

THE WOUND HEALING POTENTIAL OF AERIAL ROOTS OF *RHAPHIDOPHORA AUREA* (LINDEN EX ANDRE) CLIMBED OVER *LAWSONIA INERMIS*

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ABSTRACT

The effect of an herbal formulation and a formulation of the ethanolic extract of *Rhaphidophora aurea* (Money plant) intertwined over *Lawsonia inermis* (Mehandhi) was evaluated on the Albino Wistar rat incision wound models. The parameters studied included rate of period of wound contraction, period of epithelialization, skin breaking strength and histopathological study of the rat tissue was carried out to know the extent of collagen formation in the wound tissue. The treated animals showed significant reduction in the wound area and faster rate of epithelialization. Statistical analysis indicated dose dependent significant ($p < 0.05$) healing responses in *in vivo* models. Histopathological studies of the tissue obtained from the formulation treated group revealed that the activity was more comparable to that standard betadine. Thus, topical application can be successfully formulated for the wound healing activity.

Keywords: Incision wound model, *Rhaphidophora aurea*, Money plant, Histopathological study.

INTRODUCTION

Wounds are the outcome of damage of the skin that disrupts the outer soft tissue. Healing is a process which is fundamentally a connective tissue response, initial stage of this process involves acute inflammatory phase followed by the synthesis of collagen and other extracellular macromolecules which are later remodeled to form scar (Rupali Nandanwar, 2010). The main goals of the researches in wound healing are to evaluate the influence of various measures in wound management programs on healing and to screen drugs that encourage healing process efficiently.

Various plant products have been used in treatment of wounds over the year. Phytoconstituents derived from plants need to be identified and screened for antimicrobial activity for management of wounds (Rupesh Thakur, 2011). The plant extracts are more efficacious, free from undesirable side effects compared to the pure active principle and due to totality of constituents rather than single molecule (Barua, 2009). Wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinone and other biomolecules (Chaudhari, 2006; Sumitra, 2009). The plant *Rhaphidophora aurea* is a popular ornamental foliage plant. It has got a characteristic host - guest relationship in that it twines over other trees and grows by sucking its nutrients. Phytochemical constituents of *Rhaphidophora aurea* climbed over *Lawsonia inermis* revealed the presence of alkaloids, flavonoids, saponins, phenols, glycosides, anthraquinone and anthocyanins (Arulpriya, 2011). The leaves of *Lawsonia inermis* are used in the form of a decoction or ointment in the treatment of burns, skin inflammations, wound and ulcers. Apart from antifungal and antibacterial activity (Sakarkar, 2004) the ethanol extract of *Lawsonia inermis* and lawsone have greatly contributed towards wound healing activity ($p < 0.05$) when taken orally and topically in the form of ointment.

The present study was to investigate the wound healing activity of the herbal formulation and a formulation of the ethanolic extract of *Rhaphidophora aurea* intertwined over *Lawsonia inermis* (MM). The ointments prepared are designated G4, G5 and its preparation explained under methodology.

MATERIALS AND METHODS

Collection of plant materials

Aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis* (MM) was collected from Palakkad District and the botanical identification was carried by Dr G.V.S.Murthy, Joint Director, Botanical survey of India, Coimbatore.

Extraction

Defatted plant material 300g was extracted conventionally by refluxing with ethanol for 12 hours and the extracts was concentrated using Equitron rotary flash evaporator. The yield (9g) obtained in this extraction was preserved in refrigerator for further use.

Preparation of Topical Formulation and incorporation of extract

The fresh turmeric roots and *Tanners cassia* were allowed to shade dry and crushed into small pieces and powdered. Sandal powder was prepared from original sandal wood. Fresh *aloe vera* was used for the formulation.

Formulation F3: 1600mg of MM ethanol extract were mixed with 120mg of *Tanners cassia* flower powder, 120mg of sandal wood powder, 120mg of *Aloe vera* and 40mg of turmeric powder. This mixture was stored in a proper container and preserved

Formulation F4: 240mg of *Tanners cassia* flower powder, 240mg of sandal wood powder, 240mg of *Aloe vera* and 80mg of turmeric powder were mixed together to make up the formulation F4

Ointment preparation

The drug formulation for topical administration was prepared in the form of ointment. The vehicle was prepared by melting the wool fat, hard paraffin, yellow soft paraffin and cetostearyl alcohol and designated as G3. The vehicle was mixed with 200mg of F3 and slightly warmed in the flame; this ointment was designated as G4. The same procedure was adopted for F4 and the ointment designated as G5.

Animals and Experimental groups

Healthy young adult Albino Wistar rats weighing 180-220g were randomly divided into five groups of four animals. Before commencing the experiment each animal was assigned a unique identification marking with paint like head, tail, body and unmark.

Group I (G1) - Control

Group II (G2) - Wound + Standard (Betadine)

Group III (G3) - Wound + Vehicle

Group IV (G4) - Wound + F3

Group V (G5) - Wound + F4

Housing and feed

Animals were facilitated with standard temperature (23 ± 2°C)-controlled environment (12 h: 12h (light: dark cycle)) and have a humidity of 40%. The standard laboratory animal food pellets with water *ad libitum* feed was supplied to animals during the study period.

Wound creation

The predetermined area for wound infliction at the back of the animal was prepared for surgery by removing hairs. Group 1 animals did not receive any treatment. Before wound creation G2, G3, G4 and G5 group rats were anaesthetized by ketamine chloride. The particular skin area of the animal was shaved 1 day prior to the experiment

INCISION WOUND MODAL

Incisions wound models were made through the skin at a distance of about 5cm length with sterile scalpel blade. The parted skin was sutured with surgical thread at 1 cm intervals using a curved needle (no: 42). The continuous thread on both wound edges was tightened for good closure of the wounds and the wounds were left undressed. The ointments were administered topically to the animals of respective groups until 11th day. The animals were sacrificed on 11th day and the skin breaking strength of the healed wound was measured with INSTRON universal tensile testing system. The ethical committee of KMCH College of Pharmacy, Coimbatore-48 approved the protocol for these experiments under number KMCRET/PhD03/2010-11.

Evaluation

Wound contraction, which contributes to wound closure is expressed as a reduction in percentage of the original wound size studied, starting from the day of operation until the day of complete epithelialization and evaluated to calculate the degree of wound healing.

Percentage of wound contraction

The progressive reduction in the wound area was monitored by graph paper and the percentage of wound healing was computed at the beginning of experiments and the next 4, 7 and 9 days.

$$\% \text{ of wound area} = \frac{\text{Wound area in the day of X}}{\text{Wound area in the first day}} \times 100$$

Where X = day 1, day 4, day 7 and day 9

$$\% \text{ of wound healing} = 100 - \% \text{ of wound area}$$

Skin tensile strength

The animals were sacrificed on the 14th day and the healed tissue along the normal skin strips of 70mm length were cut out from the animal. This was preserved by normal saline, then loaded between the upper and lower holder of the INSTRON universal tensile testing machine 5500R/6021. The total breaking strength was measured in gram force. The tensile strength is calculated by the following equation

$$\text{Tensile strength} = \frac{\text{Total breaking load}}{\text{Cross-sectional area}}$$

Histopathological studies

The removed tissues from the animals were preserved separately in 10% formalin and dehydrated through alcohol, cleaned in xylene and embedded in parasin mixed paraffin wax (melting point 55-57 °C). Serial section of 5 µm were cut and stained with Hematoxylin and Eosin stains. The section was examined under light microscope (LABOMED, Germany) and photomicrographs were taken.

Statistical analysis

All the data were expressed as mean ± S.E.M. The data on percentage wound healing, wound contraction and tensile strength was statistically analyzed using Two-way analysis of variance (ANOVA) followed by Dunnet's test. The values of p < 0.05, p < 0.01, p < 0.001 were considered statistically significant.

RESULTS

Wound contraction

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 almost 80% complete (Darby, 1990). 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 (Priya, 2004). The results of the wound healing studies are presented in Table 1 and 2. It is well evident for the results that both the formulation G4 and G5 showed significantly wound healing (> 90%) on the ninth day, compared with standard (~99%).

Table 1: Effect of herbal formulation and a formulation of the ethanol extract of aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis* on wound contraction (Mean ± SEM) in incision model

Groups	Day 1	Day 4	Day 7	Day 9
G1	100	71 ± 12.1	52.5 ± 9.6	13 ± 8.7
G2	100	72.5 ± 7.5	23.5 ± 12.5a***	1 ± 1.2a**
G3	100	53 ± 13.2a***b***	26 ± 11.5a***b ^{ns}	10 ± 2.56a ^{ns} b**
G4	100	72.5 ± 9.6a ^{ns} b ^{ns}	12.5 ± 18.9a***b**	5 ± 10a ^{ns} b*
G5	100	71 ± 6.2a ^{ns} b ^{ns}	53.5 ± 10.8a ^{ns} b***	7.5 ± 15.0a*b*

*P < 0.05, **P < 0.01, ***P < 0.001, ns – not significant
a - G1 vs G2, G3, G4, G5 and b - G2 vs G3, G4 & G5

Table 2: Effect of herbal formulation and a formulation of the ethanol extract of aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis* on wound healing (Mean ± SEM) in incision model

Groups	Day 1	Day 4	Day 7	Day 9
G1	0	29	47.5	87
G2	0	26a ^{ns}	73.6a***	98.8a**
G3	0	47a***b***	74a***b ^{ns}	90a*b ^{ns}
G4	0	27.5a ^{ns} b ^{ns}	87.5a***b***	95a*b ^{ns}
G5	0	29a ^{ns} b ^{ns}	46.5a ^{ns} b***	92.5a*b*

*P < 0.05, **P < 0.01, ***P < 0.001, ns – not significant
a - G1 vs G2, G3, G4, G5 and b - G2 vs G3, G4 & G5

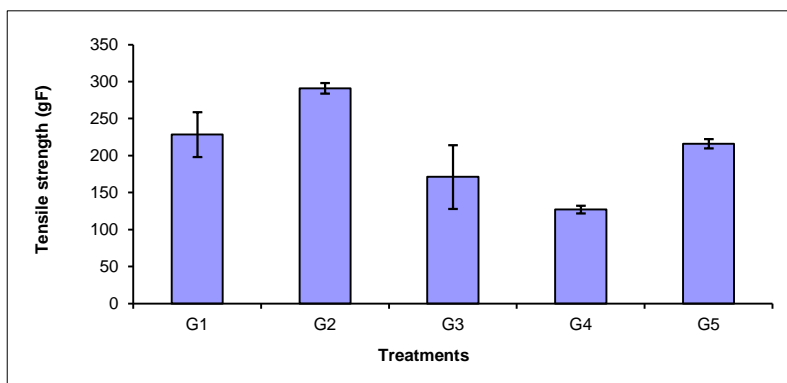
Tensile strength

The results of the measurement of skin breaking strength on the 14th day of post operation in incision wound model are depicted in Figure 1 and Table 3. The skin breaking strength of the G3 ointment treated group was less significant and the G5 ointment treated group of higher breaking strength, compared with the standard (G2).

Table3: Effect of herbal formulation and a formulation of the ethanol extract of aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis* on tensile strength (Mean ± SEM) in incision model

Groups	Tensile strength (gf)
G1	228.60 ± 30.36
G2	290.98 ± 7.10a
G3	171.30 ± 43.13b
G4	127.27 ± 5.23ab
G5	216.20 ± 6.22b

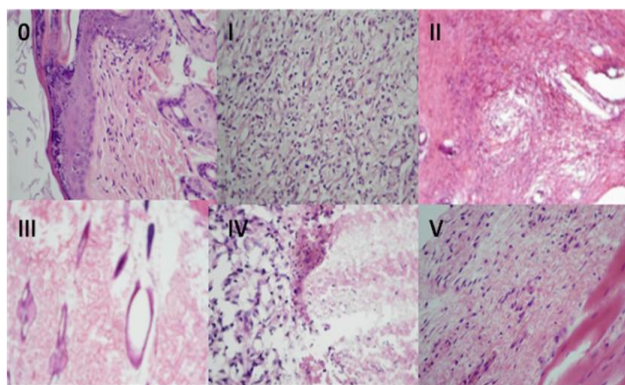
a,b are significant at p<0.05
a - G1 vs G2, G3, G4, G5 and b - G2 vs G3, G4 & G5



Graph 1: Tensile strength rate of herbal formulation and a formulation of the ethanol extract of aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis*

Histopathological observations

The multiple sections studied in histopathological examination of the tissues of the wound area treated with the vehicle, standard, control, G4 and G5 ointments in incision wound models are shown in image 1.



0-Normal skin, I-Group I, II-Group II, III-Group III, IV-Group IV, V-Group V

Image 1- Histopathological examination of herbal formulation and a formulation of the ethanol extract of Aerial roots of *Rhaphidophora aurea* (Linden ex Andre) intertwined over the *Lawsonia inermis*

DISCUSSION

The nature and mechanism of incision wound healing has been and continues to be of interest to clinicians and wound biologists. There is an increasing interest in finding herbal extracts with wound healing efficacy although the use of such extracts for treating cuts and wounds is a common practice in traditional medicine (Odimegwu, 2008). Pharmaceutically as well as biologically, the herbal extracts promote fast wound healing than control and non medicated group in different *in vivo* studies.

Comparing the formulations G4 and G5, G4 showed significant healing activity. This may be due to the presence of emulsifying agents and phytochemical constituents like alkaloids, flavonoids, anthraquinone, anthocyanin, saponins, phenols, glycosides, terpenoids and tannins in the formulation (Arulpriya, 2011; Priya, 2004; Araujo, 2001; Arunkumar, 2009; Balakrishna, 2011; Kate Fallick) Tannins act as free radical scavengers, triterpenoids and flavonoids promote wound healing due to their astringent and antimicrobial property, and saponins due to their antioxidant and antimicrobial activity, which appear to be responsible for wound contraction and elevated rate of epithelialization. Flavonoids possess

potent antioxidant and free radical scavenging effect, enhancing the level of antioxidant enzymes in granulation tissue (Shenoy, 2009).

Sterols and phenols are responsible for wound healing due to free radical scavenging and antioxidant activity, which are known to reduce lipid peroxidation, thereby reduce cell necrosis and improving vascularity (Baravkar, 2008). Comparing wound healing activity of G4 and G5, G4 was found to show better activity. This may be attributed to the additional metabolites like phenols, glycosides, etc in G4.

During the experimental studies the animals were closely observed for their attitude; in tested groups, the animals licked the ointments applied to each other which probably might have reduced the concentration of the ointment applied. This may be a reason for the less healing activity of treated groups of rats on day 4 and day7 (Table 2).

On the 9th day, G2 showed better healing activity as compared to the others (Table 2 and 3). This may be because, the antimicrobial agent povidone iodine (Betadine), is a complex of iodine, the bactericidal component, with polyvinylpyrrolidone (povidone), a synthetic polymer. Choice of povidone-iodine solution for treatment of wounds, especially the chronic wounds most often seen in physical therapy practice, is made complex by two factors. First, although there is a large body of research into various aspects of povidone-iodine use in wound care, the results are not always germane to the types of wound treatment most often provided by physical therapists (Burks, 1998).

Comparing the healing activity of G3, G4 and G5, G3 showed less activity which may be because G3 ointments contain only wax and fat. These do not have any phytochemical properties but they have only good emulsion properties. The vehicle had good emulsion properties with standard fat and therefore they can be employed as potential substitutes in the formulation of topical preparations (Florence, 2010).

The treated animals demonstrated significant ($p < 0.05$) skin breaking strength. The tensile strength of a wound is determined by the rate of collagen synthesis and more so, by the maturation process where there is a covalent binding of collagen fibrils through inter and intra molecular cross linking (Malviya, 2009). The breaking strength ultimately depicts the tensile strength, thus showing a significant increase in the tensile strength of the skin tissues in animals of treated G4 and G5.

The increase in tensile strength of treated wounds may be due to an increase in collagen concentration and stabilization of the fibroblast facilitating wound healing. This increase in collagen synthesis may be due to the antioxidant effect of the ethanol extract of aerial roots of *Rhaphidophora aurea* (Linden ex Andre) climbed over *Lawsonia inermis* (G4) (Arulpriya, 2012) which enhances wound healing.

The results of the Histopathological examination have supplemented the studies on wound healing. The principal morphological difference between the two types of incision is found at the junction of newly forming connective tissue and pre-existing dermal collagen.

The control group (G1) showed no regenerating epithelium and moderate inflammation fibroblast proliferation. Wound treated with standard (G2) showed conspicuous granulation tissue formation of squamous epithelium and matured epidermis. Vehicle (G3) showed normal epidermis, dermis with appendages with no inflammation and no fibroblast proliferation. G4 and G5 treated wounds showed normal epidermis and epithelization, in which the epidermis was well organized with proliferation of fibroblasts. It was noted from the results that G4 based topical agents caused wound contraction and coverage of wound bed with granulation tissue at a faster rate than G5. In G4 and G5 treated groups, no scar was observed on the dermis its indicating complete healing. The skin showed matured epidermis and fibroblast in dermis which are proof of complete wound healing.

Flavonoids, (Tsuchiya, 1996) triterpenoids (Scortichini, 1991) are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization. Phytoconstituents present in G4, either due to the individual or additive effect may have speeded up probably the proliferation phase of wound healing, which is contributed by increased extent of fibroblasts.

CONCLUSION

The results of the present study have led to conclusion that the formulation (G4) exhibited more prominent wound healing activity than herbal formulation (G5). The presence of major phytoconstituents like flavonoid, terpenoids, saponins and tannins in G4 and the synergistic interaction of the constituents and might have promoted wound healing activity. This study provides scientific evidence to the ethno medicinal future of the aerial roots of *Rhaphidophora aurea* (Linden ex Andre).

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