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WOUND HEALING ACTIVITY OF DIFFERENT EXTRACT OF *CALOTROPIS GIGANTEAN LINN*

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ABSTRACT

In traditional medicine *Calotropis gigantean Linn* is a well-known plant in the traditional medicine of Rajasthan. Asclepiadaceae is used in folk medicine for pain, burns, eczema, skin diseases (Ring worm), Anti-bacterial and anti-fungal and wound-healing activities. Based on its traditional use, this plant was selected for evaluation of its wound healing potential. Preliminary wound healing activity was performed on different extracts and based on the findings; Petroleum ether, chloroform, methanol and distilled water extract was selected for further studies. The extract of *Calotropis gigantean Linn* promoted wound-healing activity significantly in all the three wound models studied. Topical application twice a day for 16 days significantly increased the wound contracting ability of the extract ointment (5% and 10%) and it was comparable to the reference standard nitrofurazone ointment in the excision wound model. The tensile strength in healing of incised wounds and dead space wound was found to be significantly higher in the group of animals treated with extract ointments (5% and 10%) and extracts (200mg/kg body weight) respectively. To investigate the effects of *Calotropis gigantean Linn* on wound healing activity in rats by excision, incision and dead space wound healing models in rats.

Key words: *Calotropis gigantean Linn*, Excision wound model, Tensile strength Incised wounds, Dead space wound.

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INTRODUCTION

The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collogenation and tissue remodeling. The wound may be defined as 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.¹ *Calotropis gigantean Linn* (Asclepiadaceae) is a glabrous or hoary laticiferous shrubs or small tress, commonly known as Swallow-wort or Milkweed. The latex contain the cardiac glycosides, calotopin, uscharin, calotoxin, calactin and uscharidin,gigantin. 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

MATERIAL AND METHOD

Plant material:-

Calotropis gigantean Linn (Asclepiadaceae) whole plant from collected in the State of Rajasthan, from the till region of Pratap nagar area of Udaipur, India and authenticated as *Calotropis gigantean Linn* by Depts. Botany, Nagpur University Nagpur, voucher specimen No.9111.has been deposited to Botany Department.

Acute dermal toxicity (fixed dose procedure):-

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.434³. whole plant extract *Calotropis gigantean linn* 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).

Animals used:-

Wistar Albino rats (150 – 180 Gms) were selected for these studies. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water adlibitum.

Preparation of extract:-

The whole plant was shade dried and reduces to fine powder by mechanical grinder (coarse).The dried powder (1kg) was successively extracted with organic solvent in a soxhlet extractor by using different solvent like petroleum ether, chloroform, methanol and distilled water. The

solvent from each extract were completely removed under reduced pressure to obtain semisolid mass.

Ointment Formulation:-

Two types of Ointment formulation were prepared from the extract 5% w/w, 10% (w/w), where 5g and 10 g of the extract was incorporated in 100 g of simple ointment base B.P respectively. Nitrofurazone ointment (0.2% w/w, Smith line – Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

Wound healing activity

Excision wound:-

Ten Groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500m full thickness of skin from the depilated area, the wound was left undressed to open environment.⁴ Then the drugs, i.e., the reference standard, (0.2% w/w) Nitrofurazone (NFZ) ointment, simple ointment B.P., Petroleum ether, Chloroform, Methanol & Distt.Water, extract ointment (5% w/w), and Petroleum ether, Chloroform, Methanol & Distilled Water extract ointments (10% w/w) were applied twice day till the wound was completely healed⁵. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day. (Table 1)

Incision wound:-

Ten groups with six animals in each group were anaesthetized and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5 cm intervals; surgical threads (No.000) and a curved needle (No.11) were used for stitching. The continuous threads on both wound edges were tightened in the same manner as has already described above. The extract ointments and the Nitrofurazone ointment were administered once daily for 18 days; when wound were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured with a tensiometer⁶. (Table 2)

Dead Space Wound:-

Nine groups of Wistar albino rats (150-200g) were used. Dead space wounds were made by implanting, subcutaneously, 2.5 x 2.5 cm polypropylene tube beneath the dorsal paravertebral lumbar skin. Control animals received 2 ml of 1% Carboxyl methyl cellulose (CMC), orally, while the test groups Extracts Petroleum ether, Chloroform, Methanol & Distilled Water (200 mg/kg, respectively) orally once daily for 10 days. On the 11th post-operative day, the granuloma tissue formed on the dead space wound was excised. Wet weight was recorded and tensile strength determined⁷. The granuloma was dried in an oven at 60° C and the dry weight noted. (Table 3)

Statistical analysis:-

Data are expressed as mean \pm SEM and subjected to students't- test by comparing with the control.

RESULT AND DISCUSSION

The extract of *Calotropis gigantean Linn* promoted wound-healing activity significantly topical application twice a day for 16 days increased the wound contracting ability of the extract ointment (5% and 10%) and it was comparable to the reference standard nitrofurazone ointment in the excision wound model. (TABLE 1)

Table.1: Effect of *Calotropis gigantean Linn* extract and Nitrofurazone on Excision model

Post wounding days	Wound Area (mm ²)			
	Simple ointment (Control)	Nitrofurazone ointment (0.2 % w/w)	Extract ointments (5 % w/w each)	Extract combination (10 % w/w each)
1	531 \pm 33.6 (0)	515 \pm 36.8 (0)	509 \pm 39.8 (0)	522 \pm 21.0 (0)
2	507 \pm 18.6 (3.9)	456 \pm 36.8 (11.2)	436 \pm 14.8 (14)	436 \pm 18.8 (16)
4	462 \pm 13.8 (12.2)	314 \pm 12.6* (38.3)	388 \pm 18.6* (23)	330 \pm 19.9* (36)
6	423 \pm 30.1 (20.0)	269 \pm 14.7* (47.6)	261 \pm 19.4** (49)	203 \pm 15.3** (60)
8	387 \pm 14.8 (26.6)	190 \pm 11.4** (62.5)	105 \pm 9.8** (78)	162 \pm 12.5** (80)
10	343 \pm 23.6 (34.9)	109 \pm 8.6** (77.3)	42 \pm 5.9** (91)	43 \pm 9.6** (91)
12	267 \pm 14.3(49.2)	77 \pm 6.3** (84.6)	27 \pm 1.8** (94)	14 \pm 7.4** (97)
14	213 \pm 11.3(59.4)	34 \pm 1.6** (93.0)	10 \pm 0.7** (97)	0.0** (100)
16	187 \pm 14.3(64.3)	09 \pm 1.9** (98.0)	0.0** (100)	-----
18	173 \pm 15.1(67.7)	0.0** (100)	-----	-----

Values are mean \pm S.E of 6 animals in each group. Figures in parent theses indicate percentage of wound contraction.

*P< 0.01., **P<0.001 Vs respective control by students *t*-test.

The tensile strength in healing of incised wounds with extract ointments (5% and 10%) was found to be excellent (Table 2).

Table. 2: Effect of Extracts and Nitrofurazone on incision wound model

Group	Treatment	Tensile strength
1	Simple ointment (control)	341±14.6
4	<i>Calotropis gigantean</i> Linn Extract ointment (5% w/w)	537 ± 12.8*
5	<i>Calotropis gigantean</i> Linn Extract ointment (10w/w)	573 ± 11.8*
10	Nitrofurazone ointment(0.2% w/w)	556 ± 16.9*

Values are mean ± S.E of 6 animals in each group. Tensile strength measured at the end of 9th day. *p< 0.001 Vs respective control by student's t-test.

Wet granulation and Dry granulation in dead space wound was found to be higher in the group of animals treated with extracts (200mg/kg body weight) (Table 3).

Table. 3: Effect of Extracts on Dead Space Wound in Rats (Mean ± S.E., n = 6)

Treatment	Wet Granuloma Weight (mg)	Dry Granuloma Weight (mg)	Tensile Strength (g)
Control (1% CMC, 2ml, p.o)	222.6 ± 10.2	33.6 ± 1.8	376.3 ± 18.3
<i>Calotropis gigantean</i> Linn Extract 200 mg/kg	474.6 ± 21.4*	104.8 ± 7.3*	542.7±33.7*

* P < 0.001 Vs control by student's "t"-test

CONCLUSION

The extract of *Calotropis gigantean* Linn promoted wound-healing activity significantly in all the three wound models studied. Topical application twice a day for 16 days significantly increased the wound contracting ability of the extract ointment (5% and 10%) and it was comparable to the reference standard nitrofurazone ointment in the excision wound model. The tensile strength in healing of incised wounds and dead space wound was found to be significantly higher in the group of animals treated with extract ointments (5% and 10%) and extracts (200mg/kg body weight) respectively. To investigate the effects of *Calotropis gigantean* Linn on wound healing activity in rats by excision, incision and dead space wound healing models in rats.

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