

Wound Healing Activity of *Sida acuta* in Rats

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Abstract: Effects of topical administration of methanolic extract of *Sida acuta* ointment was studied respectively on two types of wound models in rats, (i) the excision and (ii) the incision wound model. The ointment of the methanolic extract of *Sida acuta* produced significant response in both of the wound types tested. 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. The extract facilitates the healing process as evidenced by increase in the tensile strength in the incision model. The results were also comparable to those of a standard drug nitrofurazone.

Key words: Wound Healing, *Sida acuta*.

Introduction

Sida acuta (Malvaceae), is an erect perennial shrub found throughout the hotter parts of India and Nepal. It is used for various medicinal purposes such as liver disorders, diuretic & abortifacient, in Ayurvedic preparations, asthma, fever, headache (migraine), cough, cold, ulcer, anthelmintic, snake bite, urinary diseases, female disorders, antifertility agents and sedative. Various parts of this plant are being used in the traditional systems of medicine to treat different rheumatic arthritis, analgesic, anti-inflammatory.

However, there were no reports on both ethnobotanical and pharmacological profile of this plant. Hence, the present study was made to evaluate the wound healing potential.^{1, 2}

Material and Methods

Plant Extract and standard used:

The dried plant materials were, pulverized by a mechanical grinder, sieved through 40 mesh. The powdered materials were extracted with methanol using soxhlet extraction apparatus. This methanolic extract was then concentrated and dried under reduced pressure. The methanol free semisolid mass thus obtained was used for the experiment. Two types of

ointment formulation were prepared from the extract; 5% (w/w) and 10% (w/w), where 5 g and 10 g of the extract were incorporated in 100 g of simple ointment base B.P.³ respectively. Nitrofurazone ointment (0.2% w/w, Smithkline – Beecham) was used as a standard drug for comparing the wound healing potential of the extract.

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 *ad libitum*.

Excision wound Model⁴

Four 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 500mm² full thickness of skin from the depilated area, the wound was left undressed to open environment. Then the drugs, i.e., the reference standard, (0.2% w/w) nitrofurazone (NFZ) ointment, simple ointment B.P., *Sida acuta* extract ointment (5% w/w), and Extract ointment (10% w/w) were applied

once daily 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.

Incision wound model

Four groups with six animals in each group were anaesthetized and two Para vertebral 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 experiment⁶. 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 NFZ ointment were administered once daily for 9 days; when wound were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured with a tensiometer⁷.

Measurement of Healing

Tensile strength, the force required to open a healing skin wound, was used to measure healing. The instrument for this measurement is called tensiometer was designed on the same principle as the thread tester used in the textile industry. It consisted of a 6 x 12 inch board with one post of 4 inch long fixed on each side of the longer ends. The board was placed at the end of a table. A pulley with bearing was mounted on the top of one of the posts. An alligator clamp with 1 cm width, was tied on the tip of the post without pulley by a piece of fishing line (20-lb test monofilament) so that the clamp could reach the middle of the board. Another alligator clamp was tied on a piece of fishing line with a 1 – L polyethylene bottle tied on the other end. Before testing, the animal was anaesthetized with ether in an open mask. The sutures of the wound were cut out with a pair of scissors. The animal was then placed on a stack of paper towels that could be adjusted so that the wound was on the same level of the tips of the posts. The clamps were then carefully clamped on the skin of the opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer piece of fishing line was placed on the pulley and the position of the board was adjusted so that the polyethylene bottle was freely hanging in the air.

Water was added to the polyethylene bottle at a rapid but constant rate by siphon from a large reservoir (20-L Bottle) until the wound began to open up. The amount of water in the polyethylene bottle was weighed and considered as the tensile strength of the wound.

Statistical analysis:

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

Results and Discussion

The measurements of the progress of the wound healing induced by the NFZ ointment (0.2% w/w), *Sida acuta* ointment (5% w/w) and *Sida acuta* ointment (10% w/w) the respective control groups (i.e.simple ointment treated groups) in the excision wound method are shown in Table.1. It is observed that the wounds contracting ability of the *Sida acuta* ointment (5% w/w) significantly greater than that of the control. In the case of NFZ ointment and *Sida acuta* ointment treated groups it was found to be 18 \pm 2 d.

In the incision wound studies, there was a significant increase in tensile strength of the 10-d old wound due to treatment with *Sida acuta* ointment and the reference standard NFZ ointment when compared with the respective control. Measurements of the tensile strength are shown in Table.2. The tensile strength of the NFZ ointment-and the *Sida acuta* ointment treated groups were almost the same.

The process of wound healing occurs in four phases (i) coagulation, which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition⁸. Any agent, which accelerates the above process is a promoter of wound healing. Plant products have been show to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing due to the presence of active terpenes, alkaloids and flavonoids^{9,10}. An essential oil from the leaves had broad-spectrum antifungal activity comparable to that of 0.5% hamycin a glycosidal mixture extract of *centella asiatica* has been reported to be responsible for enhanced repair only in incised wounds¹¹ and in stimulating collagen in human skin fibroblast cells¹². The wound healing property of *Sida acuta* appears to be due to the presence of its active principles which accelerates the healing process and confers breaking strength to the healed wound.

TABLE1: EFFECT OF PLANT EXTRACT AND NITROFURAZONE OINTMENT ON WOUND HEALING BY EXCISION WOUND METHOD

Post wounding days	Wound Area (mm ²) mean ± S.E. and percentage of wound contraction			
	Simple ointment	Nitrofurazone ointment (0.2% w/w)	<i>Sida acuta</i> Extract ointment (5% w/w)	<i>Sida acuta</i> Extract ointment (10% w/w)
0	529±19.3 (0.0)	518±17.4 (0.0)	513±17.9 (0.0)	536±18.4 (0.0)
2	467±18.4 (11.7)	417±21.6 (19.4)	401±14.6(21.7)	446±31.4 (16.8)
4	403±21.5 (23.8)	321±29.4* (38.0)	321±26.7*(37.4)	409±21.3* (23.7)
6	371±14.6 (29.8)	231±23.4** (55.4)	256±21.6** (50.3)	326±19.3** (39.2)
8	313±13.9 (40.8)	173±17.6** (66.7)	174±18.3** (66.0)	208±17.1** (61.2)
10	297±14.6 (45.2)	129±11.6** (75.0)	105±8.9** (79.5)	156±16.9** (70.9)
12	276±11.9 (47.8)	75±6.9** (85.5)	69±4.9** (86.5)	94±11.3** (82.5)
14	259±14.3 (51.0)	34±2.4** (93.4)	31±2.1** (93.9)	57±3.1** (89.4)
16	231±16.7 (56.3)	9±0.8** (98.2)	8±1.1** (98.4)	28±1.7** (94.7)
18	211±15.3 (60.1)	0.0** (100.0)	0.0** (100.0)	12±0.6 (97.8)
20	179±11.8 (66.1)	0.0** (100.0)	0.0** (100.0)	0.0 (100)

*P<0.01, **P<0.001 Vs Control by Students 't'-test (n=6)

Figures in parenthesis represent percentage of wound contraction.

TABLE 2 : EFFECT OF PLANT EXTRACTS AND NITROFURAZONE OINTMENT ON INCISION WOUND MODEL IN RATS

Group	Treatment	Tensile strength (g)
1	Simple Ointment	363 ± 12.9
2	<i>Sida acuta</i> Extract Ointment (5% w/w)	671 ± 22.7*
3	<i>Sida acuta</i> Extract Ointment (10% w/w)	623 ± 15.9*
4	Nitrofurazone ointment (0.2% w/w)	659 ± 27.1*

*p<0.001 Vs control by student 't'-test. Data are expressed as mean ± S.E

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