Vol 4/2 (2004) 145 - 149

JOURNAL OF NATURAL REMEDIES

Evaluation of aqueous extract of pulp and seeds of *Moringa oleifera* for wound healing in albino rats

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Abstract

Objective: To study the effect of aqueous extract of the dried pulp and seeds of *Moringa oleifera* on wound healing in albino rats. Materials and methods: 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. Results: 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. Conclusion: From the results obtained, it may be concluded that the aqueous extract of *Moringa oleifera* has significant wound healing property. Also it can be concluded that these prohealing effects may be due to their high content of crude proteins, zinc and some anti-microbial component.

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

1. Introduction

Moringa oleifera (Family: Moringaceae, English:drumstick tree, Sanskrit: shrigru) has been an ingredient of Indian diet since several centuries. *Moringa oleifera* 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 this plant are used as vegetable and can be rubbed on the temples for headache while the root and root bark are regarded as anti-scorbutic and can be used externally as counter irritants [1]. The juice of leaves mixed with honey is used for the treatment of eye disease [2]. This plant has also been reported for its anti-tumor [3], hypotensive [4], antioxidant, radio protective [5], anti-inflammatory and diuretic property [6]. In view of these the present study has been under taken to evaluate in detail the wound healing effect of the aqueous extract of seeds and pulp of *Moringa oleifera* in albino rats.

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2. Materials and methods

2.1 Plant material

The fresh fruits of *Moringa oleifera,* commonly known as drumstick were collected from the local market in Feb. 2003 and authenticated from the Dept. of Botany, R. L. Institute of Science, Belgaum.

2.2 Preparation of extract

In present study percolation method was used for extraction [7]. The pulp and seeds were separated and dried under shade for about a week. Air-dried, powdered seeds and pulp (1 kg) was added with 3000 ml of boiling water. After mixing thoroughly it was macerated in a suitable percolator for 2 h. The percolation process was continued at moderate rate by gradually adding boiling water until the extraction process was completed (indicated by fade coloured menstrum).

The percolate was evaporated on a hot water bath, not more than 800 ml; cooled and alcohol were added as a preservative to the concentrated percolate. The extract obtained was solid greasy residue (Yield 38.9%). 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.

2.3 Test compounds and reagents

Hydroxyproline (Loba chemicals, Bombay), Methyl red, HCL, Sodium Hydroxide pellets (L. R. Pune, Chemicals), Chloramine-T (LR), P-dimethyal amino benzaaldehyde (Loba chemicals, Bombay), Citric acid monohydrate (LR), Glacial acetic acid, Sodium acetate trihydrate (LR), Toluene (LR), Methyl cellulose (Thomas Baker Chemicals Co. Bombay), Perchloric acid (Thomas Baker Chemicals Co. Bombay).

2.4 Animals

Healthy male albino rats, weighing between 150-250 gm were used. They were purchased from Shri Venkatswara Enterprises, Banglore and group housed (Four per cage), in a temperature regulated environment with lights on between 7:00 AM and 8:00 PM, fed with Pellet rodent diet and water were available ad libitum unless otherwise stated.

The Institutional Animal etics committee approved all animal handeling and experimental protocols.

2.5 Wound healing studies

Animals were divided into two groups (control and test) of six animals each. They were starved for 12 h prior to wounding with access to water. The first group served as control and given the vehicle (2 % gum acacia) orally and 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.

2.6 Wound models

2.6.1 Resutured incision

The method of Ehrlich and Hunt was adopted [8]. 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 8^{th} post wounding day, sutures were removed and the breaking strength was determined on 10^{th} post wounding day by continuous constant water flow technique of Lee [9].

2.6.2 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. Granuloma formation was induced by implanting sterile cotton pellets and grass piths.

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 sterile 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. Exicision 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 15 mm in length and 8 mm 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 Lee [9]. The piece 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 Woessner [10].

2.6.3 Excision wound

A circular wound of about 2.5 cm diameter on depilated ethanol sterilised dorsal thoracic region of rats under light ether anaesthesia and observed through out 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.

2.7 Statistical analysis

Results expressed as Mean ± S.E. were evaluated for statistical significance by unpaired Student's *t* test. Values of $p<0.05$ were considered statistically significant.

3. Results

Table 1a and 1b depict the effect of the aqueous extract of *Moringa oleifera* on various wound-healing parameters using different wound models. Pharmacological studies indicated a significant increase in the tensile strength of drug treated group in incision wound model

Table 1a
Effect of aqueous extract of *Moringa oleifera* on wound healing in incision and dead space wound models

Wound model	**Re-sutured incision**	**Dead Space**		
Parameters Studied	**Breaking strength (g)**	**Breaking strength (g)**	**Hydroxyproline (µg/300mg w. w.)**	**Granuloma weight (g / 100 b.w.)**
Control	279.66±0.24	209.0±5.70	5.23±0.20	0.017± 0.0035
Aqueous extract	360.50±8.03*	263.0±6.54*	7.63±0.13*	0.140± 0.0214*

Data (Mean±S.E.) N=6, *p<0.001

when observed on 10th post wounding day (Table 1a). 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. From the histopathological study it was observed that collagen formation was more in drug treated group as compared to control.

Studies using excision wound model showed significant decrease in the epithelialization period. Epithelialization 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 1b)

4. 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 relevant clinically since most of the surgical wounds resemble it. Therefore, in the present study three wound models were selected to investigate the effect of *Moringa oleifera* aqueous seed and pulp extract on various events of healing.

Excision wound

As these wound mainly heal by contraction and epithelialization the monitored parameters in this model include - a) wound closure at different time intervals b) Time required for complete epithelialization c) Scar area. In the present study water extract has significantly promoted the healing, as indicated by decreased time duration for complete wound closure. It is cleared from the results obtained that the water extract has increased the wound contractions indicated by significantly decreased scar area on complete wound closure as compared to the control.

Resutured incision and dead space wounds:

The increased breaking strength in granulation tissue, both resutured incision wound and granulation tissue is obviously due to increased collagen content. Accordingly hydroxyproline (marker of collagen) was significantly increased in the treated group as compared to control. This was further corroborated by histopathological studies of granulation tissue, which revealed marked increase in collagen content in the treated group.

Based on the present study the prohealing mechanism of *Moringa oleifera* aqueous extract cannot be proposed. However, prohealing activity could be attributed to its antimicrobial activity, which has been reported earlier [11,12]. In the present study the wounds were not infected macro-scopically. It is difficult to comment on such an activity contributing for healing.

Table 1b
Effects of aqueous extract of *Moringa oleifera* on excision wound model.

Wound Model		Control	Aqueous Extract
Wound closure (Days)		18.5±0.22	15.0±0.56*
Mean Scar Area (mm^2)		42.33±2.40	27.66±1.87*
% of Wound	4	11.91±1.75	26.54±1.33*
Contraction by Day	8	62.26±1.67	74.55±0.28*
	12	71.06±1.09	86.27±1.12*
	14	78.25±0.75	91.28±1.27*
	16	83.52±1.78	99.75±0.70*
	18	91.49±0.42	
	20	99.79±1.10	

Data (Mean±S.E.) N=6, *p<0.001 *vs.* Control

Since systemic administration of zinc sulphate has been reported to promote healing the prohealing effect of *Moringa oleifera* as observed in the present study could be due to its high content of zinc as reported earlier [13,14]. It is well known that protein is essential for healing process and hypoprotenimia has been reported to retard the healing process [15]. One kg of mature seeds of *Moringa Oleifera* has been reported to contain crude protein- 332.5g, crude fat- 412.0g, and carbohydrate- 211.2g. Though, Lysine, threonine and Valine were deficient, the content of Methionine + Cysteine (43.6 g / Kg) was exceptionally higher [16]. The prohealing effect of *Moringa oleifera* as observed in the present study could be attributed partly to its high content of protein. An attempt has been made in the present study to investigate the possible influence of systemically administered *Moringa oleifera* aqueous pulp and seed extract on healing of excision, resutured incision and dead space wounds. However, further studies are needed to establish the clinical utility of the aqueous extract of the plant.

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