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Research Article

Wound healing activity of methanol extract of leaves of *Machilus macrantha* Nees

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Abstract

The methanol extracts of *Machilus macrantha* Nees. (Family: Lauraceae) was evaluated for their wound healing activity in rats using both excision and incision wound models. The effects of test samples on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation and wound breaking strength. The results showed significant activity of wound healing with methanol extracts of *Machilus macrantha* leaves when compared with standard reference. Nitrofurazone (0.2%w/w) in simple ointment I.P. was used as reference standard. The present work substantiates its validity of the folklore use.

1. INTRODUCTION

Wound healing is the process of repair that follows injury to the skin and other soft tissues. It involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. Each phase of normal wound healing, namely, hemostasis, inflammation, proliferation and remodeling is distinct although the wound healing process is continuous, with each phase overlapping the next. Several medicinal plants like *Curcuma longa, Azadirachta indica, Phyllanthus emblica, Ocimum sanctum, Aloe vera, Terminalia arjuna* etc. have been used since ancient time for treatment of cuts, wounds and burns[1].

Machilus macrantha Nees. (Family: Lauraceae) is an evergreen tree grow up to 30 meter high. It occurs mainly in W. Peninsula, Ceylon and India. In India, it mainly found in Bihar, Karnataka, Maharashtra, Assam etc. up to an altitude of 2100 M. Locally, it is recognized by different names as Gulmavu, Golum, Pisara, Kollarmavu and Uravu[2]. Ethnomedicinally, the barks of plant is used in the treatment of asthma, tuberculosis and rheumatism, while leaves are applied externally on ulcer[3]. The various tribes of Kunbhi using the leaf paste over fresh cuts and wounds and claim for its promising activity. The plant was studied for possible anti-inflammatory[4] and anti-arthritic activities[5]earlier. Wound healing activity of leaves of the plant, *Machilus macrantha,* yet has been not explored. Hence, the present study was undertaken to find out rationale on wound healing activity of the leaves for its traditional claim.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves and barks of the plant, *Machilus macrantha* were collected from the forests of Lonavala of Pune district, Maharashtra, India during the month of November 2008 and identified by the taxonomists of the Botanical Survey of India, Pune, Maharashtra, where a voucher specimen (Voucher specimen No. Santosh-1) has been deposited for further reference. After authentication, plant materials were collected in bulk, washed, shade dried and pulverized in a mechanical grinder to obtain coarse powder. It was stored in a well closed container till usage for the further studies.

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2.2 Preparation of Extract

Dried leaves powder (500 gm) of plant was extracted with methanol using soxhlet apparatus for 48 hours. Extracts were concentrated by rotary vaccum evaporator under reduced pressure. Percentage yield of extract was calculated on air dried basis[6]. The dried extract was evaluated for the presence of various phytoconstituents using standard procedures.

2.3 Preparation of Test Extracts Ointment

10%w/w test extract ointment (TEO) was prepared by incorporating methanol extract of leaves with simple ointment I.P[7].

2.4 Standard Reference

Nitrofurazone (0.2%w/w) in simple ointment I. P. was used as reference standard.

2.5 Animals

Healthy Wistar albino rats (150–250 g) of either sex and of approximately the same age were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pellet diet (M/s Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The experimental protocols were subjected to scrutiny of Institutional Animal Ethics Committee for experimental clearance (no. 1025/C/07/CPCSEA).

2.6 Acute Dermal Toxicity Study

The study was carried out to determine the therapeutic dose of methanol extract of leaves of *Machilus macrantha* as per OECD guidelines[8]. The animals were divided in to 3 groups (Each group with 6 animals) viz. control, standard and one group for testing methanol extract. The control group was treated with simple ointment I.P. The standard group was treated with Nitrofurazone ointment (0.2%w/w) while test group was treated with Test Extract Ointment (TEO, 10 % w/w).

2.7.1 Excision Model

The rats were inflicted with excision wounds as described by Morton and Malone[9] and suggested by Kamath *et al*[10]. The rats were anaesthetized with ketamine hydrochloride (0.5 ml/kg b.w., i.p.). Using a round seal, a full thickness skin was excised to get a

wound size of 500 mm^2 and 2 mm depth on the dorsal thoracic central region, 5 cm away from ears of anaesthetized rats. After haemostasis, animals were placed in the cages and treated topically with TEO (10%w/w) and standard Nitrofurazone ointment (0.2%w/w). The wound closure rate was assessed by tracing the wound on days 4, 8, 12 and 16 after wounding days using transparent paper and a permanent marker. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound[11]. Wound healing rate was measured using following formula,

% of wound closure= Wound area on day '0'-Wound area on day 'n' x 100

Where, the wounding day was considered as '0'

2.7.2 Incision Model

The rats were anaesthetized prior to and during creation of the wounds, with ketamine hydrochloride (0.5 ml/kg b.w., i.p.). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long and 2 mm depth were made through the skin and cutaneous tissue on the back[12]. After the incision, the parted skin was sutured 0.5 cm apart using a surgical thread and curved needle. The wounds of animals in the different groups were treated with topical application of ointments as described above for the period of 10 days. The wounding day was considered as 0. The wounds were left undressed. Ointments were topically applied to the wound once a day. The sutures were removed on 8th post wound day and continued the application of the ointments. The wound breaking strength was measured on the 10th day evening after the last application[13]. Tensile strength was calculated using the following formula

Tensile strength = Breaking strength (g)/Cross sectional area of skin (mm^2)

2.8 Statistical Analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's *t*-test. A *P* value < .05 was considered to be significant. All the values were expressed as Mean ± SEM.

3. RESULTS AND ANALYSIS

The preliminary phytochemical screening of methanol extract of leaves of *Machilus macrantha* showed the presence of steroids, triterpenoids, alkaloid and tannins while carbohydrates, flavonoids and amino acids were absent (Table 1).

The results of wound healing effects of *M. macrantha* showed significant wound healing activity in both excision and incision wound models. In excision wound model, the mean percentage closure of wound area was calculated on the 4, 6, 8, 11, 14 and 16th post-wounding days. as shown in Table 2.

TEO (10%w/w) has shown significant wound healing activity, which was comparable to that of standard reference, Nitrofurazone (0.2%w/w). However, the rate of contraction is less when compared to standard. On 14th day, complete healing of wound (100%) was observed with standard reference and 93.48% healing of wound with TEO (10%w/w) as compared to control. The control (ointment base) has shown 74.24% healing. The period of epithelialization was 16.23 ± 0.98 days for the 10% TEO treated group of animals as against 13.5 ± 1.54 for the standards drug-treated group. The methanol extract-treated animals showed faster epithelialisation of wound as compared to control group. The percentage of wound closure was 100 ± 00 in the case of standard reference Nitrofurazone on 14th day of treatment, whereas the methanol extract showed similar effects on 16th day.

In incision wound model, TEO (10%w/w) treated animals showed increase in breaking strength (478.55 ± 12.63∗∗), when compared to the control (327.5 ± 16.58). The mean breaking strength was also significant in animals treated with standard drug (491.21 ± 16.26∗∗) Results are tabulated in Table 3.

Table 1: Preliminary phytochemical screening of different extracts of *M. macrantha* leaves

Test	Result
Carbohydrate	--
Steriods	+
Triterpenoids	+
Flavonoids	--
Saponins	--
Tannins	+
Alkaloids	+
Glycosides	--
Amino acids	--

(+): Present; (−): Absent

Table 2: Effect of Methanol extracts of *M. macrantha* leaves on percentage (%) wound closure (excision wound model)

Group	Treatment	Percentage (%) wound closure						Period of epithelialization (No. of days)
		4th days	6th days	8th days	11th days	14th days	16th days	
I	Control	23.52 ± 1.21	37.72 ± 1.58	51.92 ± 1.71	71.28 ± 2.23	74.24 ± 1.18	83.56 ± 1.03	23.16 ± 0.71
II	Nitrofurazone Ointment (0.2% w/w)	48.53 ± 2.87*	74.23 ± 3.32**	84.8 ± 1.26**	96.54 ± 1.29**	100 ± 00**	-	13.5 ± 1.54**
III	TEO (10%w/w)	28.88 ± 1.95	48.34 ± 1.86*	79.78 ± 1.91*	86.37 ± 1.01**	93.48 ± 2.22**	100 ± 00**	16.23 ± 0.98**

Values are expressed as mean ± S.E. (N = 6). All columns are significant using ANOVA
*P <0 .05, **P <0 .01 when compared to control; Dunnet's t-test.

Table 3: Effect of methanol extract of *M. macrantha* leaves on wound breaking strength (incision wound model)

Group	Treatment	Breaking strength (g)
I	Control	327.5 ± 16.58
II	Nitrofurazone Ointment (0.2% w/w)	491.21 ± 16.26**
III	TEO (10%w/w)	478.55 ± 12.63**

Values are expressed as mean ± S.E. (N = 6). All columns are significant using ANOVA.
*P <0 .05, **P <0 .01 when compared to control; Dunnet's t-test.

5. DISCUSSION

Wound healing, a complex sequence of events, is initiated by the stimulus of injury to the tissues. A positive stimulus may result from the release of some factors by wounding of tissues. Cutaneous wound repair is accompanied by an ordered and definable sequence of biological events starting with wound closure and progressing to the repair and remodeling of damaged tissue. The wound healing effects of methanol extracts may be attributed to the presence of phytoconstituents like alkaloids, triterpenoids, tannins and flavonoids in the extracts which are known to promote the wound healing process mainly due to their anti-microbial property. Tannins and triterpenoids are also known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization[14-16]. In the present laboratory all the surgical interventions were carried out under sterile conditions and animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. This is very important and researchers proved that the control microbial infection is necessary for better wound healing and its management[17,18]. Increase in the wound closing rate in excision wound model is due to enhances and fasten the rate of proliferation of epithelial cells. While increase in skin breaking strength in incision wound model is due to increases the collagen maturation. It occurs due to increase in collagen turnover by anti-lipid peroxidation activity of some phytoconstituents like tannins, flavonoids etc. It helps in slowing or preventing the onset of cell necrosis and improving vascularity. It is believed that it increases collagen viability and DNA synthesis[19]. Hence wound healing activity of methanol extract of *M. machilus* leaves may be observed due to anti-oxidant and antibacterial effect of their phytoconstituents.

6. CONCLUSION

The crude methanol extract of *Machilus macrantha* leaves showed significant wound healing activity, however, to make known due to which fraction of the extract, it is so fractionation studies and their wound healing screening studies are needed.

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