Antipyretic and Wound Healing Activities of *Moringa oleifera* Lam. in Rats

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Qualitative chemical tests confirmed the presence of phytosterols, glycosides, tannins, and amino acids in the various leaf extracts of *Moringa oleifera* Lam., whereas its seed extracts showed the presence of phytosterols, glycosides, phenolic compounds, carbohydrates and amino acids. The ethanolic and ethyl acetate extracts of seeds showed significant antipyretic activity in rats, whereas ethyl acetate extract of dried leaves showed significant wound healing activity (10% extracts in the form of ointment) on excision, incision and dead space (granuloma) wound models in rats.

Moringa oleifera lam. (Family: Moringaceae) is commonly known as drumstick. It is found widely in the sub-Himalayan range and commonly cultivated in all places of India. It is a short, slender, deciduous perennial tree; widely distributed in India, Arabia and cultivated in tropical Africa, tropical America, Sri Lanka, India, Mexico and Malaysia[1]. The whole plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac[2,3]. A thorough literature survey reveals that the aqueous extracts of roots and barks were found to be effective in preventing implantation[4], aqueous extracts of fruits have shown significant anti-inflammatory and hepatoprotective activity[5], methanolic extracts of leaves have shown anti-ulcer activity[6], and ethanolic extracts of seeds exhibited anti-tumour activity[7]. However, no systematic study on antipyretic and wound healing activities has been reported in the literature. Therefore in the present investigation, we screened the leaf extracts of *Moringa oleifera* for wound healing activity, and seed extracts for antipyretic activity in rats, since its leaves are applied externally on wounds and seeds are used as antipyretic in traditional medicine[8].

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The leaves and seeds of *Moringa oleifera* were collected from local areas in and around Hubli city and were authenticated in the Department of Botany, Karnataka University, Dharwad. Leaves and seeds were shade-dried and pulverized. Wistar male rats (180-200 g) were selected and individually housed in polypropylene cages and well-ventilated rooms in a registered animal house throughout the study. Animals were allowed access to standard laboratory feed and water *ad libitum*. Paracetamol IP and Vicco turmeric creams were used as standards for antipyretic and wound healing activity respectively.

The collected leaves and seeds of *Moringa oleifera* were shade-dried, pulverized and subjected to extraction. The powdered material (1.5 kg) was exhaustively extracted (3 cycles/h) with 95% ethanol in soxhlet apparatus by continuous hot extraction. After each extraction, the solvent was recovered using flash evaporator, and the extract was concentrated under reduced pressure.

Further, the total ethanolic extract was fractionated using petroleum ether (60-80°), solvent ether and ethyl acetate. Then the preliminary phytochemical investigation and screening of antipyretic and wound healing activities was carried out.

The antipyretic activity of ethanolic, petroleum ether (60-80°), solvent ether and ethyl acetate extracts of seeds was screened by using yeast-induced hyperpyrexia method[9]. Male Wistar rats (180-200 g) were selected and divided into six groups, each having six animals. They were maintained at constant temperature of 24-25° for 24 h before pyrexia was induced by subcutaneous injection of 1 ml of 15% brewer's yeast suspension in saline solution[10]. After 18 h of yeast injection, the extracts at a dose of 300 mg/kg were administered orally to each group as a suspension in propylene glycol. Paracetamol IP (200 mg/kg) was used as standard for comparison of antipyretic activity, and all control animals received propylene glycol. Rectal temperatures were noted at 60 min intervals.

Three wound models, viz., excision wound, incision wound and dead space wound (granuloma studies) were selected for assessing wound healing activity of ethanol and ethyl acetate extracts, as these extracts showed the presence of phytosterols and phenolic compounds, which promotes wound healing activity.

For excision wound study, male Wistar rats (180-200 g) were selected and divided into four groups, each comprising six animals. They were depilated at the desired site before wounding. They were housed individually with free access to standard food and water. The animals were starved for 12 h prior to wounding. Under light ether anaesthesia, wounding was performed aseptically. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats and observed throughout the study. The ethanolic and ethyl acetate extracts (10% w/w) were formulated as an ointment prepared by I.P. method. Then, 500 mg of the prepared ointment was applied on the wound, once daily, for 14 d starting from the day of wounding; simultaneously extracts were orally administered (300 mg/kg) in double-distilled water for 14 d. The observation of percentage of wound closure was made on 4th, 8th, 12th and 14th post-wounding days[11].

The method of Ehrlich and Hunt was adopted for incision wound study[12]. Male Wistar rats (180-200 g) were selected and divided into three groups, each comprising six animals. They were depilated at the desired site before wounding. They were housed individually with free access to standard food and water. The animals were starved for 12 h prior to wounding. Under light ether anaesthesia, wounding was performed aseptically. Two paravertebral incisions of 6 cm were made through the entire thickness of the skin on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk thread. The 300 mg/kg of extracts were orally administered in double-distilled water for 9 d starting from the day of wounding. Sutures were removed on the 8th post-wounding day and the tensile strength was determined on the 10th post-wounding day by adopting continuous constant water flow technique.

For the dead space wound model (granuloma study), male Wistar rats (180-200 g) were selected and divided into three groups, each comprising six animals. They were depilated at the desired site before wounding. They were housed individually with free access to standard food and water. The animals were starved for 12 h prior to wounding. Under light ether anaesthesia, wounding was performed aseptically. The 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, implanting sterilized grass piths so as to induce granuloma formation. Cylindrical sterilized grass piths measuring 2.5 cm in length and 0.3 cm in diameter were introduced into the pouch. Each animal received two grass piths in different locations. The wounds were sutured and mopped with an alcoholic swab. Then, 300 mg/kg of extracts were orally administered in double-distilled water for 9 d starting from the day of wounding. Excision of granuloma from the surrounding tissue was performed on the 10th post-wounding day. Granuloma surrounding the grass piths was excised and slit open. The tensile strength of piece was determined on the 10th post-wounding day by adopting continuous water flow technique[13]. All the values were expressed as mean±S.E. Statistical significance was determined using Student's *t* test[14] at a probability level of P£0.001.

The preliminary phytochemical investigation of leaf extracts showed the presence of phytosterols, glycosides, tannins, and amino acids, whereas seed extracts showed the presence of phytosterols, glycosides, phenolic compounds, carbohydrates and amino acids.

The dose of extracts was determined by up and down staircase method[15], which was found to be 300 mg/kg. The ethanolic and ethyl acetate seed extracts of *Moringa*

TABLE 1: EFFECT OF SEED EXTRACTS OF *MORINGA OLEIFERA* ON YEAST INDUCED PYREXIA IN RATS.

Time (h)	Control	Standard	EE	PE	SE	EAE
0	39.5 ± 0.17	39.3 ± 0.20	39.1 ± 0.11	39.3 ± 0.13	39.1 ± 0.13	39.2 ± 0.21
1	38.8 ± 0.12	38.6 ± 0.16	38.5 ± 0.22	38.7 ± 0.15	38.4 ± 0.16	38.2 ± 0.22
2	38.4 ± 0.13	37.8 ± 0.15	37.8 ± 0.23	37.9 ± 0.14	38.2 ± 0.15	37.9 ± 0.19
3	38.1 ± 0.21	37.1 ± 0.14	37.2 ± 0.20*	37.9 ± 0.21	37.9 ± 0.14	37.1± 0.17*

Table 1 shows the data obtained from the antipyretic activity of *Moringa oleifera*. EE is ethanolic extract, PE is petroleum ether extract, SE is solvent ether extract and EAE is ethyl acetate extract. Paracetamol IP is used as standard, each value is a mean ± standard error for group of six animals (n=6). *indicates value significantly different compared to control ($P < 0.001$).

TABLE 2: MEAN PERCENTAGE CLOSURE OF EXCISION WOUND AREA ON THE FOLLOWING POST WOUNDING DAYS

Day	Control	Vicco turmeric (Standard)	Ointment containing 10 % leaf extract of	
			EE	EAE
4th	23.98 ± 0.86	52.33 ± 0.16	44.78 ± 0.35	47.07 ± 0.24
8th	53.48 ± 0.96	87.33 ± 0.25	86.58 ± 0.37	86.65 ± 0.35
10th	69.75 ± 0.75	96.85 ± 0.35	89.64 ± 0.25	94.12 ± 0.39
12th	78.90 ± 0.56	97.98 ± 0.42	92.89 ± 0.13	96.12 ± 0.16
14th	87.91 ± 0.42	99.90 ± 0.32	96.69 ± 0.45	99.87 ± 0.42*

Table 2 shows the data obtained from wound healing activity of *Moringa oleifera* on excision wound model. EE is ethanolic extract and EAE is ethyl acetate extract. Vicco turmeric is used as standard, each value is a mean ± standard error for group of six animals (n=6). *indicates value significantly different compared to control ($P < 0.001$).

TABLE 3: MEAN TENSILE STRENGTH OF RESUTURED INCISION WOUND ON 10TH POST WOUNDING DAY

Day	Tensile strength (g ± S.E.)		
	Control	EE	EAE
10th	241 ± 1.02	439.17 ± 1.11	473.80 ± 1.23*

Table 3 shows the data obtained from wound healing activity of *Moringa oleifera* on incision wound model. EE is ethanolic extract and EAE is ethyl acetate extract. Each value is a mean ± standard error for group of six animals (n=6). *indicates value significantly different compared to control ($P < 0.001$).

TABLE 4: MEAN GRANULOMA TENSILE STRENGTHS ON 10TH POST WOUNDING DAY

Day	Tensile strength (g ± S.E.)		
	Control	EE	EAE
10th	180.00 ± 0.98	345.00 ± 0.86	355.83 ± 0.89*

Table 4 shows the data obtained from wound healing activity of *Moringa oleifera*. EE is ethanolic extract and EAE is ethyl acetate extract. Each value is a mean ± standard error for group of six animals (n=6). *indicates value significantly different compared to control ($P < 0.001$).

oleifera exhibited significant antipyretic activity, whereas petroleum ether and solvent ether fractions did not show any significant activity as in Table 1. The mean percentage closure of excision wound area is shown in Table 2; ethyl acetate extract showed significant percent closure of excision wound. The contraction of excision wound was promoted from the 4th day of treatment till the 14th day. The healing of wounds in case of rats treated with ethyl acetate extract was found to be quicker than the control, which was also comparable with standard.

The results of incision wound model showed tensile strengths of 241, 439.17, and 473.80 g in control, ethanolic and ethyl acetate extracts respectively (Table 3), whereas granuloma studies showed tensile strengths of 180, 345 and 355.83 in control, ethanolic and ethyl acetate extracts respectively (Table 4). A significant increase in the tensile strength of rats treated with ethyl acetate extract as compared to control suggests that *Moringa oleifera* promoted wound healing activity.

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