements for Registration of Pharmaceuticals for Human Use (***ICH***) guidelines. The optimized gel formulation was filled in the collapsible tubes and

stored at different temperature and humidity conditions, viz. 25°C ± 2°C/ 60% ± 5% RH, 40°C ±2°C/ 65% ± 5% RH, for a period of three months and studied for various parameters.

2.5. Selection of experimental animal

Healthy albino rats of either sex and of approximately the same age, weighing about 150-200 g were used for the study. Albino rats were selected from the animal house of the department of pharmacy, MJP Rohilkhand University, Bareilly and animal were kept at an ambient temperature of 25°C ± 2°C/60% ± 5% RH with 9-12 hour light and dark cycle. Animals were fed with standard diet and water *ad libitum*. Animals were housed in polypropylene cages in groups for at least one week before using them for the experiment.

2.6. Model for wound healing activity

Excision wound model

The animals were divided into 3 groups with six animals in each group (n=6) and were anesthetized by open mask method with 3–5% light ether anesthesia, before the creation of the wound. The particular skin area was shaved 1 day before to the experiment. An excision wound was inflicted by cutting away the skin measuring 300mm^2 full thickness and 2mm depth from a predetermined shaved area.[11] The wounds were left undress to the open environment and the animals were carefully observed for any infection and those that showed any sign of infection was separated, excluded from the study and was replaced. Haemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline solution. The wounded animals were housed separately in different cages. Wound area was measured instantly by placing a transparent tracing paper over the wound and tracing it out.[11,12] The tracing paper was then placed on a 1-mm^2 graph sheet and traced out. The wound area was measured out on respective days (0th, 4th, 8th, 12th, 16th day and 20th day) and the percentage wound contraction was calculated.

The period of epithelization was calculated as the number of days required for falling off of the dead tissue remnants without any residual raw wound. For the determination of epithelization period, animals (n=6) were divided into 3 separate groups in a similar way as shown above in the excision wound model.

2.7. The protocol of experimental animals

Animals were divided into three groups, each group consisting of 6 rats. Table 1.

2.8. Evaluation of wound healing in *In-vivo* models

Skin irritation test

The ointment was evaluated for primary skin irritation test on experimental animals (shaved back of the rats) to evaluate the safety of ointment.[13]

2.9. Measurement of the wound area

The progressive changes in wound area were monitored by a camera on predetermined days (0^{th}, 4^{th}, 8^{th}, 12^{th}, 16^{th} day and 20^{th} day). Later on, the wound area was measured by tracing the wound on a graph paper.[14,15] It was calculated by the following formula.

$$\textbf{Percentage of wound contraction} = \frac{Healed\ area}{Total\ area} \times 100$$

2.10. Measurement of wound contraction

Wound contraction, which contributes to wound closure and restoration of the functional barrier**.** Contractions, which contribute to wound closure, was studied on alternate days from Day 1 to last day, i.e. starting from the day of operation till the day of complete epithelialization by tracing the raw wound on a transparent sheet wound contraction was calculated as a percentage of the reduction in original wound area size. It was calculated by the following formula.

$$\textbf{Percentage of wound contraction} = \frac{WAO - WAD}{WAO} \times 100$$

Where, WAO = wound area on day zero; WAD = wound are on corresponding days.

2.11. Determination of period of epithelialization

Falling of incrustation leaving no raw wound behind was taken as the endpoint of complete epithelization and the days required for this was taken as a period of epithelization.[16]

2.12. Measurement of wound index

Wound index was measured daily with an arbitory scoring system depicted in table 2.[11]

2.13. Data analysis: All data were presented as Mean ± SD. To compare data among groups, one way ANOVA (Analysis of Variance) factor analysis was performed. Probability $P<0.05$ was considered statistically significant.

3. RESULTS

3.1. Formulation aspects

The ointment was prepared from the 60% hydroalcoholic extract of *Martynia annua, Aloe vera, Calendula officinalis*. Hence further evaluation studies are carried out for the same **Table 3**.

3.2. Percentage wound contraction for the optimized formulation

From the table 1 it reveals the improvement of wound healing induced by polyherbal ointment formulation. On the 4th day the wound area reducing in the control treated group was 6.288±4.295% and in PHF treated group were 7.565±3.594%. On the 20th day the wound area reducing in the control treated group was 92.928±1.401% and in the polyherbal treated group was 97.696± 0.839%. So the polyherbal treated group shows more effective when compared to control treated group represent in **Graph 1** and wound contraction percentage shown in **Table 4.**

3.3. Period of epithelialization

The values of the period of epithelization for the standard, control and the treated groups are depicted in **Table 5** and **Graph 2**.

3.4. Wound index

Wound index was measured daily with an arbitrary scoring system and reported in **Table 6.**

3.5. Stability Studies of formulation

There was no change in the physical appearance of the optimized formulation as mentioned in **Table 7. A** and **7. B** Stability data of polyherbal formulation at 25 °C and 40 °C**.**

Table 1: The protocol of experimental animals.

S.no.	Group	Treatment
1.	Group I	Treatment with simple ointment base BP served as control or vehicle **control group** Treated.
2.	Group II	Treated with standard drug i.e. Povidone-iodine ointment served as **a standard group**.
3.	GroupIII	Treated with **the polyherbal formulation** in the form of ointment serve as a **treatment or polyherbal formulation (PHF) group.**

Table 2: An Arbitory Scoring System For Measurement Of Wound Healing Index.

Gross change	Wound index
Complete healing of the wound	0
Incomplete but healthy healing	1
Delayed but healthy healing	2
Healing has not yet been started but the environment is healthy	3
Formation of pus-evidence of necrosis	4
Total	10

Table 3: Formula For Polyherbal Formulation.

S.No.	Name of Ingredients	Quantity in percent	Quantity Taken
1	Hydroalcoholic extract	6%	1.8g
2	Sodium lauryl sulfate	1%	0.3g
3	Propylene glycol	12%	3.6ml
4	Stearyl alcohol	25%	7.5g
5	White petrolatum	25%	7.5g
6	Purified water	31%	9.3ml

Table 4: Percent Wound Area Reduction Values After Treatment of Rats With Polyherbal Ointment on Various Days Of Treatment (Mean±Sem).

Treatment Days	Treatment groups		
	Control	Test	Standard
4	6.288±4.295	7.565±3.594	08.946±3.032
8	45.886±2.886	47.915±3.749	50.423±7.000
12	57.503±2.061	66.043±6.448	68.666±4.695
16	72.200±5.362	88.402±4.796	90.143±3.215
20	92.928±1.401	97.696±0.839	99.116±0.865

S.E.M = Standard Error mean n=6* and their profiles are given in **Figure 1**.

Table 5: Summary of Mean (±Sem) Total Gross Wound Epithelialization Time.

Treatment groups	Epithelialization time in days (Mean±SEM)
Treated with standard ointment (standard)	21.16±0.30
Treated with simple ointment(control)	23.5±0.34
Treated with Polyherbal ointment (test)	21.83±0.30

S.E.M. = Standard error of mean, n=6*

Table 6: Measurement of Wound Index.

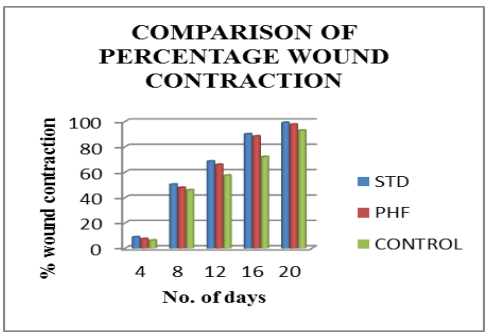
S.No.	Experimental groups	Wound index
1	Control	2
2	Standard	1
3	Test	1

Table 7.A: Stability Data of Polyherbal Formulation at 25 °c.

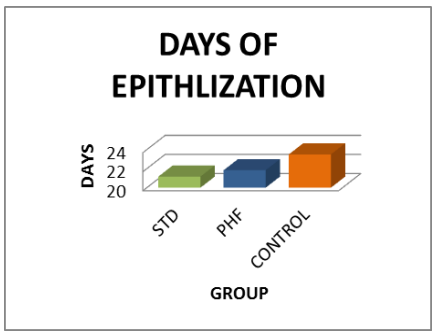
Days	pH ± S.D	Homogeneity	Grittiness	Report
0	7±0.10	***	Nil	Complies
15	6.7 ± 0.02	***	Nil	Complies
30	6.6 ± 0.17	***	Nil	Complies
60	6.6 ± 0.11	**	Nil	Complies
90	6.6 ± 0.15	**	Nil	Complies
S.D = Standard Deviation, n=6*				

Table 7: B: Stability Data of Polyherbal Formulation At 40 °C.

Days	pH ± S.D	Homogeneity	Grittiness	Report
0	6.7±0.12	***	Nil	Complies
15	6.5 ± 0.04	***	Nil	Complies
30	6.6 ± 0.01	**	Nil	Complies
60	6.6 ± 0.02	**	Nil	Complies
90	6.6 ± 0.01	**	Nil	Complies
S.D = Standard Deviation, n=6*				



Graph 1:- Comparison of percentage of wound contraction.



Graph 2:- days of epithelialization.

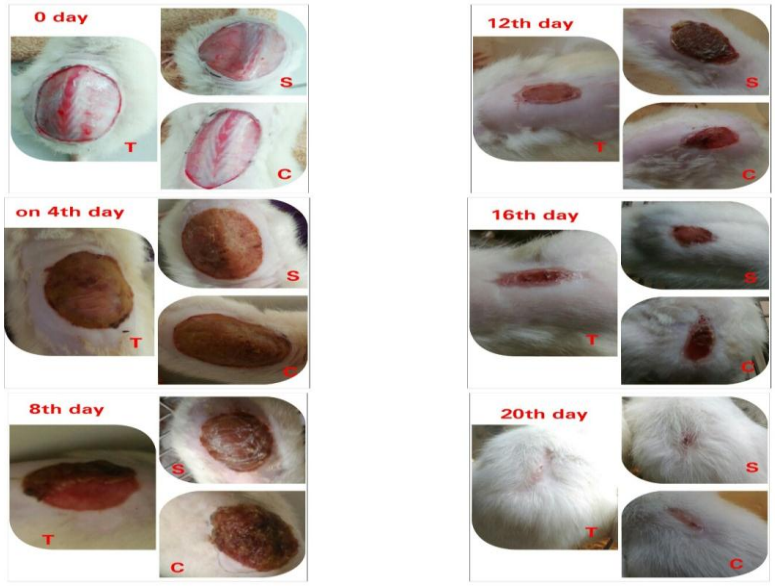


Figure 1: Percentage of Wound Contraction Treated with Polyherbal Formulation, Control, and Standard.

T=test ointment (polyherbal formulation); **S**= standard ointment; **C**= control ointment

4. DISCUSSION

The polyherbal formulation is tested for skin irritation testing and having no irritation cause with the comparison with control group animals. Standard treated group consist of six animals which are treated with Povidone-iodine 2% ointment daily for 20 days. It is one of the most frequently prescribed drugs for the treatment of wound healing. It is observed that the area of the wound is significantly decreased. Our study showed the polyherbal treated group has good improvement of wound healing activity and lower the area of wound fastly as compared to the control treated group. On 4^{th} day % of wound contraction by polyherbal treated animals was 7.565±3.594%, on 8^{th} day % of wound contraction was 47.915±3.749%; on 12^{th} day % of wound contraction was 66.043±6.448%; on 16^{th} day % of wound contraction was 88.402±4.796%; on 20^{th} day % of wound contraction was 97.696±0.839%. The formulation of the polyherbal ointment (test group) might have an influence on the higher activity compared with control treated group. The days of epithelialization were also lowered as compared to the control treated group of animals and near the standard treated group of animals. Period of epithelialization for polyherbal treated group was 21.83±0.30 and 23.5±0.34 for control treated group which was higher when compared to the polyherbal treated group and showed effective wound healing as compared to the control treated group. Environmental Stability of polyherbal formulation was good. Wound index was 1; p=0.110 for polyherbal treated group and standard treated group which was less as compared to the control treated group.

On the basis of results obtained in the present investigation is possible to conclude that the polyherbal formulation of the extract of Aloe vera, Martynia annua, calendula officinalis has significant wound healing activity in rats and the findings of the results justify the wound healing properties of the polyherbal formulation.

5. CONCLUSION

The present study design to evaluate the effect of polyherbal formulation on wound by using excision model. This model was used for the study of rate of contraction of wound and epithelization. Using the leaves of ***Martynia annua***, flowers of ***Calendula officinalis*** *and* leaves of ***Aloe vera*** for the hydroalcohol extract mixed in 2:1:1 proportion, to obtain the best formulation in order to increase the acceptability and adoptability of herbal medicine for wound healing. In the traditional system of Indian medicine, plant formulations and

combined extracts of plants are chosen rather than individual ones. It is known that Ayurvedic herbals are prepared in a number of dosage forms, in which mostly all of them are PHF.[17,4] Even so the active phytochemical constituents of individual plants have been well established, they commonly present in minute amount and always, they are insufficient to achieve the desirable therapeutic effects.

On the 4th day the wound area reducing in the control treated group was 6.288±4.295% and in PHF treated group were 7.565±3.594%. On the 20th day the wound area reducing in the control treated group was 92.928±1.401% and in the polyherbal treated group was 97.696± 0.839%. So the healing activity significantly enhance in the PHF treated group as compared to control treated group So, we found that PHF treated group is significantly higher from control treated group and control treated group is significantly lower from polyherbal treated group.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Future prospect

The following points are the recommended from this study and scope for future work.

- The following wound healing activity should be further studied with different formulation strength different dosage form and different extracts of plants parts.
- Polyherbal formulation should be tested for its effects on other wound healing model for example Incision wound model, Burn model, Dead space model, Blister wound model**,** Chick chorioallantoic membrane (CAM) Assay, Fibroblast assay and diabetic wound etc.
- The antimicrobial activity of plant extracts also tested. It would be prudent to test a range of extracts of different polarities and activity.

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REFERENCES

1. Thakur R, Jain N, Pathak R, Sandhu SS. Practices in wound healing studies of plants. Evidence-based complementary and alternative medicine, 2011; 2011.

2. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. Journal of International Medical Research, Oct, 2009; 37(5): 1528-42.
3. Hemamalini K, Ramu A, Mallu G, Srividya VV, Sravani V, Deepak P, Reddy UV. Evaluation of wound healing activity of different crude extracts of Anogeissus acuminata and Gymnosporia emerginata. Rasayan Journal of Chemistry, 2011; 4(2).
4. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacognosy reviews, Jul, 2014; 8(16): 73.
5. Hosamani KM, Sattigeri RM, Patil KB. Studies on chemical compounds of Martynia annua syn. M. diandra seed oil. Journal of Medicinal and Aromatic Plant Sciences, 2002; 24(1): 12.
6. Lodhi S, Singhai AK. Preliminary pharmacological evaluation of Martynia annua Linn leaves for wound healing. Asian Pacific journal of tropical biomedicine, Dec 1, 2011; 1(6): 421-7.
7. Boudreau MD, Beland FA. An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera. Journal of Environmental Science and Health Part C., Jul 1, 2006; 24(1): 103-54.
8. Muley BP, Khadabadi SS, Banarase NB. Phytochemical constituents and pharmacological activities of Calendula officinalis Linn (Asteraceae): a review. Tropical Journal of Pharmaceutical Research, 2009; 8(5).
9. Preethi KC, and Kuttan R. Hepato and reno protective action of Calendula officinalis L. flower extract. Experimental biology, 2009; 47(3): 163-168.
10. Rajput RT, Gokil kJ, and singh p. Development of wound healing herbal formulation "herbal wound guard", International Journal of Scientific and Research Publications, 2015; 5(3): 1-5.
11. Nayak S, Sandiford S, and Maxwell A. Evaluation of the wound-healing activity of ethanolic extract of Morinda citrifolia L. leaf. Evidence-based Complementary Alternative Medicine, 2007; 1–6.
12. Sutar N, Garai R, Sharma US, Sharma UK, Jaiswal A. Anthelmintic activity of Platycladus orientalisleaves extract. International Journal of Parasitology Research, Jan 1, 2010; 2(2): 1.
13. Trailokya D, Jiban D, Bipul N, and Suvakanta D. Formulation and evaluation of an herbal cream for wound healing activity. international journal of pharmaceutical science, 2014; 6(2): 693-697.

14. Sharma S, Sikarwar MS. Wound healing activity of ethanolic extract of leaves of Eclipta Alba. Pharmacognosy Magazine., 2008; 4(13): 108-111.
15. Patil MV, Kandhare AD, Bhise SD. Pharmacological evaluation of ethanolic extract of Daucus carota Linn root formulated cream on wound healing using excision and incision wound model. Asian Pacific Journal of Tropical Biomedicine, Feb 1, 2012; 2(2): S646-55.
16. Govindarajan R, Vijayakumar M, Rao CV, Shirwaikar AN, Mehrotra S, Pushpangadan P. Healing potential of Anogeissus latifolia for dermal wounds in rats. Acta Pharm., Dec 1, 2004; 54(4): 331-8.
17. Jayakumar RV. Herbal medicine for type-2 diabetes. International Journal of Diabetes Dev Ctries, 2010; 30: 111-112.