Wound Healing Potential of Alcoholic Extract of *Mimosa pudica* Linn. Leaves

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Summary

The present work was attempts to study wound healing activity of the Ethanolic extract of leaves of *Mimosa pudica* Linn. belong to family *Mimisace*. The ethanolic leaves extract of *Mimosa pudica* was evaluated for its wound healing activity in rats using excision and burn wound models. Extract treated animals exhibited 73% & 92% respective (5% & 10%w/w) formulations, reduction in the wound area when compared to control which was 28%. In the excision model the extract-treated wounds were found to epithelialise faster and the rate of wound contraction was higher, as compared to control wounds. This was further supported by histopathological studies. The wound contraction Studies revealed that the wound contractions increase with an increase in the herbal extracts concentration. Mupirocin used as standard in both models. *Mimosa pudica* promotes significant wound healing in diabetic rats and further evaluation of this activity in humans is suggested.

Keywords- *Mimosa pudica,* Burn wound, Excision wound, collagen fibers, epithelization.

Introduction

Wound healing processes are 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 [1]. The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration [2].

Wound healing are conveniently classified into any of three types, healing by first intention, healing by second intention and healing by third intention, depending on the nature of the edges of the healed wounds. Whereas the edges of wounds healed by first intention are smoothly closed that no scar is left, wounds healed by second intention involve formation of granulation tissues which fill up the gaps between the wound edges and are associated with significant loss of tissue, leaving little scars. Wounds healed by third intention are usually those wounds left for three to five days until granulation bed falls before they are sutured resulting in extensive scars formation [3]. Four distinct stages of wound healing have also been identified-inflammatory, debridement, proliferation, and remodeling/maturation stages. Wound healing processes are known to be influenced by among other factors by infections, nutritional status, drugs and hormones, type and sites of wound, and wasting diseases like diabetes. In fokelore medicine, medicinal plants have been used widely in facilitating wound healing with high degree of successes. This has inspired many researches which are aimed at validating the claims and discovering mechanisms which possibly explains the potentials of these herbs on wound repair processes [4].

Mimosa pudica (Lajjalu) has been extensively used in ayurveda, unani & homoeopathic medicine and has become cynosure of modern medicine. It is known in sensitive or humble plant & popular name is lajjavanti & chuimui. Punjabi name of this plant is lajwanti [5]. *Mimosa pudica* posses a wide area of therapeutic activity likes vulnerary, diuretic, antispasmodic, emetic, constipating and febrifuge. They used in haemorrhage, dysentery, inflammation, burning sensation & wounds. It is also used in jaundice, asthma, conjunctivitis, cut wounds, and glandular swelling [6-10].

The present study has been undertaken to examine the wound healing activity of the leaves of *Mimosa pudica* in excision and burn wounds models.

Materials and Methods

Preparation of leaves extract- The leaves of *Mimosa pudica* Linn were collected from local market, Udaipur. Powdered leaves were charged into soxhlet apparatus and successive hot continuous extraction was carried out using solvents such as Petroleum ether (600-800 ^{0}C), and ethanol. The drug was extracted with each solvent. Each time before extraction with the next solvent, the powdered material was air dried. Each extract was concentrated by distilling the excess solvent to obtain the crude extract [11,12].

Animals- The study was approved by Institutional Animal Ethical Committee. Healthy Sprague Dawely rats weighing 150-180 g were maintained on the standard rodent fed and water *ad libitum.* The excision & burn wound models were used to evaluate wound healing activity of *Mimosa pudica* extract. The animals were randomly distributed into four groups of 6 each in excision and of 6 each in burn wound models.

Excision wound model- Animals were anaesthetized with diethyl ether by open mask method and shaved on both sides of the back with an electric clipper. The area of wound to be created was outlined on the back of the animals with methylene blue using a stainless steel stencil. The entire wound was left open. Animals were closely observed for any infection and those which showed signs of infection were separated, excluded from the study and replaced. Animals were divided into four groups of 6 each. The normal controls (group 1) were applied with ointment base two times a day, drug treated group (group 2) were applied 5 % w/w & drug treated group (group 3) 10%w/w ointment in soft paraffin base leaves extract of *Mimosa pudica* two times a day, positive control group rats (group 4) mupirocin ointment two times a day. The treatment was done topically in all the cases. Wound areas were measured on days 1, 5 and 11 for all the groups using a transparency sheet and a permanent marker. Recording of the wound areas were measured on graph paper [13,14].

% of wound closure

$$= \frac{\text{Wound area on day 0} - \text{Wound area on day } \mathbf{n}}{\text{Wound area on day 0}} \times 100$$

n= numbers of days (0^{th}, 5^{th}, 11^{th},).

Burn wound model- Partial thickness burn wounds were inflicted on overnight- starved animals under ketamine (100 mg/kg, i.m.) & xylazine (16mg/kg, i.m.) anesthesia by pouring hot molten wax (2g) at 80^oc for 10 sec. The wax was poured on the shaven back of the animal through a cylinder of 220 mm^2 circular opening. The wax was allowed to remain on the skin till it gets solidified.

Immediately after the injury and on subsequent days, the drugs or base was applied topically; animals were divided into four groups of 6 in each group. The normal controls (group 1) were applied with ointment base two times a day, drug treated group (group 2) were applied 5 % w/w & drug treated group (group 4) 10%w/w ointment in soft paraffin base extract of *Mimosa pudica* two times a day, positive group rats (group 5) mupirocin ointment two times a day. The treatment was done topically in all the cases. Wound areas were measured on days 1, 5 and 11 for all the groups using a transparency sheet and a permanent marker. Recording of the wound areas were measured on graph paper [15].

The parameters observed in the study were as follows:-

a) Epithelialization period- It was monitored by nothing the number of days remained for the eschar to fall off from the burn wound surface without leaving a raw wound behind.
b) Wound contraction- It was noted by following the progressive changes in wound are planimetrically, excluding the days of woundling. Measurement of wound contraction same as excision wound model.

Histopathological studies -On 11th day, the regenerated tissue from the healing wounds was collected and placed in 10% buffered formalin for histopathological studies. Sections from the regenerated tissues were examined for epithelialization, inflammation, collagen, and fibroblasts.

***Statistical analysis*-** The means of wound area measurements between groups at different time intervals were compared using one way ANOVA Post hoc test. It was used to examine the mean difference in wound healing between the groups in excision and burn wound models using Graph Pad software.

Results

Effect on excision wound model- From these animal studies it can be concluded that significant increase in the wound healing activity was observed in leaves extract treated rats. In excision wound model, animals of groups 2, 3 and 4 showed a decrease in the epithelialization period and increased percentage of wound contraction when compared with the animals of groups 1, (Table- 1). Treatment of drug extract (10%) showing reduced days of epithelialization period & % of wound contraction compare to drug extract (5%). On day 11, the extract-treated animals (groups 2 and 3) showed wound contraction by 73% & 92% compared with 28% in wounds of the control group (groups 1). The wound contraction results of extract-treated animals were comparable with positive controls (95%). Comparatively analysis revealed that the high dose of leaves extract (10%) was significantly more effective in reducing the epithelialization period compare to the low dose (5%).

Effect on burn wound model- Like the excision wound model, application of mimosa pudica leaves extract formulations (5%&10%) and mupirocin standard drug topically shortened the period of epithelialization significantly and also produced significant in wound contraction-50% (days) when compared to control. Comparatively analysis of different groups indicates that the high dose of leaves extract were more effective in reducing the epithelialization period compared to the low dose (Table-2).

Table 1- Effect of *Mimosa Pudica* leaves extract on of %wound contraction and epithelization period in excision wound.

Group	Day-1	Day-5	Day-11	Period of epithelization(days)
Group-1 (Normal Control)	215±1.770	12.2±2.1	28.6±4.4	21.16±0.3072
Group-2 (Treated with drug 5%)	216±1.210	44.2±7.6	73.2±5.9*	17.66±0.2246
Group-3 (Treated with drug 10%)	212.3±1.110	54.6±7.5	92.1±4.1	16.50±0.2108*
Group-4 (positive control with mupirocin)	218.5±1.664	60.3±1.9*	95.5±4.4*	14.50±0.2236*

Shows significant as compared to normal control ($p<0.001$).
[Values are mean± SE from 6 rats in each group]

Table 2- Effect of *Mimosa Pudica* leaves extract on of %wound contraction, days of 50%wound contraction and epithelization period in burn wound.

Group	Day-1	Day-5	Day-11	Wound contraction 50% (days)	Period of epithelization in (days)
Group-1 (Normal Control)	219±1.990	14.1±3.1	19.1±3.3	8.50±0.364	40.6±0.5
Group-2 (Treated with drug 5%)	218.2±1.554	10.1±0.6	29.8±3.2*	6.30±0.3183	39.6±6.1
Group-3 (Treated with drug 10%)	217±1.770	10.7±4.2	34.9±2.4	5.66±0.1430*	29.0±1.4*
Group-4 (positive control with mupirocin)	219.4±1.993	14.2±1.5	43.1±12.6	4.76±0.1406*	25.4±1.5

Shows significant as compared to normal control ($p<0.001$).
[Values are mean± SE from 6 rats in each group]

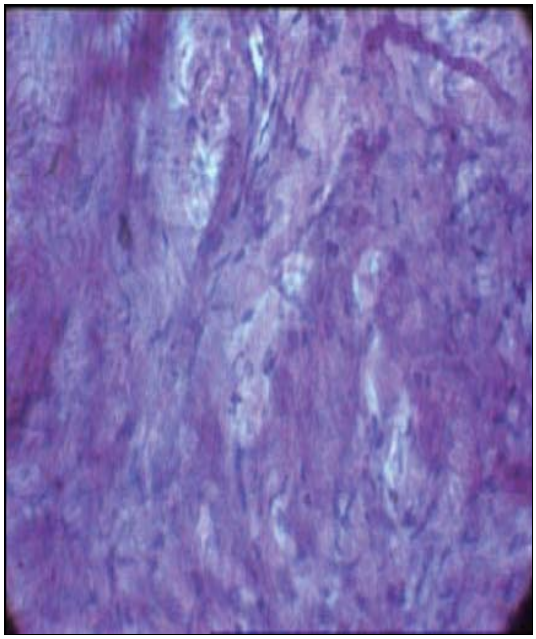
Histopathological studies-Microscopic examination of the sections prepared from the wounds of normal, control and treated groups exhibited the following characteristics.

Normal: The tissue is composed of dense collagen fibers, fibroblasts with round to oval nuclei and blood vessels.

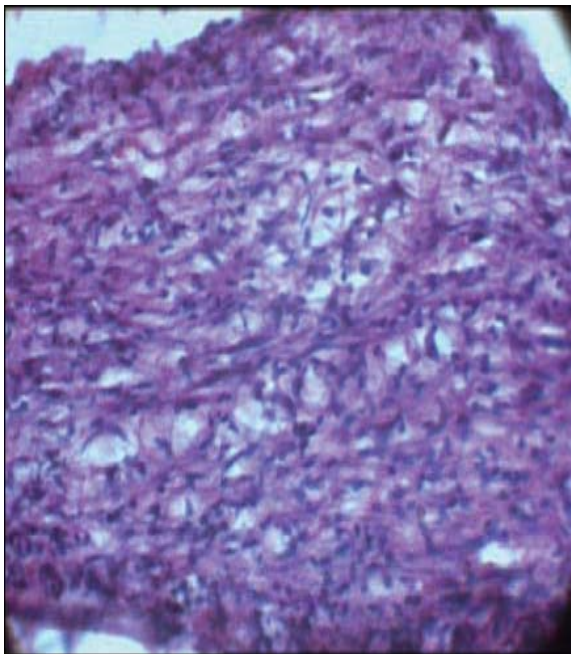
Control: The tissue showed densely inflamed connective tissue with chronic inflammatory cells between the collagen fibers; this shows incomplete wound healing. Many thin walled blood vessels are present.

Treated groups: Tissues showed fibrous connective tissue with scattered inflammatory cells and fibroblasts. There was a progressive collagenation with few thin walled blood vessels with small lumina. Epithelialization tissues were observed. Parameters like fibroblasts, collagen, and neo-vascularization were higher in the treated group compared with the control. These results show that the wound healing was faster in the treated group compared with the control group. Formulations (5%&10%) containing higher concentrations of the extracts showed dense fibrous tissue with thick collagen bundles, fibroblasts and scattered inflammatory cells. The appearance was almost identical to that of normal tissues. A comparison of histopathological studies of regenerated tissue sections of normal, control and wounds treated with the ointment-based formulation are shown in(Fig.1)

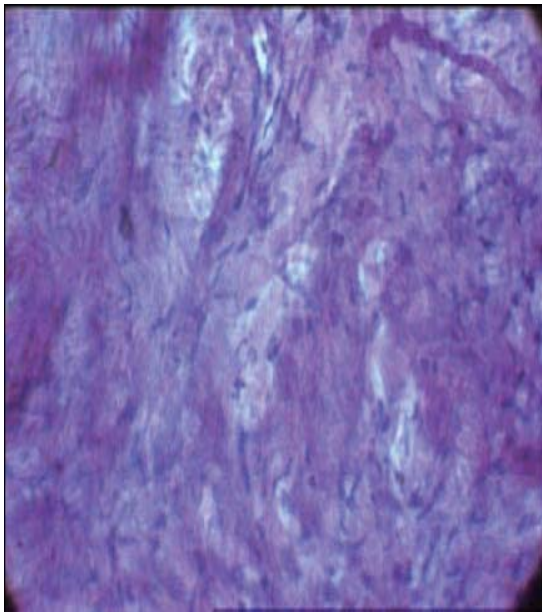
Fig.1- Histology of regenerated tissue of open wounds in normal, & treated group as on the 11th day at 400 ^{xs}



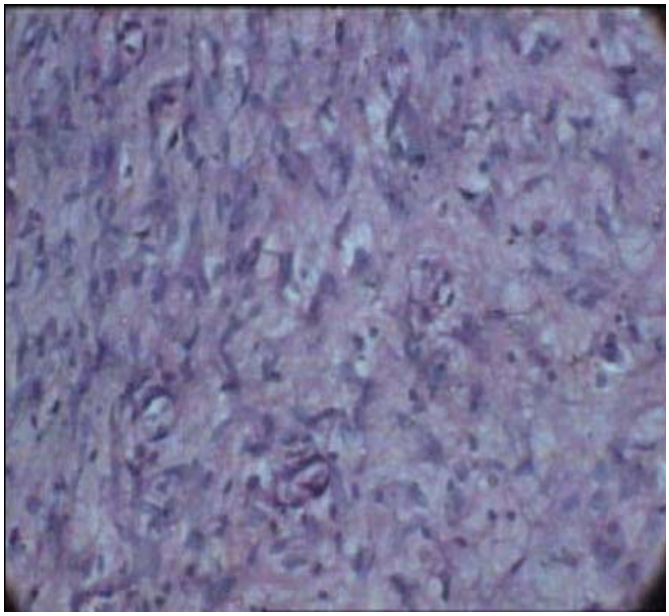
Normal



Control



Treated



Positive control

Discussion

The excision wound model was carried out to study the topically applied *mimosa pudica* leaves extract on wound healing and contraction, Increase in the wound healing activity was observed in leaves extract treated rats. On 5th day, animals of group 4 showed greater percentage of wound contraction when compared with the animals of all groups. The same pattern was observed on 11th day also. The wound contraction results of extract-treated animals were comparable with positive controls.

The wound contraction studies revealed that the wound contraction increases on increasing the herbal extract concentration. The present study shows a significant improvement in burn wound contraction in the rats treated with leaves extract ointment preparation. It can be said that extract of *mimosa pudica* could be a cheap and effective adjuvant to other topical agents for attaining faster healing of wounds, without complications. In the past, many studies have been done using natural products for the treatment of burns wounds, but these were mainly aimed at controlling infection. Anti-inflammatory activity of certain natural products could also play a part in the healing of burn wound. Although the present study did not explore the exact mechanism of proheling of *mimosa pudica* it could be attributed to both anti-inflammatory and antiseptic properties. Based on these, we propose that extract of *mimosa pudica* could significantly enrich the assortment of topical medications available for the treatment of burns. The histopathological studies showed that certain parameters, namely collagen, fibroblasts and neovascularization, were slightly higher in the treated groups compared with the control groups of animals, indicating faster remodeling of wounds. Therefore, from the above observations, it can be concluded that these topical formulations containing herbal extract possess good wound healing activity.

The present study demonstrates that *mimosa pudica* leaves extract applied topically promotes healing of wound contraction in excision and burn wound models. These preliminary results further suggest that *mimosa pudica* facilitates healing by increasing the rate and extent of wound closure.

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