# WOUND HEALING POTENTIAL OF THE LEAF EXTRACTS OF *Barleria cuspidata* HEYNE EX NEES.

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## Summary

The methanolic extracts of the leaves of *Barleria cuspidata* were investigated for their wound healing potential in the form of an ointment with two different concentrations and evaluated for wound healing potential. Both concentrations of the methanolic extract ointments showed significant responses in wound types tested when compared with the control.

**Keywords**: *Barleria cuspidata,* wound healing.

## Introduction

The plants of the *Barleria* genus comprises of a number of species of small shrubs which are much branched, prickly shrub, up to 1.5m in height. They have 3 to 5 sharp, pale colored spines, in the leaf axil all over the plant. The flowers occur in an upright spike at the top of the plant. The flowers are yellow, tubular, with long projecting stamens [1, 2]. Traditionally the leaves of many different species of this genus *Barleria* are used in toothaches and in healing of cuts and wounds [3, 4]. However the wound healing potency of the plants has not been clinically evaluated so far.

The present communication reports the wound healing potency of the ointment prepared from the methanolic extracts of the leaves on excision and incision wound models in albino rats.

## Materials and Methods

### Plant material

The whole plant of *Barleria cuspidata* Heyne ex Nees family Acanthaceae was collected from Ranchi and Dhanbad. It is found throughout the year, but flowering occurs in winter season. The plant was authenticated by Botanical Survey of India, Kolkata (Herbarium no. - CNH/I-I (37)2006/TechII/668). The herbarium has been submitted to the Dept. of Pharm. Scs., B.I.T., Mesra, Ranchi.

The leaves of the plant were separated and washed with tap water to remove dirt. They were then dried in shade at room temperature for 15 days. To ensure complete drying the leaves were kept in hot air oven at 45$^{o}$C for 5 minutes. Then the dried leaves were crushed with hand and then powdered in a mortar pestle and sieved (mesh #20).Then the powdered leaves were stored at room temperature in airtight container.

**Extractions and standards used**

The dried and powdered leaves of *Barleria cuspidata* were subjected to successive hot extraction in a Soxhlet apparatus with solvents of increasing polarity viz. Petroleum ether (60-80), Chloroform, Ethyl acetate and Methanol. The average time period for extraction was 48 hours. The individual extracts were filtered and concentrated on Rotary Vacuum evaporator [5, 6].

Preliminary phytochemical tests were carried out for the presence or absence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Saponins, Terpenes and Tannins [7 - 9]. The methanolic extract was found to contain the maximum number of phytoconstituents. Thus the methanolic extract was chosen for further pharmacological activity.

The powdered leaves were extracted with methanol by continuous hot extraction process for 72 hrs in soxhlet apparatus, using reflux condenser. Then the extract was separated by filtration. Fresh solvent was added and extracted for further 3 hrs. The extracts were concentrated by vacuum distillation on water bath. Further removal of solvent was carried out by keeping the extracts in vacuum desiccators to get a semisolid mass[10].

Two types of ointment formulations with different concentration of the extract were prepared viz. 10% w/w ointment where 10 g of the extract of the leaves was incorporated in 100g of simple ointment base B.P and 15% w/w ointment where 15 g of the extract of the leaves was incorporated in 100g of simple ointment base B.P [11]. Nitrofurazone ointment 0.2%w/w obtained from GSK. Ltd., India, was used as the standard drug for comparing the wound healing potential of the extract in different animal models.

**Acute toxicity study**

Acute toxicity study was carried out as per staircase method [12]. Albino mice of either sex weighing 20 – 25 g were used to determine $LD_{50}$. They were fasted overnight prior to the acute experimental procedure. Tween 80 (5% w/v) was used as the vehicle in which the different doses of the leaf extract was dissolved and were administered intraperitoneally. The test was done in stepwise manner till 2000mg/Kg. No mortality was found till this dose level.

$LD_{50}$ was thus found to be > 2000mg/Kg body weight. About 1/10$^{th}$ of this was taken as the therapeutic dose.

**Animals**

Male inbred albino rats weighing between 150-180g were procured from Animal House of Birla Institute of Technology, Mesra, Ranchi and were housed in polypropylene cages with one animal in each cage. They were kept under controlled environmental conditions of 25 ± 2 $^{o}$ C 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 and acclimatized for at least a week before the commencement of the experiment.

**Treatment Protocol**

The Animals were depilitated and wounded under light ether anaesthesia, semi aseptically. The experimental protocols were approved by the Institute Animal Ethics committee.

Group- I: Simple Ointment base was applied and served as vehicle control.

Group- II: Nitrofurazone ointment (0.2%w/w) was applied once daily.

Group- III: 10%w/w ointment of *Barleria cuspidate* leaf extract was applied once daily.

Group- IV: 15%w/w ointment of *Barleria cuspidata* leaf extract was applied once daily.

**Excision wound model[13]**:

Four groups of animals containing six in each group were anesthetized by open mask method with anesthetic ether. The rats were depilitated on the back and a predetermined area of 500mm$^2$ full thickness skin was excised in the dorsal inter scapular region. Rats wound were left undressed to the open environment; this model was used to monitor wound contraction and epithelisation time. The reference standard drug (0.2% w/w nitrofurazone ointment), simple ointment; 10%w/w and 15%w/w ointment of the methanolic extract of the leaves of *B. cuspidata* were applied everyday to the specific groups till the wound was completely healed. The progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e the measurement of wound on graph paper was expressed as unit (mm$^2$).Wound contraction was expressed as percentage reduction of original wound size.

All the above mentioned treatments were started from the day of operation and continued till the 20$^{th}$ day of healing. On 2$^{nd}$, 4$^{th}$, 8$^{th}$, 10$^{th}$, 12$^{th}$, 14$^{th}$, 16$^{th}$, 18$^{th}$ and 20$^{th}$ days the wound area of each rat was traced on a graph paper and measured with the help of planimeter. The number of days required for falling of scar without any residual raw wound, gave the period of epithelization.

**Incision wound model[13].**

Four groups of animal containing six in each group were taken. The animals were anaesthetized under light ether anaesthesia. One full thickness paravertebral incision of 5 cm length was made including the cutaneous muscle of the depilated back of each rat. After the incision was made the parted skin was kept together and stitched with suture 1 cm apart. The continuous threads on both wound edges were tightened for good adaptation of wound and it was left undressed. The ointment of the leaf extracts standard drug nitrofurazone ointment and simple ointment were applied twice daily to the respective groups of animals, until complete recovery.

**Tensiometer and determination of tensile strength**

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 healing. Tensiometer consists of a 6×12 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 edge of a table. Pulley with bearing was mounted on the top of one arm. An alligator clamp with one cm width was tied on the tip of the 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.

On the 9th day after wounding the sutures were removed and the tensile strength was measured on 10th day. For measuring the tensile strength the rats were again anaesthetized and each rat was placed on a stack of towels on the middle of the board. The amount of the towels could be adjusted in such a way so that the wound **was on the** same level as the tips of the arms. The clamps were then carefully clamped on the skin of the opposite edges of the wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally to the polyethylene bottle. The position of the board was adjusted so that the bottle receives a rapid and constant rate of water from a large reservoir, until the wound began to open. The amount of water in the polyethylene bottle was weighed and equated as the tensile strength of the wound. The tensile strength induced by the extract and by nitrofurazone ointment treated wounds was compared with that of the vehicle control.

## Results and Discussion

For excision wound model the effect of topical application of methanolic extract on wound contraction has been tabulated in Table 1. It was seen that the contraction rate is significantly higher ($p<0.001$) than the control on all the days of treatment. It is observed that the wound contraction ability of the extract ointment in different concentrations was significantly greater than that of the control (simple ointment treated group). The 15% w/w extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 15% w/w extract ointment treated group (18th days for 100% contraction which was almost similar to that of the Nitrofurazone treated group). 10% w/w extract ointment treated group of animals showed significant wound contraction from the 18th day onwards and achieved 100% with the wound closure time of 20th day. 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 15% w/w extract treated group and the nitrofurazone ointment treated group were comparable to each other. The 10% w/w extract ointment treated group showed a lesser but significant increase in the tensile strength compared to the control group ($P<0.001$). Thus, both concentrations of the extract as well as the standard drug showed a significant increase in tensile strength in the 10th days old wound.

After a preliminary investigation of the wound healing property of *Barleria cuspidata* Heyne ex Nees and interpreting the results it is found that, effect of the topical application of methanolic extract on wound contraction of open wound showed that it has increased the rate of contraction on post wounding days as compared to the control simple ointment base. Similarly topical application of methanolic extract on the incised and sutured wound increased its tensile strength as compared to control.

The methanolic extract of the leaves of this plant possess wound healing activity and thus justifies its use in folklore medicine. However there is scope for further thorough investigations and biochemical estimations in order to further substantiate this preliminary finding.

**Table 1.** Evaluation of *Barleria cuspidata* leaves extract and the standard drug on wound healing by excision wound method in Rats.

| **Post wounding days** | **Wound area ($mm^2$) (mean± S.E)and percentage of wound contraction** | | | |
|---|---|---|---|---|
| | **Simple ointment base** | **Nitrofurazone ointment (0.2%w/w)** | ***Barleria* extract ointment (10% w/w)** | ***Barleria* extract ointment (15% w/w)** |
| 0 | 526±3.1 | 512±2.7 | 519.88±5.73 | 515.83±7.75 |
| 2 | 438±2.2 (16.7%) | 414±4.1 (19.1%) | 430.00±5.71 (14.20%) | 415.50±4.96 (16.9%) |
| 4 | 392±3.4 (25.5%) | 306±2.6** (40.20%) | 371.00±9.02* (25.80%) | 352.33±6.04* (33.53%) |
| 6 | 314±4.2 (35.2%) | 233±2.8** (54.50%) | 310.50±2.47* (38.00%) | 285.00±2.25* (43%) |
| 8 | 306±3.9 (41.8%) | 189±1.6** (63.10%) | 249.16±5.87** (50.20%) | 198.83±3.26** (60.23%) |
| 10 | 289±0.8 (45.1%) | 108±2.2** (78.90%) | 210.16±6.61** (58.03%) | 132.33±7.62** (73.53%) |
| 12 | 268±2.7 (49.0%) | 64±1.8** (87.50%) | 140.66±11.44** (72.21%) | 72.83±9.66** (85.43%) |
| 14 | 242±1.6 (54.0%) | 30±2.2** (94.10%) | 82.00±3.30** (83.6%) | 38.66±11.16** (92.27%) |
| 16 | 218±0.8 (58.5%) | 8±0.2** (98.40%) | 42.43±4.19** (91.60%) | 12.00±4.48** (97.6%) |
| 18 | 196±2.4 (62.7%) | 00±00** (100%) | 16.06±2.82** (96.79%) | 00±00** (100%) |
| 20 | 187±3.6 (64.4%) | 00±00* (100%) | 00±00** (100%) | 00±00** (100%) |

The result were statistically significant compared with simple ointment base and P-values were calculated by students t-test (n=6); * P< 0.01, ** P< 0.001

**Table 2. Effect of *Barleria cuspidata* (10% and 15%w/w) ointment and standard drug on incision wound model in rats.**

| **Treatment** | **Tensile strength(g)(mean ± SE)** |
|---|---|
| Simple ointment | 260.16±10.11* |
| Nitrofurazone ointment | 399.16±6.45* |
| Barleria extract (10%w/w) | 350.00±20.41** |
| Barleria extract (15%w/w) | 290.52±10.34** |

Results were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.1, **P<0.001

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