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Evauation of *in vivo* Wound Healing Activity of *Moringa oleifera* Bark Extracts on Different Wound Model in Rats

[1]Lambole Vijay and [2]Upendra Kumar
[1]Singhania University, JhunJhunu, Rajasthan, India
[2]Azamgarh College of Pharmacy, Azamgarh, U.P. India

Abstract: *Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae and is used traditionally for several medicinal purposes. The present study was designed to investigate the wound healing potential of bark extracts of *Moringa oleifera* in wistar albino rats.

Key words: Excision wound, incision wound, dead space wound model, aqueous extract, ethanolic extract, *Moringa oleifera*

INTRODUCTION

The process of wound healing occurs in three stages, inflammation, proliferation and remodeling. The basic principles of wound healing like minimizing tissue damage, debriding non-viable tissue, maximizing tissue perfusion and oxygenation, proper nutrition and moist wound healing environment have been recognized for many years (Barua *et al*., 2009). Great numbers of drugs are being used from simple and less expensive analgesics to complex chemotherapeutic agents in the management of wound healing (Prasad and Rao, 1995). Appropriate method for healing of wounds is essential for the restoration of damaged anatomical continuity (Singh *et al*., 2006).

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Nadkarni, 1976; Ramachandran *et al*., 1980). The scientific classification of *Moringa oleifera* shows that it comes from Kingdom: Plantae, Division: Magnoliphyta, Class: Magnoliopsida, Order: Brassicales, Family: Moringaceae, Genus: *Moringa*, Species: *M. oleifera* (Fahey, 2005) which is at the moment distributed all over the world (Lockett *et al*., 2000).

Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological and hepato-renal disorders (The Wealth of India, 1962; Singh and Kumar, 1999; Morimitsu *et al*., 2000; Siddhuraju and Becker, 2003).

Hence, in the present study, an effort was made to establish wound healing potential of the bark extracts ointment of the plant on different wound healing models.

MATERIALS AND METHODS

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.

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 powdered drug was defatted by extracting with pet-ether (60-80°C) followed by extraction of ethanol (yield: 6.3%, w/w) with Soxhlet extractor. The extracts thus obtained were 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.

Phytochemical analysis: The ethanolic and aqueous extracts were tested qualitatively for different Phyto-constituents using various chemical tests (Pharmacopoeia of India, 1996).

Animals: The healthy Wistar albino rats of either sex, weighing 150-200 g, were housed under standard environmental conditions of temperature and humidity (25±0.50°C) and 12 h light/dark cycle were utilized for the studies. The animals were fed with standard pellet diet

Corresponding Author: Lambole Vijay, Singhania University, JhunJhunu, Rajasthan, India Tel: +91-9725975989

and water *ad libitum*. The animal studies were performed in the institute with due permission from Institutional Animal Ethical Committee (registration No. VBT/IAEC/10/12/33).

Study design: The animals were randomly allocated into four 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 and 4 applied topically with aqueous and ethanolic extract of *Moringa oleifera* in 5% w/w ointment base, respectively.

Dosing schedule: For assessment of wound healing activity extracts were 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 each of extract ointment and Povidone iodine ointment was applied once daily to treat different groups of animals, respectively.

Experimental wound models

Excision wound: The excision wound was created on rats (Morton and Malon, 1972) by cutting away a 4.9 cm^2 full thickness of skin from a predetermined area on the back of selected rat (Patil and Kulkarni, 1984; Diwan *et al*., 1982). The excised wound was left open. 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 mm^2 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:

$$\text{Percentage of wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$

Number of days required for falling of eschar without any residual raw gives the period of epithelialization (Lodhi *et al*., 2006). Incision wound: Two paravertebral incisions of 6 cm length were made through the skin and muscles at a distance of about 1.5 cm from the midline on either side of the vertebral column. After the incision was made the parted skin is kept together and stitched at 0.5 cm intervals interrupted sutures using surgical thread and a curved needle. All the groups were treated in the same manner as per protocol. The sutures were removed on day 9 and the breaking strength of the wound was measured on day 10 by continuous and constant water flow technique (Lee *et al*., 1968). 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 (Patil *et al*., 2004). 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. Granuloma tissue from one tube was used for the estimation of hydroxyproline (Neuman and Logan, 1950). Granuloma from the other tube was cut into pieces measuring 15 mm in length and 5 mm in breadth and used for determination of wound tensile strength.

Statistical analysis: Values are expressed in Mean±SEM. Results were statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnet's t-test using computerized Graph Pad Prism version 5.0, Graphpad Software, USA. A p-value <0.001 was considered extremely significant.

RESULTS

Wistar albino rats of either sex weighing between 180 and 200 g were topically treated with aqueous and ethanolic extracts ointment (5% w/w) in excision, incision and dead space wound healing models. Rats of standard group were treated with povidone iodine ointment topically. Topical application of *Moringa oleifera* aqueous and ethanolic extracts ointment in excision wound model increased the percentage of wound contraction. Epithelization time were decreased. In incision wound and dead space wound breaking strength of wounds and hydroxyproline content was increased.

The preliminary phytochemical analysis: The preliminary phytochemical analysis of the ethanolic extract of *Moringa oleifera* showed the presence of steroids and triterponoids, saponins, alkaloids and carbohydrates. The aqueous extract of *Moringa oleifera* indicated the presence of saponins, carbohydrates and alkaloids.

Excision wound model: Topical application of *Moringa oleifera* increased the percentage of wound contraction and completed wound healing by 20th day which indicates rapid epithelization and collagenization. In fact, Topical application of *Moringa oleifera* aqueous and ethanolic extracts in 5% w/w ointment base accelerated the progression of wound healing by 12th day, i.e., (90.17±0.54) and (88.17±0.47) $p<0.001$ compared with

Table 1: Effect of *Moringa oleifera* extracts topically on excision wound healing model

Groups	Wound contraction on day (%)					Period of Epithelization (days)
	4th	8th	12th	16th	20th	
Control (Simple oint base)	13.50±0.56	36.33±0.91	65.17±0.83	85.50±0.61	94.00±0.44	21.17±0.30
Standard Povidone iodine oint (5% w/w)	25.67±0.80	54.83±0.94	85.67±0.49	93.83±0.40	97.17±0.54	17.83±0.30
Moringa oleifera Aqueous extract oint (5% w/w)	29.67±0.66	60.00±0.57	90.17±0.54	97.83±0.40	100.00±0.0	13.83±0.47
Moringa oleifera Ethanolic extract oint (5% w/w)	27.67±0.42	57.00±0.44	88.17±0.47	95.17±0.54	98.00±0.44	15.83±0.30

Values are Mean±SEM (n = 6), oint: Ointment

Table 2: Effect of *Moringa oleifera* extracts topically on incision wound healing and dead space wound model

Groups	Incision wound model, wound breaking strength (g)	Dead space wound, granuloma breaking strength (g)	Dead space wound, hydroxyproline content (μg mL^{-1})
Control (Simple oint base)	388.3±0.98	262.2±4.00	33.63±1.17
Standard Povidone iodine oint (5% w/w)	492.8±2.37	412.0±5.85	48.60±0.41
Moringa oleifera Aqueous extract oint (5% w/w)	556.3±1.28	521.7±2.47	65.03±0.80
Moringa oleifera Ethanolic extract oint (5% w/w)	519.7±1.28	445.0±4.83	54.38±0.34

Values are Mean±SEM (n = 6), oint: Ointment

control (65.17±0.83). It also reduced the epithelization time from 21.17 to 13.83 and 15.83 days, respectively, compared with control. Standard Povidone iodine ointment also showed significant effect (Table 1).

Incision wound model: The breaking strength of the incision wounds was increased in aqueous extract ointment and ethanolic extract ointment treated groups to significant extent, i.e., 388.3±0.98 in control was increased up to 556.3±1.28 with aqueous extract and up to 519.7±1.28 with ethanolic extract. The results are also better to standard Povidone iodine ointment (Table 2).

Dead space wound: In the dead space wound study, there was a significant increase in granuloma breaking strength in aqueous and ethanolic extract ointment treated groups 521.7±2.47 and 445.0±4.83 when compared to control 262.2±4.00 (Table 2). There was significant increase in hydroxyproline content in aqueous and ethanolic extract treated groups 65.03±0.80 and 54.38±0.34 when compared to control 33.63±1.17. The results are superior to Standard Povidone iodine ointment which also showed significant results as compared to control.

DISCUSSION

Wound healing is an orderly progression of events that establish the integrity of the tissues. Many studies have shown that plant products are preferred in wound healing (Jagetia *et al*., 2003).

In excision wound healing study from the observed values it were assumed that *Moringa oleifera* extracts ointment (5% w/w) shows better and faster healing as compared to control group. During the initiation of the study day "0" the wound closure with *Moringa oleifera* extracts ointment were slow. As the study progress the wound healing efficiency increases shows complete healing on day 18-21 which is superior than group 2 (wounds treated with standard Povidone iodine ointment) but the control group took more time to complete wound closure. The study was carried out till fall of eschar leaving no scar behind. This shows healing potential of *Moringa oleifera* extracts ointment with better and faster wound closure.

The result obtained for breaking strength shows higher tensile strength for *Moringa oleifera* extracts ointment treated wounds as compared to wounds of control group. The increase in tensile strength may be due to promotion of collagen formation which significantly contributing to effective and better wound healing.

The *Moringa oleifera* extracts ointment also promotes healing markers as compared to that of control group. In addition to this increase in granuloma breaking strength and hydroxyproline content strongly emphasize the positive wound healing potential of aqueous and ethanolic extract ointment of *Moringa oleifera*. Accordingly hydroxyproline (marker of collagen) was significantly increased in treated group as compared to control and standard.

In conclusion, the present study demonstrated that the aqueous and ethanolic extracts of *Moringa oleifera* promote wound healing activity in animals as a preclinical study. The aqueous extract showed remarkable wound healing activity and it may be suggested for treating various types of wounds in animal and human beings. Further studies with purified phytoconstituents might be needed to comprehend the complete mechanism of wound healing activity of *Moringa oleifera.*

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CONCLUSION

Moringa oleifera bark extracts ointment accelerated wound healing in rats. The aqueous extract showed remarkable wound healing activity. Further studies with purified constituents might be needed to comprehend the complete mechanism of wound healing activity of *Moringa oleifera.*

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