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**Research Article**

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# Wound healing activity of aqueous extract of *Barringtonia acutangula* fruits in albino rats

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**ABSTRACT**

*The present study was carried out to evaluate the wound healing potential of crude aqueous extract of Barringtonia acutangula fruit (AEBA) ointment in experimental animals. All experiments were conducted following standard procedures. The crude extract ointment was administered topically in graded doses of 5% w/w, 10% w/w and 20% w/w for evaluating the wound healing potential in excision wound and incision wound model for fourteen days. Povidone iodine ointment 5% w/w was used as standard. Dose dependent activities resulted in both the wound models when compared to the standard (povidone iodine) and the control. Topical application of AEBA 20% w/w in excision wound model decreased significantly the wound area by 14th day, i.e. 67±2.12 compared with control 238.5±1.17. Similarly in incision wound model there is significant (232.66±2.20) increase in breaking strength of wounds when compared with control (107.5±15.09). Histological examination further supported wound healing activity of AEBA (20% w/w). There was mild healing was observed in 5%w/w & 10%w/w of AEBA and no healing was observed in negative control group. So, AEBA accelerated wound healing activity in rat and thus supports its traditional use.*

**Keywords:** *Barringtonia acutangula*, Excision**,** Incision wound, Histopathology

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## INTRODUCTION

Wound healing is a complex phenomenon for the regain or restoration of disrupted anatomical continuity and disturbed functional status of the skin [1], accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelization [2]. The basic principle of optimal wound healing is to minimize tissue damage, provide adequate tissue perfusion, oxygenation, proper nutrition, moist wound healing environment [3].The main aim of wound therapy is to enhance wound healing in the shortest time possible, with minimal pain, discomfort and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration [4].

*Barringtonia acutangula* is an evergreen tree belonging to family Barringtoniaceae or Lecythidaceae found throughout India. Fruits are bluntly quadrangular, long, broadest in the middle, broader angle and rounded [5]. The ethnomedicinal uses of fruits include colic, intestinal worms, wounds, ulcers, leprosy, syphilis, cough, bronchitis, dysmenorrhoea and eye troubles etc [6]. Fruits of *Barringtonia acutangula* exhibits hypoglycemic activity [7], anti-inflammatory activity [8]. Present study was carried out to assess the wound healing properties of aqueous fruits extract of *Barringtonia acutangula* in simple ointment base.

## EXPERIMENTAL SECTION

**Plant source**

The plant fruit material for this study was collected from local area of Barpali, (Dist-Bargarh, Odisha). The fresh fruits were washed thoroughly 2-3 times with running tap water and once with sterile water. Then fruits were shade dried without any contamination. The dried fruit was powdered by using grinder and passed through the mesh no 30 or 40 to get the uniform size.

**Preparation of plant extract**

The shade dried coarse powder of fruit (100 g) was subjected to continuous hot extraction with solvent petroleum ether, methanol and aqueous successively by using a Soxhlet apparatus for 16-18 hrs. The solvent was removed under pressure and the extracts were concentrated under vacuum at 40-60°C [9].

**Preparation of ointment**

Simple ointment containing the aqueous extract of fruit of *Barringtonia acutangula* was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin ointment base. The batches of the ointment containing 5%, 10%, 20% w/w of aqueous extract were prepared for the study. Povidone iodine ointment (5%w/w) was used as the standard drug for comparing the wound healing potential of the extract in excision and incision animal model [10].

**Animals**

Albino rats (Wistar strain) of either sex (150-200 g) were obtained from School of Pharmaceutical Sciences, Bhubaneswar. The study protocol was approved by Institutional Animal Ethics Committee (Registration No.1171/C/08/CPCSEA).

The animals were kept under control environmental conditions at 25±2º C temperature and 45-55 % relative humidity with natural light/dark cycle and allowed free access to food (Standard pellet diet, Hindustan Lever Ltd., India) and water. They were acclimatized for at least a week before the commencement of the experiment.

**Treatment Protocol**

The Animals were depilated and wounded under light ether anesthesia, semi aseptically. The animals were divided into five groups of six animals each and treated as follows.

Group-I: Simple ointment base was applied and served as control
Group-II: Povidone iodine ointment (5%w/w)
Group-III: 5% w/w aqueous fruit extract of *B.acutangula*
Group-IV: 10% w/w aqueous fruit extract of *B.acutangula*
Group-V: 20% w/w aqueous fruit extract of *B.acutangula*

**Wound healing study**

Screening for wound healing activity was performed by excision wound model and incision wound model without infection. All the test sample and standard drug were applied topically.

**Excision wound model**

Each group was anesthetized by open mask method with mild anesthetic ether. The rats were depilated on the back and a predetermined area of 500mm$^2$ full thickness skin was excised in the dorsal inter scapular region. The areas of the wounds were measured (sq mm) immediately placing a transparent polythene graph paper over the wound and then tracing the area of wound on it taken as initial wound reading. All the test samples were applied once daily. The wound area of each animal was measured on days 0, 2, 4, 6, 8, 10, 12 and 14 after inflicting the wound. Wound contraction (WC) was calculated as a percentage change in the initial wound size [11].

The % of wound contraction (WC) = [(Initial wound size-specific day wound size)/Initial wound size] X 100.

**Incision wound model**

The animals were anaesthetized under light ether anesthesia. One full thickness paravertibral incision of 1.5cm length was made including the cutaneous muscles depilated back of each rat. After the incision the parted skin was

kept together and stitched with black silk at both the end of the created wounds. The test samples were applied in a similar manner as excision wound model [12].

**Tensiometer and determination of tensile strength**

The tensile strength of wound represents the effectiveness of wound healing. Usually wound healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the completeness of the healing. The tensile strength was measured by using tensiometer on 14 post wounding day [13].

**Histopathology**

The histopathology study of the newly formed skin on the wounds was carried out on 14$^{th}$ post wounding day by fixing the skin in 10% formalin. Paraffin sections (5-10µ) were prepared stained with hematoxylin and eosin, and finally mounted in DPX medium. Histopathological examination were performed to study the process of epithelization on the excised wound and to find out evidence of granuloma, dysplasia, oedema and malignancy in the skin under examination [14].

**Statistical Analysis**

The means of wound area measurement and wound breaking strength between groups at different time intervals were compared by using one-way ANOVA followed by Dunnet's 't' tests [15].

## RESULTS AND DISCUSSION

Wound healing depends on the repairing ability of tissue, type and extent of damage and general health of tissue [2]. A therapeutic agent selected for wound treatment ideally improves one or more phases of healing without producing side effect [16].

In the current study, the wound healing activity of the aqueous fruit extracts of *Barringtonia acuntangula* (AEBA) was evaluated using excision and incision wound models in rats. In the excision wound model it was observed that there is dose dependant healing on 14th post wounding days. Complete wound healing took place on 14th day for both treated (20% AEBA) and standard (povidone iodine) group, whereas in the control group wound persisted beyond 14th day indicating better wound healing activity of aqueous fruit extract of the plants (Table-1). The % of wound contraction on 14th day by AEBA at 5%, 10% and 20% w/w were found to be 75.66%, 81.14%, 86.34% respectively. However on same post wounding day solvent control and standard group registered 51.65% and 90.92% wound contraction respectively (Fig-1). In incision wound model there is significant increase in skin tensile strength at 10% w/w and 20% w/w ointments of AEBA and standard as compared to control group animals (Table No-2).

**Table-1 Effect of Aqueous fruit extract of *Barringtonia acutangula* (AEBA) in excision wound**

| Treatment | Wound area in mm$^2$ mean ± SEM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | O Day | 2$^{nd}$ Day | 4$^{th}$ Day | 6$^{th}$ Day | 8$^{th}$ Day | 10$^{th}$ Day | 12$^{th}$ Day | 14$^{th}$ Day |
| Control | 493.33±2.40 | 483.50±2.97 | 468.33±2.81 | 446.00±2.58 | 419.16±2.41 | 384.00±1.57 | 320.00±1.15 | 238.50±1.17 |
| Povidone Iodine 5% w/w | 494.00±1.65 | 469.33±1.38** | 434.66±1.40** | 387.83±1.74** | 310.16±1.88** | 195.50±2.60** | 101.00±5.07** | 44.83±3.56** |
| 5% w/w AEBA | 490.33±3.11 | 478.66±3.07 | 461.16±3.02 | 433.00±3.13* | 395.83±2.78** | 338.50±3.06** | 246.16±3.40** | 119.33±2.83** |
| 10% w/w AEBA | 494.16±1.49 | 476.66±1.52 | 454.00±1.41* | 410.33±1.45** | 353.16±1.70** | 267.83±2.35** | 179.00±1.75** | 93.16±1.62** |
| 20% w/w AEBA | 490.50±2.29 | 469.00±2.47** | 437.16±2.34** | 390.33±2.72** | 317.83±2.62** | 212.66±1.62** | 109.66±2.40** | 67.00±2.12** |

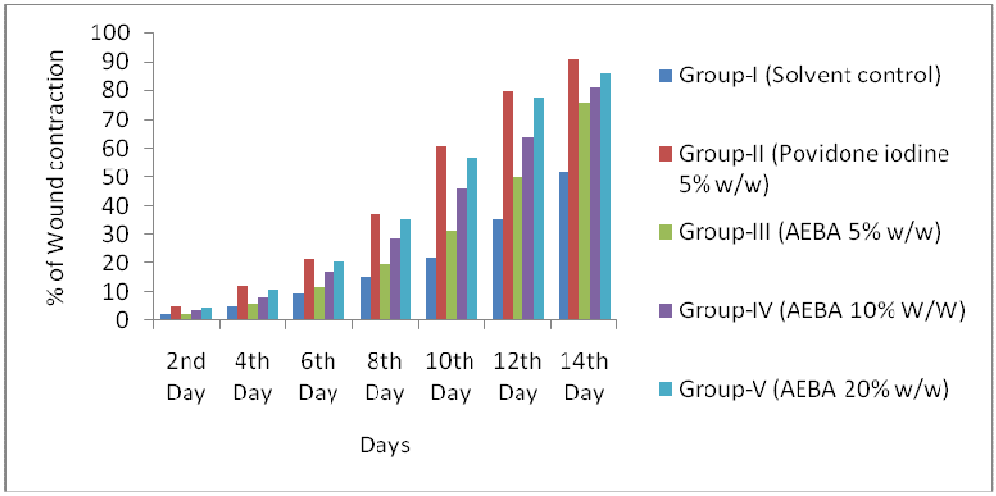
*n=6; P value: *p< 0.05, **p<0.01*

**Table-2 Effect of Aqueous fruit extract of *Barringtonia acutangula* (AEBA) on tensile strength of incised wound in rats**

| Groups/Treatment | Tensile strength (g/cm$^2$) Mean ± SEM |
|---|---|
| Group-I(Solvent control) | 107.50±15.09 |
| Group-II(Povidone iodine 5% w/w) | 231.16±3.04** |
| Group-III(AEBA 5% w/w) | 137.83±3.70* |
| Group-IV(AEBA 10% w/w) | 208.00±6.60** |
| Group-V(AEBA 20% w/w) | 232.66±2.20** |

*n=6; P value: *p< 0.05, **p<0.01*

**Fig-1 Effect of Aqueous fruit extract of *Barringtonia acutangula* in excision wound (% wound closure)**




In histopathological studies, a greater degree of epithelialization, collagen and fibroblastic deposition was observed in 20% w/w of AEBA and standard drug treated group animals. Inflammatory cells are not present. The intact portion of the skin reveals normal histology of skin (Fig- 2e & 2b). But in 5% and 10% w/w of AEBA mild healing was observed. There is incomplete formation of epithelial cells, mild oedema with lymphoid cells and fibrous tissues are not fully formed (Fig- 2c & 2d). In the control group, wounded area skin shows ulcer with inflammation exudates. There is mild increase in fibrous tissue as well as granulation tissue and congested blood vessels. Wound healing is incomplete (Fig-2a).

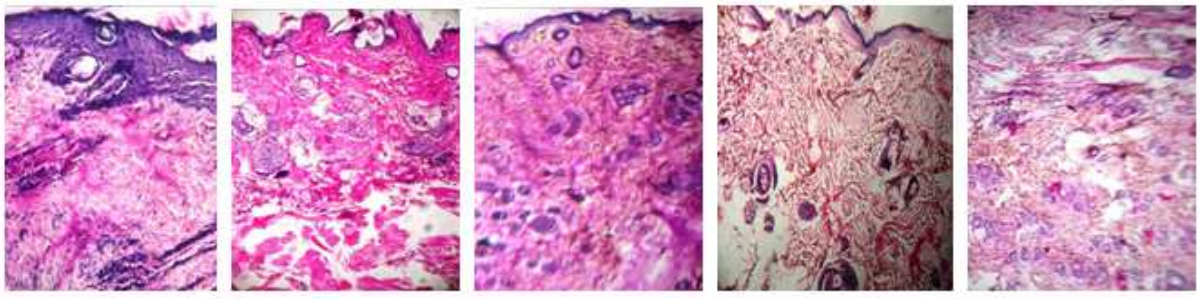


**Fig-2(a)** **Fig-2(b)** **Fig-2(c)** **Fig-2(d)** **Fig-2(e)**

Fig-2 Histopathology of wounded area skin on Day 14 post wounding with once daily topical application of drug (a) Control, (b) Positive control (Povidone iodine 5%w/w), (c) AEBA 5% w/w, (d) AEBA 10% w/w (e) AEBA 20% w/w

The fruit of *Barringtonia acuntangula* showed the presence of triterpinoid sapogenins[17]. Generally tannins, triterpinoids and flavonoids promote wound healing. Triterpinoid and flavonoids accelerate wound healing due to their astringent and antimicrobial properties [18]. So the wound healing activity of fruits part may be contributed to presence of such compounds in the fruits of the plant.

## CONCLUSION

The aqueous extract of *Barringtonia acuntangula* fruits (AEBA) showed significant wound healing activity when compared to controls. Wound contraction and increase tensile strength in 14$^{th}$ post wounding day of AEBA 20% w/w showed significant wound healing activity.

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