EVALUATION OF WOUND HEALING ACTIVITY OF SIDA SPINOSA IN RATS

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Summary

Sida spinosa belongs to the family of Malvaceae. It is a folkloric medicine for a variety of ailments including gonorrhea. However there is no scientific report on wound healing activity. The ethanolic leaf extract of sida spinosa (5% w/w and 10% w/w) are in the form of an ointment was used for evaluating the wound healing potential in excision and incision model. The results are significants. In the excision model the extract treated wounds were found to epithelialize 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 0.2 % w/w ofnitrofurazone.

Key words: wound healing, sida spinosa, nitrofurazone, incision wound, excision wound.

Introduction

Natural products, mainly the plant-derived constituents, have long been used as sources of drugs.

Natural products are also of great interest in the process of drug discovery, due to their large diversity in nature, permitting the identification of lead molecules of greater interest for the development of new therapeutic agents.[1]Though the traditional Indian system of medicine has a long history of use of plant as drug, they lacked adequate scientific documentation, particularly in light of modern scientific knowledge[2]. The sida spinosa leaves are reported to treat the pain, arthritis, asthma, bronchitis, burning sensation, haemorrhoids, intermittent fever and general debility, gonorrhea, gleet and scalding urine. Root is used as a tonic for diaphoretic. A decoction of it is said to be given as a demulcent in irritability of bladder,it also has aphrodisiac property [3-7]. 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.[8-9]

Material and Methods

Plant Extract and standard used:

The dried plant materials were, pulverized by a mechanical grinder, sieved through 40 mesh. Thepowdered materials were extracted with methanol using soxhlet extraction apparatus. This methanol 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[10] 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 Model[11]

Four groups with six animals in each group wereanaesthetized with ether. The rats were depilated onthe back. One excision wound was inflicted by cutting away a 500mm2 full thickness of skin from thedepilated area, the wound was left undressed to openenvironment. Then the drugs, i.e., the referencestandard, (0.2% w/w) nitrofurazone (NFZ) ointment,Simple ointment B.P., *Sida spinosa*extract ointment (5%w/w), and Extract ointment (10% w/w) were applied once daily till the wound was completely healed[12]. Thismodel was used to monitor wound contraction andwound closure time. Wound contraction wascalculated as percent reduction in wound area. Theprogressive changes in wound area were monitoredplanimetrically by tracing the wound margin on graphpaper every alternate day.

Incision wound model

Four groups with six animals in each group wereanaesthetized and two Para vertebral long incisionswere made through the skin and cutaneous muscles ata distance of about 1.5 cm from the midline on eachside of the depilated back of the rat. Full asepticmeasures were not taken and no local or systemicantimicrobials were used throughout the experiment[13].No ligature was used for stitching. After the incisionwas made, the parted skin was kept together andstitched with black silk at 0.5 cm intervals; surgicalthreads (No.000) and a curved needle (No.11) wereused for stitching. The continuous threads on bothwound edges were tightened in the same manner as hasalready described above. The extract ointments and theNFZ ointment were administered once daily for 9days; when wound were cured thoroughly the satureswere removed on the ninth day and tensile strengthwas measured with a tensiometer[14].

Measurement of Healing

Tensile strength, the force required to open a healing skin wound, was used to measure healing. Theinstrument for this measurement is called tensiometerwas designed on the same principle as the thread testerused in the textile industry. It consisted of a 6 x 12inches board with one post of 4 inch long fixed on eachside of the longer ends. The board was placed at theend of a table. A pulley with bearing was mounted onthe top of one of the posts. An alligator clamp with 1cm width, was tied on the tip of the post without pulleyby a piece of fishing line (20-lb test monofilament) sothat the clamp could reach the middle of the board.Another alligator clamp was tied on a piece of fishingline with a 1 – L polyethylene bottle tied on the otherend. Before testing, the animal was anaesthetized withether in an open mask. The sutures of the wound were

Cut out with a pair of scissors. The animal was thenplaced on a stack of paper towels that could beadjusted so that the wound was on the same level ofthe tips of the posts. The clamps were then carefullyclamped on the skin of the opposite sides of the woundat a distance of 0.5 cm away from the wound. Thelonger piece of fishing line was placed on the pulleyand the position of the board was adjusted so that thepolyethylene bottle was freely hanging in the air.

Results and Discussion

The progress of the wound healing induced by sida spinosa ointment (5%w/w)and sida spinosa ointment treated groups simple ointment treated groups (control) and 0.2%w/w of nitrofurazone ointment (standard drug) treated groupof animals are shown in table no 1.Its observed that wound healing contraction ability of sida spinosa (5%w/w) was significant greater than the control. The sida spinosa (5%w/w) incorporated ointment treated group showed significant wound healing from second day onwards. Which was comparable to that of the standard drug (0.2%w/w of nitrofurazone) treated group of animals. The wound closure time was lesser as well as percentage of wound contraction was muchmore withsida spinosa (5%w/w)ointment (on 18th day for 100% contraction which was almost similar to the of 0.2%w/w of nitrofurazone treated group)

Table No 1:Effect of leaf extract of Sida spinosa Linn and Nitrofurazone on Excision model

Post wounding days	Wound area (mm^2)			
	Simple ointment (control)	Nitrofurazone ointment (0.2%w/w)	Extract ointment (5% w/w)	Extract ointment (10% w/w)
0	530 ± 33.6(0)	516 ± 36.8 (0)	519 ± 24.0 (0)	521±12.8
2	509 ± 18.6(3.9)	458 ± 36.8 (11.2)	456 ± 19.8 (12.1)	495.3±15.46
4	465 ± 13.8(12.2)	318 ± 12.6* (38.3)	339 ± 17.9 (34.7)	404.5±16.36*
6	424 ± 30.1(20.0)	270 ± 14.7* (47.6)	289 ± 13.3* (44.3)	370±12.97**
8	389 ± 14.8(26.6)	193 ± 11.4** (62.5)	201 ± 14.5** (61.3)	246.2±12.71**
10	345 ± 23.6(34.9)	110 ± 8.6** (77.3)	135 ± 10.1** (73.9)	185 ±11.2**
12	269 ± 14.3(49.2)	79 ± 6.3** (84.6)	86 ± 6.4 (83.4)	64.5±4.33
14	215 ± 11.3(59.4)	36 ± 1.6** (93.0)	39 ± 4.8** (92.5)	32.33±0.731
16	189 ± 14.3(64.3)	10 ± 1.9** (98.0)	12 ± 0.9** (97.7)	12.17±0.595
18	171 ± 15.1(67.7)	0.0** (100)	0.0** (100)	0.0** (100)

Values are mean ± S.E of 6 animals in each group. Figures in parentheses indicate percentage of wound healing.

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

In the incision wound studies, there was a significant increase in tensile strength of the 10-days old wound due to treatment with *Sida spinosa* 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 spinosa*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 deposition[15] . Any agent that accelerates the above process is a promoter of wound healing[16]. Plant products have been show to possess good therapeutic potential as antibacterial activity due to the presence of active alkaloids,steroids, flavonoids and glycosides[17]. The ethanolic leaf extract of sida spinosa had broad spectrum antifungal activity comparable to the amphotericin B[18]. So may be promoter of wound healing also due to above Phytoconstituents. The wound healing property of *Sida spinosa* appears to be due to the presence of its active principles which accelerates the healing process and confers breaking strength to the healed wound.

Table No 2: Effect of leaf extract of Sida spinosa Linn and Nitrofurazone on incision wound model in rats

Group	Treatment	Tensile strength
1	Simple Ointment	360 ±11.5
2	*Sida Spinosa* Extract Ointment (5 % w/w)	661±21.5*
3	*Sida spinosa* Extract Ointment (10 % w/w)	624±12.1*
4	Nitrofurazone ointment	660.2±17.3*

*p <0.001 Vs respective control by students t-test. Data expressed as mean±S.E

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