

EVALUATION OF WOUND HEALING ACTIVITY OF CALOTROPAIN – P

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ABSTRACT

In the present study the isolated compound 'Calotropain – P' was employed for its wound healing activity. For the assessment of wound healing activity, Swiss Wister stain rats were employed and the various parameters such as excision wounds, incision wounds and dead space wounds were studied. In excision wound model, treatment was continued till the complete healing of the wound, in incision and dead space wound model the treatment was continued for 10 days. For topical application, 5% w/w ointment of 'Calotropain-P' was prepared in 2% sodium alginate and in excision and incision wound models. The healing of the wound was assessed by the rate of wound contraction, period of epithelialisation, skin breaking strength, granulation strength and histopathology of the granulation tissue. It was found that the isolated compound Calotropain-P' possesses significant wound healing activity.

Key words: Wound healing activity, Calotropain-P.

INTRODUCTION

Calotropis procera (family-Asclepidaceae) commonly known as 'Arka' in India, is a widely grown plant in tropics of Asia and Africa. Various parts of this plant have been widely used in traditional systems of medicine for various medicinal purposes. An ethanol extract of the flower of this plant is reported to have anti-microbial, anti-inflammatory, antipyretic, analgesics¹, anticancerous² and anti-malarial³ activities. Likewise, water, ethanol, acetone and some other organic solvent extracts of this plant possess insecticidal⁴, larvicidal⁵, anti-bacterial and anti-parasitic⁶ activities. Keeping in view of the medicinal importance of this plant it was thought worthwhile to investigate wound healing activities of the isolated protein fraction 'Calotropain-P'.

MATERIALS AND METHODS

Collection and preparation

The latex of *Calotropis procera* was collected from Mayurbhanj district (Orissa, India) during the month of

March to July. A specimen of this plant was authenticated in the Botanical survey of India, Calcutta, (India). The protease component Calotropain-P was isolated from the aqueous extract of the latex of the plant by a simple and economical precipitation process (patent application for both the process and the product pending).

Animals Used

Studies were carried out using Swiss albino mice (15-20gm) of both sex and Wister albino rats (150-180g) of either sex for the test of LD₅₀ value and wound healing activity respectively. They were obtained from the animal house, S.I.P.S, Jharpokharia, Orissa, maintained in well ventilated animal house. All animals had free access to standard diet and drinking water. The institutional ethical committee approved in-vivo and in-vitro experiments.

Extract (Calotropain-P) and LD₅₀ Values

The isolated compound 'Calotropain-p' was dissolved in glass distilled water and used for the pharmacological investigations. The mice were randomly distributed in different groups of six

animals each. The LD₅₀ value was estimated by following ‘Up and Down’ stair case method. 45mg/kg body weight (b.w) was taken as the therapeutic dose of the compound ‘Calotropain-P’.

Wound Healing Activity

Excision, incision and dead space wound models were used to evaluate the wound-healing activity.

Excision wound⁷

Under light ether anesthesia, each animal was secured to operation table in its natural position. A circular wound of about 500 sq. mm was made on depilated ethanol sterilized dorsal thoracic region of the rats. The animals were divided into four groups of six each. The animals of group I were left untreated and considered as the control, group II served as reference standard and treated with 1% w/w framycetin sulphate cream (FSC), animal of group III and IV were 5% of ‘Calotropain-P’ ointment. The ointment was topically applied once a day, starting from the day of the operation, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wounds were traced on mm² graph paper on 4, 8, 12, 16 and thereafter on alternate days until healing

was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

Incision wound

In incision wound model, 6cm long Para vertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat as described by Ehrlich and Hunt et al⁸. The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into four groups of six animals each. The animals of group I were left untreated and considered as the control, the group II served as reference standard and received 1% w/w framycetin sulphate cream (FSC) ointment, animals in group III and IV were treated with 5% ‘Calotropain-P’ ointment. The ointment was topically applied once in a day. The sutures were removed on the 8th post wound day. The skin breaking strength of the wounds was measured on the 10th day as described in the method of Lee et al⁹.

Dead space wound

The animals were divided into three groups of 6 rats in each group. Group I served as the control, which received 1ml of 1% gum tragacanth/kg, b.w., i.p. The animals of group II and III received 7mg/kg ‘Calotropain-P’ i.p. Under light ether anesthesia, dead space wounds were created by subcutaneous implantation of sterilized cylindrical glass piths (2.5 cm X 0.3 cm), one on either side of the dorsal paravertebral surface of the rat¹⁰. The granulation tissues formed on the grass piths were excised on the 10th post

Table 1: Effect of ‘Calotropain-P’ on Excision Wound Model in Albino Rats

	Percent Wound Closure			
	4 th Day	8 th Day	12 th Day	16 th Day
Control	13.32±1.45	26.80± 3.33	53.80±3.87	73.85±2.56
Calotropain-P	23.10± 3.12	55.64±3.74*	79.08±4.06* *	94.85±0.96**

Values are expressed in mean ± SEM, n=6, *Significant (P<0.01), **Highly significant (P<0.001).

Table II: Effect of ‘Calotropain-P’ on Tensile Strength in Different Wound Models and Blood clotting time

Group	Tensile Strength		
	Incision wound	Granuloma tissue	Blood clotting time(Sec)
Control	276.83±0.71	186.86±6.07	46.23±2.35
Calotropain-P	463.68± 1.48**	297.04± 6.12**	20.30±0.85**

Values are expressed in mean ± SEM, n=6. *Significant (P<0.01), **Highly significant (P<0.001).

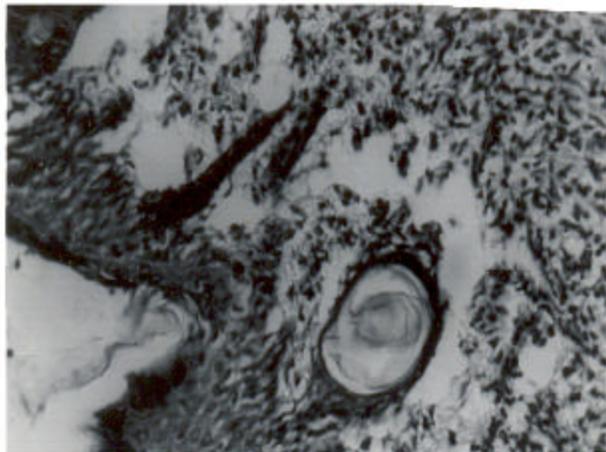


Fig. 1: Histopathological Examination of Liver Section of Control Group

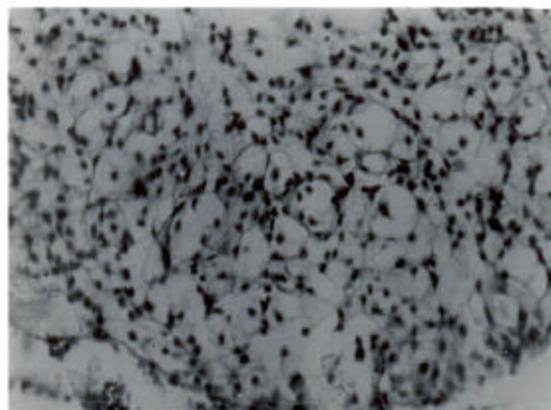


Fig. 2: Histopathological Examination of Liver Section of Calotropain-P treated Group

wounding day and the breaking strength was measured. Simultaneously, granulation tissue so harvested was subjected to hydroxyproline estimation following the method of Woessner et al¹¹, and histological study to evaluate the effect of the extracts on collagen formation.

Evaluation of Haemostatic Activity

The method of Jaiprakash et. Al.¹² was adopted for the evaluation of haemostatic activity of 'Calotropain-P' by employing Wistar albino rats. The animals were anesthetized by means of pentobarbital sodium with an usual intraperitoneal dose 7mg/kg of body weight. Occasionally a second dose of 1/3 to 1/2 of this amount was injected. The abdomen was opened by a crucial incision and the liver was gently lifted out. A piece of liver was cut from a portion of edge with a sharp scissors, leaving a cut surface 10 to 20 mm long and 3 to 6 mm wide. Distilled water was applied to the cut surface and the bleeding time was then determined. The length of the bleeding time was determined by gently blotting with pieces of filter paper at 2 to 3 s intervals. The end point was rather sharp, being indicated by a blood clot clinging to the filter paper, but with little or liquid blood wetting it. The above procedure was adopted for the test group also. Six rats were employed and average value was calculated.

Statistical Analysis

All results are expressed as mean \pm SEM. Significance of the difference between control and drug treated groups were determined by student 't' test. Differences of $P < 0.001$ were considered statistically significant.

RESULTS

Significant promotion of wound-healing activity was observed in 'Calotropain-P' in all the three wound models such as excision, incision and dead space wound. In excision wound model, the mean percentage closure of wound area was calculated on the 4, 8, 12 and 16 post wounding days as shown in Table 1. The percentage of wound closure on 'Calotropain-P' in 16th days was 94.85 ± 0.96 mm² where as in case of standard drug 1% w/w framycetin sulphate cream it was 100%. In incision wound model, Calotropain-P showed increase in breaking strength (463.68 ± 1.48) when compared to control (276.83 ± 0.71). The mean breaking strength was also significant in animals treated with standard drug FSC (510.90 ± 0.42). In dead space wound model, histological studies of the granulation tissue of the control group of animal showed more aggregation of macrophages with few collagen fibres. (Figure 1). In case Calotropain-P treated animal groups, moderate collagen deposition, macrophages and

fibroblasts were noticed Figure 2, whereas the Calotropain-P treated animal group evidenced significant increase in collagen deposition showing lesser macrophages and fibroblasts. The effect of 'Calotropain-P' has shown significant $P < 0.001$ reduction in blood clotting time, which was 20.30 ± 0.85 sec as compared to control (46.23 ± 2.35 sec) Table II

DISCUSSION

The result showed that the compound 'Calotropain-P' exhibited significant wound closure in excision wound model on 16th day and significant increase in tensile strength of incision wound as compared to control. The results shown that the Calotropain-P' exhibited significant increase in tensile strength of granuloma tissue. The histopathology explained the lesser number of inflammatory cells and increase in the bulk of collagen in test group then the control group. The study also included the haemostatic activity, which is the very first phase of wound healing process and it is useful in promoting the wound healing property. In conclusion, the significant increase in tensile strength and the prominent haemostatic activity exhibited by the compound 'Calotropain-P' could concluded the wound healing property.

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