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Research article

WOUND HEALING ACTIVITY OF LATEX OF *CALOTROPIS GIGANTEA*

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ABSTRACT

The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. The stages of wound healing are inflammatory phase, proliferation phase, fibroblastic phase and maturation phase. The Latex of *Calotropis gigantean* (200 mg/kg/day) was evaluated for its wound healing activity in albino rats using excision and incision wound models. Latex treated animals exhibit 83.42 % reduction in wound area when compared to controls which was 76.22 %. The extract treated wounds are found to epithelize faster as compared to controls. Significant ($p<0.001$) increase in granuloma breaking strength (485±34.64) was observed. The Framycetin sulphate cream (FSC) 1 % w/w was used as standard.

Keywords: *Calotropis gigantea*, Wound healing, Excision wound, Incision wound, Framycetin sulphate cream.

INTRODUCTION

The wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collegenation and tissue remodeling. In India, there has been interest in the potential of medicinal plant for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda[1].

Calotropis gigantea Linn. (Asclepiadaceae) is a glabrous or hoary, laticiferous shrubs or small trees, commonly known as THE SWALLOW-WORT or MILKWEED. Latex contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin and uscharidin; gigantin. Latex also contains the protease calotropin DI and DII and calotropin FI and FII. The latex contains some poisonous constitute due to which it has somewhat caustic effect on the

mucous membrane and tender skin, and may secondary dermatitis[2].

The latex is used as bitter, heating, oleaginous, purgative, cures, leucoderma, tumours, ascites. The latex is also used as caustic, acrid; expectorant, depilatory, anthelmintic; useful in leprosy scabies ring worm of the scalp, piles, eruptions on the body, asthma, enlargement of spleen and liver, dropsy; applied to painful joint swellings[3]. In the latex calotropin, gigantin and uscharin show digitalis-like action on the heart. The latex also used to induce abortion, infanticide[2].

MATERIALS AND METHODS

Plant source

Latex of *Calotropis gigantea* Linn. was collected from local area of Mandsaur region by making the incision on plant.

Chemicals

Framycetin sulphate cream (FSC) (1%w/w), diethyl ether, ethanol, sterilized cotton were used.

Animals

Healthy wistar albino rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard diet and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions (12/12 hr light/dark cycle; 25ºC ± 30ºC, 35- 60% RH).

Acute dermal toxicity – fixed dose procedure[4]

The acute dermal toxicity study was carried out in adult female albino rats by "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Latex of the plant *Calotropis gigantea* was applied topically at dose level 2000 mg/kg.

Selection of dose

For the assessment of cutaneous wound healing activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (200 mg/kg/day).

Grouping of animals

Animals were divided in to three groups, each group consisting of 6 rats.

Group I : Received no treatment and served as control

Group II : Received application of standard drug ointment i.e. Framycetin sulphate cream (FSC) (1 %w/w)

Group III : Received application of latex of *Calotropis gigantea* (200 mg/kg/day)

Wound healing activity

Excision and incision wound models were used to evaluate the wound-healing activity of latex of *Calotropis gigantea.* The study was approved by the Institutional Animal Ethical Committee of B. R. Nahata College of Pharmacy,

Mandsaur (M.P.), registered under CPCSEA, India.

Excision wound model[5]

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300 mm^2 and 2 mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The latex was applied at a dose of 200 mg/kg/day for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker.

Incision wound model [6, 7]

The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The latex was applied at a dose of 200 mg/kg/day for 10 days. The sutures were removed after 8 days, on tenth day the tensile strength was measured by continuous constant water supply technique.

Statistical Analysis

The means of wound area measurement and wound breaking strength between groups at different time intervals were compared using one-way ANOVA, followed by Tukey's tests.

RESULTS AND DISCUSSION

During study of wound healing in normal rats following results were obtained:

Acute toxicity studies showed that drug was found to be safe up to maximum dose of 2g/Kg body weight of the animal. In studies using excision wound model, the latex treated group III showed significantly greater wound healing as compared to control animals (Table 1 & Fig. 1-3).

Table 1. Effect of Latex of *C. gigantea* on Excision Wound [Wound Area (mm^2)]

Day	Group I	Group II	Group III
0	285.83 ± 10.362	226.67 ± 7.032	186.67 ± 9.545
4	255.00 ± 11.547**	175.83 ± 5.833**	153.33 ± 9.972**
8	213.33 ± 9.098**	145.83 ± 5.974**	120.83 ± 8.604**
16	68.33 ± 2.472**	27.50 ± 2.141**	30.83 ± 2.386**

n=6; values are in mean ± SEM, **Significant $p<0.001$

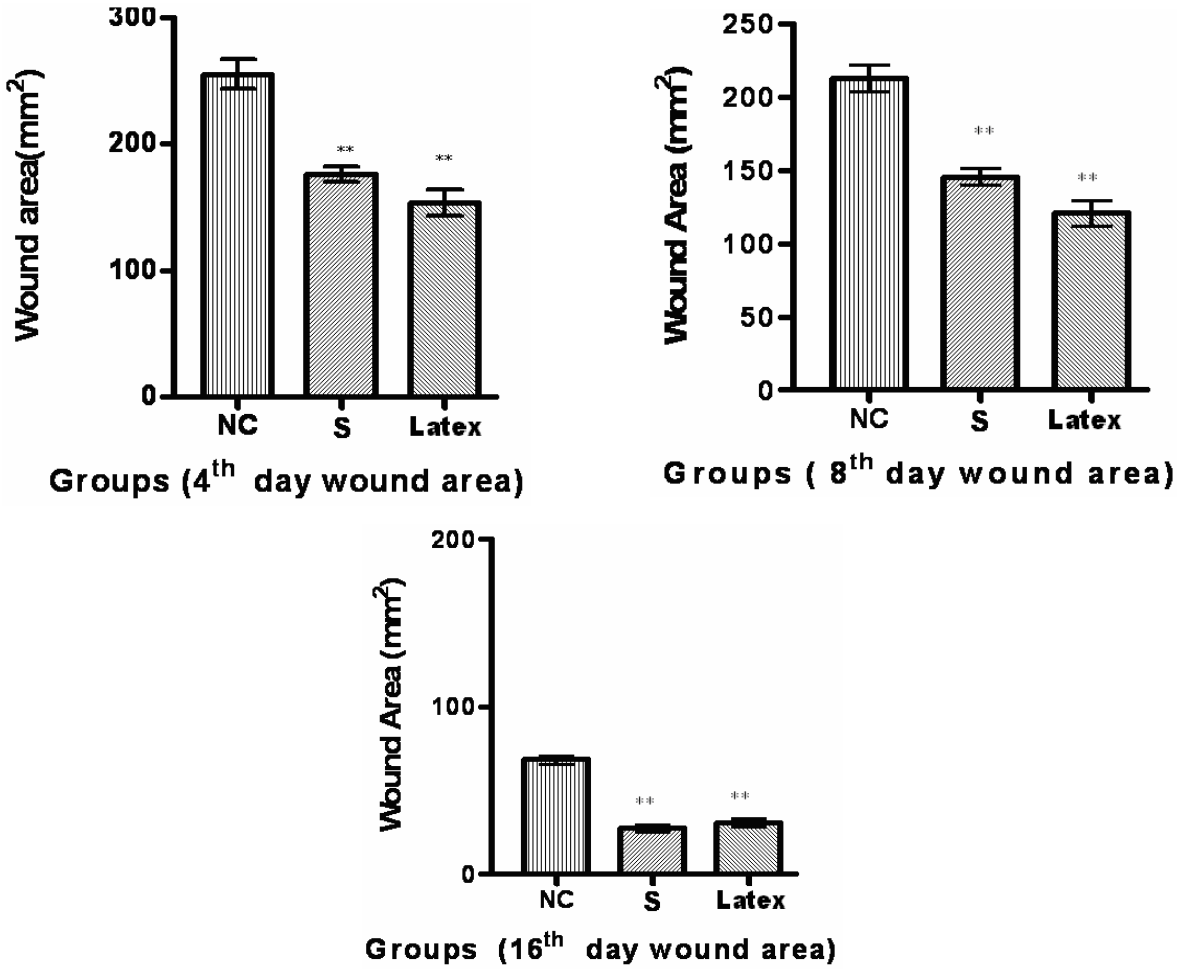

Fig. 1-3 : Comparison of wound area between groups at different days (NC=negative control, S=standard)

The standard drug treated animals in normal animals were showed significantly greater wound closure as compared to control and latex treated animals (Table 2).

Percentage wound closure

$$\text{Percentage Wound Closure} = \frac{\text{(Initial area of Wound - N}^{TH}\text{ day area of wound)}}{\text{(Initial area of Wound)}} \times 100$$

Table 2 : Effect of latex of *C. gigantea* on excision wound (% wound closure)

Day	Group I	Group II	Group III
0	0	0	0
4	10.78 %	22.42 %	17.86 %
8	25.36 %	35.68 %	35.27 %
16	76.22 %	87.86 %	83.48 %

In incision wound model, significant increase was observed in the skin tensile strength of latex treated group on 10th post wounding day (Table 3).

Table 3 : Effect of latex of *C. gigantea* on wound healing in incision wound

Groups	Incision wound breaking strength (g)
Group I	293.17 ± 31.90
Group II	421 ± 81.14*
Group III	485.17 ± 34.64***

n=6; values are in mean ± SEM, ***Very Significant p<0.0001, *Significant p<0.05

Our present study emphasized on our indigenous medicinal plant of Asia and Africa i.e. *Calotropis gigantea* R.Br. In present study incision wounds healing by granulation, collagenation, and tensile strength was measured indirectly to assess the collagen content and maturation. The results indicate that latex of *C. gigantea* R.Br. significantly promoted collagen as compared to that of control.

Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing as drugs which, influence one phase may not necessarily influence another. Hence in our study we have used two models to assess the effect of latex on various phases of wound healing.

CONCLUSION

The wound healing activity of latex of *Calotropis gigantea* R.Br. was studied by using excision and incision wound model and the latex showed the significant wound healing activity as like as standard FSC (Framycetin sulphate cream).

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