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Research Article

# WOUND HEALING EFFICACY OF HERBAL OINTMENT CONTAINING *OLDENLANDIA HERBACEA* ROXB. ON EXCISION WOUNDED ANIMALS

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**ABSTRACT**

Objective: The present study is aimed to evaluate the curative effect of herbal ointment containing *Oldenlandia herbacea* Roxb. against excision wound. Methods: Animals weighing 150 -200g were divided into five groups each comprising of six rats: Group I served disease control, Group II served as excision wounded control and Group III, IV served as excision wounded animals were treated with 10% and 20% of herbal ointment (HO) containing *Oldenlandia herbacea* Roxb. applied topically for 14days respectively and group V served as excision wounded animals treated with reference ointment soframycin. The healing of wound was assessed by the rate of wound contraction, level of hydroxyproline, hexosamine, tissue and serum protein, ascorbic acid, Deoxyribonucleic Acid (DNA), Ribonucleic Acid (RNA), Superoxide Dismutase (SOD), Lipid Peroxidation (LPO). Results: The topical application of herbal ointment treated groups showed increase in hydroxyproline, hexosamine, protein, DNA, RNA and SOD, Ascorbic acid and significant decrease in the wound contraction, LPO. Conclusion: The results concluded that the *Oldenlandia herbacea* Roxb has the efficacy in the management of wound healing.

**Keywords:** Wound; Antioxidants; Herbal Ointments; *Oldenlandia herbacea* Roxb.

## INTRODUCTION

Wound represents a major health problem both in terms of morbidity and mortality. Wound is characterized by the loss of epithelial integrity, disruption of normal structure and function of skin and its underlying tissues[1].Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical events leading to reestablishment of strength in injured tissue and provide adequate tissue perfusion, oxygenation and nutritional support[2].

Wounds have affected humans since pre-historic times and the treatment of healing is an art as old as humanity[3]. Due to poor hygienic conditions and increasing life expectancy coupled with modern way of life, wound particularly chronic wounds highly affect a growing number of elderly people and seriously reduce the quality of life. Now a days, lot of wound healing drugs and antibiotic creams are available in the market, but it can produce several adverse effects especially allergic reaction, edema formation and unhealed scar formation and delayed re epithelialization etc. So, there is a need to develop an alternative human friendly drug. Hence the research on wound healing is rapidly increased in modern biomedical sciences.

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases[4]. Moreover, Indian folk medicine comprises numerous prescriptions for therapeutic purposes such as healing of wounds, inflammation, skin infections, leprosy, diarrhorea, scabies, venereal disease, ulcers, snake bite[5], etc. More than 80% of the world's population still depends upon traditional medicines for various skin diseases[6]. Herbal medicines in wound management involve disinfection, debridement and providing a moist environment to encourage the establishment of the suitable environment for natural healing process[7]. Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns[8].

*Oldenlandia herbacea* Roxb. belonging to Rubiaceae family, is an erect, glaborus annual herb found in temperate and tropical regions. Decoction of the herb can be used for the treatment of rheumatic arthritis and swellings. The herb is boiled with oil, and the oil is used to cure elephantiasis and pains in the body. The leaves have been used as an expectorant in asthma. The aerial part of this plant used for wound healing[9]. Hence the present study was aimed to assess the wound healing efficacy of *Oldenlandia herbacea* Roxb. on excision wounded albino rats.

## MATERIALS AND METHODS

### Collection of Plant Materials

Aerial parts of the *Oldenlandia herbacea* Roxb. were collected in and around Tiruchirappalli district, Tamil Nadu, INDIA. The material was identified and authenticated with the specimen (Voucher No–S002) deposited at Rapinet Herbarium, St. Joseph's College, Trichy.

### Preparation of plant extract

Aerial parts of the *Oldenlandia herbacea* Roxb. were shade dried and powdered coarsely using electrical blender. 200g of the plant powder was mixed with six parts of water. Then it was boiled until it was reduced to one third and filtered. Then the filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to the preparation of wound healing ointment.

**Preparation of herbal ointment**

The wound healing ointment was prepared by mixing aqueous extract of *Oldenlandia herbacea* Roxb. at the concentration of 10 and 20% of (w/w) using ointment base[10].

**Experimental animals**

Healthy adult Wistar strain of albino rats of either sex, weighing 150- 200 g were used as experimental models. Animals were kept in ventilated cages and fed with standard rat chow pellet obtained from Sai Durga Food and Feeds, 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 five groups each containing of six animals. GROUP I was served as Normal control GROUP II was served as Excision wounded animals without treatment, GROUP III and IV were served as Excision wounded animals treated with Herbal ointment (HO) at 10% and 20 % (0.5g applied topically for 14 days), GROUP V was served as Excision wounded animals with Standard Drug Soframycin Ointment (SO) (0.5g applied topically for 14 days).

**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 2cm areas of skin in length, 0.2cm in depth were created by using surgical scissors. Homeostasis achieved by blotting the wound with cotton swab soaked in normal saline. All the rats were given regular dressing changes and kept for observation[11].

**Measurement of Wound Contraction**

An excision wound was traced by following the progressive changes in wound area excluding the day of wounding. The size of wounds was traced on a transparent paper in every day, throughout the study period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated[12]. The percentage of wound contraction was calculated by the following formula:

$$\% \text{ Wound Contraction} = \frac{\text{Initial Wound Size } \times 100}{\text{Initial Wound Size}}$$

**Parameters studied**

After the experimental period, the animals were sacrificed by cervical dislocation and the blood and tissue samples were collected for analyzing biochemical parameters such as hydroxyproline[13], hexosamine[14], DNA[15], RNA[16], tissue protein[17], lipid peroxide[18], superoxide dismutase[19] and Ascorbic acid[20].

**Statistical analysis**

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

**RESULTS**

**Effect of Herbal Ointment on Rate of Wound Contraction by Excision Wounded Animals**

The rate of wound contraction on post wounding days was presented in Table 1. The size of the wound was measured at $0^{th}$ day, $7^{th}$ day and $14^{th}$ day. A moderate reduction in the size of the wound was observed in the animals treated with herbal ointment at the dose level of 10%. At $14^{th}$ day, a complete wound closure was observed in the animals treated with herbal ointment at 20% when compared to standard.

**Effect of Herbal Ointment on Hydroxyproline, Hexosamine, DNA and RNA by Excision Wound**

Table 2 depicted the levels of Hydroxyproline, hexosamine, DNA and RNA of the wound tissues. Those levels were significantly reduced in Group II animals. Upon treatment with herbal ointment, those levels were revered back to normal.

**Effect of Herbal Ointment on tissue Protein by Excision Wound**

Table 3 demonstrated that the protein levels were significantly lower in the HO treated groups (groups III, IV), when compared to excision control (group II).

**Effect of Herbal Ointment on LPO by Excision Wound**

Table 4 represents the significant elevation in the level of LPO was noted in the wound tissue of excision wound control animals and upon treatment with herbal ointment the level was decreased.

**Effect of Herbal Ointment on SOD and Ascorbic Acid by Excision Wound**

The level of enzymatic antioxidant SOD and Non-enzymatic antioxidant Ascorbic acid was found to be lower in excision wounded animals whereas treatment with herbal ointment raised the levels of both SOD and Ascorbic acid in excision wounded rats (Table 5).

**DISCUSSION**

Wound Contraction is the process where the wound contracts, narrowing or closing the wound. Fibroblasts appear in the wound after 2-3 days, yet myofibroblasts predominate at day 12 when wound contraction is 80% complete[21]. Wound contraction, the process of shrinkage of area of the wound depends on the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue[22]. In the present study, herbal ointment treated animals were found to contract much faster. Increased rate of wound contraction in herbal ointment treated animals might be due to increase in proliferation and transformation of fibroblast cells into myofibroblasts. And also, the effect of the herbal ointment on wound contraction may be due to the presence of flavonoids and saponins which are responsible for the release of cytokines, increased synthesis of collagen and angiogenesis.

Collagen is a major protein of extracellular matrix and is the component that contributes wound strength and also it plays a crucial role in homeostasis. Breakdown of collagen liberates the hydroxyl proline. Measurement of hydroxyl proline could be as an index for collagen turn over. The decreased level of hydroxyl proline shows the defective proliferation and activation of fibroblast which affects the collagen synthesis. The increased

amount of hydroxy proline in a Herbal ointment treated groups (II, III) indicates the faster rate of wound healing[23].

Glycosaminoglycans and proteoglycans are synthesised by fibroblasts in the wound area. These substances form a highly hydrated gel like ground substance, a provisional matrix on which collagen fibres are embedded. Increased hexosamine level in herbal ointment treated animals were associated with a stability of collagen fibres by enhancing electrostatic and ionic interaction. DNA is a diffusible oligonucleotide appears to be a stimulus and essential for fibroplasias of cells. Decreased level of DNA in excision wound control indicates disturbances in the cellular proliferation and RNA indicates the low level of transcription. The increased level of DNA and RNA in herbal ointment treated groups indicates the rapid formation of granulation tissue in response to injury and prophylactic action on protein synthesis[24].

**Table 1: Effect of Herbal Ointment on Rate of wound contraction in excision wounded Rats**

| Group | $0^{th}$ day ( $cm^2$) | $7^{th}$ day ( $cm^2$) | $14^{th}$ day ( $cm^2$) |
|---|---|---|---|
| I | - | - | - |
| II | 2.36 ±0.51$^{*a}$ | 1.27 ± 0.28$^{*a}$ | 0.70±0.02$^{*a}$ |
| III | 2.24 ±0.08$^{**b}$ | 1.10 ± 0.16$^{**b}$ | 0.3±0.03$^{**b}$ |
| IV | 2.21 ±0.06$^{**b}$ | 1.05 ±0.07$^{**b}$ | 0.1±0.02$^{**b}$ |
| V | 2.10 ±0.04$^{*c}$ | 0.90 ±0.04$^{*c}$ | 0. 45±0.02$^{*c}$ |

$cm^2$ – Centimeter square, Values are expressed as mean ± SEM n=6, $^{a*}$P< 0.05 statistically significant when excision wound control (Group II) compared with normal group (Group I),$^{b**}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with excision wound control groups(group II),$^{c*}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with standard drug treated(Group V).

**Table 2: Effect of Herbal Ointment on Hydroxyproline, Hexosamine, DNA and RNA in Excision Wounded Rats**

| Groups | Hydroxyproline (mg/g Tissue) | Hexosamine (mg/g tissue) | DNA (mg/g tissue) | RNA (mg/g tissue) |
|---|---|---|---|---|
| I | 78.56 ±2.10 | 72.18±1.89 | 20.25±1.25 | 23.7±0.08 |
| II | 43.89± 2.05$^{*a}$ | 13.13±0.82$^{*a}$ | 6.1± 0.29$^{*a}$ | 11.33± 0.39$^{*a}$ |
| III | 49.11 ± 1.59$^{**b}$ | 32.67 ± 1.20$^{**b}$ | 11 ± 0.15$^{**b}$ | 14 ± 0.30$^{**b}$ |
| IV | 69.56 ± 1.95$^{**b}$ | 57± 1.51$^{**b}$ | 15.68± 0.12$^{**b}$ | 17.54 ± 0.25$^{**b}$ |
| V | 76.44±2.05$^{*c}$ | 70.67± 2.11$^{*c}$ | 20.08 ± 0.17$^{*c}$ | 23.5 ± 0.15$^{*c}$ |

mg/g Tissue – Milligram per gram tissue, Values are expressed as mean ± SEM n=6, $^{a*}$P< 0.05 statistically significant when excision wound control (Group II) compared with normal group (Group I),$^{b**}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with excision wound control groups(group II),$^{c*}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with standard drug treated(Group V).

**Table 3: Effect of Herbal Ointment on Tissue protein in Excision Wounded Rats**

| Groups | Tissue protein (mg/g tissue) |
|---|---|
| **I** | 1.8± 0.04 |
| II | 0.50± 0.02$^{*a}$ |
| III | 0.94± 0.08$^{**b}$ |
| IV | 1.51± 0.15$^{**b}$ |
| V | 1.65± 0.09$^{*C}$ |

mg/g tissue – Milligram per gram tissue. Values are expressed as mean ± SEM n=6, $^{a*}$P< 0.05 statistically significant when excision wound control (Group II) compared with normal group (Group I),$^{b**}$P< 0.05 statistically significant when HO treated (Group III, IV) compared with excision wound control groups(group II),$^{c*}$P< 0.05 statistically significant when HO treated (Group III, IV) compared with standard drug treated(Group V).

**Table 4: Effect of Herbal Ointment on LPO in excision wounded Rats**

| Groups | LPO (milli moles of MDA produced/g tissue) |
|---|---|
| I | 3.65± 0.12 |
| II | 5.80 ±0.19$^{*a}$ |
| III | 4.32 ±0.14$^{**b}$ |
| IV | 2.85±0.10$^{**b}$ |
| V | 3.20 ±0.12$^{*C}$ |

millimoles of MDA produced/g tissue – Millimoles of malondialdehyde produced per gram tissue. Values are expressed as mean ± SEM n=6, $^{a*}$P< 0.05 statistically significant when excision wound control (Group II) compared with normal group (Group I),$^{b**}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with excision wound control groups(group II),$^{c*}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with standard drug treated(Group V).

**Table 5: Effect of Herbal Ointment on SOD and Ascorbic acid in excision wounded Rats**

| Groups | SOD (mg of epinephrine oxidized /g tissue) | Ascorbic acid (mg/g tissue) |
|---|---|---|
| I | 45.62± 1.81 | 3.98± 0.14 |
| II | 14.21± 1.94$^{*a}$ | 1.68± 0.03$^{*a}$ |
| III | 24.14± 2.58$^{**b}$ | 2.63 ± 0.05$^{**b}$ |
| IV | 43.08 ± 1.38$^{**b}$ | 3.39 ± 0.08$^{**b}$ |
| V | 43.41± 1.88$^{*c}$ | 3.74 ±0.09$^{*c}$ |

mg of epinephrine oxidised/ g tissue – Milligram of epinephrine oxidised per gram tissue. mg/g tissue – Milligram per gram tissue. Values are expressed as mean ± SEM n=6, $^{a*}$P< 0.05 statistically significant when excision wound control (Group II) compared with normal group (Group I),$^{b**}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with excision wound control groups(group II),$^{c*}$P< 0.05 statistically significant when HO treated (Group III,IV) compared with standard drug treated(Group V).

Protein is required as part of the inflammatory process, in the immune response and in the development of granulation tissue. The low level of protein content in excision wounded controls signifies the delayed wound healing. Concomitant increase in the total protein content in herbal ointment treated animals signifying active synthesis and deposition of matrix proteins in the granulation tissues. Protein deficiency inhibits wound remodeling. The remodeling process increases the strength of healed tissue[25].

Lipid peroxidation is oxidative deterioration of poly unsaturated fatty acids which leads to cellular injury and also generate the peroxide radicals. The cytokine cascade activated after a wound injury which stimulates phagocytic cells that results in the formation of oxygen free radicals and lipid peroxidation. In group II animals showed an elevation in LPO which indicates the scavenging capacity of the wounded tissues. Decreased level of lipid peroxide in the herbal ointment treated groups indicates the anti-lipid peroxidative effect of herbal ointment containing *Oldenlandia herbacea* Roxb.

The superoxide dismutase (SOD) level were found to be increased in the herbal ointment treated groups (III, IV) when compared to excision wound control group (II) which indicates that the tissue damage was being repaired by the scavenging activity appear to be a reflex mechanism to guard against the extra cellular oxygen derived free radicals. Low level of SOD in untreated animals showed to increased tissue damage and inhibit the healing process in control group. Thus, SOD enhanced wound healing may be due to the free radical scavenging action of the plants as well as enhanced antioxidant enzyme level in the granulation tissue[26].

Ascorbic acid is act as a antioxidant. And it plays a major role in the hydroxylation of amino acid residues (proline and lysine) in pro collagen, which is necessary for its release and subsequent conversion to collagen. In the production of collagen, ascorbic acid stimulates the functions of neutrophil and the process of angiogenesis. These combined effects of ascorbic acid promote rapid wound healing. Ascorbic acid deficiency causes alterations in the production of collagen fibers which is due to decreased tensile strength of fibroblast. Increased level of vitamin C in HO treated group (III, IV) indicates the rapid wound contraction by enhanced tissue repair and scavenging free radicals.

## CONCLUSION

From the results, it can be concluded that the aqueous extract of aerial parts of the *Oldenlandia herbacea* Roxb. has efficacy on healing the wounds and also stimulates the collagen turn over, angiogenesis etc. in wound tissue. However, it needs further evaluation in clinical settings before consideration for the treatment of wounds. In future, isolation and characterization of active constituents will becarried out to understand the complete mechanism of wound healing activity of *Oldenlandia herbacea* Roxb.

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