

Experimental evaluation of *Albizia lebbbeck* Benth. stem bark for wound healing activity in mice

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(Received: August 13, 2010; Accepted: September 21, 2010)

ABSTRACT

The present study was aimed to evaluate the wound healing activity of extract of bark part of *Albizia lebbbeck*. It is well-known plant in Indian traditional medicines. On the basis of traditional use and literature references, this plant was selected for wound healing potential. The methanolic extract of bark parts of *Albizia lebbbeck* was examined for wound healing activity in the form of ointment in three types of wound models on mice: the excision, the incision and dead space wound model. The extract ointments showed considerable response in all the above said wound models as comparable to those of a standard drug Betadine ointment in terms of wound contracting ability, wound closure time, tensile strength and dry granuloma weight. Histological analysis was also consistent with the proposal that *Albizia lebbbeck* bark extract exhibits significant wound healing potential.

Key words: *Albizia lebbbeck*, Wound healing activity, Betadine, Methanolic extract.

INTRODUCTION

The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistries found in plants for their therapeutic properties. Each drug extracted from the plants is substance with a particular therapeutic action¹. The usage of herbal plants as traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effects².

Albizia lebbbeck Benth. (Bengali name: *Shirish, Koro*; Family: Leguminosae) is a deciduous tree with compound leaves, flat oblong fruits, round cream colored seeds, grows wild and planted in almost all districts of Bangladesh³. The plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa. Bark is pale with glabrous young shoot. The bark is bitter, cooling, alexiteric, anthelmintic, cures "vata", diseases of the blood, leucoderma, itching, skin disease, excessive

perspiration, inflammation, and good in rat bite. The bark is good for ophthalmia. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea⁴. Ethanolic extract of pods possesses antiprotozoal, hypoglycemic and anticancer properties. The methanolic extract of the pod was investigated for antifertility activity⁵⁻⁶. The plant extracts also evaluated in allergic rhinitis⁷, memory and learning of mice⁸, analgesic and anti-inflammatory⁹. All parts of the plant are recommended for the treatment of snake-bite¹⁰. It is reported to possess anxiolytic¹¹, anticonvulsant¹²⁻¹³, antifertility¹⁴ and antidiarrhoeal activities¹⁵. Research on plants with medicinal properties and identification of the chemical components responsible for their activities have corroborated the traditional uses of ancient healing wisdom and lore and have proven the enduring healing potential of many plant medicines even in today's hi-tech community.

Normal wound healing response begins the moment the tissue is injured. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated¹⁶. Impaired wound healing may cause severe health-related complications, such as infections and tissue necrosis. These ailments have spurred the search for wound healing agents derived from ethno medicinal sources¹⁷.

Current methods used to treat wounds include debridement, irrigation, antibiotics, tissue grafts, proteolytic enzymes, and corticosteroids, which possess major drawbacks and unwanted side effects. Therefore, the development of potent wound healer drugs with fewer side effects is necessary. Although *Albizia lebbbeck* bark has traditionally been used in the treatment of many types of skin disease and inflammatory conditions in India., in villages people use plant extract to treat their wounds without any experimental knowledge. Literature search revealed that there is no experimental basis to prove the wound healing activity of *Albizia lebbbeck* bark. Therefore, this study has been undertaken to assess the wound healing activity of methanolic extract of *Albizia lebbbeck* bark in mice.

MATERIAL AND METHODS

Plant material

The stem bark of *Albizia lebbbeck* was collected from mature trees and its botanical identification was confirmed from National Botanical Research Institute Lucknow. A voucher specimen NBRI/CIF/Re./08/2008/32 was deposited in the herbarium of NBRI Lucknow. The plant material was dried in shade, powdered and sieved through 40-mesh size and material was stored in well-closed container.

Extraction and preparation of formulation

Bark of *Albizia lebbbeck* Benth was finely pulverized (100 g) and extracted in Soxhlet apparatus for 24 h with methanol then concentrated and dried under reduced pressure. The extract was weighed and the yield obtained (13.70% w/w). The semisolid mass (dark brown colour) was obtained

and used as ingredient for 5% ointment preparation. About 5 g of semisolid extract was incorporated into the 100 g of simple ointment base B.P. Simple ointment base was used as control group. Extract ointment was used ones daily to treat different groups of animals.

Animals

Healthy Albino mice of either sex (35–45 g) with no prior drug treatment were used for all the present *in-vivo* studies. The animals were fed on a commercial pellet diet (Hindustan Lever, Bangalore, India), and water ad libitum. The animals were acclimatized to laboratory hygienic conditions for 10 days before starting the experiment. Animal study was performed with due permission from institutional animal ethical committee.

Wound healing activity

Grouping of animals

Screening for wound healing activity was performed for incision, excision and dead space wound model. 54 animals of either sex weighed between 35 and 40 g were divided into three groups based on treatment type in each model consisting of six animals as follows: group I – simple ointment base; group II – 5% Betadine (Win-Medicare, India) ointment and group III – 5% methanolic extract ointment of *Albizia lebbbeck*. The hairs on the skin of white surface of animals were removed by using a suitable depilatory (Anne-French hair removing cream).

Excision wound model

Circular wounds of approximately 10mm diameter were inflicted on the cleared skin by cutting under ether anesthesia. The areas of the wounds were measured (sq. mm) immediately by using vernier calipers. This was taken as the initial wound area reading. Group-I animals served as control (negative control), which received ointment I.P. Group-II served as standard (positive control) to which Betadine (5% w/w in ointment I.P.) was applied topically. Group-III animals served as test, which were treated with the extract (5% w/w) in a similar manner. All the test samples were applied once daily. The wound area of each animal was measured on 1st, 4th, 7th, 10th, and 13th post wounding day (Table 1). The wound closure was measured at regular intervals of time to see the percentage of

wound closure and epithelialization time that indicates the formation of new epithelial tissue to cover the wound. The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization.

Incision wound model

All animals were anaesthetized before wound creation and paravertebral long incisions were made through the skin at the distance of about 1.5 cm from midline on the depilated back of mice. No local or systemic antimicrobials were used throughout the experiment. All groups were treated same as in excision model, the both edges kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then simple ointment base, extract ointment and standard ointment were applied daily up to 7 days; when wounds were cured thoroughly the sutures were removed on the day 8th and tensile strength¹⁸, of cured wound skin was measured using tensiometer (Table 2). The skin breaking strength is expressed as the minimum weight (in grams) of water necessary to bring about the gapping of the wound.

Dead space wound model

This model was used for the study of granuloma tissue. Animals were anaesthetized by ether anesthesia and wound was made by implantation of cotton pellets (10 mg), (2.0×0.5), one on side, in the lumbar region on the dorsal surface in each animal. On the 8th post-wounding day, granuloma tissue formed on implanted cotton pellets was dissected out carefully. Granuloma tissue from one part was dried (60°C) and weighted, while the other part of granuloma tissue¹⁹⁻²⁰ was used for determination of tensile strength (Table 3).

Histopathological Studies

Wound tissue specimens from control, test and standard groups were taken after complete healing of excision wound. After usual processing, 6-mm thick sections were cut and 10% of neutral formalin solution was used to fix the granulation tissues for 24 h and dehydrated with a sequence of ethanol- xylene series of solutions. The inflicted

materials were embedded with paraffin at 40-60 °c. Microtome sections were taken at 10µm thicknesses. The processed sections were stained with haematoxylin eosin²¹⁻²² and observed under microscope.

Wound healing evaluation parameters

Wound contraction and epithelialization time

An excision wound margin was traced after wound creation by using transparent paper and area measured by vernier calipers. Wound contraction was measured in each 3 days interval, until complete wound healing and expressed in percentage of healed wound area. The epithelialization time²³ was measured from initial day.

Measurement of tensile strength

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. Sutures were removed on the day 8 after wound creation 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, which is based on method of Kuwano²⁴. In this method wound breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen.

Dry granulation weight

From the collected tissue 8th day skin sample of dead space wound (granulomas) was excised from cotton pellets and granuloma mass was dried and weighed, while the other part of granuloma tissue was used for determination of tensile strength.

Histopathological studies

The healing tissues obtained on the 13th day from all three groups of animals of the excision wound model were processed for histological study. Sections were qualitatively assessed under the light microscope and observed in respect of fibroblast proliferation, collagen formation, epithelialization and blood vessels.

Statistical analysis

Treated group was compared with the

control group. The results were analyzed statistically using Student's t-test to identify the differences between the treated and control. The data were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The percentage wound contraction²⁵ was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

Wound area was measured by tracing the wound margin using vernier calipers in each 3 days interval and healed area was calculated by subtracting from the original wound area. On day 7, the wound contraction of standard and test were found to be significant ($P < 0.05$) in comparison to control group. On day 10, wound was completely

healed for standard group while for test group it was also almost at complete healing stage. On day 13, test group healed 97.6% and control group showed 73.8% healing. It was also observed that epithelialization period of test and standard group were less in comparison to control group (Table 1). The time required for complete epithelialization of the excision wound is an important parameter to assess the wound healing process.

The studies on excision wound healing model reveal that all the three groups showed day to day decrease in wound area. However, on 13th post wounding day, control animals group-I showed 26.2 ± 1.68 of unhealed wound area whereas group-II standard group animals, showed that of $.04 \pm 0.02$ and the test group-III exhibited that of 2.4 ± 0.68 wound area. When compared with the control, the activity of extract was found to be highly significant ($P < 0.05$).

Table 1: Effect of Methanolic Extract Ointment of *Albizia lebbek* Benth on Excision Wound Model

S. N.	Treatment	Period of epithelizaion	% wound contraction, post wounding day (Mean \pm SD, n=6)				% Un-healed Wound area mm ² , 13 th post wounding day 13 th Day Mean \pm SD
			4 th Day Mean \pm SD	7 th Day Mean \pm SD	10 th Day Mean \pm SD	13 th Day Mean \pm SD	
1	Control	22 nd days	26.55 \pm 1.62	31.38 \pm 1.04	62.49 \pm 0.61	73.8 \pm 1.68	26.2 \pm 1.68
2	Standard	11 th days	47.8 \pm 1.83	73.28 \pm 0.75	97.48 \pm 0.63	99.6 \pm 0.02**	.04 \pm 0.02
3	Test (Extract treated)	13 th days	41.7 \pm 0.73	55.34 \pm 0.86	86.88 \pm 0.75	97.6 \pm 0.68**	2.4 \pm 0.68

$P < 0.05$, ** $P < 0.001$ vs. control.

Table 2: Effect of Methanolic Extract Ointment of *Albizia lebbek* Benth on Incision Wound Model

S. NO.	Treatment	No. of Animals	Tensile strength in gm/cm on 8 th post wounding day (Mean \pm SD, n=6)
1	Control	6	169.62 \pm 2.71
2	Standard	6	304.87 \pm 1.25*
3	Test (Extract treated)	6	254.87 \pm 1.54*

$P < 0.05$ vs. control.

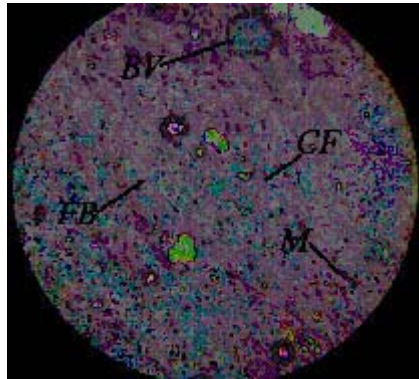


Fig. 1A: Histological section of the granuloma tissues of Control animal showing incomplete healing with less epithelialization arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate incomplete healing of wound

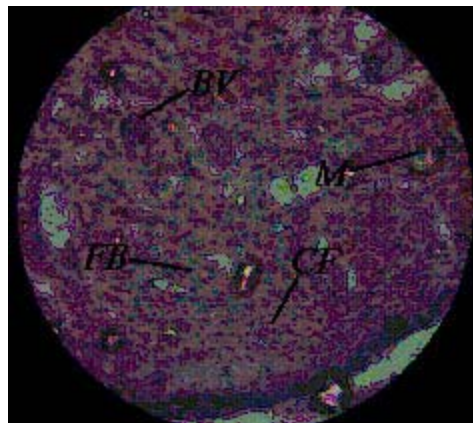


Fig. 1B. Histological section of the granuloma tissues of Standard Treated animal showing complete healing with enhanced epithelialization arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate complete healing of wound

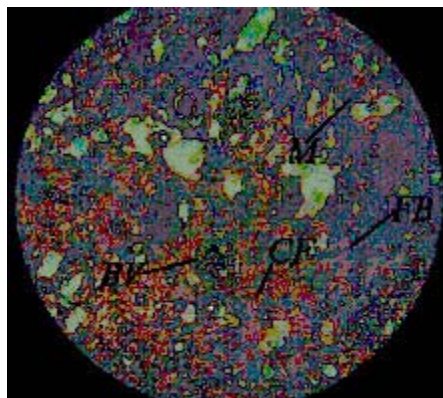


Fig. 1C: Histological section of the granuloma tissues of Methanol Extract Treated animal showing complete healing with moderate epithelialization and collagenation arrow showing Macrophage (M), Blood Vessels (BV) and Collagen formation (CF) indicate complete healing of wound

The promotion of wound healing activity is also well gazed by its tensile strength of the incision wound. Generally wound-healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibers. The tensile strength of the test group was found to be (254.87 ± 1.54) which was higher than that of control group (169.62 ± 2.71) of animals and slightly lesser than that of standard group (304.87 ± 1.25) of animals on 8th post wounding day.

The effect of topical administration of the test group and control treated group on dead space

wound model was assessed by increase in the weight of granulation tissue and increase tensile strength. The data is depicted in table-3. This indicates enhanced collagen maturation by increased cross-linking of collagen fibers. The increased weight of the granulation tissue also indicated the presence of higher protein content. Among these treated animals the response was shown to be the best in test animals.

Histology of the wound tissue of the control animals showed the presence of acute inflammatory cells, fibroblastic connective tissue and very little number of blood vessels (Fig. 1A). The lesser

Table 3: Effect of Methanolic Extract Ointment of *Albizia lebbbeck* Benth on Dead Space Wound Model

S. No.	Treatment	No. of Animals	Granulation Tissues dry-weight, mg/100gm (Mean \pm SD, n=6)	Tensile strength in gm/cm on 8 th post wounding day (Mean \pm SD, n=6)
1	Control	6	17.12 \pm 0.85	159.12 \pm 1.75
2	Standard	6	43.62 \pm 0.47*	317.37 \pm 2.13*
3	Test (Extract treated)	6	34.12 \pm 0.85*	284.37 \pm 1.10*

P<0.05 vs. control.

epithelialization and lesser collagen formation indicated incomplete healing of the wound in control animals. Where as, in the sections of standard treated group animals (Fig. 1B) increased collagen deposition was observed. The sections of the granuloma tissue of the animals treated with methanolic extract showed moderate epithelialization, fibrosis, collagen formation and increased number of blood vessels (Fig. 1C)

CONCLUSIONS

The study has showed that the methanolic extract ointment of *Albizia lebbbeck* effectively stimulates wound contraction; increase tensile strength of incision and dead space wounds as compared to control group. This was demonstrated by a significant increase in the rate of wound

contraction and by enhanced epithelization. Significant increase (P<0.05) in tensile strength, collagen levels and weight of granulomas were observed, which were further supported by histopathological studies and gain in granuloma breaking strength. This indicated improved collagen maturation by increased cross-linking while an increase in dry granuloma weight indicated accumulation of higher protein content. These finding could justify the pronounced wound healing activity of this plant.

ACKNOWLEDGEMENTS

The authors are thankful to Bhopal Institute of Technology & Science-Pharmacy, Bhojpur Road, Bangrasia, Bhopal for providing facilities to carry out the research work.

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