

ORIGINAL RESEARCH ARTICLE

Evaluation of Wound Healing Activity of Stem Bark of *Albizzia lebeck***Rajesh Kumar Upadhyay^{*}, Neelam Balekar, Dinesh Kumar Jain, Neetesh Kumar Jain**

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ABSTRACT

Introduction: *Albizzia lebeck*, commonly known as siris, is used in folklore medicine in skin diseases, disease of blood and other different diseases. There was no scientific evidence justifying the use of *Albizzia lebeck*, therefore the present study was aimed at evaluation of wound healing activity of the plant.

Materials and methods: In the present study the bark of *Albizzia lebeck* was studied for wound healing activity by incorporating the aqueous and ethanolic extract (5% and 10% w/v in normal saline was applied topically and 250mg/kg and 500mg/kg doses was given orally). Wound healing activity was studied in three types of model in Wistar rats viz. excision, incision and dead space wound model. In case of the excision wound model wound contraction and period of epithelization was studied and incision wound model was evaluated by determining tensile strength while dead space wound model were evaluated by determining weight of granuloma and hydroxyproline content.

Results: Treatment of wound with ethanolic extract 10% w/v in normal saline topically exhibited significant ($P < 0.001$) wound healing activity in all three models. The ethanolic and aqueous extracts were quantitatively analyzed and total alkaloids, total flavonoids and total phenols was found to be greater in ethanolic than aqueous extract.

Conclusion: The ethanolic extract exhibited better wound healing activity probably due to alkaloids, phenols and flavonoids constituents.

Key words: *Albizzia lebeck*, Excision wound, Incision wound, dead space wound, Hydroxyproline content.

INTRODUCTION

Wound is a clinical entity and is as old as mankind, often possesses problem in clinical practice. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover that healing agents and keep up pace with problems encountered^[1].

Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns^[2].

Albizzia lebeck (Mimosacea), commonly known as siris has been reported to contains, d-catechin, friedelin, leucanthocyanidin, tannins, albizzia saponin A, B, C, anthones, phenolic glycoside and procyanidin^[3,4].

The plant is well known for its anti-microbial^[5], anti-inflammatory and analgesic^[6], anti-allergic activity^[7], *in vitro* antibacterial effect^[8] and anti-

microbial^[9]. However, the literature survey revealed that no systematic study had been carried out on wound healing activity. Hence, in the present study, an effort was made to establish wound healing potential of the plant using animal models.

MATERIALS AND METHODS**Materials:**

The stem bark of *Albizzia lebeck* was procured from local garden of Indore M.P. Authentication of plant on basis of pharmacognostic study and organoleptic characteristics was done by Botanical survey of India Pune. A voucher specimen no. (RAJUPALBL3) of stem bark of *Albizzia lebeck* has been deposited in museum of Department of Botany, Botanical Survey of India.

Preparation of aqueous and ethanolic extract:

The dried coarsely powdered bark was extracted with petroleum ether (60–80 °C) to remove fatty matters. For aqueous extract the defatted marc were subjected to hot decoction. The solvent was filtered through muslin cloth. The total aqueous extract was concentrated using rotary evaporator. For the preparation of ethanolic defatted marc was subjected to Soxhlet extraction with 90% ethanol. The total ethanolic extract was concentrated using rotary evaporator [10].

Phytochemical analysis:

The aqueous and ethanolic extracts were tested qualitatively for presence of different phytoconstituents using various phytochemical tests and screened quantitatively for content of total alkaloid [11], total phenols by Folin-Ciocalteu method [12], and total flavanoid content [13,14].

Animals:

The Wistar rats of either sex weighing 150–200 g were used. Animals were housed under standard condition of temperature (25 ± 2 °C), 12h/12h light/dark cycle and fed with standard pellet diet (Godrej Agrowet Ltd. Indore, India) and water *ad libitum*. The animals were allowed to acclimatize for one week before the experiments. All experimental protocols were approved by the IAEC under the supervision of CPCSEA.

Acute oral toxicity:

The acute oral toxicity testing of the ethanolic and the aqueous extract was carried out by using AOT 425.

Wound healing activity:

Treatment schedule for excision wound model, Wistar rats were divided into several groups as follows;

- Group I : Topical control (saline 0.9%)
- Group II : Oral control (0.5% Na-CMC)
- Group III : Standard I (Povidone iodine ointment, topically)
- Group IV : Standard II (Turmeric powder 0.5 g/cm², topically)
- Group V : Standard III (Ascorbic acid 500 mg/kg, p.o.)
- Group VI-VII : ALAE (250 and 500 mg/kg, p.o.)
- Group VIII-IX : ALEE (250 and 500 mg/kg, p.o.)
- Group X-XI : ALAE (5% and 10%, topical)
- Group XII-XIII : ALEE (5% and 10%, topical)

Treatment schedule for incision wound models, Wistar rats were divided into several groups as follows;

- Group I : Topical control (saline 0.9%)
- Group II : Standard (Povidone iodine ointment, topically)
- Group III : ALEE (5%, topical)
- Group IV : ALEE (10%, topical)

Treatment schedule for dead space wound models, Wistar rats were divided into several groups as follows;

- Group I : Topical control (saline 0.9%)
- Group II : Standard (Povidone iodine ointment, topically)
- Group III : ALEE (5%, topical)
- Group IV : ALEE (10%, topical)

Excision wound model:

The rats were anesthetized by administering ketamine-xylazine in combination (ketamine 60 mg/kg and xylazine 5mg/kg i.p.). A full thickness of the excision wound of circular area (approximately 600mm²) was made on the shaved back of the rats 30 minutes later the administration of ketamine-xylazine injection. The wounding day was considered as day 0 (**Figure 1**). The treatment was started as per treatment schedule for excision wound model. The wounds were monitored and the area of wound was measured on 0, 3, 6, 9, 12 and 15th post-wounding days (**Figure 2**). The period of epithelization was calculated as the number of days required for falling of the dead tissue without any residual raw wound [15,16].

Wound healing rate [17]

$$\% \text{ of wound closure} = \frac{\text{Wound area on day 0} - \text{Wound area on day } n}{\text{Wound area on day 0}} \times 100$$

Where n = number of days 3rd, 6th, 9th, 12th and 15th day.



Figure 1: A circular excision wound on the day 0



Figure 2: A completely healed excision wound after 19 day treatment

Incision wound model:

The rats were anesthetized by administering ketamine-xylazine in combination. Incision wounds of about 6 cm in length and about 2 mm in depth were made with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine-xylazine injection. The parted skin was kept together and stitched with sterilized curved needle and black silk at 0.5 cm intervals. The wounds of animals in the different groups were treated as per treatment schedule for incision wound model, for the period of 10 days. The wounding day was considered as day 0 (Figure 3). When wounds were cured thoroughly, the sutures were removed on the 9th post-wounding day (Figure 4) 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 11th day^[18].

Tensile strength was calculated using the following formula^[19].

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$



Figure 3: Incision wound on the day 0



Figure 4: A completely healed incision wound after day 9

Dead Space Wound Model:

Dead space wounds were created by subcutaneous implantation of sterilized cotton piths of 10mg on the right side groin and axilla on the ventral surface of each rat. Treatment was given as per described in dead space wound model. The granulation tissues formed on the cotton piths were excised carefully on the 10th post wounding day under light ether anesthesia. The tissue was dried overnight at 60 °C and the dry granulation tissue weight was recorded on 11th day^[16,20]. The samples were prepared and the absorbance of the solutions was determined spectrophotometrically at 557 nm. The hydroxyproline values may be determined directly from the standard curve^[21].

Statistical Analysis:

Data were expressed as mean \pm standard error of mean and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Tukey's-Kramer multiple comparison test. P <0.05 were considered significant.

RESULTS

Phytochemical analysis:

The preliminary phytochemical screening of plant extract revealed the presence of tannins, alkaloids, flavonoids, saponins, terpenoids, phytosterols and volatile oils. Quantitative tests indicated that the stem bark ethanolic extract is highly rich in flavonoids, tannins, lipid and alkaloids (Table 1).

Excision wound study:

The wound healing contracting ability of animals treated with ethanolic extract 5% and 10% topically was found to be significantly higher (P<0.001) on day 9, 12 and 15 as compared to the control. The epithelization period was also found to be least i.e. 18.2 and 16.2 days in case of animals treated with ethanolic extract 5% and 10% respectively. Povidone iodine (5% w/w) also showed significant effect (P<0.001) as compared

with control which is found to be equivalent to the animals treated with 10% topically (Table 2).

All other treated groups i.e. ALAE (250 and 500 mg/kg p.o.), ALEE (250 and 500 mg/kg p.o.) and ALAE (5% and 10% topical) were found non significant when compared with control (Table 2).

Incision wound study: The tensile strength of the incision wounds were found to be significantly ($P < 0.001$), ALEE-T 5% (507.9 ± 12.20) and ALEE-T 10% (565.6 ± 9.70) against control (399.5 ± 11.0) (Table 3).

Dead space wound study: In dead space wound study the groups treated with 5% and 10% topical,

significantly increased weight of granuloma by 50.20 mg and 61.34 mg respectively against control 30.89 mg. The L-hydroxyproline content was also found to be significantly increased in group treated with 5% and 10% ($P < 0.001$) as compared to control (Table 3).

Table 1: Quantitative determination of phytoconstituent in aqueous and ethanolic extract

S. No	Phytoconstituents	ALAE	ALEE
1	Total alkaloids (g %)	2.14	2.48
2	Total flavonoid content (mg quercetin equivalents/g of extract)	8.15	12.15
3	Total phenolic content (mg tannic acid equivalents/g of extract)	15.08	24.56

Table 2: Effect of ethanolic and aqueous extracts of *Albizia lebbek* bark on wound contraction of excision wound

Treatment & Doses	Percentage wound contraction (mean \pm SEM)					Period of epithelization (days)
	Day3	Day6	Day9	Day12	Day15	
Control -T	09.51 \pm 5.87	22.26 \pm 7.60	33.61 \pm 5.11	52.71 \pm 5.16	67.85 \pm 3.50	25.4 \pm 0.60
Control -O	11.27 \pm 1.23	26.79 \pm 4.93	48.95 \pm 4.19	65.62 \pm 4.39	71.07 \pm 3.63	24.2 \pm 0.37
Std.-I (Povidone-iodine)	16.86 \pm 2.0	45.45 \pm 2.0 ^a	80.80 \pm 1.12 ^c	94.11 \pm 0.39 ^c	99.95 \pm 0.12 ^c	15.2 \pm 0.30 ^c
Std.-II (Turmeric powder)	13.63 \pm 2.12	34.62 \pm 3.41	70.54 \pm 2.37 ^c	88.76 \pm 1.80 ^c	98.60 \pm 4.30 ^c	16.8 \pm 0.58 ^c
Std.-III (Ascorbic acid)	13.20 \pm 2.71	44.43 \pm 3.45	71.71 \pm 2.09 ^b	83.47 \pm 1.8 ^c	91.89 \pm 1.13 ^c	18.2 \pm 0.58 ^c
ALAE-O (250mg/kg)	09.40 \pm 1.10	20.68 \pm 2.70	31.33 \pm 3.25	49.19 \pm 1.55	71.02 \pm 2.55	23.2 \pm 0.58
ALAE-O (500mg/kg)	12.75 \pm 2.48	31.16 \pm 4.60	37.44 \pm 3.86	62.80 \pm 6.15	70.11 \pm 4.59	21.8 \pm 0.37 ^a
ALEE-O (250mg/kg)	12.06 \pm 0.82	25.87 \pm 6.10	45.96 \pm 3.91	60.57 \pm 7.64	85.17 \pm 2.54 ^a	21.0 \pm 0.44 ^c
ALEE-O (500mg/kg)	10.97 \pm 3.61	24.86 \pm 3.14	45.94 \pm 3.26	61.45 \pm 5.09	74.77 \pm 3.80	21.2 \pm 0.3 ^a
ALAE-T (5% w/v in saline)	13.05 \pm 1.72	25.06 \pm 1.76	34.80 \pm 1.15	47.98 \pm 2.54	64.09 \pm 3.65	22.8 \pm 0.37 ^a
ALAE-T (10% w/v in saline)	13.56 \pm 3.71	28.38 \pm 3.70	42.21 \pm 4.35	56.30 \pm 3.37	70.03 \pm 1.49	19.8 \pm 0.37 ^a
ALEE-T (5% w/v in saline)	14.80 \pm 5.40	44.16 \pm 3.7 ^a	70.06 \pm 5.80 ^c	84.62 \pm 1.36 ^c	90.77 \pm 0.94 ^c	18.2 \pm 0.58 ^c
ALEE-T (10% w/v in saline)	15.11 \pm 1.82	44.58 \pm 2.28 ^a	79.00 \pm 5.89 ^c	91.98 \pm 2.9 ^c	98.42 \pm 2.60 ^c	16.2 \pm 0.54 ^c

ALEE = *Albizia lebbek* ethanolic extract, ALAE = *Albizia lebbek* aqueous extract, T = topical, O = Oral, All data are reported as mean \pm SEM (n=5) ^ap<0.05, ^bp<0.01, ^cp<0.001, when compared to control

Table 3: Effect of ethanolic extract of *Albizia lebbek* bark on incision wound and dead space wound model

Group	Treatments (Topically)	Incision wound	Dead space wound	
		Tensile strength on 10 th day (g)	Granuloma wt. (mg/100g)	Hydrpxyproline content (μ g/ml)
I	Control-T	399.5 \pm 11.0	30.89 \pm 0.85	1.52 \pm 0.083
II	ALEE-T (5% w/v in saline)	507.9 \pm 12.2 ^a	50.20 \pm 3.40 ^a	2.72 \pm 0.24 ^a
III	ALEE-T (10% w/v in saline)	565.6 \pm 9.7 ^a	61.34 \pm 1.39 ^a	5.01 \pm 0.12 ^a
IV	Standard-T (Povidone-Iodine)	608.7 \pm 9.8 ^a	72.10 \pm 3.37 ^a	5.12 \pm 0.21 ^a

ALEE = *Albizia lebbek* ethanolic extract, T = topical,

All data are reported as mean \pm SEM (n=5) ^ap<0.001, when compared to control.

DISCUSSION AND CONCLUSION

Wound healing is a complex and dynamic process of restoring cellular structure and tissue layers in damaged tissue as closely as possible to its normal state. Wound contracture is a process that occurs throughout the healing commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage [22].

Granulation tissue found in the final part of proliferative phase is composed of fibroblast collagen edema and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content. The ethanolic extract of *Albizia lebbek* demonstrated a significant increase in the hydroxyl-proline content of the granulation indicating increased collagen turnover. Collagen

the major component which strengthens and supports extra cellular tissue is composed of the amino acid, hydroxyproline, which has been used as biochemical marker for tissue collagen [23].

The preliminary phytochemical analysis of the alcoholic bark extract of *Albizia lebbek* showed the presence of tannins, alkaloids and flavonoids. Any of the observed phytochemical constituents present in *Albizia lebbek* may be responsible for wound healing activity. Recent activity has shown that phytochemical constituents like flavonoids are known to promote the wound healing process mainly due to their astringent and antimicrobial properties, which appears to be responsible for wound contraction and increased rate of epithelization [24].

The wound healing property of *Albizzia lebbek* may be attributed to the phytoconstituents present in the plant and the quicker process of the wound healing could be function of either individual or additive effect of phytoconstituents^[25].

Topical application of ethanolic extract of *Albizzia lebbek* increased the tensile strength of incision wound may be due to increase in collagen concentration and stabilization of the fibres^[26]. Wound healing activity of plant extract may also be subsequent to an associated antimicrobial effect^[27,28].

The present study has demonstrated that an ethanolic bark extract of *Albizzia lebbek* has properties that render it capable of promoting accelerated wound healing compared with controls. Wound contraction increased tensile strength, hydroxyproline content antimicrobial activity support further evaluation of *Albizzia lebbek* in the tropical treatment and management of wounds.

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