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PRECLINICAL ASSESMENT OF WOUND HEALING ACTIVITY OF SEED EXTRACT OF AZADIRACHTA INDICA

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INTRODUCTION: Herbal medicines which formed the basis of health care throughout the world since the earliest days of mankind are still widely used and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value is still growing, although this varies broadly between countries¹. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as remedial agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Regulation of development and exportation is therefore essential, together with international cooperation and coordination for their conservation so as to ensure their availability for the future. The United Nations Convention on Biological Diversity states



ABSTRACT: Medicinal plants are important for pharmacological research and drug development. **Aim and Objective:** The aim and objectives of present study was to evaluate the wound healing activity of seed oil of *Azadirachta indica* in Excision and Incision wound models in rats. **Result Discussion:** The study thus demonstrated the wound healing activity of Neem seed oil and found to be effective in the functional recovery of the wound. It promotes wound contraction of excision wound as compared to control group.

that the conservation and sustainable use of biological diversity is of critical importance for meeting the food, health and other needs of the growing world population, for which purpose access to and sharing of both genetic resources and technologies are essential ^{2, 3}.

A wound is a type of injury which happens relatively quickly in which skin is torn, cut, or punctured (an open wound), or where blunt force trauma causes a contusion (a closed wound). In pathology, it specifically refers to a sharp injury which damages the epidermis of the skin.

MATERIAL AND METHOD:

Extraction of Neem Seed Oil: Neem seed were purchased and authenticated by Department of Pharmacognosy and Phytochemistry, CPS Puri. The raw materials were dried by using an oven with temperature of 60°C, ground to powder and then macerated and extracted with the use of water. The Aquous method of Extration was selected as It Is Economical and Environment Friendly.

Animal Selection: Albino rats of 6-8 weeks old and 160-180g body weight were offered by Dadhichi College of Pharmacy, Cuttack. All rats were kept at room temperature and allowed to accommodate in standard conditions at 12 hr light and 12-hr dark cycle in the animal house. Animals were fed with commercial pellet diet and water *ad libitum* freely throughout the study ⁴. The Experimental Procedure was approved by IAEC (Institutional Animal Ethical Committee of DCP, Bearing No 1200/po/Re/S/08/CPCSEA.

Preparation of Ointment (Control): 10 g of blank petroleum jelly B.P (obtained from the Formulation Unit, Department of Pharmaceutics, DCP) was weighed into a beaker and then melted in a in a thermostatic water bath. The required quantities of antibiotics (Gentamycin) were weighed, added to the molten ointment base and then homogenized by trituration and stored in a tight glass bottle.

Experimental Design: Rats were divided into 3 groups each containing 5 animals as follows

TABLE 1: EXPERIMENTAL DESIGN FOR EXCISIONWOUND MODEL

Group	Group Specification	Intervention		
Group I	Simple base ointment	Only with simple		
		base Ointment		
Group II	Standard	Gentamycin (1%)		
Group III	Test	Neem Seed Oil		

Wound Induction: A full thickness of the excision wound of 177 mm2 created along the markings

using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical interventions were carried out under sterile condition. After 24 h of wound creation, the ointments was applied gently to cover the wounded area once daily until complete healing wound area and wound contraction, epithelializtion period ^{5, 6}.

Estimation Parameter:

Measurement of Wound Contraction: The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size.

All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded. Mean of all healed wounds was determined.

Percent wound contraction = Healed area / Total area \times 100

Data of all the parameters were analyzed using the Graph pad 5.0 software. Analysis of Variance (ANOVA); one way ANOVA followed by Dunnets comparison test was performed. The values were expressed as Mean \pm SEM. P value<0.05 was considered as significant ^{7,8}.

Compounds	^{3th} Day	^{6rd} Day	^{9th} Day	12th Day	15th Day	18th Day	^{21th} Day
	% WC	% WC	% WC	% WC	% WC	% WC	% WC
Simple base	147.43±0.2	95.05±0.28	41.86±0.28	28.28±0.13	14.52 ± 0.07	5.31±0.07	3.90±0.28
ointment	(16.71)	(46.30)	(76.35)	(84.02)	(91.79)	(97)	(97.80)
Standard	147.43±0.2	78.55±0.28	28.28±1.33	17.35±1.13	1.33 ± 0.64	0.38±0.19	0.00 ± 0.00
	(16.71)	(55.62)	(84.02)	(9020)	(99.25)	(99.78)	(100)
Neem seed	149. ±0.64	78.57±0.39	41.87 ± 0.04	23.77±1.33	11.35±0.95	4.16±0.21	1.33 ± 0.07
oil	(15.46)	(55.61)	(76.34)	(86.57)	(93.59)	(97.65)	(99.25)

TABLE 2: PERCENTAGE WOUND CONTRACTION IN EXCISION WOUND MODEL



FIG 1: EFFECT OF NEEM SEED OIL ON % WOUND CONTRACTION IN EXCISION WOUND MODEL

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RESULT AND DISCUSSION: More than 99% healing at 21th days were observed in 97% of cases in Group I, 100% in Group II, 99% in Group III, which shows statistical insignificance of healing in Group II & III. Beneficial effects of therapy were obvious from 18th onwards. Among these animals pus and discharge decreased and granulation tissue began to appear by 2nd week.

Antimicrobial effects of Neem oil have been demonstrated against Streptococcus mutans and Streptococcus faecalis. It has shown faster wound closure of punch wounds by re-epithelialization of the epidermis and increased migration of various cells including myofibroblasts, fibroblasts, and macrophages in the wound bed. Multiple areas within the dermis showed extensive neo-vascularization as well.

CONCLUSION: The use of *A. Indica* seed oil in Indian traditional systems of medicine for wound healing has been justified by this work. The neem seed oil showed highly significant pro-healing effect almost equivalent to standard drug, which may be partly due to the anti-inflammatory activity, proliferation of fibro collagenous 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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CONFLICT OF INTEREST: The author Declears There is no Conflict of Interest

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