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Research Paper

WOUND HEALING POTENTIAL OF A HERBOMINERAL SKIN OINTMENT: A COMPARATIVE STUDY

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In the present study the ointment is a combination of herbs and minerals where the chief constituents are copper sulphate, mercury, sulphur, lead oxide, and a herb named kampillaka (*Mallotus phillipinensis)* prepared in a buffer ghee at a ratio of ¼:1:1:2:4:16. After trituration of minerals it converts into black sulphide of mercury (kajjali) and later mixed with herb and ghee keeping two important concepts of ointment formation in consideration that is, fusion method and geometrical dilution method. For a comparative study of this herbomineral ointment with framycetin, the wounds were developed on the dorsolateral flank of the albino mice. All albino mice were kept into two groups one to be treated with herbomineral ointment and other with framycetin. They were kept in polypropylene cage and maintained in standard laboratory conditions of temperature (22 ± 2^0C) and light dark cycle of 12:12 hours. Later, the test animals were examined for temperature, body weight, and feeding habits. The healing of wound was calculated by a prescribed standard formula. Dressing of the wound was also made at regular intervals. The mean of the data were placed in the table considering all cardinal features. The statistical analysis showed better result in herbomineral ointment in comparison to the framycetin.

Keywords: Herbomineral ointment, Framycetin, Wounds, Dressing, Healing

INTRODUCTION

Herbal therapy is one of the most ancient sciences of the world and is practiced widely in Asian subcontinents (Patwardhan *et al*., 2004). It depends mainly upon herbal and herbomineral formulations while herbominerals are said to be more effective in low doses after proper purifications and preparation methods (Mishra, 2003). However, the herbal formulations still needs authentic justifications (Nagappa and Cheriyan, 2001). Several reports regarding wound healing potential of herbal and herbominerals like Septilin a proprietary preparation having *Balsamodendran mukul and Rubia cardifolia*

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(Udupa *et al*., 1989) and *Centella asciatica* (Suguna *et al*., 1996) *Leucasla endulaefolia* (Saha *et al*., 1997), *Anogeissus latifolia* (Govindrajan *et al*., 2004), and leaf extracts of *Hypericum hookerianum* (Mukherjee and Suresh, 2000), *Opuntia ficus indica* (Park and Chun, 2001), *Clerodendrum splendens* (Stephen *et al*., 2010) have been produced with the time.

Wounds are sequels of injuries which result into opening or breaking of the skin (Meenakshi *et al*., 2006). In other words wound is break in the epithelial integrity of the skin which may be accompanied by disruption of the underlying structures and normal tissues resulting into contusion, hematoma, laceration or an abrasion (Enoch and Jhon, 2005). Injuries always followed by healing via three phases inflammatory, proliferative and remodeling to get the proper strength and appearance (Sumitra *et al*., 2005). In all the systems of medicines, external application of ointments is the effective means of wounds healing because of their lubricous, buffering, regenerative, anti-inflammatory, antiseptic, antimicrobial, tensile strength improving capacity, moisture holding capacity, protein and collagen synthesis capacity. Ointments are semisolids or soft preparations for external application containing active drugs mixed with a fatty oily or paraffin bases.

Most of the countries in the world either developing or under developed non healing wounds due to infections and poor hygienic conditions are major health problems (Senthil *et al*., 2006). Herbomineral formulations may be an ideal choice due to their claim about shortening of the healing time and minimization of undesired consequences.

In the present investigation an effort has been made to study the comparative potential of an ayurvedic and allopathic formulated ointment. The idea and formula of ointment was taken from an ayurvedic text Yogratnakara (chapter vrana-sodhana ropan). The ingredients of the ointment are Tutha (copper sulphate), Parada (mercury), Gandhaka (sulphur), Murdasankha (lead oxide), Kampillaka (*Mallotus phillipinensis*) and ghee at a ratio of ¼:1:1:2:4:16 respectively (Vaidaya lakchamipati, 2010). These ingredients has also been claimed for the antibacterial and healing potential in ancient several ayurvedic texts and supported by modern scientists also (Reddy, 2007).

MATERIALS AND METHODS

Collection of Ingredients: In the present study all ingredients of the ointment were taken from laboratory of Post Graduate department of Government Ayurvedic College Patna. Proper authentication was done under keen observation of laboratory experts.

Preparation of Ointment: Ingredients were purified by standard established techniques (Mishra, 2003). Then mercury was mixed with sulphur and triturated till formation of an ideal kajjali (black sulphide of mercury). There are two important concepts of ointment formation that is fusion method and geometrical dilution method. Keeping this in view firstly ghee was melted, after that solid mass of mixture of herb and minerals were mixed in the ghee to follow geometrical dilution method. For the comparative study, Framycetin cream was taken as another healing agent. Framycetin is a famous antiseptic cream and used frequently to treat the different infected or uninfected abrasions of the skin.

Test Animals and Its Grouping: All experiments were made in laboratory, situated in the department of physiology at Bihar Veterinary

College Patna after getting due permissions from head of PG Department of G.A.C.H.Patna and IAEC (Institutional Animal Ethics Committee) of the BVC Patna. Total 14 albino mice were purchased through registration number 1365\c\10\CPCSEA dated 13.08.2010 from Indian Veterinary Research Institute Bareilly, UP.

All albino mice were kept into two groups, seven of them treated with test herbomineral ointment and rest seven by a cream containing framycetin antibiotic of aminoglycoside group. For acclimatization purpose animals were kept in animal house for 10 days. During this period daily feed intake, general behavior and all the essential health examinations were done. The albino mice were housed in polypropylene cage and maintained in standard laboratory conditions of temperature (22 ± 2°C) and light–dark cycle of 12:12 h. The animals were fed with set standard formula during the experiment.

Excision Wound Model: The dorsolateral flank of every albino mice was shaved carefully. Washing was done with spirit and Betadine solution followed by local anesthesia with injectable xylocaine 2% in premeasured and assessed area. After that wound was created by excision of full thickness of skin in anaesthetized area with the help of BP Blade and curve scissors 1.5 cm far from the vertebral column. Haemo-stasis was achieved by blotting the wound with a cotton swab soaked in normal saline. For suppuration the wounds were kept open for seven days leading to its conversion into septic wound. While dressing at alternate day, an overall health of the animal was examined. Contractions, which contribute to wound closure, were studied on alternate days from day one (the date when dressing started) to complete closure. The wound was covered with transparent plastic sheet followed by tracing with permanent marker pen. Then area was calculated through placing the sheet on graph paper and counting the small squares. Squares up to half were counted as complete square and less than half were omitted from counting. Healing of wounds were mainly achieved through the process of wound contraction and scar formation. The contraction of the wound provides rough idea about scar formation and regeneration (Yannas, 2005c). The percentage contraction of the wound was calculated according to the following formula given by Kumar and Tyagi (1972) and used by Verma (2000), Kumar (2003) and Pallavi and Durgaprasad (2008).

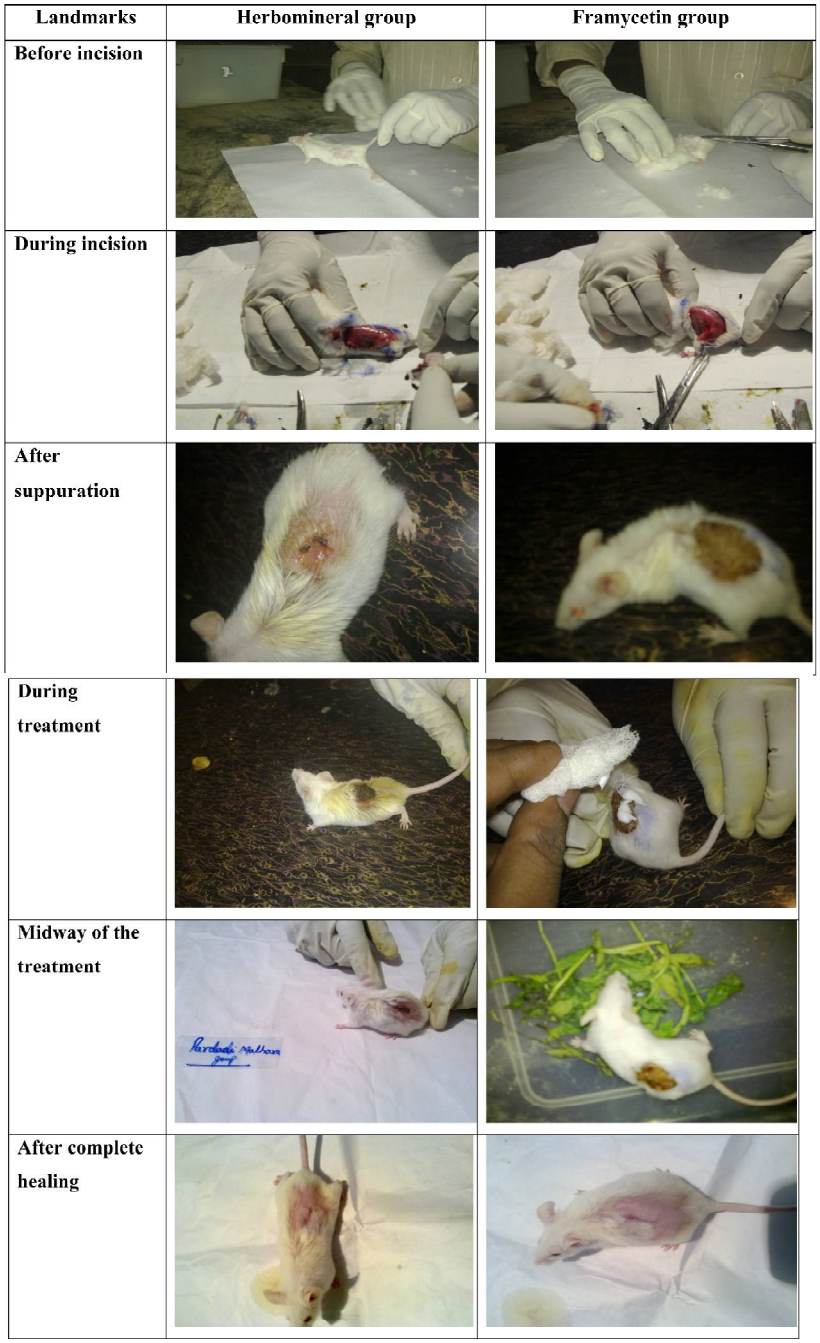
$$\frac{(a-b)}{A}\times 100$$

where *a* = area of the wound at the beginning of a period, *b* = area at the end of period, *A* = initial area.

STATISTICAL DATA ANALYSIS

All the data recorded individually and the mean were drawn. The entire experiment was divided into five segments that are before incision, after incision, after suppuration, during dressing and after healing respectively. Before incision for a period of three days all animals were kept under close observation. After incision for a period of seven days animals were left untreated. The after suppuration period is of seven days which makes the wounds completely septic. After that the dressing with above mentioned ointments was started. For the presentation of data this segment is subdivided into four another sub segments that is 5 days, 10 days, 15 days, 20 days respectively (as shown in Figure 1).

Figure 1: Showing Different Stages of Creating Wounds, Treatment and Healing in Albino Mice



Note: Before incision: Both group of Albino mices were shaved on dorsolateral flank and locally anaesthetized; During incision: Wounds were created 1.5 cm far from the vertebral column with the help of BP blade and curve scissors; After suppuration: The wounds show septic symptoms after seven days of incision; During treatment: Dressing started on both group of animals that is dressing by herbomineral ointment and framycetin ointment; Midway of the treatment: Encouraging results were observed by herbomineral treatment in comparison with framycetin; and After complete healing: Healing in case of herbomineral ointment was found better than framycetin.

RESULTS AND DISCUSSION

Healing is a natural phenomenon but always need proper caring and management. Mean of data regarding temperature, daily feed, body weight were almost same in both groups before incision. During suppuration marked elevation in temperature with decreased body weight and daily feed intake was observed in both groups. The mean of temperature-102.8°F, body weight-42gm, and daily feed intake-6.7% of body weight was obtained in herbomineral group while 103.1°F-temperature, 46 gm body weight and daily feed intake 6.8% of body weight was obtained in framycetin group. After a gap of 7 days every wound were examined and observed with edema, inflammation, pus pockets, discolorations, bad odors which supports the concept of ayurveda regarding septic changes in untreated wounds.

Temperature is one of the important sign of infected wound which became almost normal in herbomineral group at the end of 2nd sub-segment (10th day of dressing) while in framycetin group that was 102.1°F on that time. Normal range was achieved in framycetin treated group at the end of 3rd sub-segment (Table 1). Body weight was also regained up to at the end of 3rd sub segment in herbomineral group while in framycetin group normal body weight which was at the beginning of experiment cannot be regained up to end of the trial (Table 2). Daily feed was decreased equally in both groups during suppuration. Normal feeding that was equal to 9.8%of the body weight before incision was regained in herbomineral group at the end of 3rd sub-segment of dressing but in framycetin group it was not seen till end of experiment (Table 3).

The rate and process of healing is represented by contractions because they provide information regarding proliferation and scar formation which are essential art of wound healing. In this study, contraction was maximum between 5-10 days in herbomineral group while in framycetin group it was maximum between 15-20 days showing delayed healing (Table 4). In albino mice treated with herbomineral ointment, total closure was observed in 15 days@0.74mm^2/day while in Framycetin in 21 days @0.55mm^2/day (Table 5). It may be due to special potency of herbomineral ointment and composition because success of wound healing depends on sufficient nutrients

Table 1: Showing Body Temperature of the Test Animals Before and After Treatment)

	Herbomineral Group	Framycetin Group
Before Incision	99.8 °F	99.8 °F
After incision	100.2 °F	100.2 °F
After suppuration	102.8 °F	103.1 °F
During dressing	102.8 °F	102.9 °F
5th Day	101.1 °F	102.3 °F
10th Day	99.6 °F	102.1 °F
15th Day	99.7 °F	99.8 °F
20th Day	99.7 °F	99.8 °F
After Healing	99.7 °F	99.8 °F

Table 2: Showing Body Weight of the Test Animals Before and After Treatment		
	Herbomineral Group	Framycetin Group
Before Incision	55 gm	60 gm
After incision	54 gm	57gm
After suppuration	42 gm	46 gm
During dressing		
5th Day	48 gm	46 gm
10th Day	51 gm	50 gm
15th Day	58 gm	50 gm
20th Day	60 gm	52 gm
After Healing	61 gm	54 gm

Table 3: Showing Daily Feeding (% of Body Weight) of Test Animals Before and After Treatment		
	Herbomineral Group	Framycetin Group
Before Incision	9.8%	9.9%
After incision	9.1%	9.0%
After suppuration	6.7%	6.8%
During dressing	5.6%	5.8%
5th Day	7.8%	7.1%
10th Day	8.8%	7.4%
15th Day	9.9%	7.3%
20th Day	9.8%	7.4%
After Healing	9.9%	8.1%

Table 4: Showing Percent Area Contraction of the Wounds in Test Animals During Treatment		
	Herbomineral Group	Framycetin Group
Before Incision	–	–
After incision	–	–
After suppuration	–	–
During dressing	–	–
5th Day	41.02 %	11.78 %
10th Day	38.06 %	22.03 %
15th Day	29.91 %	34.15 %
20th Day	–	21.34 %
After Healing	–	10.67 %

Table 5: Showing Days/Duration for Complete Closure of the Wound in Test Animals	
Herbomineral Group = 15 days	Framycetin Group=21 days

Table 6: Showing Days/Duration for Complete Closure of the Wound in Test Animals	
Herbomineral group=14.28%	Framycetin Group=42.85%

being supplied to the wound site (Mayer-Ingold, 1993). It is also an indication of rapid healing potential in comparison to framycetin group. 14.28% mortality was seen in herbomineral group while in framycetin group 42.85% mortality was founded during dressing period (Table 6). It indicates that herbomineral ointment has some systemic effects also who prevented the animals from post incision stress and invasion of infections inside the body also. No hypersensitivity or allergic reactions were observed during study in both groups.

CONCLUSION

According to the results reported here, herbomineral ointment prepared with standard methods was found to have better activity on the wound healing in experimental animals compared to other framycetin groups. Herbomineral ointment was found effective in every step of the experiment. After this study it can be concluded that composition of this herbomineral ointment may be helpful to treat the superficial wounds of skin.

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