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Research Article

Wound Healing Activity of *Indigofera Enneaphylla* Linn

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ABSTRACT

Indigofera enneaphylla Linn is a well-known plant in Indian traditional medicine. In the present investigation, wound healing potential of alcoholic extract of whole plant of *Indigofera enneaphylla Linn* was evaluated by excision and incision in animal wound model. The formulated ointment containing the above extract showed significant response in both of the wound models when compared to those of a standard drug, nitrofurantoin in terms of wound contracting ability, wound closure time and tensile strength.

INTRODUCTION

Indigofera enneaphylla Linn (Fam:Leguminosae) is an under-shrub widely grown throughout India. The plant has many folk uses such as diuretic, antiscorbulic, 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 diseases. The antibacterial activity of plant extracts against different microorganisms was examined and reported[3]. On the basis of its traditional use, this plant was selected for evaluation of its wound healing activity.

Proper and timely wound healing is a vexing problem faced by all clinicians. In majority of patients normal healing establishes tissue integrity quickly and effectively. Wound healing involves a highly dynamic integrated series of cellular, physiological and biochemical processes, which occur in living organism. Repair through regeneration is very common in unicellular and the lower metazoan animal groups while it is highly restricted in the higher animals[4].

MATERIALS AND METHODS

The whole plant of *Indigofera enneaphylla* Linn was collected from Tirunelveli district, Tamilnadu during the month of July-August and authenticated by a Botanist from Annamalai University, Annamalai Nagar and a voucher specimen was deposited in the Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences, Trichy, for future reference.

Preparation of extracts

The air-dried plant parts were reduced to a 40 mesh powder and were extracted successively with petroleum ether (60-80° C) ethyl acetate and ethanol (90% w/v) using soxhlet extractor. All the extracts were concentrated to dryness under reduced pressure and controlled temperature. Preliminary phytochemical screening was carried out for all the above extracts by following standard procedure [5]. The alcoholic extract alone was selected for the study of wound healing activity, since this extract showed the presence of maximum amount of phytoconstituents (Table No 1). The extract with different concentrations (0.5% and 1% w/w) were formulated as an ointment by using simple ointment base BP[6]. Swiss albino rats (150-180g) were used for the present investigation. The animals were provided with food and water *ad libitum*. The formulated ointments (0.5% and 1% w/w) at a quantity of 0.5g were applied once daily to treat different group of animals, while simple ointment base and 0.2% w/w nitrofurazone ointment were applied in the same quantity to severe as control and standard respectively.

Excision Wound Model[7]

Excision wound model is infliction on the dorsal thoracic region 1-1.5 cm away from the vertebral column on the either side and 5 cm away from the ear. A full thickness of the excision wounds were made on the rats by removing a 500 mm^2 piece of skin from shaven backs after anaesthetized by the open mask method. After wound infection, the wound was left open to the environment. Animals were divided into five groups of 6 each. The Group 1 animals were left untreated and considered as the

control. Group 2 animals served as reference standard and treated with nitrofurazone ointment. Animals of Groups 3 and 4 were treated with 0.5 and 1% w/w of the formulated 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 in graph paper on wounding day followed by sixth, twelfth and eighteenth day (**Fig:1:A-D**).

Incision Wound Model[8]

All animals were anaesthetized before wound creation and two paravertebral long incisions were made through the skin at the distance of about 1.5cm from midline on each side of the depilated back of the rat. No local or systemic antimicrobials were used throughout the experiment. All groups were treated same as in excision model, the both edges kept together and stitched with black silk surgical thread (no.000) and a curved needle (No.11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed and then the oral dose was given once a day to the rats upto 9 days. When wounds were cured thoroughly the sutures were removed on the day 9 and tensile strength of cured wound skin was measured using tensiometer [7] (**Fig:2:A-D**)

Statistical Analysis [9]

All the data are expressed as mean ± SEM. The values obtained for the extracts were compared with control group using Students 't' test. The values of $P<0.001$ were considered to indicate a significant difference between the groups.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the plant showed the presence o**f** alkaloids, aminoacids, flavonoids, phenolic compounds, saponins, steroids, tannins and triterpenes in alcoholic extract. The results are shown in Table 1.The effect of formulated ointments, nitrofurazone ointment (standard) and simple ointment base (control) in the excision wound model were assessed by measuring the wound area and percentage of wound contraction, while in the incision wound model they were assessed by the tensile strength. The results of excision and incision wound model experiments are given in table 2 and 3 respectively. The results revealed that in excision wound study, the extract formulated ointments showed better and fast healing activity when compared with the untreated control group. The formulation with 1% extract concentration showed action (100%) similar to that of standard drug (99.70%). The formulation containing 0.5% extract concentration though showed good wound healing activity (88.02%), its action was much less than that of other two formulations. In the incision wound studies, there was a significant increase in tensile strength on day10 for both extract ointments and the standard nitrofurazone ointment when compared with the control. The effect produced by the application of the extract ointment (1%w/w) was found to be same as that obtained by the application of nitrofurazone ointment (0.2%w/w). From the above results it is revealed that the wound healing activity of the selected extract may be due to the presence of certain phytoconstituents such as alkaloids, amino acids, phenolic compounds etc.

CONCLUSION

In conclusion, the results of study showed that the alcoholic extract of *Indigofera ennaephylla* Linn. Effectively stimulates wound contraction (excision wound) and significantly increased the tensile strength of incision wounds in animal models as compared to control group. These findings could justify the inclusion of this plant in the management of wound healing.

Table 1: Preliminary Phytochemical screening of the whole plant of *Indigofera enneaphylla* Linn

Constituents	Pet- ether	Ethylacetate	Alcohol
Alkaloids	-	+	+
Aminoacids	-	+	+
Anthroquinone glycosides	-	-	-
Flavonoids	-	-	+
Oils	-	-	-
Phenolic groups	+	+	+
Saponins	-	-	+
Steroids	+	+	+
Sugars	-	-	-
Tannins	-	-	+
Triterpenes	+	+	+

+ = Present - = Absent

Table 2: Effect of *Indigofera enneaphylla* ointment on excision wound model in rats

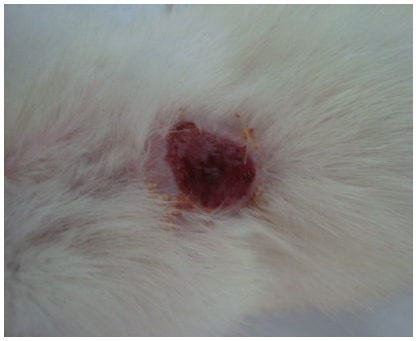
Treatment	Wound area (mm^2 ± SEM and (percentage of wound contraction)			
	Post Wounding days			
	0 day	6 th day	12 th day	18 th day
Simple ointment base	530±33.6 (0)	424±30.1 (20.0)	269±14.3 (49.2)	179±11.8 (66.1)
Nitrofurazone Ointment (0.2%w/w)	515.72±4.0480 (0.00)	286.50±8.790 (44.66)	83.00±1.421 (84.08)	0.48±0.500 (99.70)
Extract ointment (0.5%w/w)	496.74±4.890 (0.00)	304.52±6.280 (40.42)	85.00±2.882 (83.86)	6.76±0.680* (88.02)
Extract ointment (1.0%w/w)	502.64±5.106 (0.00)	286.00±3.102 (45.00)	70.25±1.314 (86.60)	0.00±0.000* (100.00)

*P <0.001 (n=6)

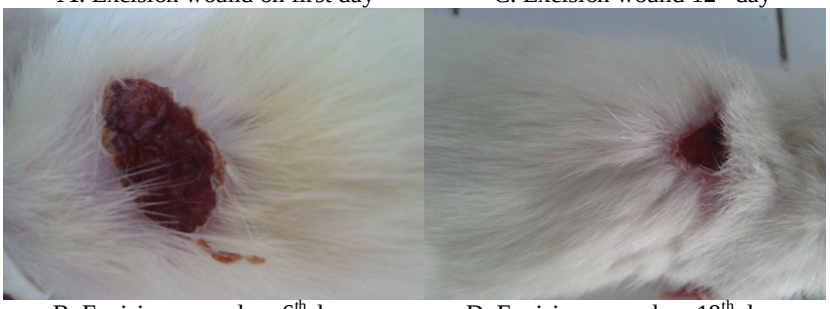
Table 3: Effect of *Indigofera enneaphylla* ointment on excision wound model in rats

Treatment	Tensile strength in grams ± SEM
Control	404±13.2
Nitrofurazone ointment (0.2%w/w)	542±10.86
Extract ointment (0.5%w/w)	534±10.58*
Extract ointment (1.0%w/w)	538±11.2*

*P < 0.001 (n=6)

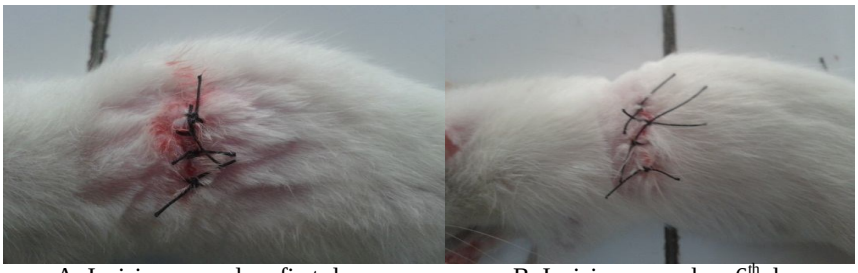


A. Excision wound on first day C. Excision wound 12th day

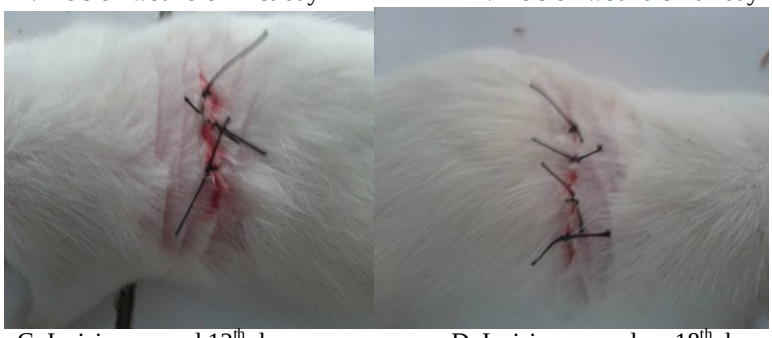


B. Excision wound on 6th day D. Excision wound on 18th day

Fig. 1: Excision wound healing: A. Excision wound on first day; B. Excision wound on 6th day; C. Excision wound 12th day; D. Excision wound on 18th day



A. Incision wound on first day B. Incision wound on 6th day



C. Incision wound 12th day D. Incision wound on 18th day

Fig. 2: Incision wound healing: A. Incision wound on first day; B. Incision wound on 6th day; C. Incision wound 12th day; D. Incision wound on 18th day.

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