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Research Article

Development and study of wound healing potential of a polyherbal formulation

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Abstract

Plants are more potent healers because they promote the repair mechanisms in the natural way. Wound healing potential of a polyherbal formulation consisting of *Calophyllum inophyllum* L, *Hibiscus sabdariffa* L and *Quercus infectoria* Olive in Excision, Incision and Dead space wounded rat models and also compared with reference ointment. Wistar strain of albino rats weighing 150 – 200g has been divided into 9 groups each comprising of 6 rats. Group – I, IV and VII served as wounded control, group – II, V and VIII are wounded and treated with PHO topically, group – III, VI and IX are wounded and treated with reference drug. The biochemical and enzymatic parameters studied were hydroxyproline, hexosamine, ascorbic acid, superoxide dismutase, lipid peroxide and protein, DNA, RNA and WBC. These parameters were increased in the rats treated with polyherbal ointment. Lipid peroxide and WBC level was decreased when compared to control and standard group. The results of the present study substantiate the traditional claims that the use of PHO prepared from plants possesses significant wound healing promoting activity and provides scientific evidence to the ethnomedicinal property of plants in the healing of wounds.

Keywords: Wound healing, Polyherbal Ointment, Antioxidant, Biochemical Parameters.

INTRODUCTION

The skin is one of the largest organs in the body that performs numerous vital function including fluid homeostasis, thermoregulation, immunologic, neurosensory and metabolic function. The skin also provides primary protection against infection by acting as a physical barrier[(1)]**.** More than 80% of the world's population depends upon traditional medicines for various skin diseases[(2)]. The wound may be defined as loss or breaking of cellular and anatomical or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation[(3)]**.** The process of wound healing consist of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with region of strength of injured tissue[(4)]. Recently, the traditional use of plants for wound healing has received attention by the scientific community[(5)]. Almost all part of the plant has been utilized in traditional medicine practices**.** Different parts of plant used for wound healing contain some active principle or components that possess anti-microbial function[(6)]. Hence in the present investigation of polyherbal ointment of *Calophyllum inophyllum* L, *Hibiscus sabdariffa* L and *Quercus infectoria* Olive has been prepared and evaluated for it wound healing efficiency.

Materials and methods

Collection of plant material

Plant materials used for this study were collected from places in and around Tiruchirappalli. The materials were identified and authenticated by Taxonomist Rev. Fr. Dr. John Britto, Director, Rapinart Herbarium, St. Joseph College, Tiruchirappalli.

Polyherbal ointment preparation

Fresh leaves of *Calophyllum inophyllum* L, *Hibiscus sabdariffa* L and dried seed of *Quercus infectoria* Olive were used for the preparation of the ointment. The plant materials were shade dried and ground into fine powder using electrical blender. It was then passed through a sieve of 80 mesh 60 g of the each plant material were taken. Mixed 50g of purified bee wax with 60 ml of gingely oil. Boiled the wax, removed from heat and added the powdered plant materials to the mixture were stirred continuously till an ointment consistency was obtained. From this ointment 1g was applied daily on wounded animals.

Experimental design

Healthy adult wistar strain of albino rats of either sex, weighing 150-200g were used as experimental models. Animals were kept in well ventilated cages and fed with standard rat chow pellet obtained from SaiDurga Feeds and Food, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Animal grouping

The rats were divided into nine groups each comprising of six animals.

GROUP I: **Excision** wounded control. (Without treatment)

GROUP II: **Excision** wounded Polyherbal Ointment (PHO) treated (1 g/ day topically)

GROUP III: **Excision** wounded Soframycin Ointment (SO) treated (1 g/ day topically)

GROUP IV: **Incision** wounded control. (With out treatment)

GROUP V: **Incision** wounded Polyherbal Ointment (PHO) treated (1 g/ day topically)

GROUP VI: **Incision** wounded Soframycin Ointment (SO) treated (1 g/ day topically)

GROUP VII: **Dead space** wounded control. (Without treatment)

GROUP VIII: **Dead space** wounded Polyherbal Ointment (PHO) treated (1 g/ day topically)

GROUP IX: **Dead space** wounded Povidone Ointment (PO) treated (1 g/ day topically)

Creation of wound

Excision wound

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 2.5cm 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 initiated [7].

Incision wound

Incision wound was created under light ether anesthesia; one paraventral incisions of 6 cm were made through the entire thickness of skin on any one side of vertebral column with the help of a sharp blade. After the incision was made the parted skins were kept together and sutured with nylon thread by 0.5cm apart. Surgical thread (NO. 000) and curved needle of (NO. 11) were used for suturing. The continuous threads of both wound edges were tightened for good adoption of wounds. Breaking strength or tensile strength represents the promotion of wound healing [8].

Dead space wound

Dead space wounds were inflicted by implanting sterilized cotton pellets (10mg) in the lumber region on the ventral surface of each rat. On the 10th post wounding day, the granulation tissue formed

on the implanted cotton pellet was carefully removed. The wet weight of the granulation tissue was noted. This granulation tissue were dried at 60°C for 12 hours and weighed and the weight was recorded. To the dried tissue added 5 ml of 6 N HCl and kept at 110°C for 24 hours. The neutralized with acid and the hydrolysate of the dry tissue was used for the determination of hydroxyproline [9].

Biochemical parameters

Estimations of Hydroxyproline[10] and Hexosamine[11]- the acid hydrolysate of the dry tissue sample were used. Granulation tissues were extracted in 5% trichloroacetic acid (TCA) incubated overnight with 10% perchloric acid. Centrifuged after incubation and washed with 5% perchloric acid. The precipitate was dissolved in warm 10% perchloricacid and subjected for the estimation of DNA [12] and RNA [13]**.** Serum protein was estimated [14]**.**

Antioxidant assay

The liver was excised out from the sacrificed animals rinsed in ice cold normal saline followed by a wash in cold 0.15MTris-HCl (pH7.4) and dried. A 10%(w/v) homogenate was prepared in 0.15 M Tris-HCl buffer. From this the lipid peroxidation was determined by analyzing the levels of thiobarbituric acid reactive substances (TBARS) [15] and the activity of superoxide dismutase (SOD) was assayed [16].

Statistical analysis

Experimental data were expressed as mean ± SEM. Statistical analysis was performed using analysis of varience between polyherbal ointment treated with control groups. Data were considered to be significant at $p<0.05$.

Results

Wound healing activity

The polyherbal ointment prepared promotes significant wound healing activity in all the three wounded models. The wound contracting ability of PHO treated Excision wounded models **(Table 1)** were found to be significantly ($p<0.05$) greater than the control animals (group-I) and reference ointment treated animals (group – III).**Table 2** shows the tensile strength value of the incision and dead space wound models, there was a significant increase in tensile strength ($p <0.05$) in wounded treated with PHO in groups- V and VIII as compared to control group-IV and VII [17].

Table: 1 Rate of Wound Contraction on Excision Wounds:

Groups	O Day cm^2	5th Day cm^2	10th Day cm^2
I (Excision control)	2.1±0.14	1.79±0.09	0.51±0.15
II(Excision PHO)	2.3±0.16 *	0.98±0.12 *	0.00±0.06 *
III(Excision SO)	2.1±0.21	1.7±0.08	0.68±0.07

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

Table: 2 Effect of PHO on Tensile Strength In Incision and Dead Space Model of Wounds:

GROUPS	TENSILE STRENGTH N/cm^2
CONTROL (group IV)	12.70 ± 3.41
PHO TREATED(group V)	18.96 ± 4.16 *
REFERENCE OINTMENT(group VI)	15.42 ± 4.21
CONTROL (group VII)	10.83 ± 3.56
PHO TREATED(group VIII)	17.51 ± 6.92 *
REFERENCE OINTMENT(group IX)	14.12 ± 4.35

*Values were expressed as mean ±SEM n=6, *p<0.05 when compared PHO treated (group V and VIII) with control group (group IV and VII).*

Biochemical parameters

Table-3 depicts the Hydroxyproline, Hexosamine, Protein, DNA and RNA levels in the granulation tissues of control and treated groups of all the three experimental models. Significant increase in the Hydroxyproline and Hexosamine content were observed in the PHO treated groups (II, V and VIII). A similar trend was observed, with significant increase in the Protein, DNA and RNA content. **Table – 4** shows the antioxidant activity of PHO. The levels of peroxide were found to be lowered and the activities of SOD were increased in PHO treated animals.

Table: 3 Effect of PHO on various biochemical parameters

Groups	HydroxyProline mg/g tissue	Hexosamine mg/100mg tissue	Protein mg/100mg wet tissue	DNA mg/100mg wet tissue	RNA mg/100mg wet tissue
I	57.51±7.82	5.34±1.16	4.14±1.52	1.87±0.81	2.54±0.94
II	77.51±6.42*	8.96±2.43*	7.25±2.09*	4.37±0.54*	4.75±0.82*
III	67.64±6.59	7.36±1.52	6.44±1.91	3.62±0.82	3.75±0.64
IV	47.26±6.92	4.96±1.04	5.81±1.04	2.51±0.66	1.87±0.84
V	84.54±6.23*	7.85±2.17*	8.32±2.14*	4.77±0.41*	4.52±0.74*
VI	64.72±7.64	6.52±2.08	6.52±2.72	3.87±0.56	3.57±0.71
VII	35.14±6.56	5.92±2.93	4.96±2.36	2.01±0.27	2.56±0.32
VIII	71.49±7.91*	8.14±1.66*	7.56±2.55*	5.11±0.74*	4.75±0.64*
IX	61.39±7.47	6.52±1.89	6.04±2.11	3.92±0.63	3.42±0.18

*Values were expressed as mean ±SEM n=6, *p<0.05 when compared PHO treated (group II,V and VIII) with control groups (group I, IV and VII).*

Table: 4 Effect Of PHO on Lipid peroxide and Superoxide dismutase activity

Groups	Superoxide Dismutase mg/mg protein	Lipid peroxidation moles/mg protein
I	5.43±0.27	5.42±o.91
II	7.56±0.43*	3.56±0.43 *
III	6.16±0.57	4.32±0.81
IV	4.51±0.32	6.53±1.07
V	8.14±0.64*	3.98±0.95 *
VI	6.04±0.37	4.57±0.29
VII	4.68±0.56	6.81±0.79
VIII	8.92±0.72 *	4.07±0.56 *
IX	6.54±0.65	5.52±0.17

*Values were expressed as mean ±SEM n=6, *p<0.05 when compared PHO treated (group II,V and VIII) with control groups (group I, IV and VII).*

Discussion

Wound healing and tissue repair are complex process that involve a dynamic series of events including clotting, inflammation, granulation, tissue formation, epithelization, collagen synthesis and tissue remodeling. Wound contraction is the process of shrinkage of the area of the wound. It is mainly dependent upon the type and extent of damage, the general state of healed and the ability of the tissue to repair. The granulation tissue of the wound is primarily composed of fibroblasts, collagen and new blood vessels. The undifferentiated mesenchymal cells of the wound margin differentiate into fibroblasts, which start migrating into the

wound gap along with the fibrin strands [18]. The increase in tensile strength of the granulation tissue indicated enhanced collagen maturation by increase cross linking [19]. The collagen is the main constituent of extracellular tissue, which is responsible for support and strength. Free hydroxyproline and its peptides are released with collapse of collagen. Increase in dry tissue also indicates the presence of elevated protein content [20]. Increased hexosamine content in the early stages of wound healing indicating the active synthesis of fibroblast, which act as ground substances (mucopolysaccharides) on which the collagen can be laid on [21]. Protein is required as part of the inflammatory process, in the immune response and in the development of granulation tissue. The decreased level of serum protein in the wound control group (group I,IV and VII) could have contributed to its delayed healing. The plant augment the protein synthesis and fastened the process of healing. The main protein synthesized during the healing process is collagen and the strength of the collagen determines wound strength [22]. Group II, V & VIII animals showed marked ($p<0.05$) rise, indicating the rate of the collagen synthesis for the regeneration of wounded tissue. The greater synthesis of RNA, DNA and protein greater will be the formation of granulation tissue in response to injury [23]. SOD is a potent antioxidant enzyme and an increase in its activity has been reported to be a reflux mechanism to guard against the extracellular oxygen derived free radical [24]. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibers, by increasing the circulation, by preventing the cell damage and by promoting the DNA synthesis [25].

Conclusion

The present study revealed that the plant used in the polyherbal ointment possesses significant wound healing promoting activity. The present finding provides scientific evidence to the ethnomedicinal property of plants in the healing of wounds. These natural sources would serve better in the treatment of wounds at a faster rate. It needs further evaluation in clinical settings before consideration for the treatment of wounds.

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