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Evaluation of Wound Healing Activity of Leaves of *bombax Ceiba*

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Abstract
Bombax Ceiba, commonly known as Silk cotton tree, is used in folklore medicine in Diabetics, Hypertension, as analgesics, leucorrhoea, sealing of secondary infection, healing of wounds and skin eruptions. There was no scientific evidence justifying the use of Leaves of Bombax Ceiba, therefore the present study was aimed at evaluation of wound healing activity of the plant. In the present study the leaves of Bombax ceiba were studied for wound healing activity by incorporating extract in simple ointment base B.P. in concentration of 2% (w/w) and 5% (w/w) .Wound healing activity was studied in two types of model in rats viz. excision and incision wound model. The results were also comparable to those of a standard drug, Povidone Iodine in terms of wound contracting ability, wound closure time, tensile strength. The statistical data indicated that the wound with ointment containing 5% w/w Methanolic extract exhibited significant (P < 0.001) wound contracting ability and period of epithelization. Significant tensile strength was observed with both the ointment formulations 2% w/w and 5% w/w. Excision wound models was supported by histopathological examination. The experimental data demonstrated that Bombax Ceiba displayed significant wound healingactivity.

Introduction

A wound may be defined as a "Disruption of normal tissue structure and function" and can be categorized by its etiology, location, or duration (Cherry and Hughes, 2000). Wound healing involves a chain of well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a definite fashion including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling.

India has a rich practice of plant-based knowledge on healthcare. A outsized number of plants/plant extracts/decoctions or pastes are equally used by tribals and folklore traditions in India for management of cuts, wounds, and burns (Kumara *et al*.,2007).

Bombax ceiba (Bombacacaea) is a tall and deciduous tree at a height of 20-40m, with smooth or buttressed trunk with pyramidal spreading branches, gray or brown bark covered with hard, black, sharp, conical spines (Prajapati and Purohith, 2003). It is found throughout the warmer parts of India and Srilanka (Singh and Panda, 2005). In Indian system of medicine 'Ayurveda', the plant is commonly known as Raktashalmali (Sanskrit). This drug is a rasayana. It is a element of dashamul kwatha.

Usually the plant exudates gum, light brown to opaque called as 'semulgum 'or' mochras'is used in vata diseases (Singh and Panda, 2005). Bark is astringent, diuretic, demulcent, diuretic, healing of

abscesses, wounds and other skin eruptions, leaves are anti-inflammatory, roots are aphrodisiac, ant diarrheal, flowers are diuretic and laxative, gum is used in hemoptysis, seeds are used in gonorrhea (Deshpandey, 2008).

A survey of literature revealed that no scientific study on the wound healing activity of leaves of this plant has been reported to validate folklore claims on this property. So the objective of the present study was to evaluate the effect of Methanolic extract of *Bombax ceiba* leaves on different parameters related to wound healing activity on albinorats.

Materials and Methods

Collection of plant material

Experiments of the present investigation were carried out with the viable and healthy leaves of *Bombax ceiba* which was procured from the Katihar medical college garden and the local market. The collected plants were identified and authenticated by a qualified Botanist.

Preparation of plant extract

The air-dried crude drug was powdered to obtain coarse powder. The powdered drug was extracted with methanolin a soxhlet extractor. The extract acquired was concentrated by recovering the solvent by Rotary Flash Evaporator. At temperature not more than 50ºC the concentrated extract was then evaporated to dryness in vacuum oven. The dried extract was stored at 2–8ºC in refrigerator and kept in tightly stoppered bottle under refrigeration until further processing at intervals.

Preliminary photochemical screening

Preliminary phytochemical screening (Kokate, 1994; Harborne, 1998) revealed the presence of glycosides, proteins, carbohydrates, saponins, phenolic compounds, tannins, gums and mucilages.

Animals

Healthy Wistar Albino Rats weighing 150 g-250 g were used for the study. Approval for experimental protocol was cleared by the Institutional Animal Ethical Committee (KMC/IEC/2016-2019/30/MD(Pharma)) of Katihar Medical College, Katihar, Bihar, India

Group 1: Simple ointment treated control group

Group 2: Animals treated with Standard (Povidone Iodine 5 % w/w)

Group 3: Animals treated with MBCLD 2% w/w (2g extract in 100g simple ointment) (Alcoholic extract ointment of Bombax Ceiba low dose 2% w/w)

Group 4: Animals treated with MBCHD 5% w/w (5g extract in 100g simple ointment) Alcoholic extract ointment of *Bombax Ceiba* high dose 5% w/w)

Acute Dermal Toxicity Studies

The skin of the animal was clean-shaven at three different positions on the dorsal side, each about 500 mm^2. The 1^{st} area was kept as control and vehicle was applied to it. 2^{nd} area was applied with MBCLD 2% w/w and the 3^{rd} area treated with MBCHD 5% w/w. After four hour, the skin was observed for signs of inflammation (Gfeller and Kobel, 1985).

Selection of dose and treatment period

Two types of ointment was formulated with different concentration of the extract viz. 2% (w/w) ointment, where 2 g of extract was integrated in 100 g of simple ointment base (Anonymous, 1953); 5% (w/w) ointment where, 5g of extracts of the leaves were fused in 100gof simple ointment base**.** Povidone Iodine ointment (5% w/w) (Betadine) (WIN MEDICARE) was used as standard drug for equating the wound healing potential of the extract in different animal model.

Excision wound model

The rats were anesthetized by administering Phenobarbitone (4mg/kg).Then the rats were depilated on the dorsal side and a predetermined area of 500 mm^2 full thickness skin was excised in the dorsal inter scapular region. The drugs were applied topically daily till the complete

epithelization, starting from the day of operation. The parameters studied were wound contraction and time of epithelization. The wounds were traced on mm^2 graph paper on the days of 2^{nd} 4^{th}, 8^{th}, 10^{th}, 12^{th} ,14^{th},16^{th},18^{th} and 20^{th} day. The wound contraction was measured at regular intervals of time to see the percentage of wound contraction and epithelization time that indicates the formation of new epithelial tissue to cover the wound. The sum of days required for falling of the scar without any residual of the raw wound gave the period of epithelization (Kokane *et al*., 2009).

Wound closure % = Wound area on day 0 - Wound area on day n/ Wound area on day 0 x 100 where *n* =number of days 2^{nd} 4^{th}, 8^{th},10^{th}, 12^{th} ,14^{th},16^{th},18^{th} and 20^{th} day.

Incision wound model

Administering ketamine (0.5 ml/kg b. w. i.p.) the rats were anesthetized then one long incision of 6 cm length on the depilated dorsal side was made through the skin till the cutaneous muscle (Udupa et al. 1995). The Rats were divided into Four groups with six animals in each group were anaesthetised and two paravertebral-long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated dorsal side of the rat. All the Animals present in the groups were treated in the same manner as mentioned in the case of the excision wound model. After the incision was made, the parted skin was kept together and stitched with black silk at 1 cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The uninterrupted threads on both wound edges were tightened for good closure of the wound. Sample drugs along with simple ointment (control) and standard drug were administered once daily for 9 days; when wounds were cured thoroughly the sutures were removed on the 9^{th} day and the measurement of tensile strength was done using tensiometer (Saha and Mukherjee, 1996; Udupa *et al .,1994)*

Tensiometer

The tensiometer comprises of a 6 x 12 inch wooden board with one arm of 4 inch long, fixed on both side of the possible longest distance of the board. The board was engaged at the edge of a table. A pulley with bearing was fixed on the top of one arm. An alligator clamp with 1 cm width was tangled on the tip of the other arm by a fishing line in such a way that the clamp might reach the middle of the board. Another alligator clamp was knotted on a longer fishing line with a polyethylene bottle on the other end. The tensile strength of a wound denotes the degree of wound healing. Generally wound healing agents promote a gain in tensile strength. The instrument used for measurement of tensile strength is called a tensiometer, as explained above. One day before performing the experiment (measurement of tensile strength) the sutures were removed from the stitched wound.

Determination of tensile strength

Tensile strength is the confrontation to breaking under tension. It indicates the extent to which the repaired tissue resists to breaking under tension and may indicate the quality of repaired tissue.

Sutures were removed on the day 9^{th} after wound formation and the tensile strength was measured. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer (Kuwano *et al*., 1994).The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 1 cm away from the wound. The amount of water in the polyethylene bag was assessed and considered as an indirect measure of the tensile strength of the wound. The mean determination of tensile strength on the two para vertebral incisions on each sides of the animals was taken as the measurement of the tensile strength of the wound for an individual animal. The tensile strength of the wounds treated with extracts were compared with the controls.

Statistical Analysis

The values are represented as mean ± S.E.M for six albino rats. Unpaired *t*-test was used for recording the P-value and significance with respect to the control group.

Histopathology

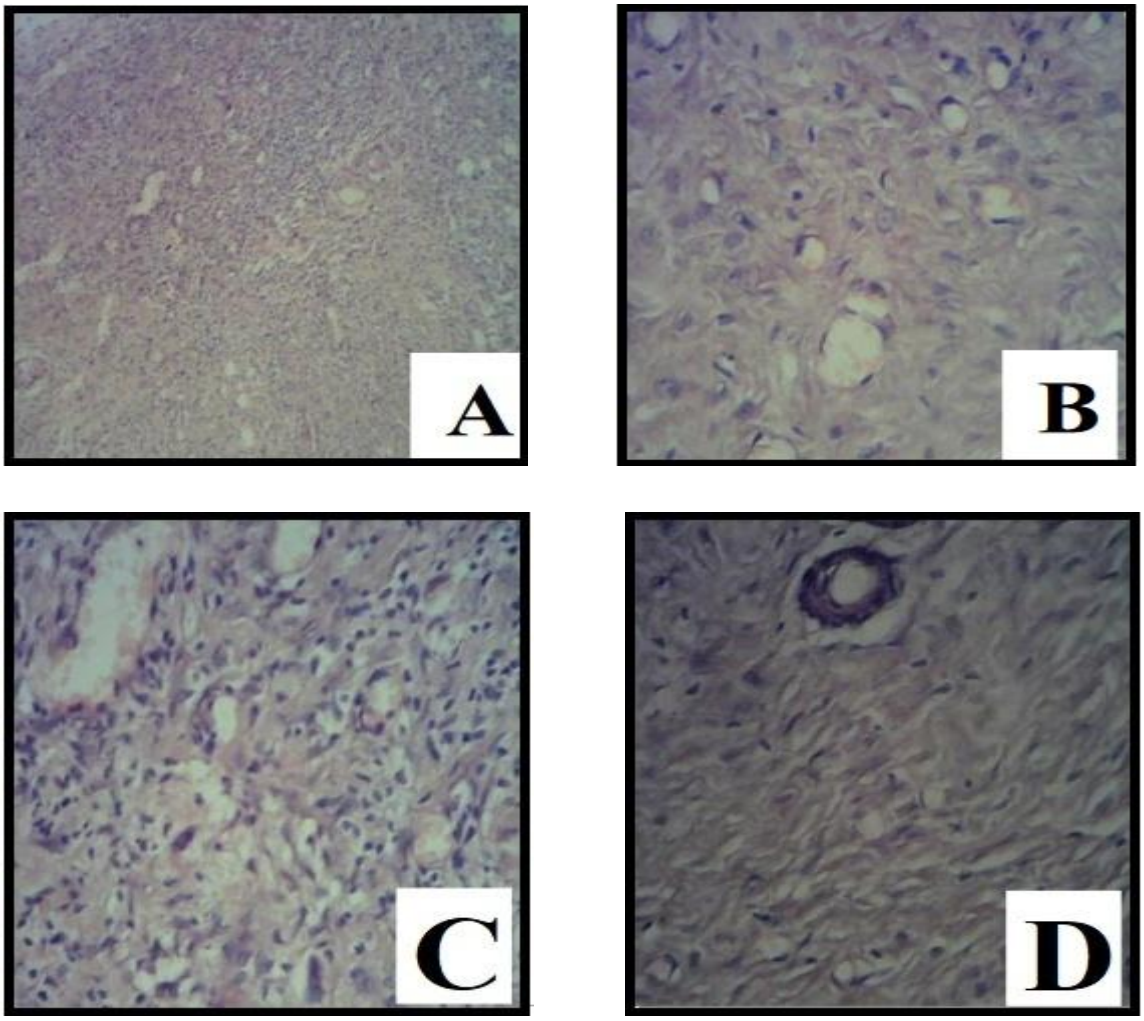
After the study period, a specimen sample of skin tissues of each group of rats were taken out from the healed wounds of the animals in the above excision wound model for histopathological examinations. The thin sections of the tissues were stained with hematoxylin and eosin solution and observed for the histological changes under microscope (Sharma S *et al*., 2007).

Results

The results of wound healing activity by excision wound model were presented in Table 1 and 2. It was observed that the wound contracting ability of the extract ointment in both concentrations were significantly greater than the control (i.e. simple ointment treated group). The wound contracting ability of animals treated with ointment containing 5% (w/w) Methanolic extract was found to be highly significant (P <0.001) on day 20 (99±1.35%) as compared to the control group (83.08±1.41%). Treatment with MBCHD produced significant (P<0.001) reduction in the period of epithelization (15.5 days).The results are presented as mean weight in gram ± SEM, the measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 3. The tensile strength of the extract ointment (MBCLD 2% w/w, MBCHD 5% w/w) treated groups showed maximum significant P<0.001 breaking strength (679.74±10.19, 794.29±10.39) and the Povidone Iodine ointment (5% w/w) treated group (653.89±10.52) showed significant higher breaking strength, P<0.001 compared with control group. Thus both the concentrations of the extract i.e MBCLD 2% w/w, MBCHD 5% w/w as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.

Histopathology

The multiple sections studied in histopathological examination of the tissues of the wound area treated with the extract ointments (2% and 5% w/w), (5% w/w) Povidone Iodine ointment, and simple ointment (control) in excision wound models are shown in Fig. 1.The control group showed mild leukocyte infiltration, fibroplasia, neovascularization and minimial collagen deposition. Scar formation and epithelial regeneration were not observed in control group indicating no wound healing. The tissue architecture in group treated with MBCHD 2% and Betadine ointment showed mild leukocyte infiltration, collagen deposition, moderate fibroplasia, moderate neovascularization and mild epithelial regeneration. There was mild infiltration of inflammatory cells and collagen deposition, severe fibroplasias, Prominant angiogenesis and moderate epithelial regeneration in rats treated with MBCHD 5% indicating enhanced wound healing process. Scar formation was well developed in groups treated with MBCLD 5%. The histological sections of the healed tissues showed that tissue regeneration is much greater in MBCLD 5%, MBCHD 2% and standard Betadine treated groups when compared to control. MBCLD 5%, MBCHD 2% and standard Betadine treated groups also revealed enhanced epithelialization, fibroplasia and neovasculari-zation indicating faster healing process.



A. Control. (Skin underlying granulation tissue),
B. Wound treated with Std. Betadine. (Granulation tissue and collagen),
C. Wound treated with MBCLD 2% Ointment. (Scar, Collagen),
D. Wound treated with MBCHD 5% Ointment. (Scar, Fibrocollagenous Tissue)

Figure 1. Plate showing slides of hematoxylin and eosin (HE) staining of the histopathological evaluation of healed tissue of animals used in excision wound model

Excision Wound Model

Table 1: Effect of Methanolic leaves extracts of *Bombax Ceiba* on wound contraction of excision wound.

Groups	Treatment	2nd day	4th day	8th day	10th day	12th day	14th day	18th day	20th day
Group1	Control (simple ointment)	32.5 ± 0.0298	37.6 ± 0.03688	43.7± 0.1917	50.1± 0.4944	56.3 ± 0.03688	62.1 ± 0.1917	76.2 ± 0.6607	83.8 ± 0.0368
Group2	Standard (Betadine ointment 5% w/w)	36.2 ± 1.335***	43.3 ± 2.119***	50.2± 3.313***	57.3 ± 0.333***	64.7± 3.313***	72.1± 0.6614***	88.5± 3.313**	96.9 ± 0.3651***
Group3	MBCLD (2% w/w)	46.3 ± 1.187**	48.5 ± 3.313***	53.7 ± 1.335***	60.1± 1.922**	66.8± 2.119***	73.8 ± 1.335***	82.5± 2.119***	97± 3.204**
Group4	MBCHD (5% w/w)	60.1 ± 0.1917**	66.6 ± 0.6614***	72.4± 0.0298***	76.6 ± 0.6607**	79.9 ± 1.335***	82.4± 2.119***	86.7± 1.187**	99.2± 0.4216***

n = 6 animals in each group. The treated groups are compared by Unpaired Student t test with the control group.
*** $P < 0.001$,** $P < 0.01$, * $P < 0.05$

Table 2: Sum of Period of Epithelialization of ointment containing Methanolic leaves extract of Bombaxceiba.

Group	Treatment	Period of Epithelialization
Group 1	Control (Simple Ointment)	21.56 ± 0.2449
Group 2	Standard (Betadine Ointment 5%)	17.67 ± 0.2236***
Group 3	MBCLD 2%	17.01 ± 0.5099**
Group 4	MBCHD 5%	15.5 ± 0.836***

n = 6 animals in each group. The treated groups are compared by Unpaired Student t test with the control group.
*** $P < 0.001$,** $P < 0.01$, * $P < 0.05$

Incision Wound Model

Table 3: Effect of topical application of ointment contains Methanolic extract of *Bombax Ceiba* on incision wound model in rats.

Groups	Treatment	Dose	Tensile strength (g) Mean weight in gram ±S.E.M
Group 1	Control (Simple ointment)		347.24±10.71
Group 2	Standard Betadine ointment (Povidone iodine)	5%w/w	653.89±10.52***
Group 3	MBCLD	2%w/w	679.74±10.19***
Group 4	MBCHD	5% w/w	794.29±10.39***

n = 6 animals in each group. The treated groups are compared by Unpaired Student t test with the control group.
*** $P < 0.001$,** $P < 0.01$, * $P < 0.05$

Discussion

In the present paper we report the wound healing potential of the Methanolic extract of the leaves of Bombaxceiba on Incison and Excision wound models. The extract of Bombax ceiba showed antimicrobial activity (Digge V.G et al 2015), analgesic and anti-inflammatory effects (DAR A et al., 2005, Anandarajagopal K et al.,2013). So, if any plant material presents antimicrobial, analgesic and anti-inflammatory activities together, it can be supposed that this material also may help to promote wound healing and contribute skin regeneration.

We Observed that, In Incision wound model the animals treated with ointment containing MBCLD 2% (w/w) and MBCHD 5% (w/w) extracts showed significantly higher (P<0.001) (679.74±10.19, 794.29±10.39) tensile strength when compared to the control group. Thus both the concentrations of extracts and standard showed a significant increase in tensile strength in 10 days old wound. Tensile strength is one of the most important factors in wound healing, and is a valuable measure that reflects the sub dermal organization of the collagen fibers in the newly deposited collagen (Jimenez PA et al.,1999).The increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibers (Suguna L et al.,2006).In excision wound model, MBCHD (5% w/w) showed significant (P<0.001) percentage of wound contraction (99±1.35%) on 20th day and significant (P<0.001) reduction in period of epithelization (15.5 ± 0.836). In excision wound models significant wound healing activity was observed in the group of animals treated with MBCLD (2% w/w) and MBCHD (5% w/w) when compared with control animals. The wound contraction is mediated by specialized myofibroblasts found in the granulated tissue (Moulin et al. 2000). So, the increase in wound contraction in MBCLD 2% and MBCHD 5% extract might be a result of the enhanced activity of fibroblasts. Histopathological studies in the

control group showed mild leukocyte infiltration, fibroplasia, neovascularization and minimial collagen deposition. Scar formation and epithelial regeneration were not observed in control group indicating no wound healing where as group treated with MBCHD 2% and Betadine ointment showed mild leukocyte infiltration, collagen deposition, moderate fibroplasia, moderate neovascularization and mild epithelial regeneration. Whereas group treated with MBCHD 5% showed mild infiltration of inflammatory cells and collagen deposition, severe fibroplasias, Prominant angiogenesis, well developed scar and moderate epithelial regeneration in rats indicating enhanced wound healing process compared to control.

Conclusion

Methanolic extracts of *Bombax ceiba* at high and low dose possesses significant wound healing activity.

Bombax ceiba can be further developed as a drug formulation as its toxicity is very low and showed better pharmacological activity in both Incision and Excision Wound models.

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