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## PRECLINICAL EVALUATION OF *MORINGA OLEIFERA* LEAVES ON INDUCED EXCISION AND INCISION WOUND MODELS

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### ABSTRACT

The purpose of this research is to screen the wound healing ability of a hydro-alcoholic extract of *Moringa oleifera* leaves. *Moringa oleifera* is frequently used to treat wide variety of skin conditions in the Ayurvedic medicine system, as well as it is also used as folk medicine in wound management. Beside this, the *Moringa oleifera* has proven scientifically for its anti-microbial, anti-oxidant, and anti-inflammatory activity. So, it can be assumed that *Moringa oleifera* may show potent wound healing activity as the three most important related activities (anti-microbial, anti-oxidant, and anti-inflammatory activity) has already been proved. *Moringa oleifera* leaves were subjected for maceration by using 70% ethanol. Two different (2.5% and 5%) doses of *Moringa oleifera* gel formulation was prepared with HPMC (gel base) to apply topically. Evaluation of ability for healing from wound was done by “Excision, and Incision wound” models. In the excision wound model, *Moringa oleifera* treated rats showed a significant improve the epithelization time and 50% wound contraction. likewise, in contrast to the control group, the rats

that received the extract of *Moringa oleifera* leaves exhibited a significant rise in breaking strength in an incision wound model. *Moringa oleifera* leaves hydroalcoholic extract gel formulations shown likely similar activities as compared to synthetic standard/market povidone-iodine as well as herbal standard Aloe vera gel. It can be concluded that *Moringa oleifera* leaves 70% ethanolic extract (2.5 % and 5% gel formulation) has potent wound healing activity.

**Keywords:** *Moringa oleifera*, Hydroalcoholic extract, Povidone-iodine, Aloe vera, Wound healing activity

## INTRODUCTION

Ayurveda is the oldest system of medicine in India, based on both philosophical and experimental principles. While synthetic drugs have numerous adverse effects, the World Health Organisation reports that between 70 - 80% of the population relies on traditional medicines, mostly derived from herbal sources worldwide.

After an injury, wound healing is the process of mending the skin and other soft tissues. Following an injury, the cells beneath the injury swell up in an inflammatory response. More collagen, or connective tissue synthesis, is produced by the dermis, the skin's lowest layer. After then, the outer skin, or epithelial tissue, will regrow [1].

Wound healing is a complex process. It has four phases- inflammatory phase (0–5 days), proliferative phase (3–14 days), maturation phase (7 days–1 year), and scar formation are a series of linked and concurrent processes that occur during the complex and aggressive process of wound healing [2]. There are

several synthetic medicines are available in the market to cure the wound; but based on their adverse effects and price, nowadays the acceptance of herbal drugs are increasing day by day [3].

The herb *Moringa oleifera* is used anti-inflammatory as well as a wound healing activity in folklore medicine system. Its acceptance as a wound healing agent is lacking of scientific research and documentation.

## MATERIALS AND METHOD

### Collection and preparation of leaves extract:

The leaves of *Moringa oleifera* were collected from the local area of Chakdaha, West Bengal, India. Fresh tender leaves were collected, washed with clean and fresh water for removing the dust particles followed by shade drying. Dried leaves were subjected for extraction. The extraction process of *Moringa oleifera* leaves were done with 70% ethanol by using maceration technique for

consecutive 48 hours. The extract has been collected and kept for evaporation under hot air oven to get the constant weight. After that the *Moringa oleifera* leaves extract was collected and stored under refrigeration at 4°C.

### Experimental animals:

For the investigation, male albino wistar rats weighing between 200 and 250 grams were utilized. The purchase of the animals came from M/S Saha Enterprise (authorized animal breeder). They had given out commercial food pellets and unlimited access to tap water. Every experiment was carried out between the hours of 10 a.m. and 6 p.m., with the animals housed (approval no: 1502/ PO/ a/ 11/ CPCSEA) in a clean, distinct polypropylene cage. The rats and rabbits were kept in a regular laboratory setting with a room temperature of 22±2°C and a 12-hour light/12-hour dark cycle, in N.S.C.B.I.P (Netaji Subhas Chandra Bose Institute of Pharmacy) approved by the CPCSEA.

### Drugs and chemicals:

Ethanol (Dr. S.C. Deb's Dilution), Distilled water, Povidone iodine ointment (Betadine 10% Ointment, Win-Medicare Pvt Ltd), Xylazine (Indian Immunologicals), Ketamine (Ciron Drugs & Pharmaceuticals Pvt Ltd), Hydroxypropyl methylcellulose (Loba Chem

Pvt Ltd), Methylene blue, Aloe vera gel (Himalaya).

### Gel preparation and Dose selection: [4]

Different herbal medications' capacity to cure wounds has been investigated in a range of topical formulations, such as gels, ointments, and creams. The vehicle selection is biased in favour of gel. This is advantageous because the aqueous composition of the gel should mix with the aqueous exudates from the wound more quickly and effectively. Furthermore, the phytochemical extract used in the different tests includes organic, hydroalcoholic, and aqueous components. Consequently, the extraction's lipophilic component would be challenging to extract from lipophilic lotions or ointments, which is why the gel was the best option. With the reduced retention and improved drug flow anticipated from a polymeric gel-based vehicle. Thus, hydroxypropyl methylcellulose (HPMC) was our choice for the gel foundation. There is a topical HPMC gel formulation ready. *Moringa oleifera* (2.5%) in HPMC (7.5%) is used as a low dose while *Moringa oleifera* (5%) in HPMC (7.5%) is used as a high dose when applied topically in an excision, incision wound model. Ten days of treatment were given to incision wound models, and until the scar came off the wound, excision wound models received treatment.

**Excision Wound Model: [5]**

200–250 grams of male wistar albino rats were collected. Five groups of six animals each were formed out of the animals. Both before the wounds formed and during the procedure, xylazine (13mg/kg) and ketamine (87mg/kg) were used to anesthetize the animals. The rats were given excision wounds. The fur on each side of the animals in the lumber section was clipped clean using an electric clipper, and the anticipated area of the wound on the animals' backs was marked out with a circular stainless-steel stencil using methylene blue. A full thickness excision wound of 500 mm<sup>2</sup> and 0.2 cm depth was created along the marks using surgical scissors and toothed forceps. The incision was totally visible.

Group I: Control group: animals in this group were given only 7.5% HPMC gel twice a day; they did not get any other care.

Group II: Standard group: twice a day, the animals in this group received USP 10%w/w povidone-iodine ointment (also known as 10% betadine ointment).

Group III: Herbal Standard Group: twice a day, aloe vera gel was given to the animals in this group.

Group IV: Test group: animals in this group were given 2.5% *Moringa oleifera* in 7.5%

HPMC gel twice a day until full epithelium was formed.

Group V: Test group: twice daily, animals in this group were given 5% *Moringa oleifera* in 7.5% HPMC gel until full epithelium was formed.

By tracing the wound with cellophane paper and a permanent marker at days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 following wounding, the contraction of the wound was ascertained. The wound areas were identified and measured on a millimetre scale graph paper. The number of days needed for eschar to collapse without any raw wound remaining was calculated and recorded based on the length of epithelium.

**Incision Wound Model: [6-10]**

The same as in the excision wound model, five groups of male wistar albino rats weighing 200 to 250 grams were formed. Much like in the previous model, rats were anesthetized both prior to and during the development of the wound. An electric clipper was used to shave the animals' dorsal fur. Ehrlich and Hunt describe making a straight paravertebral incision 6 cm long on the back's skin and subcutaneous muscle. After complete haemostasis, the wound was closed using interrupted sutures placed at equal intervals of roughly 1 cm. From the 0th to the 9th post wounding day, the animals were given their

prescribed medication daily. On the 10th post wounding day, a continuous, constant water flow technique was used to conduct the wound breaking strength test.

Three millimetres away from the edge of the incision, Allis forceps were firmly placed on both flanks of the wound. The forceps was fastened to a metal rod that was fixed on one side, and to a lightweight plastic container that was secured with a thread that crossed a pulley on the other. The container, which was attached to a continuous water flow method (100 drops/minute), continued to suspend.

The addition of weights was stopped as soon as the incision started to gap, and the weights were raised to stop more tissue injury. The weights needed to provide the necessary gaps were noted.

### Statistical Analysis:

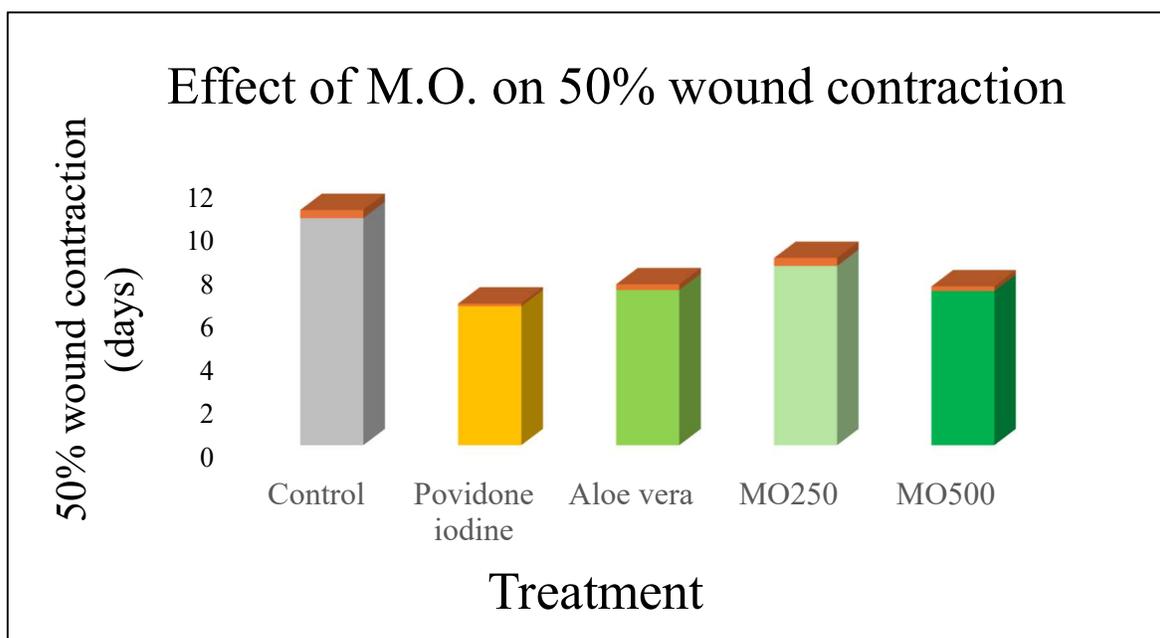
The results are presented as mean  $\pm$  S.E.M. The differences between experimental groups were evaluated using one-way ANOVA (control vs. treatment), followed by Dunnett's test, and were accepted statistically significant when  $P < 0.05$ .

## RESULT AND DISCUSSION

**Table 1: Effect of *Moringa oleifera* (M.O.) on period of epithelialization and 50% wound contraction in excision wound model**

Treatment	Period of epithelialization(days)	50% wound contraction
Control (7.5% HPMC gel)	20.556 $\pm$ 0.527	10.462 $\pm$ 0.376
Povidone iodine	13.662 $\pm$ 0.485***	6.405 $\pm$ 0.123***
Aloe vera gel	15.703 $\pm$ 0.441***	7.152 $\pm$ 0.264***
M.O. (2.5%) in 7.5% HPMC gel	17.866 $\pm$ 0.307***	8.258 $\pm$ 0.372***
M.O. (5%) in 7.5% HPMC gel	15.434 $\pm$ 0.214***	7.103 $\pm$ 0.215***

All values are mean  $\pm$ SEM, n=6, \*\*\* p <0.001 vs control



**Figure 1**

All values are mean  $\pm$ SEM, n=6, \*\*\* p <0.001 vs control

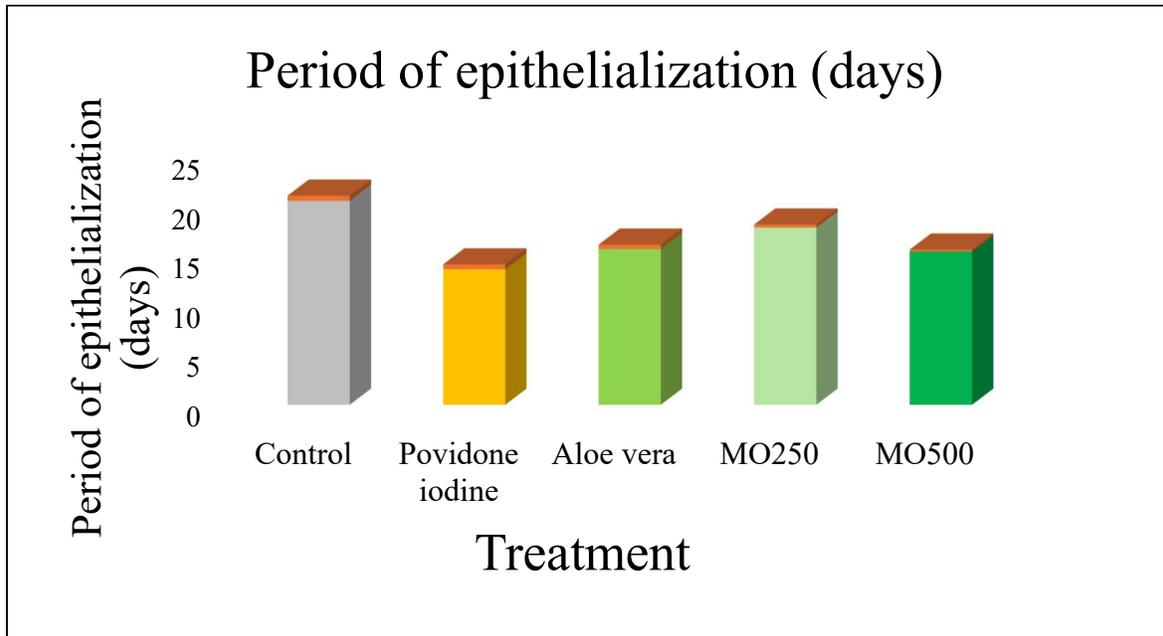


Figure 2

All values are mean ±SEM, n=6, \*\*\* p <0.001 vs control

Table 2: Effect of *Moringa oelifera* (M.O.) breaking strength in incision wound model

Treatment	Breaking strength
Control (7.5% HPMC gel)	257.38±4.23
Povidone iodine	476.78±5.95***
Aloe vera Gel	363.74±5.65***
M.O. (2.5%) in 7.5% HPMC gel	354.52±4.71***
M.O. (5%) in 7.5%HPMC gel	425.5±5.52***

All values are mean ±SEM, n=6, \*\*\* p <0.001 vs control

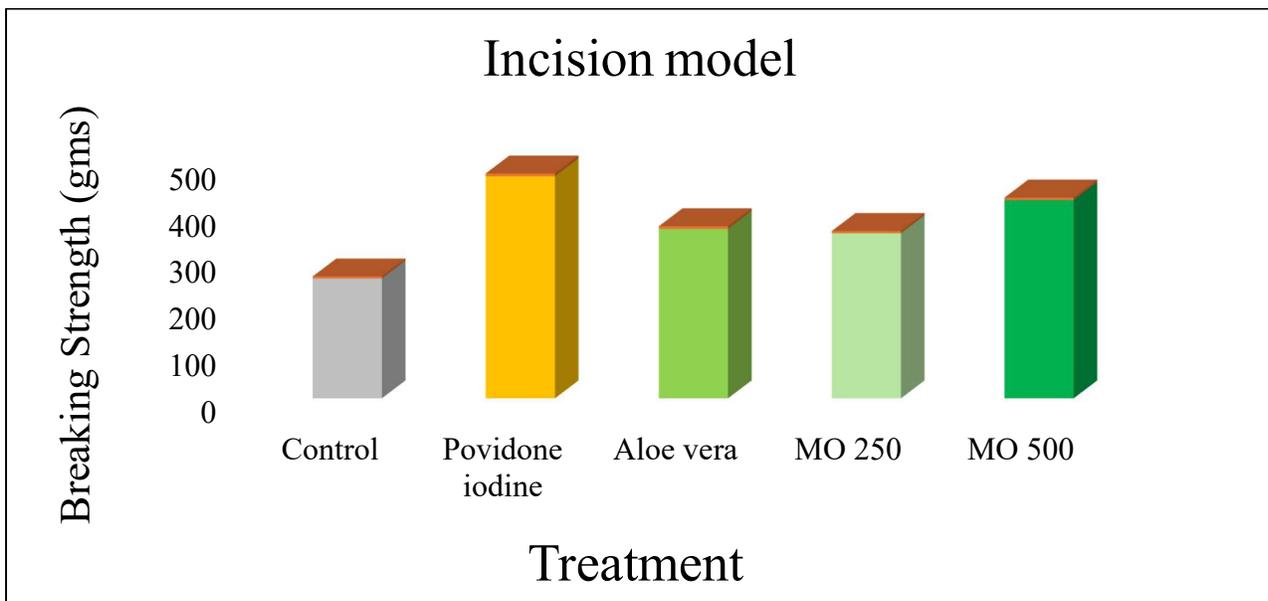


Figure 3

All values are mean ±SEM, n=6, \*\*\* p <0.001 vs control

From previous research works it has been confirmed that chemical investigation of *Moringa oleifera* leaves extract prove the presence of numerous phytoconstituents such as steroids, alkaloids, tannins and protein. Some of these chemical elements also may be responsible for the healing activity of *Moringa oleifera* [11].

By blocking the activity of phospholipase A2, steroids have anti-inflammatory properties. Phospholipase A2 is essential for the synthesis of inflammatory chemicals [12].

Alkaloids, which have nitrogen atoms in their structures, typically have antioxidant, anti-inflammatory, and antibacterial properties [13, 14].

Tannins are known for their antioxidant properties, and the medication used to demonstrate these properties is also known to exhibit varying degrees of wound-healing action. Protein is regarded as the building block of the human body. Proteins are made up of amino acids. Amino acids have the potential to speed up the healing process by accelerating cell proliferation. Additionally, amino acids support eschar's maturation.

There is proof of the antioxidant and antimicrobial properties of *Moringa oleifera*. It is anticipated that any medication with antioxidant and antimicrobial properties will also have a therapeutic impact. Its alkaloids

may be the cause of its antioxidant properties. Its anti-inflammatory properties may be attributed to the presence of steroids, tannins, and alkaloids. Since it has been shown that a herb's most active ingredient and the cause of its ability to heal wounds is tannin. All of the mentioned data point to the possibility that the strong wound-healing properties of *Moringa oleifera* leaves from the presence of tannin, alkaloids, steroids, and protein, which also distinguishes *Moringa oleifera* as a special herb.

## CONCLUSION

The purpose of the present study was to evaluate whether the phytochemicals in *Moringa oleifera* leaves would work on improving wound healing in albino wistar rats having experimentally conceived wounds. The hydroalcoholic extract of *Moringa oleifera* leaves that was obtained by maceration method, was used in two different doses of *Moringa oleifera* (2.5%, 5%) gel formulation by using HPMC as gel base, which was used for in vivo wound healing study on albino wistar rats. Hydro alcoholic extract of *Moringa oleifera* leaves demonstrated remarkable wound healing activity in excision as well as incision wound models.

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