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Exploration of Healing Promoting Potentials of Leaves of *Morus alba* L. in Albino Rats

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ABSTRACT

To establish the wound healing activity of aqueous and ethanolic extract of roots of *Morus alba.* Two models were performed to evaluate the wound healing activity i.e., Incision and Excision model. In incision model the parameter which was carried out was breaking strength of wounded skin. In excision model percentage wound contraction and period of epithelialization was established for both the extracts. Reference standard drug was *Aloe vera* ointment for comparison with other groups. From the observation in both the models, Aq. extract of *Morus alba* was found to have greater wound healing activity in terms of breaking strength in incision model and percentage wound contraction, period of epithelialization was highest in excision model compared with other groups. In conclusion, our findings suggest that aq. extract of *Morus alba* possess better healing ability than the ethanolic extract.

Keywords: *Morus Alba*, Incision, Excision, Phytochemical Analysis, Aloe Vera Ointment

1. INTRODUCTION

The wound repair process has three orderly but temporally overlaid stages: Inflammation, cell proliferation and tissue regeneration (Murti *et al*., 2011a). Wound healing is a process which 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 latter remolded to form scars (Charde *et al*., 2010). Therefore, tissue repair and wound healing are the complex processes that involve a series of biochemical and cellular reactions (Sadaf *et al*., 2006; Ayyanar and Ignacimuthu, 2009; Thomas *et al*., 2010). There are several reports stating that the extracts of several plants, used for wound healing properties (Suntar *et al*., 2010; Sadaf *et al*., 2006; Zhaia *et al*., 2009).

Some of plants possessing prohealing activity have been scientifically analyzed. The wound healing potential of Tridex procumbens, Trigonella foenumgraecum, leucas lavandulaefolia and aloe vera have shown promising healing activity (Murti *et al*., 2011b).

The mulberry tree, a plant of the family Moraceae and the genus *Morus*, has been widely cultivated to feed silkworms. The leaves and the roots of *M. alba* have also been used in traditional medicine as a cathartic, analgesic, diuretic, antitussive, sedative, hypotensive and antiphlogistic and for the treatment of edema (Nomura, 1999). The decoction of the leaves is used as a gargle for relief of inflammation of the throat. The plant contains flavonoids, coumarine and stilbene, which have hepatoprotective and free radical scavenging activity (Oh *et al*., 2002). The other uses of *M. alba* are as a hypoglycemic (El-Beshbishy *et al*., 2005). Cardioprotective (Enkhmaa *et al*., 2005) and neuroprotective agent (Abdel *et al*., 2005). The mulberry fruit has been used as a medicinal agent to nourish the blood and for the treatment of weakness, fatigue, anemia and premature graying of hair. In

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addition, some phenolic compounds from *M. alba* have been reported to have antioxidant properties. A piper dine alkaloid and some glycoproteins were isolated from the bark and leaves, which had antidiabetic effects (Kusano *et al*., 2002).

Phytochemical reports on *M. alba* L. indicates that the plant contains flavonoids, tannins, triterpenes, anthocyanins, anthroquinones, phytosterols, sitosterols, benzofuran derivatives, morusimic acid, oleanolic acid, alkoloids, steroids, saponins and phenolic compounds (El-Beshbishy *et al*., 2005; Kusano *et al*., 2002). A survey of the literature on *M. alba* revealed only a few pharmacological reports on the plant. No major investigative reports were found pertaining to its wound healing activity of roots; therefore, we undertook the present study to determine the healing promoting potential of *M. alba leaves* by using different animal models for wound healing based on exploratory behavior.

2. MATERIALS AND METHODS

2.1. Materials

Leaves of *Morus alba* L. growing in natural habitat in Panipat, Haryana, India, was collected in January, 2012 and identified by Dr. H.B. Singh, NISCAIR, New Delhi, India by carrying out macroscopic and microscopic evaluation and has been submitted in the institute for future reference purpose.

2.2. Preparation of the Leaves Extract

Dried and coarsely 500 g powdered leaves of *Morus alba* was extracted with 90% (v/v) ethanol in soxhlet apparatus for 36 hrs and aqueous extract was prepared by using maceration technique of extraction. Filter the filtrate. The filtrate was concentrated on water bath using petridish. The temperature was maintained at 55°C. The powdered extract was dried and weighed (Murti *et al*., 2010).

2.3. The Preliminary Phytochemical Analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in ethanolic and aqueous extract (Joseph and Raj, 2010; Padmaa, 2009; Murti and Kumar, 2011).

2.4. HPTLC Profile (High Performance Thin Layer Chromatography)

Chromatography was performed on 3×10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F254 (Merck, Germany). Before using, the plates were washed with methanol and activated at 110°C for 5 min. Samples were applied as 4 mm wide bands and 6 mm apart by using a Camag (Muttenz, switzerland) Linomat IV sample applicator equipped with 100 µL syringer. A constant application rate of 5 µl S^{-1} was used.

2.5. Animals

Wistar albino rats of either sex weighing between 180 and 200 g were obtained from Nitin Biologicals, New Delhi. The study was approved by the Institutional Ethics Committee for animal experimentation PDM School of Pharmacy, Safidon, Jind, Haryana, (India) (PDM/IAEC/11/11/04) and all the procedures on animals were carried out as per CPCSEA guidelines, India. These animals were used for the wound healing activity studies. The animals were stabilized for 1 week. They were maintained in standard conditions at room temperature, 60±5% relative humidity and 12 h light dark cycle. They had been given standard pellet diet and water *ad libitum* throughout the course of the study. The ethanolic and aqueous extract of *Morus alba* was administered topically to all groups of animals.

2.6. Incision Wound Model

The rats were anesthetized by administering ketamine (0.5 mL kg^{-1} b. w. i.p). Incision wounds of about 6 cm in length and 2mm 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. The wounds of animals in the different groups were treated with drug by oral administration 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 tensile 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 (Mohammed, 2006; Dnyaneshwar *et al*., 2009; Sachin *et al*., 2009; Kiran and Asad, 2008; Zhaia *et al*., 2009).

2.7. Excision Wound Model

A standard wound of uniform 2 cm diameter was formed with the aid of a round seal (Pattanayak and Sunita, 2008; Nayak *et al*., 2005; Jagtap *et al*., 2009; Pradeep *et al*., 2009). The percentage wound closure,

epithelization time and scar area on complete epithelization was measured.

2.8. Histopathological Examinations

A specimen sample of skin tissues from control and treated groups were taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin (Vinothapooshan and Sundar, 2010; Adiga *et al*., 2010; Barua *et al*., 2009) and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation.

2.9. Statistical Analysis

The mean value ± SEM was calculated for each parameter. Results were statistically analyzed by one-way-Analysis of-Variance (ANOVA) followed by post-hoc dunnet's test. $p<0.05$ was considered as significant.

3. RESULTS

3.1. Phytochemical Analysis

On preliminary phytochemical screening the extract showed that the leaves of *Morus alba* L. contain saponins, tannins, alkaloids and flavanoids while other constituents like amino acids, carbohydrate was absent. Further the presence of these chemical constituents was confirmed by HPTLC fingerprinting.

3.2. Incision Wound Model

The breaking strength of the skin in incision wounds was increased in drug treated groups to significant extent; i.e., 306.8±18.13 in control was increased up to 494.5±4.992 with M.A. AQ extract, 453.3±2.108 with M.A. ETH extract (**Table 1**). The results were also comparable to standard drug *Aloe Vera* (422.5±4.787) (**Table 1 and Fig. 1**).

3.3. Excision Wound Model

The progress of the wound healing induced by *Morus alba* extract (Aqueous and Ethanolic) treated groups, Control (Simple Ointment) treated group and povidone iodine (standard drug) treated group of animals are shown in **Table 2**. It was observed that the wound contracting ability of the both extract (32.83±0.23 and 28.00±2.31) from day 4 onwards was significantly greater than that of the control (10.83±2.24).

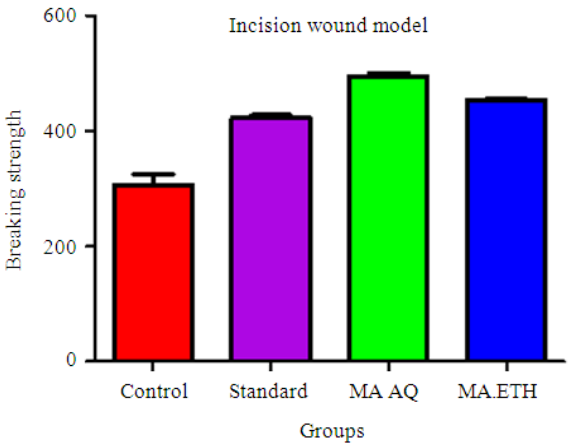

Fig. 1. Measurement of breaking strength in Incision Model

Table 1. Skin breaking strength in 10 day old albino rats by *Morus alba* (topical route)

Groups (n)	Breaking Strength (SKIN)
Control (Simple Ointment) (Topically) (6)	306.8±18.13
Standard (Aloe Vera Ointment) Topically (6)	422.5±4.787***
M.A. AQ Extract (Topically) (6)	494.5±4.992***
M.A. ETH Extract (Topically) (6)	453.3±2.108***

n = numbers of animals in each groups; *** = $p<0.05$ against control group; *** Statistical analysis applied was ANOVA (Analysis of variance) followed by post-hoc test

The percentage wound contraction of the standard drug, i.e., *Aloe Vera* ointment treated group of animals was 25.16±1.54 on day 4 compared to control. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the aqueous extract ointment treated group (12.00 days for 100% contraction which was almost better than that of the Povidone iodine (17.00 days) treated group. Ethanolic extract ointment treated group of animals showed significant complete epithelialization in 15.00±0.258 days compared to control (21.00±0.3651 days) (**Table 2, Fig. 2 and 3**).

3.4. Histopathological Studies

The various parameters studied in histopathological examination of the tissues of the wound area treated with Aq. and Ethanolic Extract of *Morus alba*, control to treated groups are depicted in **Table 3**. Treatment was found to promote keratinization, epithelization, fibrosis, collagenation and neovascularization and values were compared to standard treatment in 10 day old wound.

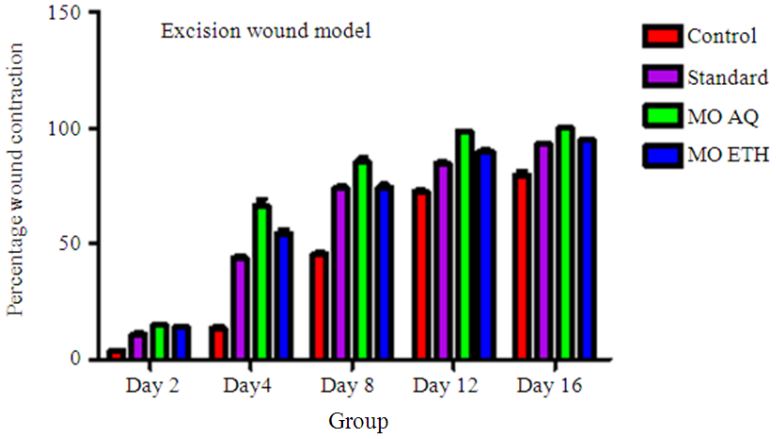

Fig. 2. Graphical representation of Percentage wound contraction

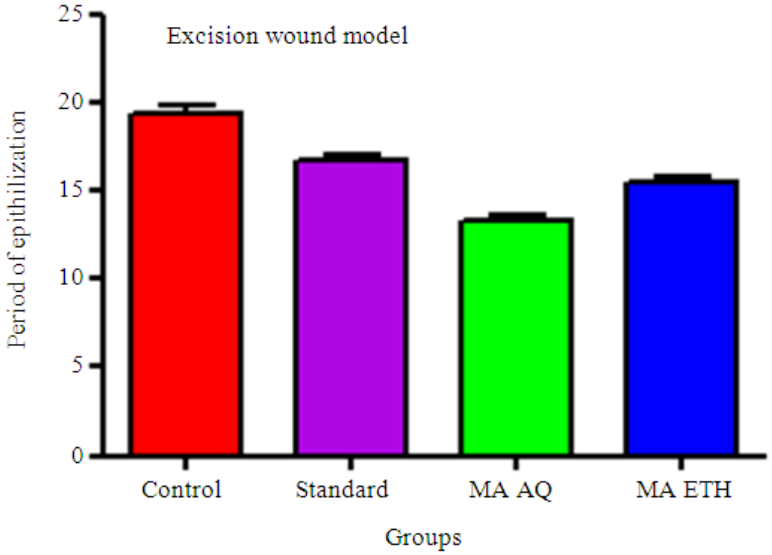

Fig. 3. Graphical representation of period of epithelialization

Table 2. Percentage wound contraction and period of epithelialization in excision wound model

Groups (6)	% Wound contraction in days					Period of epithelialization (Days)
	Day 2	Day 4	Day 8	Day 12	Day 16	
Control (Topical) (Simple Ointment) (6)	4.17±3.79	10.83±2.24	32.00±1.23	52.00±2.54	61.00±1.32	19.50± 0.3416
Aloe Vera (Topical) (6)	5.04±2.32	25.16±1.54***	55.33±3.67***	70.16±3.3***	81.83±1.23***	16.83±0.3073***
M.A. AQ. Extract (Topical) (6)	8.5±0.10	32.83±0.23***	65.33±0.64***	90.5±0.12***	100±0.00***	13.33±0.3333***
M.A. ETH. Extract (Topical) (6)	6.9±0.13	28.00±2.31***	60.00±1.23***	76.00±0.32***	99.33±1.32***	15.50±0.3416***

n = numbers of animals in each groups; *** = p<0.05 against control group; *** Statistical analysis applied was ANOVA (Analysis of variance) followed by post-hoc test

Table 3. Histological examination of granuloma wounds treated with Aq. and Ethanolic Extract and control at the end of 10 days

Parameter	Control	Aqueous extract	Ethanolic extract
Keratinization	1.2±0.19	3.6±0.63	3.7±0.62
Epithelization	1.3±0.21	3.8±0.52	4.0±0.95
Fibrosis	2.0±0.51	3.9±0.81	4.2±0.55
Collagenation	2.2±0.49	4.3±0.98	4.4±0.36
Neovascularization	1.9±0.36	4.5±0.98	4.6±0.67

All the results are reported as mean ±S.E.M. of each group of rat. One-way ANOVA and $p<0.01$ considered significant.

4. DISCUSSION

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair (Shivhare *et al*., 2010). Therefore in this study two different models were used to establish the healing potentials of aqueous and ethanolic *extracts* of *Morus alba* on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres. A healing tissue synthesizes collagen, which is a constituent of growing cell (Bhat *et al*., 2007). Increase in blood vessels and role of antioxidants were experimentally proved (Lodhi and Singhai, 2011).

From the observations, it was evident that *Morus alba* possesses a definite potential healing action. The breaking strength of the incision wounds was increased in aqueous and ethanolic extract treated groups.

In excision wound healing model the aqueous and ethanolic extract of the leaves of the plant *Morus alba* showed significant increase in percentage closure by enhanced epithelialization. This enhanced epithelialization may be due to the effect of *Morus alba* extracts on enhanced collagen synthesis. The higher breaking strength indicates better healing of wounds. Thus it supports the wound healing activity of *Morus alba*.

Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids, alkaloids, saponins and tannins (Charles *et al*., 2006; Pattanayak and Sunita, 2008) are known to promote the wound-healing process. The study reveals that both aqueous and ethanolic extracts treated groups possesses good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like, flavanoids, alkaloids, saponins and tannins present in it.

5. CONCLUSION

The aqueous and ethanolic extract of leaves of *morus alba* showed the healing promoting potentials in both the models that is incision and excision wounds.

6. ACKNOWLEDGEMENT

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6.1. Conflict of Interest Statement

We declare that we have no conflict of interest.

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