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# SCREENING OF WOUND HEALING EFFECT OF BARK OF *BARRINGTONIA ASIATICA*

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**ABSTRACT**

*Barringtonia asiatica* is used in folklore medicine in fomenting, sealing of secondary infection, healing of wounds and skin eruptions. There was no scientific evidence justifying the use of bark of *Barringtonia asiatica* , therefore the present study was aimed at evaluation of wound healing activity of the plant. In the present study the bark of *Barringtonia asiatica* were studied for wound healing activity by incorporating extract in simple ointment base B.P. in concentration of 2% (w/w) and 4% (w/w) .Wound healing activity was studied in three types of model in rats viz. excision, incision and burn wound model. The results were also comparable to those of a standard drug, nitrofurazone in terms of wound contracting ability, wound closure time, tensile strength. The statistical data indicated that the wound with ointment containing 4% w/w alcoholic 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 4% w/w. The results of histopathological examination supported the outcome of both excision and burn wound models. The experimental data demonstrated that *Barringtonia asiatica* displayed remarkable wound healing activity.

**Keywords:** *Barringtonia asiatica* ; Alcoholic extract; Excision wound model; Incision wound model; Burn wound model; Nitrofurazone.

## 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 [1]. Wound healing involves a chain of well orchestrated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodeling. India has a rich tradition of plant-based knowledge on healthcare. A large number of plants/plant extracts/decoctions or pastes are equally used by tribals and folklore traditions in India for treatment of cuts, wounds, and burns [2].

*Barringtonia asiatica* (L.) Kurz (Family – Barringtoniaceae) is a tree to 25 m tall with glossy alternate, petiolate, entire bark, obovate, 12-40 cm long, 10-20 cm broad. Flowers are large and showy, petals white, calyx green, with pinkish filaments with yellow anthers. Fruit a large fibrous drupe (up to 12 cm long), shiny green, quadrangular (square in cross section), containing a large single seed. This tree usually forms large spreading branches as well as a large, spreading buttress root system. It is common along the sea shore, edges of mangroves, lowland river margins and coastal forests. It is widespread throughout the tropical Pacific and Indian Oceans

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and widely cultivated in tropical areas. Gallic acid, saponins (including barrinin A1), hydrocyanic acid, monosaccharides, triterpenoids (bartogenic acid, 19-epibartogenic acid, and anhydrobartogenic acid) [3]. Traditional used In the Cook Islands, the seed is grated, mixed with coconut cream and rubbed onto burns and wounds. In Fiji, a decoction of the leaves is used to treat hernia. A decoction of the bark is used to treat constipation and epilepsy. In Samoa, the fruit or bark is used to treat yaws, seed to treat ringworm and the bark is used in treating tuberculosis. In Solomon Islands and Samoa it is used to stun fish [4,5].

## MATERIALS AND METHODS

### *Collection of plant material*

The bark of *Barringtonia asiatica* were collected and authenticated from tirumala hills, Tirupati in Jan, 2011. The voucher specimen has been kept in herbarium in Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal, India.

### *Preparation of plant extract*

The air-dried crude drug was pulverized to obtain coarse powder. The powdered drug was extracted with methanol in a soxhlet extractor. The extract thus obtained was concentrated by recovering the solvent by Rotary Flash Evaporator. The concentrated extract was then evaporated to dryness in vacuum oven at temperature not more than 50ºC. The dried extract was stored at 2–8ºC in refrigerator and kept in tightly stoppered bottle under refrigeration until use for the biological testing and phytochemical screening.

### *Preliminary photochemical screening*

Preliminary phytochemical screening [6,7] revealed the presence of glycosides, proteins, carbohydrates, saponins, phenolic compounds, tannins, gums and mucilages.

### *Animals*

Healthy Wistar Rats between 2-3 months of age and weighing 180-200g were used for the study. The experimental protocol was approved by the Institutional Animal Ethical Committee of Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal, India.

**Group 1:** Simple ointment treated control group
**Group 2:** Animals treated with Standard (Nitrofurazone 0.2% w/w)
**Group 3:** Animals treated with AEBA 2% w/w (2g extract in 100g simple ointment) (Alcoholic extract ointment of *Barringtonia asiatica* low dose 2% w/w)
**Group 4:** Animals treated with AEBA 4% w/w (4g extract in 100g simple ointment) Alcoholic extract ointment of *Barringtonia asiatica* high dose 4% w/w

### *Acute Dermal Toxicity Studies*

This study was carried out on rabbits and rats. The skin of the animal was shaved at three different positions on the dorsal side, each about 500 $mm^2$. The 1st area was kept as control, to which vehicle was applied. 2nd area was applied with AEBA 2% w/w and the 3rd area treated with AEBA 4% w/w. After 4 hr, the skin was observed for signs of inflammation [8].

### *Selection of dose and treatment period*

Two types of ointment formulations with different concentration of the extract were prepared viz. 2% (w/w) ointment, where 2 g of extract was incorporated in 100 g of simple ointment base, 4% (w/w) ointment where, 4g of extracts of the bark were incorporated in 100g of simple ointment base B.P. Nitrofurazone ointment (0.2% w/w) obtained from Smith Kline– Beecham Pharmaceuticals Bangalore, India, was used as standard drug for comparing the wound healing potential of the extract in different animal model.

### *Excision wound model*

The rats were depilated on the back and a predetermined area of 500 $mm^2$ full thickness skin was excised in the dorsal inter scapular region. The drugs were topically applied daily till the complete epithelization starting from the day of operation. The parameters studied were wound closure and time of epithelization. The wounds were traced on $mm^2$ graph paper on the days of 4th, 8th, 12th and 16th. The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelization 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 epithelization [9,10].
Wound closure % = Wound area on day 0 - Wound area on day n/ Wound area on day 0 x 100 where *n* =number of days 4th, 8th, 12th, and 16th day.

### *Incision wound model*

The rats were anesthetized by administering ketamine (0.5 ml/kg 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. 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 back of the rat. All 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 0.5-cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous 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 ninth day and tensile strength was measured with a tensiometer [11,12].

***Tensiometer***

The tensiometer consists of a 6 x 12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board. The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp with 1 cm width was tied on the tip of the other arm by a fishing line in such a way that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with a polyethylene bottle on the other end. The tensile strength of a wound represents the degree of wound healing. Usually wound healing agents promote a gain in tensile strength. The instrument used for measurement 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 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 9 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 [13].The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound. The mean determination of tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of the extract-treated wounds were compared with controls.

***Burn wound model***

Partial thickness burn wounds were inflicted on overnight-starved animals under pentobarbitone (30mg/kg b. w. i.p.) anesthesia by pouring hot molten wax at $80^0$C. The wax was poured on the shaven back of the animal through a cylinder of 300 $mm^2$ circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drugs or vehicle was applied topically as mentioned above [14].

***Statistical analysis***

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

**RESULTS**

The results of wound healing activity by excision wound model were presented in Table 1. It was observed that the wound contracting ability of the extract ointment in both concentrations were significantly greater than that of the control (i.e. simple ointment treated group). The wound contracting ability of animals treated with ointment containing 4% (w/w) alcoholic extract was found to be highly significant ($P < 0.001$) on day 16 as compared to the control group. Treatment with AEBA produced significant ($P<0.001$) reduction in the period of epithelization.

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 2. The tensile strength of the extract ointment (AEBA 2% w/w, AEBA 4% w/w) treated groups showed maximum significant $P<0.001$ breaking strength and the nitrofurazone ointment (0.2% w/w) treated group showed significant higher breaking strength, $P<0.001$ compared with control group. Thus both the concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound.

The results of wound healing activity by burn wound model are presented in Table 3. There was a significant increase in percentage contractibility from day 4 onwards in AEBA treated rats and also on later days the closure rate is much faster when compared with control rats. The wound contracting ability of animals treated with ointment containing (4% w/w) alcoholic extract was found to be significantly higher ($P < 0.001$) on $16^{th}$ day when compared to the control group. A better healing pattern and reduction in period of epithelization was observed in AEBA 4% w/w treated group showed highly significant ($P<0.001$) activity.

**Table 1: Effect of Alcoholic bark extracts of *Barringtonia asiatica* on wound contraction of excision wound**

| Groups | Treatment | Dose | Day 4 | Day 8 | Day 12 | Day 16 | Period of epithelization (Mean % of wound contraction ± S.E.M) |
|---|---|---|---|---|---|---|---|
| Group 1 | Control (simple ointment) | - | 3.82 ± 0.02 | 19.11 ± 0.03 | 42.13 ± 0.14 | 65.12 ± 0.75 | 24.57 ± 0.49 |
| Group 2 | Standard (Nitrofurazone) | 0.2 % w/w | 11.04 ± 1.12*** | 30.79 ± 2.49*** | 63.17 ± 0.23*** | 94.00 ± 0.41*** | 17.02 ± 0.16*** |
| Group 3 | AEBA | 2 % w/w | 8.78 ± 1.27** | 25.12 ± 3.73 | 49.13 ± 1.22** | 79.43 ± 3.84** | 20.14 ± 0.46** |
| Group 4 | AEBA | 4 % w/w | 7.64 ± 0.19** | 25.17 ± 0.14** | 65.52 ± 0.47** | 91.46 ± 0.16*** | 17.49 ± 0.14*** |

*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: Effect of *Barringtonia asiatica* extract and standard drug on incision wound model in rats**

| Groups | Treatment | Dose | Tensile strength(g) Mean weight in gram ±S.E.M |
|---|---|---|---|
| Group 1 | Control (Simple ointment) | | 356.42 ± 2.44 |
| Group 2 | Standard (Nitrofurazone) | 0.2% w/w | 584.12 ± 3.22*** |
| Group 3 | AEBA | 2% w/w | 524.62 ± 2.12*** |
| Group 4 | AEBA | 4% w/w | 546.12 ± 3.16*** |

*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 3: Effect of Alcoholic bark extracts of *Barringtonia asiatica* on wound contraction of burn wound**

| Groups | Treatment | Dose | 4th day | 8th day | 12th day | 16th day | Period of epithelization (Mean % of wound contraction ± S.E.M) |
|---|---|---|---|---|---|---|---|
| Group 1 | Control (simple ointment) | | 24.49 ± 0.09 | 34.27 ± 2.28 | 58.84 ± 1.10 | 69.92 ± 1.16 | 22.88 ± 0.25 |
| Group 2 | Standard (Nitrofurazone) | 0.2% w/w | 29.12 ± 2.81 | 54.43 ± 0.49*** | 74.65 ± 0.71*** | 82.15± 0.26*** | 19.48 ± 0.51*** |
| Group 3 | AEBA | 2% w/w | 27.14 ± 4.17 | 46.28 ± 2.78** | 52.39 ± 1.40** | 72.14 ± 1.38* | 22.18 ± 0.85** |
| Group 4 | AEBA | 4% w/w | 29.20 ± 0.29** | 34.46 ± 2.62 | 63.13 ± 0.48** | 80.25 ±1.70*** | 19.93 ± 1.42*** |

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 AND CONCLUSION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is a product of the integrated response of several cell types to injury. This sequence of physiologic events occurs by a process of connective tissue repair. These events involve four phases [15-17].

(i) Coagulation, which prevents blood loss.
(ii) Inflammation and debridement of wound.
(iii) Epithelial repair, including proliferation, mobilization, migration and differentiation.
(iv) Tissue remodeling and collagen deposition

Any agent which accelerates the above processes is a promoter of wound healing. The application of medicinal concoctions from plants to treat skin lesions, in particular, burns and wounds, has had a long tradition. Plants with wound healing activity have been reported and experimentally studied on various animal models to reveal the most active promising compounds [18].

Results obtained in the present study suggest that treatment of excision wounds with alcoholic extract of *Barringtonia asiatica* has accelerated the wound healing process. Treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. Tensile strength was measured to confirm the wound healing activity claimed for this plant. The increase in tensile strength of treated wounds may be due to increase in collagen concentration and stabilization of the fibers [12,19]. It was observed that nitrofurazone increased the collagen content of the skin ultimately and contributed to wound strength. The results showed that treatment with the alcoholic extract of *Barringtonia asiatica* on the rat dermal wound healing, increasing the tensile strength and enhancing the wound healing process. Studies on burn wound models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epithelization. The results suggest that treatment with alcoholic extract of *Barringtonia asiatica* may have a beneficial influence on the various phases of wound healing like epithelization, collagen synthesis, and wound contraction, resulting in faster healing. The wound healing potential of the *Barringtonia asiatica* extract may probably be as a result of the presence of a mixture of phytoconstituents including flavanoids and tannins. Histological findings showed that the original tissue regeneration is much greater in skin wounds treated with the extracts than in control wounds. Ointment from the bark of *Barringtonia asiatica* exhibited significant prohealing activity when topically applied on rats by affecting various stages of healing process. The result of the present study offers pharmacological evidence on the folklore use of bark of *Barringtonia asiatica* for healing wounds.

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