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Research Article

Evaluation of wound healing activity of ethanolic extract of *Azadirachta Indica* leaves on incision and excision wound models in Wister albino rats

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ABSTRACT

Background: Wound healing is complex cellular and biochemical cascade that lead to restitution of integrity and function. Recently, the traditional use of plants for wound healing has received attention by the scientific community, as traditional medicine is a source of less expensive, comprehensive medical care, especially in developing countries. *Azadirachta indica* (Neem) is well-known in India, as one of the most versatile medicinal plants having a wide spectrum of biological activity. Hence, this study was undertaken to evaluate the wound healing activity of the ethanolic extract of *A. indica* leaf in the experimentally-induced wound in rats.
Methods: The healing effect produced by *A. indica* extract was assessed by the rate of wound contraction histopathology and skin breaking strength by using excision wound model and incision wound model in Wister albino rats. This was compared with control (soft white paraffin) and standard (1% w/w framycetin sulfate ointment). The results have been analyzed by calculating the mean values, standard deviation and compared by using student t-test.
Results: The ethanol extract of leaves of *A. indica* significantly promoted the wound healing activity in both excision and incision wound models.
Conclusion: The study revealed promising wound healing activity of ethanolic extract of *A. indica* and provides a scientific rationale for the traditional use in the management of wounds.

Keywords: Traditional medicine, Framycetin sulfate ointment, Soft white paraffin

INTRODUCTION

Wound is delineated as disruption of structural and physiological continuity of living tissue. They are inescapable events of life; wound may arise due to physical, chemical, or microbial agents. Wound healing is complex cellular and biochemical cascade that lead to restitution of integrity and function, accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelization.[1] When healing takes place in a direction away from its normal course, it is common to have non-healing, under or over healing. Treatment is, therefore, aimed at either shortening the time required for healing or minimizing the undesired consequences.

The World Health Organization (WHO) has been promoting traditional medicine as a source of less expensive, comprehensive medical care, especially in developing countries. The WHO also recognized the importance of traditional medicine and has treated strategies, guidelines, and standard for botanical medicines.[2] Approximately, one-third of all traditional medicines in use is for the treatment of wounds and skin disorders, compared to only 1-3% of modern drugs.[3]

Azadirachta indica (Neem) is well-known in India for more than 2000 years, as one of the most versatile medicinal plants having a wide spectrum of biological activity. It is called as "villagers' dispensary" because of its medicinal value. Every part of the tree has been used as a household remedy against various human ailments. Various studies have been carried out and it is shown to have antipyretic, immunostimulant, antiulcer, antioxidant, hypoglycemic, hepatoprotective activity.[4]

Hence, this study was undertaken to evaluate the wound healing activity of the ethanolic extract of *A. indica* leaves in the experimentally-induced wound in albino rats.

Objectives

1. To evaluate the wound healing activity of ethanolic extract of *A. indica* leaves in Albino rats
2. To compare the wound healing activity of *A. indica* with the standard, 1% w/w framycetin sulfate cream (FSC) ointment.

METHODS

A total 36 healthy adult Wister albino rats of either sex weighing 180-250 g were randomly selected from the central animal house. Pregnant and diseased animals were excluded in this study. The study was conducted after obtaining approval from Institutional Animal Ethical Committee.

The ethanolic extract of leaves of *A. indica* (test drug) formulation was obtained from the Himalaya drug company. 5 g of alcoholic extract of *A. indica* was mixed with 95 g of soft paraffin to prepare 5% ointment (w/w).

The selected animals were housed individually and maintained at a temperature-controlled, well-ventilated animal room for a period of 7-day prior to the experimental period to allow for acclimatization to the laboratory condition. They were kept on standard pellet diet and water *ad-libitum*. The animals were starved 12 hrs prior to wounding with water *ad-libitum*. Wounding was performed aseptically under light ether anesthesia.

Wound models

The wound healing activity was carried out in two different wound models in albino rats such as excision wound model and incision wound model.

In each model, the animals were divided into three groups, each group containing six animals.

Group-I (control) received the topical application of simple ointment base (B.P)

Group-II (standard) received the topical application of framycetin sulfate cream 1% w/w

Group-III (test) received the topical application of 5% w/w ointment of *A. indica*.

Excision wound model

Under light ether anesthesia, the animals were secured to operation table in prone position. An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anesthetized rat. The particular skin area was shaved 1 day prior to the experiment. Under aseptic precautions, the skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2. Hemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline.[5] The ointments were applied topically once in a day, until the epithelization was complete starting from the day of the experiment. The parameters studied were shown in Table 1.

The percentage of wound healing was calculated of original wound size (500 mm^2) for each animal of group using the formula:[7]

$$\text{Percentage of wound closure} = \frac{\text{Wound area on day 0} - \text{wound area on respective day}}{\text{wound area on day 0}}$$

Incision wound model[8,9]

Under light ether anesthesia, the animals were secured to operation table in natural position. The skin of the back where the wound was to be made was shaved. Two paravertebral straight incisions of 6 cm each were made with the help of sharp surgical blade through full thickness of the skin on either side of the vertebral column of the rat. Care was taken to see that the incisions were at least 1 cm lateral to the vertebral column. After complete hemostasis, the wounds were closed by means of interrupted sutures with 4-0 silk thread placed at equidistant points about of 1 cm apart. Wounds were mopped with cotton swabs soaked in 70% alcohol, and animals were caged individually (Figure 1a).

The ointment containing the test formulation was applied topically once in a day. The sutures were removed on 8th post wound day, and the tensile strength (defined as the force just sufficient to disrupt the wound) of the healed wound was measured on 10th day (Figure 1b).

Statistical analysis

The data were entered in excel format and analyzed by calculating the mean, standard deviation, and the groups were compared using Student's t-test ($p<0.05$ was considered as significant).

RESULTS

The results of excision and incision wound models were showed in (Table 2). In excision wound model, the test group

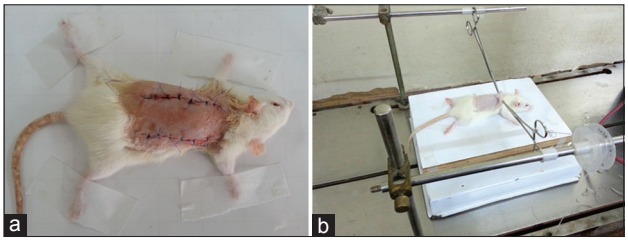

Figure 1: Incision wound model. (a) Incision wound model showing sutured two paravertebral straight incisions. (b) Determination of tensile strength by using continuous water flow technique of Lee

Table 1: The parameters studied in excision and incision wound models.

Wound model	Attribute	Parameters studied	Methodology	Made on post wounding day
Excision	Physical	Percentage of wound contraction	Planimetry*	0, 4, 8, 12, 16 and subsequently on every alternate day till complete epithelisation
		Epithelization time (days)	Observed the day on which scab falls	Noted on falling of scab without raw area
		Scar area of excision wound (mm^2)	Planimetry	Studied on complete epithelization
	Histological[6]	Cellular elements, fibroblast and collagen fibers	Microscopy	4, 8, and 12
Incision	Mechanical	Tensile strength measurement	Continuous water flow technique of Lee[9]	10

*Retracing the wound from butter paper on to mm scale graph paper and then number of squares were counted

Table 2: The parameters studied and results of excision and incision wound models.

Groups	Excision wound model							Incision wound model
	Percentage of wound contraction					Epithelisation period (days)	Scar area (mm^2)	Tensile strength (g)
	4th day	8th day	12th day	16th day	18th day			
Control	18.84±0.54	38.54±0.65	59.24±0.73	76.77±0.55	90.45±0.52	22.83±0.47	40.50±1.70	205.61±6.87
Standard	29.15±0.75	54.22±0.65	78.50±0.57	90.54±0.78	99.03±0.33	18.33±0.49	31.66±2.01	383.05±11.23
Test	26.94±0.36	51.22±0.64	75.86±0.37	88.66±0.51	97.21±0.40	19.66±0.33	34.16±2.03	325.97±13.36

sq.mm=Square millimeter, G=Grams

showed significant increase in the percentage of wound contraction when compared to control group (Figure 2).

The mean time for complete epithelization in test group (19.66±0.33) was statistically decreased ($p<0.01$) compared to control group (22.83±0.47) and statistically increased ($p=0.01$) compared to standard group (18.33±0.49).

The mean scar area was significantly decreased ($p<0.01$) in test group compared to control group; but was increased when compared to the standard group.

Histology of excision wounds in test group showed significantly increased fibrocollagenous tissue deposition anti-inflammatory activity when compared to control group (Figure 3).

In incision wound model, there was a significant increase ($p<0.01$) in the tensile strength of test group compared to the control group and comparable to the standard group.

DISCUSSION

Wound healing is one of most important defense mechanism of the body as proper healing is essential for restoration of disrupted anatomical continuity and disturbed functional state.

The finding of the present study clearly indicates that the test drug (*A. indica*) significantly promoted healing of excision wound as evidenced by enhanced wound closure rate (97.21% on 18th day), decreased time duration (19 days) and mean scar area (34.16 mm^2) of complete epithelization. The study also showed wound healing was promoted by abundant proliferation of connective tissues with angiogenesis.

In incision wound study, the test drug promoted healing of resutured incision wound as evidenced by significant increase ($p<0.01$) in the tensile strength of test group. The increased tensile strength might be due to increased proliferation and transformation of fibroblast cells into myofibroblasts.

The study of Barua et al.[10] states that in the excision wound model, the mean percent of closure of wound increased significantly ($p<0.01$) from "0" day until 21st day which was 95.65% in case of *A. indica* treated group in comparison to the control group (75.15% on 28th day post wounding) and in the incision wound model, significant increase ($p<0.05$) in tensile strength was observed in the methanolic extract of *A. indica* compared to the control group.

The study results of Pandey et al.[11] showed wound contraction rate (83.93±1.38), epithelization period (15.86±0.33), tensile strength and hydroxyproline content were significantly increased for volatile oil of *A. indica* treated group as compared to control group. It also explained that terpenoids, source of *A. indica*, which play an important role in wound healing because the terpenoid strengthen the skin, increase the concentration of antioxidants in wounds,

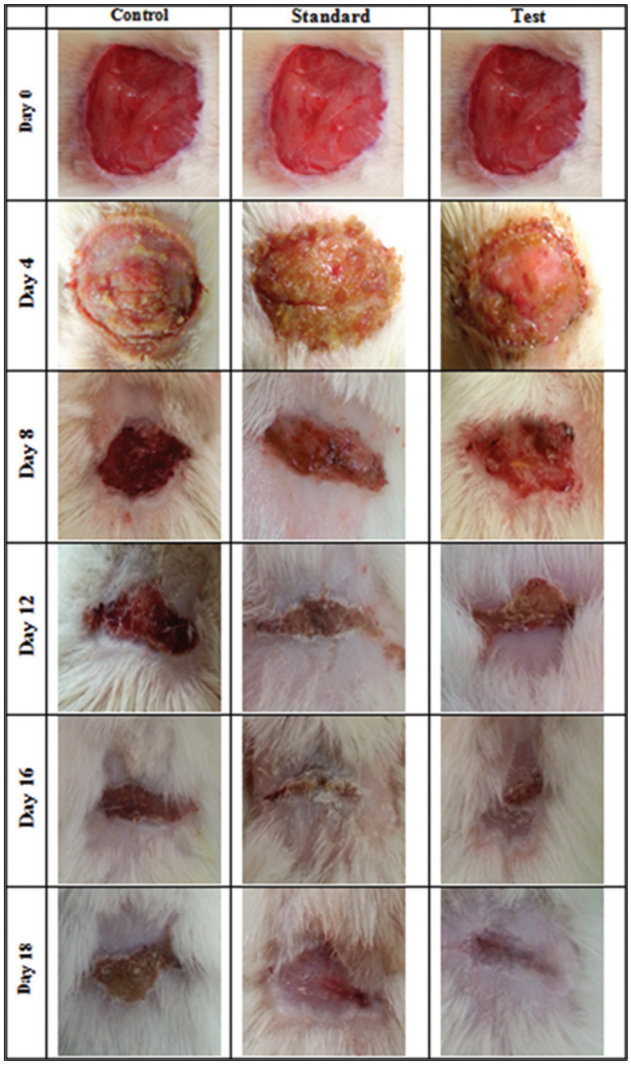

Figure 2: Photograph of Excision wounds showing wound contraction

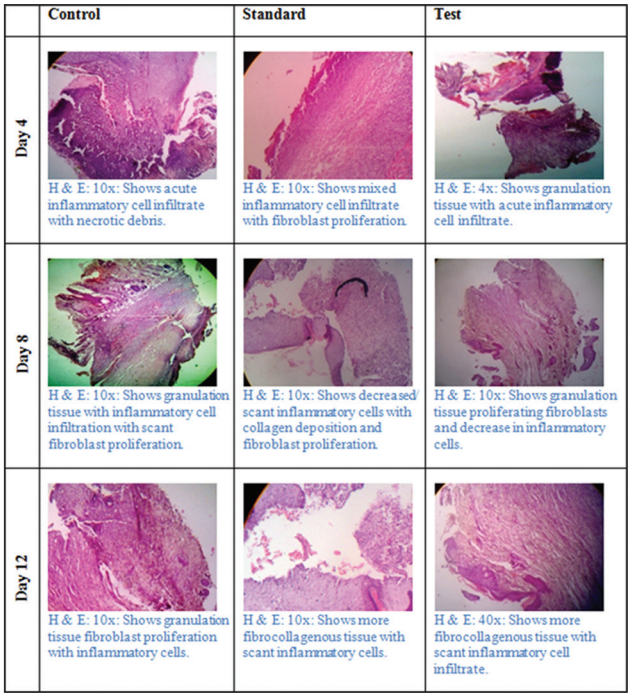

Figure 3: Histology of Excision wounds

and restore inflamed tissues by increasing blood supply.[12] The previous studies showed *A. indica* plant possesses Anti-oxidant activity,[13] antibacterial activity,[14] and Anti-inflammatory activity[15] which may help to promote wound healing.

About 5% ointment of *A. indica* showed significant wound healing activity but when compared to the standard, 1% w/w FSC ointment, it takes more time for complete epithelization. Increased percentage concentration of *A. indica* or combined with other wound healing herbs may decrease the duration of wound healing and will be a better agent than standard as phytochemicals are not only cheap and affordable but are also safe.

The findings of the present study if extrapolated to the clinical situation, it appears that *A. indica* when used in a surgical incision or clean excision wounds could promote healing independent of their antibacterial activity.

CONCLUSION

The use of *A. indica* in Indian traditional systems of medicine for wound healing has been justified by this work. The ethanolic extract of *A. indica* leaves showed highly significant pro-healing effect almost equivalent to standard drug, which may be partly due to the anti-inflammatory activity, proliferation of fibrocollagenous tissue and angiogenesis properties. Hence, it can be used as a wound healing agent if it is confirmed by clinical trials, which would be cost effective. As animal studies cannot be directly compared with effects on humans, there is a need for clinical evaluation in humans to confirm this effect.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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