ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

EVALUATION OF WOUND HEALING POTENTIAL OF AQUEOUS EXTRACT OF BARRINGTONIA ACUTANGULA FRUIT IN RATS WITH BACTERIAL INFECTION

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Received on 19-01-2016 *Accepted on 29-02-2016*

Abstract

Objective: To investigate the wound healing activity of aqueous extract of *Barringtonia acutangula* fruits (AEBA)

Methods: Two models were performed to evaluate the wound healing activity i.e. infected excision and infected incision models. The wound area was infected with a loop full of inoculums of mixed microorganisms comprising of *Staphylococcus aureus* and *Escherchia coli*. In incision model the parameter which is carried out was breaking strength of the wounded skin. In excision model the percentage wound contraction and period of epithelialization were evaluated. Reference standard drug was Povidone iodine ointments for comparison with other groups.

Results: From the observation in both two infected wound models, aqueous extract produced significant wound healing activity in order of 20% w/w > 10% w/w > 5% w/w in terms of breaking strength in incision model and percentage wound contraction in excision model.

Conclusions: The results indicate that the aqueous extracts of *Barringtonia acutangula* fruits (AEBA) in the form of 20% w/w ointment produced significant wound healing activity in presence of infection.

Key words: Infected excision wound, infected incision wound and histopathology.

Introduction

Poor hygienic conditions are the cause for Wound infections in developing countries. Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeuroginosa, Salmonella typhi and Klebsiella pneumoniae are the principal pathogens of wound infection [1]. The adverse effects and drug resistance developed in conventional antimicrobial drugs commonly exist in the treatment of infectious diseases. In order to overcome this problem, recent attention turns toward herbal formulation [2].

Barringtonia acutangula (family Barringtoniaceae) is a medicinally valuable plant mostly found in Meghalaya, Assam, West Bengal, Bihar, Odisha, Madhya Pradesh and Deccan peninsula, Bangladesh, Myanmar and Sri Lanka [3]. Fruits are bluntly quadrangular, broadest in the middle, slightly narrowed towards and truncate at each end, crowned by the small persistent calyx [4]. Traditionally fruit is used in diseases of the blood, bronchitis, sore eyes, headache, hallucinations, abdominal colic, syphilis, nasal catarrh, wound, ulcer, leprosy, cough, dysmenorrhoea etc [4-6]. Aqueous extract of fruit at dose of 400 mg/kg significantly decreases blood glucose levels in STZ induced hyperglycaemic rats [7]. The ethanolic fruit extract also shows anti-inflammatory property on wistar rats [8]. Thorough literature survey reveals that there is lack of scientific report on wound healing activity of *Barringtonia acutangula*. The present study is an attempt to investigate wound healing activity of its aqueous extract of *Barringtonia acutangula* fruits in excision and incision infected wound model in rats.

Materials and Methods

Collection and preparation of plant extract

The plant material for the proposed study was collected from local area of Barpali (Dist-Bargarh, Odisha). The fruit was dried under shade and powdered by the help of mechanical process. The shade dried coarse powder of fruit (100 g) was subjected to continuous hot extraction with solvent petroleum ether, methanol, 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 methanol extract was 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 different animal model [10].

Animals

Albino rats (Wistar strain) of either sex (150-200g) 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 controlled 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 anaesthesia, semi-aseptically. Then they were divided into 5 groups of 6 animals each & 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 AEBA ointment

Group-IV: 10% w/w AEBA ointment

Group-V: 20% w/w AEBA ointment

Wound healing study

Both infected excision wound model and infected incision wound model was followed to screening for wound healing activity. All the test sample and standard drug were applied topically.

Infected excision wound model

Each animal was anesthetized by open mask method with mild anaesthetic ether. 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 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. This was taken as the initial wound area reading. The wound area was infected with a loop full of inoculums of mixed microorganisms comprising of *Staphylococcus aureus* (gram +ve) and *Escherchia coli (gram –ve).* The mixed microorganisms were prepared by mixing 1ml of each from 106 cfu/ml of *S.aureus* and *E.coli* cultures. It was left for 48 hr to develop the infection and then the treatment was started in following manner to the different groups, till the wound was completely healed. 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, 12]. % of wound contraction (WC) = [(Initial wound size-specific day wound size)/Initial wound size] X 100.

Infected incision wound model

The animals were anaesthetized under light ether anaesthesia. One full thickness para vertebral 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. All the rats were infected, left for 48 hrs for infection to set in and the test sample was applied in a similar manner as in infected excision wound model [12, 13].

Tensile strength

The tensile strength of wound represents the effectiveness of wound healing. The tensile strength was measured by using tensiometer on 14 post wounding day [14].

Histopathology

The histopathology study of the skin (newly formed on the wounds) was carried out on 14th post wounding day by fixing the skin in 10% formalin. Paraffin sections (5-10µ) were prepared stained with haematoxylin and eosin, and finally mounted in DPX medium.

Histopathological examination were performed to study the process of epithelialisation on the excised wound and to find out evidence of granuloma, dysplasia, oedema and malignancy in the skin under examination [15].

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-test [16].

Result and Discussion

The wound healing process takes place by itself and does not require much help, but various risk factors such as infection and delay in healing brought attention to promote this process.

Topical application of aqueous fruit extract of *Barringtonia acuntangula* at the wound site produced significant wound healing activity.

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.[14]

Current study using excision infected wound model revealed that the aqueous fruit extract of *Barringtonia acutangula* (AEBA) significant increase in wound healing activity in dose dependent healing on 14th post wounding days.

At 20% w/w it showed highly significant wound healing activity ($p<0.01$) as compared to control. The percentage of wound contraction on 14th day by AEBA at 5%, 10% and 20% w/w were found to be 41.10%, 45.58%, 56.79% respectively.

However on same post wounding day control and standard group 37.43% and 65.15% wound contracture respectively (Table-1 & Fig-1). In infected incision wound model also there is significant increase in skin tensile strength ($p<0.01$) by 20% w/w ointment of AEBA and standard as compared to control group animals (Table-2).

Table-1: Effect of aqueous extract of *Barringtonia acutangula* fruit in infected excision wound.

Treatment	Wound area in mm^2 mean ± SEM							
	O Day	2nd Day	4th Day	6th Day	8th Day	10th Day	12th Day	14th Day
Control	496.83± 2.44	504.66± 1.81	496.66± 1.85	483.66± 1.94	466.66± 4.40	437.05± 4.46	382.16± 5.14	310.83± 2.44
Povidone Iodine 5% w/w	491.66± 2.53	498.15± 2.60	475.83± 0.87**	443.33± 2.56**	391.13± 2.48**	303.83± 3.15**	213.66± 3.44**	171.33± 3.48**
5% w/w AEBA	496.05± 0.56	491.33± 0.42**	476.83± 1.99**	467.16± 0.65**	451.33± 0.84*	426.66± 1.25	373.66± 1.60	292.16± 1.49*
10% w/w AEBA	493.12± 1.71	484.66± 1.72**	474.66± 2.88**	448.13± 3.13**	414.33± 3.68**	367.25± 4.00**	305.35± 4.10**	268.31± 4.78**
20% w/w AEBA	488.83± 2.68	493.83± 2.52*	468.83± 3.21**	442.83± 2.12**	400.66± 2.26**	326.83± 2.38**	244.33± 3.75**	211.22± 3.91**

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

Fig-1: Effect of aqueous extract of *Barringtonia acutangula* fruit in infected excision wound (% wound closure).

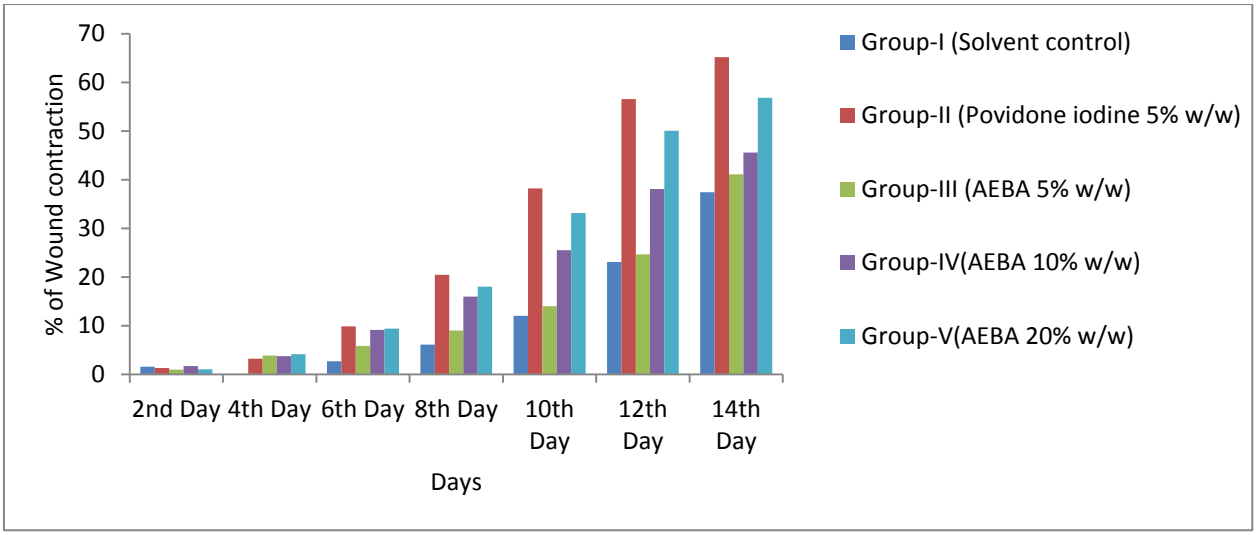


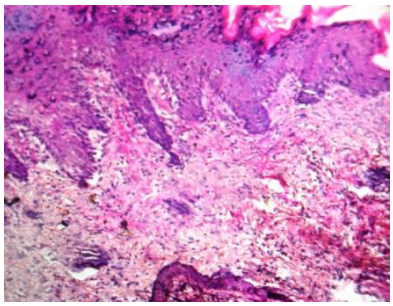
Table-2: Effect of aqueous extract of *Barringtonia acutangula* fruit (MEBA) on tensile strength of infected incised wound in rats.

Groups/Treatment	**Tensile strength (g/cm^2) mean±SEM**
Group-I(Solvent control)	88.33±10.54
Group-II(Standard)	215.83±4.16*
Group-III(AEBA 5%w/w)	114.33±3.76*
Group-IV(AEBA 10% w/w)	176.5±3.83**
Group-V(AEBA 20% w/w)	206.66±7.26**

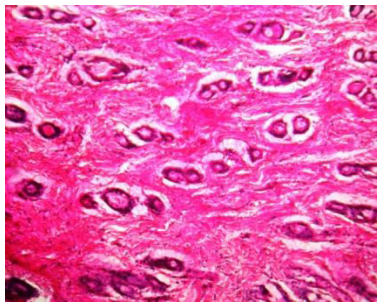
n=6; P value: **p<0.01

In histopathological studies of granulation tissue, there is increased number of fibroblast, granulation tissue, collagen tissue and epithelialisation in case of povidone iodine 5% w/w and AFEBA ointment 20% w/w. However 5% w/w & 10% w/w AFEBA showed mild healing as compared to control group (Fig-2 a,b,c,d,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.



(a)



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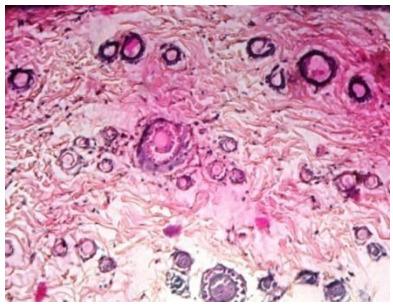

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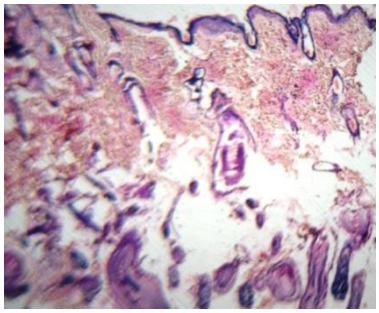
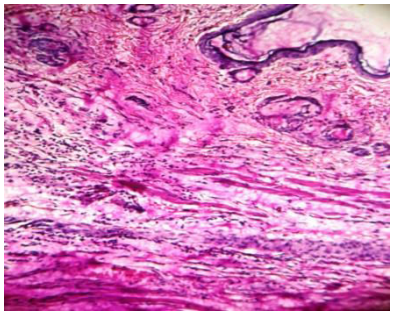


(d)



(e)

Preliminary phytochemical screening of AEBA showed presence of alkaloid, saponin & polysaccharides. Flavonoids and triterpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation [17-19].

In Povidone iodine (5% w/w) treatment group granulation tissues and blood vessels are formed along with fibroblasts and collagens. Also there is no evidence of oedema. Similarly in case of MEBA (20% w/w) there is complete formation of granulation tissue, epithelial layer, collagen and regular arrangement of Fibroblast. Blood capillaries are formed uniformly with no evidence of inflammation and malignancy. However in case of MEBA (10% w/w) treated group there is formation of mild fibrous tissue and minimal oedema. Blood vessels are congested due to increase number of red blood cells. There is no evidence of granulation tissue. There is no formation of granulation tissue as well as epithelial tissue in case of MEBA (5% w/w) treated group. Inflammatory exudates with mild oedema are also seen. In case of control group Fibroblasts are not formed; granulation tissues and blood vessels are absent.

Conclusion

When a wound occurs and is exposed to external environment, it is more prone to be attacked by microbes, which invade through the skin and delay the natural wound healing process. The use of AEBA for various skin infections is justified by this work, as it showed significant activity against *S. aureus* and *E. coli* organisms. The external application of the extract on wound prevented the microbes to invade through the wound. Thus, the protection of wound occurs against the infection of the various organisms.

Acknowledgement

We acknowledge the secretarial assistance of Mr Tapas Ranjan Satpathy in preparing the manuscript.

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