EVALUATION OF WOUND HEALING POTENTIAL OF CRUDE LEAVE EXTRACTS OF CROTALARIA PALLIDA AITON. IN WISTAR RATS.


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ABSTRACT

The present study was an attempt to investigate the wound healing potential in two different types of wound models in albino rats viz., incision and excision. of various extracts of Crotalaria pallida leave. The different crude extracts such as ethanol, ethyl acetate, n-butanol and petroleum ether are obtained by successive soxhlet extraction. The ethanolic extract showed significant increase in wound contraction and formation of scar in excision wound model. The extract showed significant increase in the breaking strength of resutured incision wound as compared to control group (p<0.05). The result of the present study indicate that ethanolic extract of C.pallida has more significant wound healing property than the other three extracts in excision and incision wound model.

KEYWORDS: Crotalaria pallida; Excision and incision wound model.

INTRODUCTION

In tribal areas different plant drugs used to treat various skin disorders including wound. The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a in a physiologic environment conducive to repair and regeneration.[1] Some medicinal plants have been employed in folk medicine for wound care. Wound may often possess in problems in clinical practices. Healing
of wounds is one of the most complex biological process after birth, as a result of interplay of different tissue structure and a large no of resident and infiltrating cell type constituted. Even no synthetic drug has been attributed in market for direct healing of wounds. WHO has been promoting traditional medicine as a source of less expensive, especially in developing countries and also recognized the traditional medicine. The literature survey reveals that there are no reports on the wound healing activity of the leaf extracts of *Crotalaria pallida*. This prompted the authors to undergo the present study. The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *Crotalaria pallida* (family: fabaceae) also known as jhunjhunuka (Oriya) is frequently distributed. And extensively used traditionally by the tribal people. *Crotalaria pallida* Aiton is a species that belongs to the Fabaceae family, popularly known as “rattle or rattlesnake” due to the sound of their fruits when dry. Crotalaria is one of the largest genera in tropical Africa. The genus includes 690 species that are mainly situated in Africa and Madagascar. The species have also been found throughout in India. This is an erect shrub, annual short-lived perennial herb of 1.5 m or more tall. Taproot white or brown and stem grooved, solid, glabrous. Leaves trifoliolate, alternate spiral, stalked, leaflets elliptic, more than 2 cm long/ wide, hairy on upper surface, margin entire, apex obtuse base acute, pinnately veined. Flowers bisexual, grouped together in a terminal raceme, stalked, petals 5, yellow. Fruit a rounded. This species is used in traditional medicine, the plant is used to treat urinary problems and fever, a poultice of the roots is applied to swelling of joints and fever and its leaves as vermifuge. Mikirs of Assam take about 20 ml. extract of leaves in early morning to kill intestinal worms. Powder of leaf and root bark with the leaf of Wrightia tinctoria & Tragia involucrate is made to a paste with water and applied externally for skin diseases. Pharmacological studies have demonstrated it also presents anti-inflammatory, antimicrobial, antioxidant, antibacterial & antifungal functions, *Crotalaria pallida* extracts as a putative HIV-protease inhibitor.

**MATERIALS AND METHODS**

**Collection of Plant Material**

The leaves of *Crotalaria pallida* were collected from the tribal belts of the local area of Baipariguda of Koraput district (India) in the month of November 2011. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-198/11, dated 16.12.2011). After authentication leaves were
collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder and stored in a closed air tight container for further use.

**Preparation of Extracts**

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 750 g coarse powder was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.[13,14,15]

**Phytochemical investigation**

The crude ethanol, ethyl acetate, n-butanol and petroleum ether extracts of the leaf of *Crotalaria pallida* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis.[14,15]

**Animals**

Healthy adult wisterstrain of albino rats weighing approximately 180 to 250gms were used. They were housed in standard conditions of temperature (25±2 °C), 12 hours light per day cycle, relative humidity of 45-55% in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar, Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

**Wound healing Activity**

**Wound model**

**Excision wound**

For the excision wound study, animals were divided into 5 groups of six rats in each group. Group-I served as control and applied with Vaseline, Group-II, Group-III, Group-IV and Group-V were treated with ethanol, ethyl acetate, n-butanol and petroleum ether extracts.
respectively. An impression was made on the dorsal thoracic central region 5mm away from the ears, by using a round seal of 2.5 cm diameter as described by Morton and Malone\cite{16}. The skin of the impressed area was excised to the full thickness to obtained area of about 500 mm2 under light ether anaesthesia in aseptic condition. The animals were housed individually. The methanol extracts in simple ointment base (5% w/w) were applied on the wound once a day for 18 days starting from the day of wounding. The percentage wound closure was observed on 4th, 8th, 12th, 16th, 18th post wounding day. Epithelization time (in days) and size of the scar area was noted.

**Incision wound**

Incision wound model was performed according to Ehrlich and Hunt\cite{17}. The animals were divided into 5 groups of six rats in each group, and kept in separate cage. Group-I served as control, received only 2% gum acacia suspension (1 ml/kg, p.o), ethanol, ethyl acetate, n-butanol and petroleum ether extracts (250 mg/kg) were given orally once a day to group-II, III, IV and V respectively for 10 days. Under light ether anesthesia, the animals were secured to operation table in its natural position. Two paravertebral straight incisions of 6 cm each were made through the entire thickness of the skin, on either side of the vertebral column with help of sharp blade. Removal of the sutures was done on 8th post wounding day. Tensile strength was determined on both wounds by continuous constant water flow technique of Lee. \cite{18}

**Statistical analysis**

The results are reported as Mean±SE. Statistical analysis was done using ANOVA (Tukey-MultipleComparison Test). When probability (p) was less than 0.05 was considered as significant.\cite{19}

**RESULT AND DISCUSSION**

Table-1: Effect of extracts of *Crotalaria pallida* leave on the breaking strength in incision wound

<table>
<thead>
<tr>
<th>Sl no.</th>
<th>Group</th>
<th>Breaking strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>246.14 ± 23.06</td>
</tr>
<tr>
<td>2</td>
<td>petroleum ether</td>
<td>327.18 ± 11.36</td>
</tr>
<tr>
<td>3</td>
<td>ethyl acetate</td>
<td>332.22 ± 12.52</td>
</tr>
<tr>
<td>4</td>
<td>n-butanol</td>
<td>347.16 ± 14.37</td>
</tr>
<tr>
<td>5</td>
<td>ethanol</td>
<td>426.68 ± 17.26*</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6)* p<0.05 vs control
The preliminary phytochemical screening showed that the different solvent extracts of *C. pallida* contain the alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins were present in all the solvent extract & carbohydrates absent. In the study using excision wound model, animals treated with ethanol extract of *C. pallida* leave showed significant decrease in epithelization period as evidenced by shorter period for fall of eschar as compared to control group (p<0.05) Fig-1. The extract also facilitated the increase in rate of wound contraction than control group. The petroleum ether extract treated animal (Group-II)
showed wound contraction by 72.23%. The ethyl acetate extract treated animals (Group-III) showed wound contraction by 76.43%. The n-butanol extract treated animal (Group-IV) showed wound contraction by 83.53%. The ethanol extract treated animal (Group-V) showed wound contraction by 87.63% as compared with the control (Group-I) by 62.27% in all the extract. Fig-2 The result of present study reveals that ethanolic leave extracts of *Crotalaria pallida* possess a prominent prohealing activity in incision wound model. This was demonstrated by significant increase in the skin. Ethanol extract treated groups (p<0.05) on 10th post wounding day are presented in Table-1.

CONCLUSION

In the present study, wound healing activity of *Crotalaria pallida* was studied and the results of the present study suggest that local application and systemic administration of ethanol extract of the leaf has shown more significant wound healing activity in excision and incision wound models and support the popular use of plant to open wound in folk medicine. The wound healing property of *C.pallida* has been attributed to its antimicrobial effects. The presence of phytoconstituents like flavonoids, terpenoids, saponins, phenols, steroids and tannins either individually or combined together may exhibit the synergistic effect towards healing of wounds. However, further investigation employing isolation of constituents and screening models are needed for further confirmation of wound healing potential of *Crotalaria pallida* leave. Thus the traditional use has been pharmacologically validated.

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