IN VIVO WOUND HEALING POTENTIAL OF *TERMINALIA CATAPPA* (INDIAN ALMOND) LEAVES EXTRACT ON RATS

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ABSTRACT

Terminalia catappa linn is known to have a barrage of medicinal uses such as anti-inflamatory, hepatoprotective, antioxidant, antiparasitic. The methanolic extract of Terminalia catappa leaves, in the form of an ointment with two different concentrations (10% and 15% w/w ointment of extract in simple ointment base) was evaluated for wound healing potential in an excision wound model in rats. The results were comparable to standard drug Nitrofurazone ointment. It was observed that the wound contracting abilities of 10% and 15% extract ointments were significantly ($P < 0.05$) greater than that of the control. The wound closure time was less and the percentage of wound contraction was much more with the 15%w/w extract ointment treated group. On 14th day 100% contraction was observed which was almost similar to that of the nitrofurazone ointment group. 10%w/w extract ointment group of animals showed significant wound contraction from the 14th day onwards and achieved 100% with the wound closure time of 16th days. Both concentrations of the methanolic extract of Terminalia catappa ointment showed significant responses when compared with the control group. Thus methanolic extract of Terminalia catappa leaves proves to be a potential wound healing agent.

KEYWORDS: - *Wound healing, Terminalia catappa, Nitrofurazone Ointment.*

INTRODUCTION

Terminalia species are native from Africa and are now widely spread out in tropical and sub-tropical regions [1]**.** The leaves bark and fruit of the tree *Terminalia catappa L.* (*Combretaceae*) have been commonly used as a folk medicine for antidiarrhea, antipyretic and haemostatic purposes [2]**.** The leaves of *T. catappa* have been used for the prevention and treatment of hepatitis and liver-related diseases [3]. The leaves of *T. catappa* contain many hydrolysable types of tannin, such as punicalagin, punicalin, terflavins A and B tergallagin, tercatain, chebulagic acid, geraniin, granatin B, and corilagin [4]**.** Punicalin and punicalagin showed inhibited HIV replication in infected H9 lymphocytes with little cytotoxicity and also in purified HIV reverse transcriptase [5].

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system [6].An important aspect of the use of traditional medicinal remedies and plants in the treatment of burns and wounds is the potential to improve healing and the same time to reduce the financial burden [7]. Wound healing occurs in three stages: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction [8]. In angiogenesis, new blood vessels grow from endothelial cells. In fibroplasias and granulation tissue formation, fibroblasts grow and form a new, provisional extracellular matrix by excreting collagen and fibronectin. Collagen, the major component which strengthens and supports extracellular tissue, contains substantial amounts of hydroxyproline, which has been used as a biochemical marker for tissue collagen. In epithelialization, epithelial cells proliferate and spread across the wound surface. Wound contraction occurs as the myofibroblasts contract. Platelets release growth factors and other cytokines [9]. Present study was conducted to evaluate the wound healing effect of *Terminalia catappa* leaves extract in Wistar rats.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Terminalia catappa* were collected in area free of pesticides and other contaminants from the local vendor of district Sagar, Madhya Pradesh and shade dried. The plant was identified and authenticated from Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.). The voucher specimen of the plant was deposited at the department for future reference (Voucher specimen no. **Bot./Her./B/2829**).

Preparation of Extract

The powder leaves (150 g) was extracted by stirring using a magnetic stirrer with 600 ml of methanolic at 30 °C for 4 h. The extract was filtered through Whatmann filter paper no. 41 for removal of peel particles. The residue was re-extracted with 500 mL of MeOH and filtered. The extracts were pooled and concentrated under vacuum at 40°C using a Speedvac system (SC110A, Savant, USA).

Animals

Wistar rats (150-200g) were used in this study. They were given water *ad libitum* and fed with commercial food pellets. The experimental protocol was approved by the Institutional Animal Ethics Committe of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals). Following overnight starving, animals were anesthetized with local anesthesia xylocaine and suitably wounded after shaving the area to be operated to bear excision wound. The wounds were not dressed and no systemic or local antimicrobial agents were used.

Excision Wounds

The surgical materials were sterilized and dorsal fur of the animals was shaved with an electric clipper. The rats were anesthetized with (Xylocaine®) 2% Jelly, Astra Zeneca Pharma India ltd and anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using stencil. A full thickness of the excision wound of circular area of 500mm^2 and 2mm depth was created along the markings using toothed forceps, scalpel and pointed scissors. Wound contraction was monitored by measuring wound area, planimetrically, on alternate days till the wounds were completely healed.

Formulation of ointment (British pharmacopoeia, 1996)

(a) Preparation of 20g simple ointment (B.P.) base. Wool fat (1g), hard paraffin (1g), cetostearyl alcohol (1g) and white soft paraffin (17g) was mixed and heated gently with stirring then cooled.
(b) 2 gm 50% methanolic extract of *Terminalia catappa* was added separately to 20gm of base (10% ointment).
(c) 3 gm 50% methanolic extract of *Terminalia catappa* was added separately to 20gm of base (15% ointment).

Drug administration

24 animals were divided into groups of four and treated as follows:
Group 1: Simple ointment base was applied once daily and served as vehicle control.
Group 2: Standard drug nitrofurazone ointment (0.2% w/w) was applied once daily served as positave control.
Group 3: *Terminalia catappa* 50% methanolic extract ointment (10% w/w) was applied once daily.
Group 4: *Terminalia catappa* 50% methanolic extract ointment (15% w/w) was applied once daily
All the above mentioned treatments were started from the day of wound creation and continued till 20th day of healing. The wound closure rate was assessed by tracing the wounds on day 2nd, 4th, 6th, 8th, 10th, 12th, 14th, 16th, 18th and 20th. Post wounding using transparency paper and a permanent marker. The changes in healing of wound i.e. measurement of wound on graph paper was expressed as unit (mm^2). Wound contraction was expressed as percentage reduction of original wound size.

Statistical analysis

Results obtained from three wound models have been expressed as mean±SEM. The data was evaluated by one way ANOVA followed by Dunnett's t-test, $P < 0.05$ was considered as significant.

RESULTS & DISCUSSION

The least rate of wound healing was seen in control group, which received simple ointment. Treatment with standard group heals the wound in a faster rate than other group, but complete healing was obtained on day 12. The upper layer of wound was surgically removed and subjected to histological studies. Histological examination of the haematoxylin and eosin stained tissue of the rat wounds treated with extract and nitrofurazone ointment have led to reduce scar formation and enhanced fibroblast proliferation, angiogenesis, keratinisation and epithelialisation as compared to vehicletreated group or control group. The measurement of the progress of the wound healing induced by the standard, ointments and control in the excision wound model were shown in **Table 1**. It was observed that the wound contracting abilities of 10% and 15% extract ointments were significantly ($P < 0.05$) greater than that of the control. The earlier wound contraction rate of the methanolic extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue [10].

Table 1:- Evaluation of *Terminalia catappa* leaves extract ointment (10% and 15% w/w) and Nitrofurazone (0.2%w/w) Ointment in wound healing by excision wound method in rats

Post – wounding (days)	Wound area (mm^2) (means ±S.E.) and percentage of wound contraction			
	Simple ointment (control)	**Extract ointment (10%, w/w)**	**Extract ointment (12%, w/w)**	**Standard nitrofurazone ointment (0.2%, w/w)**
0	510.2±3.01	489.2±2.1	499.1±2.8	475.6±1.65
2	402.06±2.7 (21.1%)	399.8±2.5* (18.3%)	375.1±2.4* (24.9%)	389.2±2.6* (18.2%)
4	336.4±2.24 (34.1%)	326.0±2.3** (33.4%)	256.0±2.2* (48.8%)	222.3±2.25** (53.25%)
6	275.2±3.20 (46.1%)	156.1±3.2** (68.1%)	105.0±1.5** (78.9%)	108.9±1.54** (77.2%)
8	242.0±2.99 (52.6%)	112.8±2.75** (78%)	31.8±1.02** (93.7%)	56.1±1.07** (88.3%)
10	201.5±2.51 (60.1%)	94.2±2.3** (80.8%)	8.3±.91** (98.3%)	11.9±0.99** (97.5%)
12	180.4±2.26 (64.7%)	15.0±3.9** (97%)	2.5±0.5** (99.5%)	9.7±0.87** (97.8%)
14	155.3±1.60 (70%)	8.1±2.7** (98.4%)	0±0** (100%)	0±0** (100%)
16	146.4±1.37 (71.3%)	0±0** (100%)	0±0** (100%)	0±0** (100%)

CONCLUSION

Wound healing process consists of different phases such as granulation, collagenization, collagen maturation and scar maturation which are concurrent but independent to each other. Hence in this excision wound models study conclude that methanolic extract ointment at both concentrations (10%,15%) exhibited significant wound healing activity. This was demonstrated by a significant increase in the rate of wound contraction and enhanced epithelization of excision wounds. This may be due to the effect of *Terminalia catappa* leaves extract on increased collagen synthesis.

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