International Journal of Research Instinct
(Peer-reviewed Journal)

ISSN ONLINE 2348-2095
Research Article

Effect of Poly Herbal Ointment On Excision Wounds in albino rats

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ABSTRACT

Wound is the interruption of the continuity in tissue resulting from the opening or break of the skin. Wound healing is an integrated cellular and biochemical process of restoring normal structure and functions of damaged tissue. Healing is a natural phenomenon by which the body itself can overcome the damage to the tissue but the rate healing is very slow and chance of microbial infection is high. India has a rich tradition of plant based knowledge on healthcare system. Several herbs and medicinal plants proved to be a wound healers were identified and formulated for treatment and management of wounds. In the present study attempt was made to investigate the wound healing potential of a polyherbal ointment (*Anisomeles malabarica* (L.) R.Br.ex Sims., leaves of *Desmodium gendeticum* L. and leaves of *Albizia lebbeck* (L.) Benth.) on excision wounds in albino rats. Animals weighing 150 -200g were divided into four groups each comprising of six rats: Group I served as excision wounded control and Group II, III served as excision wounded rats were treated with two different doses (5% and 10%) of poly herbal ointment applied topically for 14days and group IV served as excision wounded animals treated with reference ointment soframycin.The healing of the wound was assessed by the rate of wound contraction, estimation of hydroxyl proline, hexosamine, tissue protein, DNA, RNA and enumeration of WBC and platelet count. The topical application of polyherbal ointment treated groups showed increase in hydroxyl proline, hexosamine, tissue protein, DNA, RNA and significant decrease in wound contraction, WBC count and platelet count. In conclusion, these results suggested the potency of polyherbal ointment in the management of wound healing.

Keywords

Wound healing, soframycin DNA, RNA, , polyherbal ointment

1. INTRODUCTION

Wound is such a kind of injury wherein either skin or any other external surface gets torn and are considered as unavoidable events of life. Physical as well as chemical andthermal or even microbial and immunological factors may be responsible for causing wound resulting in the loss of epithelial continuity with or without the underlying connective tissue loss **(Raina *et al* ., 2008).**Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue. Healing requires the collaborative efforts of various tissues and cell lineages. It involves aggregation of platelets, clotting of blood, fibrin formation, and an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelialisation. The number of chemical agents or drugs are used for the treatment of wound healing but it can cause different kinds of side effects for long term use. So there is need to develop a new wound healing drug without undesirable side effects .

Herbal drugs play a role in the management of various disorders; most of them speed up the natural healing process of humans. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional. Methods practiced such as ayurveda, unani and sidhha. Medicinal components from Plants an important role in conventional as well as western medicine. Numerous medicinal plants and their formulations are used for various disorders in ethano-medical practices as well as traditional system of medicine in India.

Three common plants namely *Albizzia lebbeck* Benth , *Desmodium gengeticum* L. belonging to Fabaceae family and *Anisomeles malabarica* (L.) R.Br.ex Sims. beloning to Lamiaceae family were selected under study. In earlier literature reported that those three plants have used for the treatment of allergic reactions , inflammation , wound infection and liver disorders etc.,

Several formulation based on each one of their components are reported in Ayurveda texts and medicinal claims for wound healing property. Individually as well, these components described several therapeutic values. So the aim of the present study was under taken to evaluate the wound healing potentials of poly herbal ointment on excision wounded animals.

2. MATERIALS AND METHODS

2.1.COLLECTION OF PLANT MATERIALS

Plant materials used for this study were collected in an appropriate seasons , identified and authenticated with the specimen deposited at Rapinat Herbarium, St. Joseph College, Trichy.

2.2. PREPARATION OF PLANT EXTRACTS

Fresh leaves of the all the selected plant materials were shade dried and powdered coarsely using electric blender. 200g of the each plant powder was mixed with six parts of water. Then it was boiled and extracted until it was reduced to one third and filtrated. Then the filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to the preparation of wound healing activity.

2.3. POLYHERBAL OINTMENT PREPARATION

The ointment was prepared by mixing the aqueous ectract of *Anisomeles malabarica* (L)R.Br.ex Sims., *Albizia lebbeck* Benth. *and Desmodium gangeticum* L. In an equal ratio. The poly herbal ointment was used at concentration of 5% and 10%.

2.4.ANIMAL GROUPING

The rats were divided into four groups each containing of six animals. GROUP I is Normal Excision wounded animals without treatment , GROUP II and III is Excision wounded animals treated with POLYHERBAL OINTMENT (PHO) 5% and 10% (0.5g) applied topically for 14 days , GROUP IV is Excision wounded animals with Standard Drug SOFRAMYCIN OINTMENT (SO) (0.5g) applied topically for 14 days.

2.5.CREATION OF WOUND

Excision wound (Mortone *et al.*,1972)

An excision wound was created on the dorsal side of rats. The dorsal sides of rats were shaved with a razor blade. Excision wound of size 2cm areas of skin in length, 0.2cm in depth were created by using surgical scissors. Haemostasis achieved by blotting the wound with cotton swab soaked in normal saline. After 2 hours the dead tissue were excised and the treatment was started. All the rats were given regular dressing changes and kept for observation.

2.6.MEASURMENT OF WOUND CONTRACTION (Sadaf *et al.*, 2006)

An excision wound was tracted by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper in every days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 200mm^2, as 100%, by using the following formula as

$$\% \text{ wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

3. STATISTICAL ANALYSIS

All the results were expressed as mean ± S.E. The data were statistically analyzed by one – way analysis of variance (ANOVA) and P values <0.05 were considered significant.

4. PARAMETERS STUDIED

After the experimental period , the animals were sacrificed by cervical decapitation and the blood and tissue samples were collected for analysing the haematological parameters like total leukocyte **(Armour** *et al*., **1965)** and platelets count **(Usha and Swaroop** *et al***, 1996)** and biochemical parameters such as hydroxy proline (**(Woessner, 1961)** , hesoxamine **(Wagner, 1979)** , DNA**(Giles and Myers, 1965)** , RNA **(Yoichi Endo, 1970) and** Tissue protein **(Lowry** *et al***, 1951).**

5. RESULTS AND DISCUSSION

Table 1: RATE OF WOUND CONTRACTION ON NORMAL AND DIABETIC EXCISION WOUNDED ANIMALS

The rate of wound contraction on post wounding days on normal excision model were presented in Table 1.

Groups	0^{th} day (cm^2)	7^{th} day (cm^2)	14^{th} day (cm^2)
I	1.9 ±0.12	1.6 ± 0.08	0.7 ±0.05
II	2.0 ±0.08*	1.3 ± 0.06*	0.3 ±0.03*
III	2.0 ±0.06*	1.1 ±0.07*	0.1 ±0.03*
IV	2.1 ±0.03	1.3 ±0.04	0.5 ±0.02

Values are expressed as mean ± SEM n=6, *P< 0.05, when compared PHO treated (group II,III) with control groups(group I).

Wound contraction involves a complex and superbly orchestrated interaction of cells, extra cellular matrix and cytokines. In the present study, PHO treated animals were found to contract much faster. Increased rate of wound contraction in PHO treated animals might be due to increase in proliferation and transformation of fibroblast cells into myofibroblasts. **(Radhika *et al.*, 2012).**

Table 2 LEVELS OF HYDROXYPROLINE AND HEXOSAMINE IN THE PHO TREATED, UNTREATED AND STANDARD DRUG TREATED WOUNDED ANIMALS

Groups	Hydroxy proline (mg/g Tissue)	Hexosamine (mg/g tissue)
I	28.27 ± 0.15	5.30 ± 0.14
II	47.02 ± 0.19*	8.45 ± 0.20*
III	51.64 ± 0.49*	11.60 ± 0.40*
IV	38.13±0.20	9. 33 ± 0.35

Values are expressed as mean ± SEM n=6, *P< 0.05 , when compared PHO treated (group II ,III) with control groups (group I).

The level of Hydroxy proline and hexosamine is increased in PHO treated animals compared to untreated animals.

Hydroxy proline , a major compound for the synthesis of collagen. It is a biochemical marker for the determination of tissue collagen. The increase amount of hydroxy proline in a PHO treated groups (II,III) substantiates the healing potential of herbal ointment. Increased level of hydroxy proline content indicates the enhanced collagen synthesis, and also initiate the regeneration and the repair of wounded tissue. So, the poly herbal ointment enhance the collagen stability and also enhance the wound healing process **(Haritha yadav *et al.*, 2012 and Nithya *et al.*, 2011).** Hexosamine is a matrix molecule, and they act as ground substrate for the formation of extracellular matrix. Hexosamine content was increased in PHO treated animals which can reflect the stabilization of collagen molecule by enhancing electrostatic & ionic interaction. Increased hexosamine level in early stages of wound healing indicating the active synthesis of fibroblast, which act as ground substances (mucopolysaccharide) on which the collagen can be laid on. **(Nithya *et al.*, 2011).**

Table 3 LEVEL OF TISSUE PROTEIN IN THE PHO TREATED, UNTREATED AND STANDARD DRUG TREATED WOUNDED ANIMALS

Groups	Tissue protein(mg/g tissue)
I	0.8 ± 0.01
II	1.50 ± 0.10*
III	1.85 ± 0.15*
IV	1.25 ± 0.09

Values are expressed as mean ± SEM n=6, *P< 0.05 , when compared PHO treated groups (II, III) with control groups (I).

The result showed increase in the level of protein in the PHO treated groups (groups II, III) , when compared to control (group I). Protein is essential for enhancing the wound healing. The protein deletion appear to delay wound healing by prolonged the inflammatory phase by inhibit fibroplasia, collagen proteoglycan synthesis and also inhibit the remodelling. The protein deficiency inhibit wound healing. Protein is required for the part of inflammatory process, in the immune response and the development of granulation tissue . Increased protein content in the PHO treated animals signifying active synthesis and deposition of matrix protein in the granulation tissue and enhance the healing process **(Douglas mackay ND *et al.*,2003).**

Table 4 : Level of DNA in the PHO treated, Untreated and standard drug treated wounded animals:

Groups	DNA (mg/g tissue)	RNA (mg/g tissue)
I	0.28 ± 0.004	0.61 ± 0.007
II	1.05 ± 0.005*	1.56 ± 0.003*
III	1.35 ± 0.003*	2.08 ± 0.003*
IV	1.10 ± 0.003	1.25 ± 0.003

Values are expressed as mean ± SEM n=6, *P< 0.05 , when compared PHO treated (group II ,III) with control groups (group I).

Increased level of DNA indicating the rapid tissue repair and proliferation. Decreasing level of DNA content indicates a decreased rate of cell proliferation. Drug that inhibit lipid peroxidation is belived to increase the viability of collagen fibrils enhance the circulation, and prevent cell damage by promoting the DNA synthesis significant increase DNA synthesis in PHO treated animal (II,III) enhance rapid regeneration of tissue and result in the increase in wound contraction.

Low level of RNA content indicates the low level of transcription, and also decreased the protein content in control group to disturb the healing process. The PHO prevent tissue damage and enhance increased synthesis of DNA, RNA and Proteins are responsible for the formation of granulation tissue in response to injury **(Rasik *et al.*, 1999)**.

Table No.5: Enumeration of WBC in the PHO treated, Untreated and standard drug treated wounded animals:

Groups	WBC (Thousands of Cells/cubic mm)
I	9.14 ± 0.02
II	8.10 ± 0.02*
III	10.62 ± 0.01*
IV	9.53 ± 0.05

Values are expressed as mean ± SEM n=6, *P< 0.05, when compared PHO treated (group II, III) with control groups (group I)

White blood cells help with wound healing by removing tissue debris and dead cells from the site of the injury **(Mike 2010).** The significant increase in WBC count in Group I rats is due to the infection at the wound site. The white blood cells will ensure that the healing process remains in the inflammatory phase. Pathogenic microbes compete with macrophages and fibroblasts for limited resources and may cause further necrosis in the wound bed.

On contrast, the reduction in WBC count in group II, III,IV rats is primarily due to the antibacterial action of phytoconstituent present in the poly herbal ointment.

Table No . 6: Enumeration of Platelets in the PHO treated, Untreated and standard drug treated wounded animals:

Groups	Platelets (Millions of cells/cumm)
I	407.67 ± 0.88
II	306.33 ± 0.67*
III	287.00 ± 1.15*
IV	220 ± 0.98

Values are expressed as mean ± SEM n=6, *P< 0.05, when compared PHO treated (group II, III) with control groups (group I).

Increased platelet count in Excision wounded animals indicates the cellular injury. Treatment with PHO enhance platelet aggregation and there by generating rapid tissue repair in the group (II,III). It releases multiple growth factors including PGDF, chemotacting agent, TGF Beta for the stimulation of the deposition of the extracellular matrix.

Low level of Platelets in the treated group indicates in the untreated group shows the clotting of blood and remains in unhealed conditions. The platelets are the natural healing agents which our body generates to cure itself. Platelets stimulate fibroblast proliferation so that it may reduce the healing time. Platelets express and release substances that promote tissue repair and influence processes such as angiogenesis, inflammation and the immune response **(Nurden *et al.*, 2008).**

6. CONCLUSION

In conclusion, the present study demonstrated that the aqueous extracts of poly herbal ointment applied topically promotes healing of wound with enhanced collagen turn over and these present findings provides scientific evidence to the ethno medicinal property of plants in the healing

of wounds.Though many synthetic medications are available in the market for wound healing but these natural sources would serve better in the healing of wounds at a faster rate with scar less.

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