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### EFFECT OF ETHANOL EXTRACT OF *RHYNCHOSIA BEDDOMEI* STEM ON WOUND HEALING IN RATS

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#### ABSTRACT

To screen the wound healing activity of ethanol extract of *Rhynchosia beddomei* stem using incision, excision wound models and evaluate histopathological changes of granuloma tissue. Material and method: The stem powder of *Rhynchosia beddomei* was extracted with 95% ethanol by continuous heat extraction and was subjected for phytochemical investigation and screened for wound healing activity. A supportive histopathological examination was determined for the pattern of lay-down for collagen. Triterpenoids, steroids, flavonoids and saponins were found to be present in ethanol extract of the *Rhynchosia beddomei*. In resutured incision wound model, ethanol extract showed significant breaking strength ( $P < 0.01$ ) compared to control. The ethanol extract promotes better wound healing by increasing the percentage wound closure and decreasing epithelization time ( $P < 0.001$ ) compared to control. The results of the present study reveal that ethanol extract of bark of *Rhynchosia beddomei* have significant wound healing activity. The pro-healing action seems to be due to the increased synthesis of collagen, it's cross-linking as well as better alignment and maturation. This may be attributed to the presence of flavonoids in this plant.

**Key Words:** *Rhynchosia beddomei*, Wound healing activity.

#### INTRODUCTION

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds. A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues, which leads to loss or breaking of cellular and anatomic or functional continuity of living tissue. Wound healing occurs in three stages: inflammation, proliferation, and remodeling. Chronic wounds are wounds that fail to heal despite adequate and appropriate care. Such wounds are difficult and frustrating to manage [1]. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major

drawbacks and unwanted side effects. *Rhynchosia beddomei* belonging to family Fabaceae found in various regions of India and India - ISO as native place. Its leaf was possess' anti-tumor activity [2,3], anti-microbial activity and anti-fungal activity<sup>4</sup>. It is also having anti-oxidative property. The literature survey revealed that no scientific study on diuretic activity of stem extract of this plant has been reported. Though there is no scientific evidence to support the wound healing effect of *Rhynchosia beddomei*, tribal men continue to use the plant in the treatment of wound. The objective of this investigation was to ascertain the scientific basis for the use of this plant in the treatment of wound, using different wound models.

#### MATERIALS AND METHODS

##### Plant material

The *Rhynchosia beddomei* stems were collected from medicinal garden of Anurag Pharmacy College. The plant and plant material were identified and authenticated by Department of Pharmacognosy, Anurag Pharmacy College and Voucher herbarium specimens was deposited

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in the Department of Pharmacognosy of our College. The plant material was dried in sun shade, pulverized, passed through sieve no.40 and stored in air tight container and used for further extraction.

### Preparation of extract

The powder was subjected to hot percolation method using Soxhlet apparatus with ethanol. The extract was dried and weighed.

### Acute toxicity study

Healthy adult albino rats of either sex, starved overnight, were divided in to six groups (n = 6) and were orally fed with increasing doses (10, 30, 100, 300, 1000 and 3000mg/kg b.w) of ethanol extract to determine the safer dose by an up and down staircase method. The animals were observed continuously for one hour, frequently for the next four hours and thereafter for 24h. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioral changes [2].

### Excision wound

This model was employed to study the rate of wound contraction and epithelization. A round seal of 2.5 cm in diameter was impressed on the dorsal thoracic central region 5 cm away from the ears. The entire thickness of the skin from demarked area was excised to get a wound measuring around 500 mm<sup>2</sup>. Animals were subjected to the treatment from '0' day till the wound completely healed or up to 21<sup>st</sup> post wounding day,

whichever was earlier. The observations of percentage wound contraction were made on 2<sup>nd</sup>, 6<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> post wounding days [3].

### Resutured incision wound

Two para-vertebral straight incisions of 6 cm each were made through the entire thickness of skin on either side at least 1 cm lateral to the vertebral column. Wounds were sutured with catgut. Sutures were removed on 7<sup>th</sup> post wounding day and the breaking strength was estimated on 10<sup>th</sup> post wounding day by continuous, constant water flow technique [4].

### Statistical analysis

All results are presented as the Mean  $\pm$  S.E.M. The test of significance was statistically analyzed using Student's t test.

### RESULTS

In the present study, animals showed good tolerance to ethanol extract of *Rhynchosia beddomei* and a dose as high as 3000mg/kg was found to be non-lethal. Hence 300 mg/kg dose (1/10<sup>th</sup> of highest safer dose) was selected in the study. The excision wound heals by contraction and epithelization. The mean percentage wound closure (Table 1) for extract was found to increase significantly at P<0.001 on 2<sup>nd</sup>, 6<sup>th</sup>, 10<sup>th</sup> and 4<sup>th</sup> post wounding days in comparison with control. Whereas, the extract showed significantly decreased period of epithelization (15.5  $\pm$  0.4 days), when compared with control (19.5  $\pm$  0.8 days).

**Table 1. Effects of orally administered ethanol extract of *Rhynchosia beddomei* on resutured incision and dead space wounds in rats**

Treatment	Incision	Dead space		
	breaking strength (g)	Dry granuloma weight (mg)	Breaking strength (g)	Hydroxyproline content ( $\mu$ g)
Control	296.7 $\pm$ 5.1	37 $\pm$ 1.8	192.5 $\pm$ 3.1	979.4 $\pm$ 0.8
Extract 375 mg/kg	350.6 $\pm$ 12.6b	99.36 $\pm$ 1.8a	315 $\pm$ 3.7a	2020.17 $\pm$ 139.3a

Values are mean  $\pm$  s. e. m. of six rats in each group. aP<0.001, bP<0.01 significantly different from control (Student's t test). In resutured incision wound model, extract showed significant increased (P<0.01) mean breaking strength (350.6  $\pm$  12.6 g) of a 10 days old wound compared to control (296.7  $\pm$  5.1) as shown in Table 2.

**Table 2. Mean percentage wound closure and period of epithelization of excision wounds following post wounding days.**

Treatment	2nd day	6th day	10th day	14th day	Period of Epithelization in days
Control	19.4 $\pm$ 0.6	42.2 $\pm$ 0.82	83.4 $\pm$ 0.97	94.9 $\pm$ 0.79	19.5 $\pm$ 0.8
Extract 375 mg/kg	21.9 $\pm$ 0.7a	56.1 $\pm$ 2.0a	96.2 $\pm$ 0.7a	99.2 $\pm$ 0.3a	15.5 $\pm$ 0.4a

Values are mean  $\pm$  s. e. m. of six rats in each group. a P<0.001 significantly different from control (Student's t test).

### DISCUSSION

The wound healing activity proposed for the extract of bark of *Rhynchosia beddomei* was in line with the observations made. It has been reported that triterpenoids possess an ability to increase the collagen

content, which is one of the factors promoting wound healing [4]. As the title plant is rich in triterpenoids, it may be responsible for the wound healing activity. Lipid peroxidation is an important process in several types of injuries like burns, infected wounds, skin ulcers, etc. Hence

any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in increase in the strength of collagen fibres by increasing the circulation, preventing the cell damage and promoting the DNA synthesis. This is suggested by the fact that there was an increase in the wound breaking and

granuloma breaking strength after the administration of the little plant extract. Thus it may be concluded that the bark of *Rhynchosia beddomei* is endowed with significant wound healing activity, justifying its use in the traditional system of medicine. Further studies are in progress to ascertain the bioactive components of the plant extract.

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