
Wound Healing Activity of *Indigofera enneaphylla* Linn

S. HEMALATHA, N. SUBRAMANIAN*, V. RAVICHANDRAN AND K. CHINNASWAMY¹
Pallavan Pharmacy College, Iyyengarkulam, Kanchipuram-631 502
¹Madras Medical College and Research Institute, Chennai.

Accepted 17 April 2001

Revised 3 April 2001

Received 14 August 2000

Indigofera enneaphylla Linn. is a well known plant in Indian traditional medicine. On the basis of its traditional use and literature references, this plant was selected for evaluation of its wound healing potential. An alcoholic extract of aerial parts of *Indigofera enneaphylla* Linn. was examined for its wound healing activity in the form of ointment in two types of wound models in rats the excision wound model and the incision wound model. The extract ointments showed significant response in both of the wound models as comparable to those of a standard drug, nitrofurazone, in terms of wound contracting ability, wound closure time and tensile strength.

Indigofera enneaphylla Linn. (Papilionaceae) is an under shrub widely grown plant throughout India. The medicinal value of the aerial plant of the plant has been claimed by the Indian system of medicine are well known. The plant has many folk uses such as diuretic, antiscorbutic, antidiarrhoeal, analgesic, in many skin infections and in burns, especially in wound healing^{1,2}. The juice of the plant is believed to be effective in venereal diseases³. The antibacterial activity of plant extract against different microorganisms was examined. The wound healing potential of *Trigonella foenumgraecum*⁴, *Leucas lavandulaefolia*⁵, Aloe gel and other topical antibacterial agents have also been reported⁶. Based on its use in wound healing in traditional practices and literature references, the present study was undertaken to evaluate the wound healing activity of an extract of aerial parts of *Indigofera enneaphylla* Linn.

The aerial parts of *Indigofera enneaphylla* was collected from Coimbatore District, Tamilnadu during the months of June-July 1998 and authenticated by the Botany Division of Captain Srinivasa Moorthy, Drug Research Institute, Chennai where a voucher specimen was deposited. The air-dried parts were reduced to a 40 mesh powder and were extracted successively with petroleum ether (60-80°) and ethanol (90% w/v) using a

Soxhlet extractor. This ethanolic extract was concentration to dryness under reduced pressure and controlled temperature (50-60°). The different concentrations (0.5, 1 and 2% w/w) of extract ointment were prepared by using simple ointment base BP⁷. Swiss albino male rats (150-180 g) were used for the present investigation. The animals were maintained at a well-ventilated, temperature controlled (30±1°) animal room for 7 days prior to the experimental period. The animals were provided with food and water *ad libitum*. The extract ointments (0.5, 1 and 2% w/v) at a quantity of 0.5 g were applied once daily to treat different groups of animals, while simple ointment base and 0.2% w/w nitrofurazone ointment were applied in the same quantity to serve as control and standard respectively.

The present investigation was evaluated in two different models in rats such as the excision and the incision wound models. In the excision wound model^{5,8} the full thickness excision wounds were made on the rats by removing a 500 mm² piece of skin from the shaven backs after anaesthetized with anaesthetic ether by the open mask method. After wound infection, the wound was left open to the environment and these rats were divided into six groups of six rats each as follows: Group I - untreated control, Group II - rats treated with a reference standard (0.2% w/w nitrofurazone ointment), Group III - rats treated with simple ointment base (control), Group IV, V and VI - rats treated with 0.5, 1 and 2% w/w of

*For correspondence
e-mail: <natesansubbu@rediffmail.com>

extract ointments respectively. Wound healing potential was monitored by wound contraction and wound closure time. Wound contraction was calculated as percentage reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper on wounding day followed by sixth, twelfth and eighteenth day.

For incision wound model⁹, six groups with animals in each group were anesthetized and two paravertbral or 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. All the groups were treated in the same manner as mentioned in the case of excision wound model. After the incision was made, the parted skin was kept together and stitched tightly with continuous black silk at 0.5 cm intervals; surgical thread (No.000) and a curved needle (No.11) were used for stitching. Extract ointments, simple ointment base (control) and standard drug were applied once daily for nine days. When the wounds were cured thoroughly

the sutures were removed on the ninth day and the tensile strength of the healed wound was measured on day 10 by continuous and constant water flow technique¹⁰. Tensile strength of the healed wound for an individual animal calculated by the mean determination of tensile strength of the two paravertbral incisions on both sides of the animals. The tensile strength of the extract-treated wounds was compared with controls. The results are expressed as mean \pm SEM and statistical significance was evaluated by using students 't' test versus control group. $P < 0.001$ implies significance.

The effect of extract ointments, nitrofurazone ointment (standard) and simple ointment base (control) in the excision wound model and in the incision wound model were assessed by measuring the wound area, percentage of wound contraction and tensile strength respectively. The data including wound area (mm^2) percentage of wound contraction and tensile strength of healed wound was furnished in Table 1. The present investigation revealed that the test extract in varying

TABLE 1: EFFECTS OF ALCOHOLIC EXTRACT OF *INDIGOFERA ENNEAPHYLLA* LINN WOUND-HEALING

Treatment	Excision wound model				Incision wound model
	Wound area (mm^2)+S.E.M and (Percentage of Wound contraction)				
	Post wounding days				Tensile strength in Grams \pm S.E.M.
0 day	6 th day	12 th day	18 th day		
Sample ointment B.P	531025 \pm 1.493 (0.00)	411.50 \pm 5.909 (22.51)	278.50 \pm 3.095 (47.57)	160.00 \pm 1.080 (68.74)	403 \pm 13.4
Nitrofurazone ointment (0.2% w/w)	515.70 \pm 4.049 (0.00)	285.50 \pm 8.800 (44.67)	82.00 \pm 1.414 (84.09)	0.50 \pm 0.500 (99.60)	545 \pm 11.2
Extract ointment (0.5% w/w)	520.75 \pm 4.940 (0.00)	324.00 \pm 5.590 (38.58)	86.50 \pm 1.500* (83.09)	9.75 \pm 0.853* (98.14)	528 \pm 10.1
Extract ointment (1% w/w)	521.50 \pm 8.700 (0.00)	305.50 \pm 6.300 (41.41)	84.00 \pm 2.880* (83.87)	6.75 \pm 0.750* (98.69)	533 \pm 10.7*
Extract ointment (2% w/w)	524.75 \pm 5.251 (0.00)	284.00 \pm 3.109 (45.29)	70.25 \pm 1.314* (86.61)	0.00 \pm 0.000* (100.00)	539 \pm 11.8*

p value vs respective control by Student's-'t' test * $p < 0.001$ (n=6)., Numbers in parenthesis denote percentage of wound contraction.

concentration in the ointment base were capable of producing significant wound healing activity on both wound models. The entire test extract ointments used in excision wound model showed significant wound healing effect on the days 12 and 18. Perusal of the Table 1 indicates that out of the three extract ointments used in the experiment, ointment prepared with 2% w/w of alcoholic extract of *Indigofera enneaphylla* Linn. has been found to have relatively more wound healing activity with 100% of wound closure on day 10 is as compared to the standard (nitrofurazone ointment, 0.2% w/w) while the other two extract ointments (0.5 and 1% w/w) did not show much difference there effects on wound closer. In the incision wound studies, there was a significant increase in tensile strength on day 10 due to treatment with either the extract ointments or the standard nitrofurazone ointment when compared to control. The effect produced by the nitrofurazone ointment (0.2% w/w) application was found to be same as that obtained with the application of the extract ointment (2% w/w) on the basis of the results obtained in the present investigation it is possible to conclude that the ointment of the extract of *Indigofera enneaphylla* Linn. has significant wound healing activity

at all the doses tested these results also support the folklore of the use of the plant for wound healing.

REFERENCES

1. Anonymous, In; The Wealth of India, Vol. 5, Publication and Information Directorate, CSIR, New Delhi, 1991, 178.
2. Nadkarni, A.K., In; Indian Meteria medica, 3rd Edn., Vol. 1, Popular Prakashan, Mumbai, 1976, 678.
3. Kritkar, K.R. and Basu, B.D., In; Indian Medicinal Plants, 2nd Edn., Vol. 1, International Book Distributors, Dehradun, India, 1935, 709.
4. Taranalli, A.D. and Kuppast, I.J., *Indian J. Pharm. Sci.*, 1996, 58, 117.
5. Saha, K., Mukherjee, P.K., Das, J., Saha, B.P. and Pal, M., *J. Ethnopharmacol.*, 1997, 56, 139.
6. Heggors, J.P., Kucukelebi, A., Stabeanu, C.J., Martin, C.R. and Winters, W.D., *Phototherapy Res.*, 1995, 9, 455.
7. British Pharmacopoeia, Vol. II, British Pharmacopoeia commission, HMSO Publication Centre, London, 1993, 1096.
8. Chitra, P., Sajithalal, G.B. and Chandrakesan, G., *Indian J. Exp. Bio.*, 1998, 36, 596.
9. Udupa, S.L., Udupa, A.L. and Kulkarni, D.R., *Fitoterapia*, 1994, 65, 141.
10. Lee, K.H., *J. Pharm. Sci.*, 1968, 57, 1238.