INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 2, 2018; 237 – 243 ; http://ijpda.com; ISSN: 2348-8948

MEDFOOD'18 [1st February 2018]

National Conference on Phytochemicals in Medicinal Plants and Food

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Research Article

Healing potential of a polyherbal ointment on experimentally created incision, excision and diabetic excision wound models

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Date Received: 23rd January 2018; Date accepted: 29th January 2018; Date Published: 17th February 2018

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. In the present study an attempt was made to investigate the wound healing potential of a polyherbal ointment comprising of aqueous extract of *Calophyllum inophyllum* Linn, *Albizia amara* Roxb and *Couroupita guianensis* Aubl on incision wounds, excision wounds and diabetic excision wounds in albino rats. Animals weighing 150- 200g were divided into twelve groups each comprising of 6 rats each. Group I,V and IX served as incision, excision and diabetic excision wounded control respectively and Group II,III,VI,VII,X and XI served as rats treated with polyherbal ointment at two different doses (10% and 20%) of polyherbal ointment applied topically for 15days, Group IV and VIII served as incision and excision wounded animals treated with reference ointment povidone and Group XII served as diabetic wounded animals treated with reference ointment silverex. The healing of the wound was assessed by the rate of wound contraction, tensile strength, hydroxy proline and hexosamine. The results suggested the potency of polyherbal ointment in the management of wound healing.

Keywords: Wound healing, Polyherbal Ointment, Biochemical Parameters.

INTRODUCTION

Wounds are inescapable events of life, which arise due to physical or chemical injury or microbial infections. The healing of wounds often deviates from normal course, under- healing, over-healing or failure of wounds. Research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Medicinal plants are coming into prominence because of the overuse of conventional medicines such as antibiotics which has resulted in development of resistance with many infectious organisms [1].

Wound refers to the disruption/opening in the epithelial tissue of the skin, caused by loss of physical, chemical, thermal and mechanical integrity and infections occurred by microbes present in the environment and delay the wound repair [2].

Wound healing process is well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds is an important biological process involving tissue repairs and regeneration. It involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue [3].

Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. With the development of scientific research methods, the significant successes reported have led to investigation into medicinal plants with a view of confirming these acclaimed properties [4].

The treatment of wounds is sometimes problematic especially in chronic wounds with a prevalence of 4.5 per 1000 population. Although there has been an enormous development in pharmaceutical drug industry, wound healing drugs are not still satisfactory because of their low availability, high cost, and various detrimental side effects. Therefore, medicinal plant derived drugs is under great demand due to a common belief that they are safe, reliable, clinically effective, low cost, globally competitive and better tolerated by patients . Since ancient times, human beings have been using many plant resources based on empirical observations without any scientific knowledge for the treatment of wounds, cuts, and burns. Biologically active compounds of these plants such as tannins, triterpenoids and alkaloids have been found to affect one or more phases of wound healing process . Therefore, isolation of principle compounds and development of novel wound healing drugs are important for possible future therapeutic applications [5].

Materials and methods

Collection of plant material:

Plant source selected for the present study is *Calophyllum inophyllum* Linn, *Albizia amara* Roxb and *Couroupita guianensis* Aubl.The aerial part of the Plants were collected, around Tituchirappalli and authenticated by Taxonomist Rev.Fr.Dr.John Britto,Director at RAPINAT herbarium of St.Joseph's College, Tiruchirappalli, Tamilnadu, India.

Preparation of Ointment:

The extract ointment was prepared from the equal proportions of *Calophyllum inophyllum* Linn, *Albizia amara* Roxb and *Couroupita guianensis* Aubl at a concentration of 10% and 20% (w/w) using simple ointment base.

In-vivo Studies:

To investigate the wound healing efficacy of herbal ointment by studying various biochemical parameters on wounded rat models. The preclinical trials have to be conducted to confirm the wound healing potential of these medicinal plants.

Experimental design

Animal Grouping:

Group I:	Incision wound control
Group II:	Incision wound will be treated with the 10% Polyherbal ointment. (0.5g/kg body weight, applied topically for 15 days)
Group III:	Incision wound will be treated with the 20% Polyherbal ointment. (0.5g/kg body weight, applied topically for 15 days)
Group IV:	Reference Ointment (Povidone , 0.5g/kg body weight, applied Topically for 15 days).
Group V:	Excision Wound Control.
Group VI:	Excision wound will be treated with the 10% Polyherbal ointment (0.5g/kg body weight, applied topically for 15 days)
Group VII:	Excision wound will be treated with the 20% Polyherbal ointment. (0.5g/kg body weight, applied topically for 15 days)
Group VIII:	Reference Ointment (Povidone, 0.5g/kg body weight, applied Topically for 15 days).
Group IX:	Diabetic Excision Wound Control.
Group X:	Diabetic Excision wound will be treated with the 10% polyherbal Ointment (0.5g/kg body weight, applied topically)

Group XI: Diabetic Excision wound will b treated with the 20% Polyherbal Ointment. (0.5g/kg body weight, applied topically)

Group XII: Reference Ointment (Silverex, 0.5g/kg body weight, applied topically).

After the experimental period, the animal will be sacrificed by cervical dislocation, blood and scar tissues samples will be collected and used for further studies.

Creation of wound

Excision wound [6]

An excision wound will be created on the dorsal side of rats. The dorsal sides of rats will be shaved with a razor blade. Excision wound of size 2.5cm^2 areas of skin in length, 0.2cm^2 in depth will be created by using surgical scissors. Haemostasis achieved by blotting the wound with cotton swab soaked in normal saline. The wound will be left undressed to the open environment, then the Poly herbal ointment (PHO) and Standard ointment (SO) will be topically applied daily on respective group of animals till the wounds completely healed. Rate of wound contraction will be studied by tracing the raw wound. (After creating the wounds, all the animals in the groups will be kept separately for first three days)

Diabetic Excision wound [7]

Rats were made diabetic by a single injection of alloxan monohydrate (120 mg/kg, i.p.) prepared in citrate buffer (0.1 M, pH 4.5), after overnight fasting. Blood was drawn from the tail vein 24 hours after the injection and the glucose level was estimated. Wounds were made on the rats showing elevated blood glucose level (>250 mg/dl). Blood glucose levels were estimated at the time of creation of the wounds.

An excision wound will be created on the dorsal side of rats. The dorsal sides of rats will be shaved with a razor blade. Excision wound of size 2.5cm^2 areas of skin in length, 0.2cm^2 in depth will be created by using surgical scissors. Haemostasis achieved by blotting the wound with cotton swab soaked in normal saline. The wound will be left undressed to the open environment, then the PHO and SO will be topically applied daily on respective group of animals till the wounds completely healed. Rate of wound contraction will be studied by tracing the raw wound. (After creating the wounds, all the animals in the groups will be kept separately for first three days)

Incision wound [8]

Incision wound will be created under light ether anesthesia; two paraventral incisions of 6 cm will be made through the entire thickness of skin on either side of vertebral column with the help of a sharp blade. After the incision will be made the parted skins will be kept together and sutured with nylon thread by 0.5cm apart. Surgical thread (NO.000) and curved needle of NO.11 are used for suturing. The continuous thread of both wound edges will be tightened for good adoption of wounds. Breaking strength or tensile strength represents the promotion of wound healing. (After creating the wounds, all the animals in the groups will be kept separately for first three days).

Physical parameters

Rate of wound contraction [9]

The rate of wound contraction will be determined by the reduction in wound size. The wound size will be measured regularly by tracing wound site with trace paper and measured graphically.

Tensile strength [10]

On the 10th day after creating the wound the animals were anaesthetised. Healing tissue along with normal skin at two ends was excised for tensile strength measurement using Tensile Testing Machine TKG-20. Strips of 1cm width and 5cm length were cut out from the excised tissue in treated and control animals and were loaded between the upper and lower holder of the machine in such a way that the effective load bearing size was 2.5 x 2.5 cm with the wound remaining in the centre. The total breaking load is measured in Newtons and the tensile strength was calculated by the following equation:

Tensile strength=Total breaking load/Cross-sectional area

Biochemical parameters

Estimations of Hydroxyproline and Hexosamine

the acid hydrolysate of the dry tissue sample were used by the following methods Woessner ,1961[11] and Wager, 1979[12].

Results and Discussion

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of amino acid is the major component of extra cellular tissue, which gives strength and support [13].

In the present study, polyherbal ointment treated excision groups (6and7) and diabetic excision groups (10and 11)were found to contract much faster than control groups(5and 9). Increased rate of wound contraction in polyherbal ointment treated animals might be due to increase in proliferation and transformation of fibroblast cells into myofibroblasts.

The process of maturation of collagen fibrils was catalyzed by the enzyme lysyl oxidase. Lysyl oxidase is the enzyme involved in the formation of cross-links, therefore play a very important role in the maturation process and in wound healing. The increased level of the enzyme activity may result in increased cross linking which leads to concurrent increase in the tensile strength of wounds [14].

Collagen is known to play important role in the process of wound contraction and gain in tensile strength depends upon increase in the collagen content. The tensile strength of Polyherbal ointment treated groups (2 and 3) is highly significant as compared to control group 1 and reference ointment group 4.

Diabetes mellitus was one of the factors affecting the normal course of wound healing. Diabetic wound healing is characterized by a delay in cellular infiltration and formation of granulation tissue, decreased wound collagen content, diminished wound tensile strength and prolonged epithelialization time. Experimental diabetes has been shown to impair wound healing by decreasing collagen concentration and formation of granulation tissue by increasing activities of protease and collagenase[15]. The blood glucose level was found to be elevated in group9,10,11,12 animals throughout the studies.

Wound healing was a fundamental response to tissue injury that results in restoration of tissue integrity which is due to the synthesis of the connective tissue matrix. Collagen was a major protein of the extracellular matrix and was the component that ultimately contributes to wound strength. Measurement of the hydroxyproline could be used as a index for collagen turnover[16].

On topical application of polyherbal ointment on excision,incision and diabetic excision wounded animals, there was a significant increase ($p \leq 0.05$) in hydroxyproline level in group of animals which received topical application of polyherbal ointment.The effect of polyherbal ointment was evidenced from the result obtained for incision groups(2 and 3),excision groups (6 and 7) and diabetic excision groups(10 and 11).

Glycosaminoglycans and proteoglycans were synthesized by fibroblasts in the wound area. Hexosamine and hexuronic acid were matrix molecules, which act as ground substratum for the synthesis of new extracellular matrix. These substance forms highly hydrated gel liked ground substance, a provisional matrix on which collagen fibres are embedded. They are known to stabilize the collagen fibres by enhancing electrostatic and ionic interactions with it and possibly control their ultimate alignment and characteristic size [17].

Hexosamine content was found to be siginificantly ($p<0.05$) increased in polyherbal ointment treated incision groups (2 and 3),excision groups (6 and 8) and diabetic excision groups (10 and 11) than compared to control groups (1,5and7).Increased hexosamine level in early of wound healing indicating active synthesis of fibroblast.

Table No:1 Rate of Wound Contraction on Post Wounding Days on Normal Excision and Diabetic Excision wounded animal models were presented below.

GROUPS	0th DAY (cm^2)	5th DAY (cm^2)	10th DAY (cm^2)	15th DAY (cm^2)
5	2.3±0.04	1.1±0.04	1.3±0.02	0.9±0.01
6	2.3±0.02*	1.6±0.01*	1.2±0.01*	0.6±0.02*
7	2.2±0.01*	1.7±0.02*	0.9±0.02*	0.4±0.01*
8	2.2±0.01	1.8±0.02	1.2±0.01	0.5±0.01
9	2.1±0.04	1.82±0.04	1.33±0.06	0.9±0.04
10	2.2±0.01*	1.4±0.02*	0.9±0.02*	0.3±0.01*
11	2.2±0.02*	1.0±0.02*	0.6±0.01*	0.0±0.01*
12	2.3±0.02	1.6±0.01	1.1±0.02	0.7±0.01

*Values were expressed as mean ± SEM n=6,*p<0.05 when compared polyherbal ointment treated groups(6,7 and 10,11)with control groups(5 and 9).*

Table 2: Tensile strength of Incision wounded animals

Groups	Tensile strength(N/cm^2)
1	9.714±2.61
2	13.446±1.98*
3	15.536±3.05*
4	11.419±2.14

Values were expressed as mean ± SEM n=6, p<0.05 when compared Polyherbal ointmenttreated groups(2 and 3)with control group 1.*

TABLE :3 Blood glucose level in diabetic excision animals.

Groups	Blood glucose
9	210.52±7.19
10	213.31±5.01*
11	214.04±5.64*
12	211.03±5.19

Values were expressed as mean ± SEM n=6, p<0.05 when compared Polyherbal ointment treated groups(10 and 11)with control group 9.*

Table No:4 Level of Hydroxyproline in the Polyherbal ointment treated, untreated and standard drug treated wounded animals:

Groups	Hydroxyproline(mg/g tissue)
1	33.6±3.14
2	40.1±3.52*
3	49.4±3.11*
4	39.4±2.44
5	29.4±2.55
6	36.8±2.46*
7	43.4±3.56*
8	33.1±2.67
9	24.8±2.07
10	31.7±1.98*
11	39.6±2.14*
12	28.4±2.65

Values were expressed as mean ± SEM n=6, p<0.05 when compared Polyherbal ointment treated groups(2,3,6,7,10 and 11)with control groups(1,5 and 9).*

Table No:5 Level of Hexosamine in The Polyherbal ointment Incision,Excision and Diabetic Excision Wounded Animals.

Groups	Hexosamine(mg/100mgtissue)
1	5.2±0.76
2	8.9±0.14*
3	11.1±0.43*
4	6.2±0.66
5	4.8±0.65
6	6.8±0.65*
7	9.4±0.73*
8	4.8±0.43
9	4.6±0.15
10	6.8±0.16*
11	8.1±0.42*
12	5.1±0.32

Values were expressed as mean ± SEM n=6, p<0.05 when compared Polyherbal ointment treated groups(2,3,6,7,10 and 11)with control groups(1,5 and 9).*

Conclusion

The present study was concluded 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.

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