

Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats

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Aqueous extract of leaves of *M. oleifera* was investigated and rationalised for its wound healing activity. The aqueous extract was studied at dose level of 300 mg/kg body weight using resutured incision; excision and dead space wound models in rats. Significant increase in wound closure rate, skin-breaking strength, granuloma breaking strength, hydroxyproline content, granuloma dry weight and decrease in scar area was observed. The prohealing actions seem to be due to increased collagen deposition as well as better alignment and maturation. From the results obtained, it may be concluded that the aqueous extract of *M. oleifera* has significant wound healing property.

Keywords: Aqueous extract, *Moringa oleifera*, Wound healing

Moringa oleifera (Family: Moringaceae, English: drumstick tree, Sanskrit: shrigru) has been an ingredient of Indian diet since centuries. It is cultivated almost all over the country and its leaves and fruits are used as vegetables. Almost all parts of the plant have been utilized in traditional medicine practices. The leaves and young buds of the plant are used as vegetable and can be rubbed on the temples for relieving headache while the root and root bark are regarded as anti scorbutic and can be used externally as counter irritants¹. The juice of leaves mixed with honey is used for the treatment of eye diseases². The leaves of the plant have also been reported for its antitumor³, hypotensive⁴, antioxidant, radio-protective⁵, anti-inflammatory and diuretic properties⁶. Traditional practitioners of Ayurveda advocate the use of leaves by rubbing on a flat stone with gradual addition of water. The paste thus prepared is used for external application in tissue injury. Keeping this in view, wound healing potential of aqueous extract of leaves of *Moringa oleifera* has been investigated on different parameters of wound healing in albino rats.

Materials and Methods

Plant material — The fresh leaves of *M. oleifera* Linn were collected in the month of July 2002 and

authenticated by Dr. A.M. Mujumdar, Head, Botany Department, at Agharkar Research Institute, Pune.

Preparation of extract — In the present study percolation method was used for extraction⁷. The leaves were separated and dried under shade for about a week. Air-dried, powdered leaves (1 kg) were added with 3000 ml boiling water. After mixing thoroughly it was macerated in a suitable percolator for 2 hr. The percolation process was continued at moderate rate by gradually adding boiling water until the extraction process was completed (indicated by fade coloured men strum). The percolate was evaporated on a hot water bath, not more than 800 ml; cooled and alcohol was added as a preservative to the concentrated percolate. The extract obtained was solid greasy residue (yield 32.4 %). Phytochemical screening of aqueous extract gave positive test for proteins, amino acids and glycoside. After qualitative chemical investigation the aqueous extract was taken for pharmacological studies.

Animals — The animals were divided into groups of six animals in each group and kept in plastic cages at 23 ± 1 °C in 12:12 hr dark:light cycle, with free access to standard pellet feed (Chakan Oil Mill, India) and water. All experiments were carried out between 0800 and 1700 hrs in a quiet laboratory at an ambient temperature. The animal experiments were conducted as per protocol approved by Institutional animal ethics committee (IAEC) and as per Indian norms laid down

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by the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi.

Wound healing studies

Animals were divided into two groups (control and test) of 6 animals each. They were starved for 12 hr prior to wounding. The first group served as control and given the vehicle (2 % gum acacia) orally. The second group received the aqueous extract by oral dose of 300 mg/kg daily for 10 consecutive days in the resutured incision and dead space wound model and for 20 days in the excision wound model. They were housed individually in the animal cages after wounding.

Acute oral toxicity study

Male Swiss albino mice weighing 18-22 g were dosed with extract and were observed for any symptoms of toxicity for 48 hrs as per OECD guidelines 425 and LD₅₀ was estimated using AOT 425 software (Westat, EPA, USA). Based on the results obtained from this study, the dose for further pharmacological studies was fixed to be 300 mg/kg, po.

Wound models

Resutured incision — The method of Ehrlich and Hunt was adopted⁸. Under light ether anesthesia two para vertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk threads with help of straight round-bodied needle. On 8th post wounding day, sutures were removed and the breaking strength was determined on 10th post wounding day by continuous constant water flow technique of Lee⁹.

Dead space wounds — Physical changes in the granuloma tissue were studied in this model. Under light ether anaesthesia, subcutaneous dead space wounds were inflicted in the region of the axilla and groin, by making a pouch through a small nick in the skin.

Cylindrical grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced into pouch to harvest the granulation tissue. Each animal received 2 grass piths and cotton pellets in different locations. The wounds were sutured and mopped with an alcoholic swab. Animals were placed into their individual cages after recovery from anesthesia. Excision of the granulomas from the surrounding

tissue was performed on the 10th post-wounding day under light ether anesthesia.

Granulation tissue surrounding the grass piths were excised and slit open. The breaking strength of piece measuring about 15mm in length and 8mm in width (obtained by trimming the rectangular strip of granuloma tissue) was determined on 10th post wounding day by a continuous constant water flow technique of Lee⁹. The pieces obtained at the end of these measurements were then preserved in 10% formalin solution for histopathological studies to evaluate the effect of the extract on collagen formation. Hydroxyproline estimation was carried out in 10-day-old granulation tissue in control as well as test group by the method of Woessner¹⁰.

Excision wound — A circular wound of about 2.5 cm diameter was made on depilated ethanol sterilised dorsal thoracic region of rats under light ether anaesthesia and observed throughout the study. Animals were housed individually; the oral dose was given once a day. The observations of percentage wound closure were made on 4th, 8th, 12th days and subsequently on every alternate day till complete wound closure occurred and scar size was noted.

Statistical analysis — Data obtained for each set of wound healing models were expressed as means $\pm$ SE and analysed by unpaired Student's *t* test. Level of significance was set at $P < 0.05$.

Results

The LD₅₀ was found to be more than 5000 mg/kg, po in acute oral toxicity testing. Effects of the aqueous extract of *M. oleifera* on various wound-healing parameters using different wound models have been shown in Tables 1 and 2. Pharmacological studies indicated a significant increase in the tensile strength of drug treated group in incision wound model when observed on 10th post wounding day (Table 1). Tensile strength of the granuloma tissue, weight of this tissue and hydroxyproline content also were significantly increased in drug treated vs. control group in dead space wound model.

Studies using excision wound model showed significant decrease in the epithelization period. Epithelization was found to be enhanced significantly by the aqueous extract as evidenced by the shorter period required for Escher dropping as compared to the control. The extract also facilitated the contraction (Table 2).

Histopathological studies revealed increased granulation tissue along with well-formed

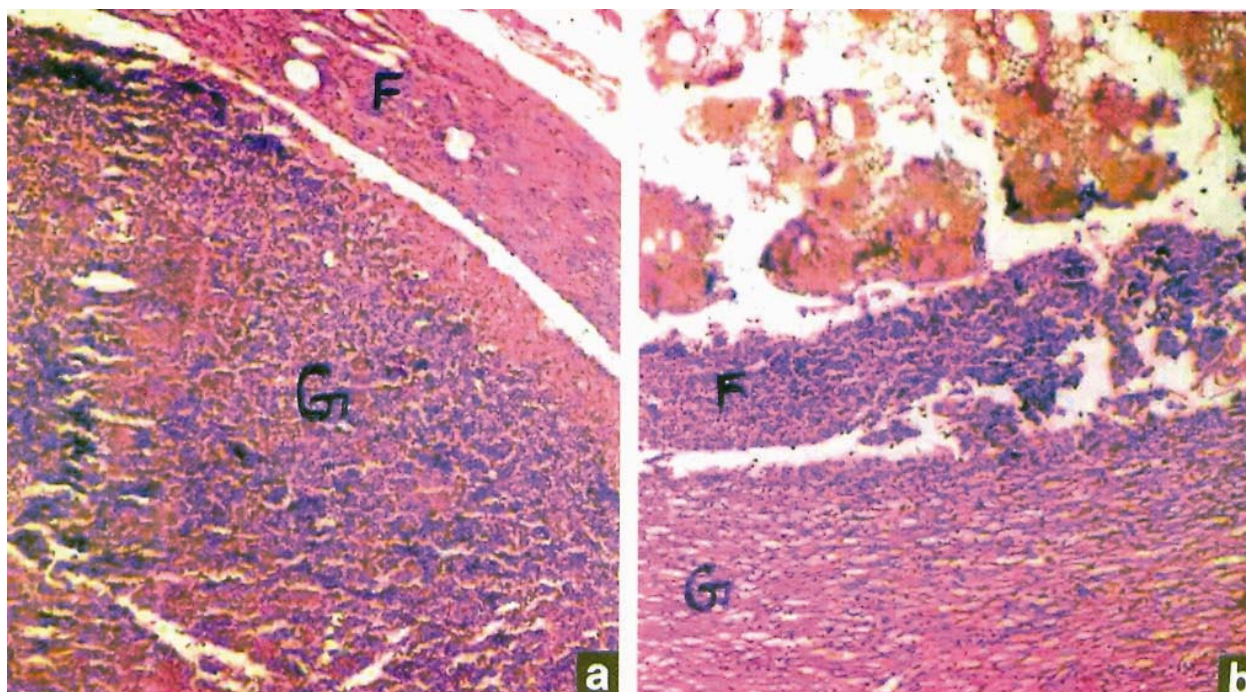


Fig.1 – Microphotograph of granuloa tissue [note abundant granulation (G) along with well formed fibrocollagenised tissue (F) in treated group (a) as compared to control (b). H&E, 100×

Table 1—Effect of aqueous extract of *M. oleifera* on wound healing in incision and dead space wound models
[Values are mean $\pm$ SE from 6 animals in each group]

	Re-sutured incision		Dead space	
	Breaking strength (g)	Breaking strength (g)	Hydroxyproline (μ g/300mg wet weight)	Granuloma weight (mg %)
Control	282.66 $\pm$ 0.24	219.0 $\pm$ 5.70	5.23 $\pm$ 0.20	36.72 $\pm$ 1.90
Aqueous extract	358.50 $\pm$ 8.03	252.0 $\pm$ 6.54	6.83 $\pm$ 0.13	45.61 $\pm$ 1.85

Data analysed by unpaired Student's *t*-test

P values: * < 0.05, ** < 0.01

fibrocollagenised tissue in aqueous extract treated group as compared to control group (Fig.1).

Discussion

The complex process of healing involves various phenomena like wound contraction, granuloma formation etc. The contribution for healing by these events depends upon the type of the wound. Wound contraction plays a significant role in healing of excision wound, while granuloma formation contributes in healing of dead space and resutured incision wounds. Resutured incision model is more

Table 2 —Effects of aqueous extract of *M. oleifera* on excision wound model

[Values are mean $\pm$ SE from 6 animals in each group]

Wound model	Control	Aqueous extract
Wound closure (days)	19.5 $\pm$ 0.22	16.0 $\pm$ 0.56*
Mean scar area (mm ²)	44.33 $\pm$ 2.40	30.66 $\pm$ 1.87*
Wound contraction % by day		
4	11.91 $\pm$ 1.75	26.54 $\pm$ 1.33*
8	62.26 $\pm$ 1.67	74.55 $\pm$ 0.28*
12	71.06 $\pm$ 1.09	86.27 $\pm$ 1.12*
14	78.25 $\pm$ 0.75	91.28 $\pm$ 1.27*
16	83.52 $\pm$ 1.78	99.92 $\pm$ 0.70*
18	91.49 $\pm$ 0.42	—
20	99.79 $\pm$ 1.10	—

Data analysed by unpaired Student's *t* test

P values: * < 0.05, ** < 0.01

relevant clinically since most of the surgical wounds resemble it. The leaves of the plant are said to contain fatty acid, vitamin E, carotenoid, selected mineral elements like Fe, Cu, Zn, Co and amino acid, along with two nitrile glycosides, Niazirin I and Niazirin II and three mustard oil glycosides¹¹. In addition, Niazinin, Niazimicin and Niaziminin were also isolated from the leaves⁴. Based on the present findings the prohealing mechanism of *M. oleifera* aqueous leaves extract cannot be proposed. However,

prohealing activity could be attributed to its antimicrobial activity, which has been reported earlier. In the present study the wounds were not infected macroscopically. It is difficult to comment on such an activity contributing for healing.

Since systemic administration of zinc sulphate has been reported to promote healing, the prohealing effect of *M. oleifera* as observed in the present study could be due to its high content of zinc as reported earlier. In conclusion, extract showed significant wound healing activity against excision, resutured incision and dead space wounds.

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