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Effect of *Moringa oleifera* Lam. on normal and dexamethasone suppressed wound healing

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ABSTRACT

Objective: To investigate aqueous extract of bark of *Moringa oleifera* for normal wound healing and dexamethasone suppressed wound healing using incision, excision and dead space wound models in Wistar rats. **Methods:** In incision and dead space the extracts were applied daily topically till the 10th post wounding days while in excision model it was till the complete epithelialization process. Standard group were treated with Povidone iodine ointment topically daily. The breaking strength, percentage of wound contraction, period of epithelialization, dry granulation weight and hydroxyproline content were observed. **Results:** The aqueous extract of *Moringa oleifera* significantly increased the wound breaking strength in incision wound model. The aqueous extract treated wounds were found to epithelize faster and the rate of wound contraction was significantly increased as compared to control wounds. Significant increase in granulation breaking strength and hydroxyproline content in dead space wound was observed. The aqueous extract significantly decreased the antihealing activities of dexamethasone in all the wound models. **Conclusions:** The results indicated that the aqueous extract of *Moringa oleifera* promotes wound healing significantly and able to overcome the wound healing suppressing action of dexamethasone.

1. Introduction

Moringa oleifera Lam. (*M. oleifera*) (family Moringaceae) is commonly known as Drumstick tree, indigenous to Northwest India, Pakistan, Bangladesh and Afghanistan. This rapidly growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics now distributed all over the world^[1,2].

Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological, hepato-renal disorders, diabetes mellitus, CNS depressant, and for antifertility effect^[3–5].

Wound is defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues^[6]. Healing of wound is a biological process that is initiated by trauma and often terminated by scar formation. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collagenation and tissue remodeling^[7]. Wound healing is fundamentally a connective tissue response. Initial stage of this process involves an acute inflammatory phases followed by synthesis of collagen and other extracellular macromolecules which are later remolded to form scars^[8].

Dexamethasone is very potent anti-inflammatory glucocorticoid used in organ transplantation and skin allografts^[9]. Glucocorticoids are known to suppress wound healing^[10]. Dexamethasone treatments strongly interfere with both the synthesis and degradation of type I and type III collagen^[11]. It is also a potent transcriptional inhibitor of human type VII collagen promoter activity in dermal fibroblasts, which leads to decreased anchoring of fibril formation^[12].

In India, there has been interest in the potential of natural products obtained from plants and animals for development of drugs with wound healing properties as taught in a popular form of Indian medicine known as Ayurveda^[13].

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There are several reports stating that the extracts of several plants have wound healing properties^[14,15]. Some plants possessing prohealing activity have been scientifically analyzed. *Tridax procumbens*, *Trigonella foenumgraecum*, *Leucas lavandulaefolia* and *Aloe vera* have shown promising wound healing activity^[16]. Hence, the present study has been undertaken to investigate the effect of aqueous extract of bark of *M. oleifera* on the different parameters of wound healing alone and in the presence of dexamethasone induced suppression of wound healing in Wistar rats.

2. Materials and methods

2.1. Plant collection and extracts preparation

M. oleifera barks were harvested during the dry season from trees grown in Surat region of Gujarat state. The family and species of *M. oleifera* were confirmed by Hemchandra North Gujarat University, Patan and barks were kept in the University Herbarium. *M. oleifera* barks were air-dried at room temperature in the Department of Pharmacology until constant weight was attained. They were kept away from direct sun light to avoid destroying active compounds. They were then pounded to coarse powder using metallic motor and pestle to ease the extraction of active compounds.

2.2. Extraction process

The aqueous extract was prepared by decoction method with drug: distilled water in ratio of 1:5 (yield: 10.4%, w/w). The extract thus obtained was concentrated by recovering the solvent by rotary flash evaporator. The concentrated extract was then evaporated to dryness in vacuum oven at temperature not more than 50°C. The dried extract was stored at 2–8°C in refrigerator.

2.3. Phytochemical analysis

The aqueous extract was tested qualitatively for different Phyto-constituents using various chemical tests^[17,18].

2.4. Animals

The healthy Wistar albino rats of either sex, weighing 150–200 g, were housed under standard environmental conditions of temperature and humidity [(25±0.5) °C and 12 h light/dark cycle] were utilized for the studies. The animals were fed with standard pellet diet and water *ad libitum*. The animal studies were performed in the institute with due permission from Institutional Animal Ethical Committee (VBT/IAEC/10/12/33).

2.5. Study design

The animals were randomly allocated into five groups with six animals each for the three experimental animal wound models. Group 1 received simple ointment base applied

topically on the wounds. Group 2 received Standard 5% w/w Povidone iodine ointment topically. Group 3 received aqueous extract of *M. oleifera* in 5% w/w ointment base. Group 4 received intramuscular dexamethasone injection and Group 5 received dexamethasone intramuscularly and treated with aqueous extract of *M. oleifera* in 5% w/w ointment base.

Dosing schedule—For assessment of wound healing activity aqueous extract was formulated in ointment by using simple ointment BP as base. 5% (w/w) ointment was applied where 5 g of extract were incorporated in 100 g of simple ointment base BP. 0.5 g of extract ointment and Povidone iodine ointment was applied once daily to treat different groups of animals, respectively. Dexamethasone was used in the dose of 0.3 mg/kg *i.m.*; full dose on the day of operation and half the dose thereafter on the alternate day till the end of the study period^[19].

2.6. Experimental wound models

All the wounding procedures were carried out under anesthesia by administering ketamine (0.5 mL/kg bw. *i.p.*).

2.6.1. Excision wound

The excision wound was created on rats by cutting away a 4.9 cm² full thickness of skin from a predetermined area on the back of selected rat. The excised wound was left open (Figure 1). Wound healing potential was determined by wound contraction and wound closure time (Period of epithelization). Wound area was measured by tracing the wound margin using 1 mm² graph paper on the day of wounding and subsequently on alternative days until healing was complete. The healed area was calculated by subtracting wound area from the original wound area. The percentage of wound contraction was calculated using the formula: Percentage of wound contraction = (Healed area / Total wound area) × 100. Number of days required for falling of scale leaving no raw wound behind indicated the period of epithelialization^[20,21].

2.6.2. Incision wound

Incision wounds of about 6 cm in length and 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm intervals. Surgical thread (No. 000) and a curved needle (No. 9) were used for stitching. The continuous thread on both wound edges were tightened for good closure of the wounds (Figure 2). The wounds of animals in the different groups were treated with drug by topical application as described above, for the period of 10 days. The wounding day was considered as day 0. When wounds were cured thoroughly, the sutures were removed on the 8th post-wounding day and the breaking strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day reported^[22].

2.6.3. Dead space wound

Physical changes in the granuloma tissue were studied in

this model. The dead space was inflicted on either side in the lumbar region through a small nick in the skin. Sterilized glass cylinder measuring (2.5×0.5) cm was introduced into the pouch. The wounds were sutured and mopped with alcoholic swabs. Animals were placed in individual cages after recovery from anesthesia. The day of the wound creation was considered as day zero. On 10th post wounding day, the granulation tissue formed on the implanted tube is carefully dissected under anesthesia. The wet weight of the granulation tissue was noted. Granuloma from the one tube was cut into pieces measuring 15 mm in length and 5 mm in breadth and used for determination of wound breaking strength. Granuloma tissue from other tube were collected and dried at 60 °C for 24 h weighed and weight was noted. The dried granulation tissue then utilized for the estimation of hydroxyproline[23].

2.6.4. Hydroxyproline estimation

Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, granuloma tissue from other tube were collected and dried in a hot air oven at 60–70 °C for constant weight and were hydrolysed in 6N HCl at 130 °C for 4 hour in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid and colour was developed with the help of Ehrlich reagent at 60 °C. The absorbance was measured at 557 nm using a spectrophotometer (Shimadzu 1700, Japan). The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline[24].

2.7. Statistical analysis

The data was statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's *t*-test with equal sample size. The difference was considered significant when *P*-values < 0.05. All the values were expressed as mean ± standard error of mean (SEM).

3. Results

3.1. The preliminary phytochemical analysis

The preliminary phytochemical analysis of the aqueous extract of *M. oleifera* indicated the presence of saponins, carbohydrates, and alkaloids.

3.2. Excision wound model

In excision wound model the aqueous treated animals showed significant increase in percentage wound contraction and significant decrease in epithelization period as compared to control. In dexamethasone treated group significant decrease in percentage wound contraction and significant increase in epithelization period were observed as compared to control (*P*<0.001). The reversal effect was observed with the extract treated animals which showed significant decrease in epithelization period and significant increase in percentage wound contraction as compared to dexamethasone treated group (Table 1 and Table 2). The results of the aqueous extract treated group in absence of dexamethasone were significantly better to standard povidone iodine (*P*<0.001).

3.3. Incision wound model

Table 1.

Effect of aqueous extract of *M. oleifera* in absence and presence of dexamethasone in excision wound model.

Post wounding days	Percentage wound contraction				
	Control simple ointment	Povidone iodine ointment	<i>M. oleifera</i> aqueous ointment	Dexamethasone injection <i>i.m.</i>	Dexamethasone injection + <i>M. oleifera</i> aqueous ointment
Day 4	13.50±0.56 ^x	25.67±0.80 ^{ax}	29.67±0.66 ^{ax}	9.33±0.33 ^a	19.50±0.34 ^{ax}
Day 8	36.33±0.91 ^x	54.83±0.94 ^{ax}	60.00±0.57 ^{ax}	28.67±0.55 ^a	42.50±0.42 ^{ax}
Day 12	65.17±0.83 ^x	85.67±0.49 ^{ax}	90.17±0.54 ^{ax}	49.50±0.76 ^a	73.33±0.76 ^{ax}
Day 16	85.50±0.61 ^x	93.83±0.40 ^{ax}	97.83±0.40 ^{ax}	69.83±0.87 ^a	88.33±0.55 ^{bx}
Day 20	94.00±0.44 ^x	97.17±0.54 ^{ax}	100.0±0.00 ^{ax}	86.00±0.57 ^a	95.50±0.71 ^{cx}

P values ^a < 0.001; ^b < 0.01; ^c < 0.05 vs. control; ^x < 0.001 vs. dexamethasone.

Table 2.

Effect of aqueous extract of *M. oleifera* in absence and presence of dexamethasone in incision, excision and dead space wound model.

Treatment	Incision wound breaking strength (g)	Excision wound epithelization period (days)	Dead space wound model	
			Granuloma breaking strength (g)	Hydroxyproline content (μg/mL)
Control simple ointment	388.30±0.98 ^x	21.17±0.30 ^x	262.20±4.00 ^x	33.63±1.17 ^x
Povidone iodine oint. (5% w/w)	492.80±2.37 ^{ax}	17.83±0.30 ^{ax}	412.00±5.85 ^{ax}	48.60±0.41 ^{ax}
<i>M. oleifera</i> aqueous oint. (5% w/w)	556.30±1.28 ^{ax}	13.83±0.47 ^{ax}	521.70±2.47 ^{ax}	65.03±0.80 ^{ax}
Dexamethasone injection (0.3 mg/kg <i>i.m.</i>)	343.80±1.62 ^a	23.83±0.30 ^a	202.50±4.42 ^a	18.38±0.53 ^a
Dexamethasone inj. (0.3 mg/kg <i>i.m.</i>) + <i>M. oleifera</i> aqueous oint. (5% w/w)	444.30±2.47 ^{ax}	22.17±0.30 ^{cy}	341.70±2.10 ^{ax}	44.03±0.45 ^{ax}

P values ^a < 0.001; ^b < 0.01; ^c < 0.05 vs. control; ^x < 0.001; ^y < 0.01 vs. dexamethasone.

A significant decrease in wound breaking strength in dexamethasone alone treated group were observed as compared to control group. Suppression of wound breaking strength by dexamethasone was effectively reversed ($P<0.001$) when treated along with aqueous extract of *M. oleifera* as shown in (Table 2). The results of the aqueous extract treated group in presence of dexamethasone were comparable to standard.



Figure 1. Excision wound model.



Figure 2. Incision wound model.

3.4. Dead space wound model

In the dead space wound study, there were significant increase in granuloma breaking strength and hydroxyproline content in extract alone treated groups and decrease in

dexamethasone treated group when compared to control. The reversal of wound suppressant effect of dexamethasone was observed in dexamethasone plus aqueous extract treated group as compared to dexamethasone alone treated group ($P<0.05$). Standard drug povidone iodine also showed significant increase as compared to control (Table 2).

4. Discussion

Wound healing process consists of different phases such as granulation, collagenization, collagen maturation and scar maturation which are concurrent but independent to each other. The use of single model is inadequate and no reference standard exists that can collectively represent the various phases of wound healing. Hence in this study three different models were used to assess the effect of aqueous extract of *M. oleifera* in presence as well as in absence of dexamethasone on various phases of wound healing.

The results of the present study revealed that aqueous extract of *M. oleifera* possesses a definite prohealing action in normal healing as well as in steroid suppressed wound healing. In excision wound healing model the aqueous extract of *M. oleifera* showed significant increase in percentage closure of excision wounds by enhanced epithelization. This enhanced epithelization may be due to the effect of *M. oleifera* extract on enhanced collagen synthesis^[25,26]. Similarly, the breaking strength of the incision wounds was increased in aqueous extract treated groups in incision wound healing model. Deposition of newly synthesized collagens at the wound site increases the collagen concentration per unit area and hence the tissue tensile strength^[27–30].

In dead space model there was a significant increase in granuloma tissue breaking strength in extract treated groups in dead space wound model. The higher breaking strength indicates better healing of wounds. Higher hydroxyproline content was seen with extract treatment. The increased amount of hydroxyproline in aqueous extract treated groups underlines increased collagen content, since hydroxyproline is the direct estimate of collagen synthesis it supports the wound healing activity of *M. oleifera*^[31,32]. The aqueous extract of the plant *M. oleifera* also antagonized the action of dexamethasone to some extent on collagen synthesis, maturation, deposition, period of epithelization and hydroxyproline content. Thus it has potential for antagonizing the antihealing effect of steroids in patient receiving steroid therapy.

Conflict of interest statement

We declare that we have no conflict of interest.

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