EVALUATION OF WOUND HEALING ACTIVITY OF DALBERGIA SISSO LEAVES IN ALLOXAN INDUCED HYPERGLYCEMIC RATS

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Abstract : With an aim to study wound healing activity of Dalbergia sissoo leaves in alloxan induced hyperglycemic rats, wounds were created after the induction of diabetes with alloxan (125 mg/kg) in albino wistar rats by intraperitoneal administration. Rats were topically treated with Dalbergia sissoo leaves extract which was formulated in the form of ointment and wound healing activity of Dalbergia sissoo leaves (5% and 10% w/w, topically) was observed. Antioxidants have been reported to play a significant role in improving the wound healing process and protecting the tissues from oxidative damage. As a result, antioxidants were detected for their presence in the extracts using ascorbic acid as standard through superoxide scavenging assay and reducing power assay. Wound which were treated with ointment containing 5% and 10% extract of Dalbergia sissoo leaves showed significant wound healing effect. Wound contraction in upto 20th days was 29.33+/−3.055 mm in control group while the contraction in group 4 (diabetic rats with wound, given extract 5% ointment) and 5 (diabetic rats with wound, given extract 10% ointment) was 41.00+/−3.606 and 26.67+/−1.528 respectively. The extract of Dalbergia sissoo leaves had properties that render it capable of promoting accelerated wound healing activity compared with the controls. On the basis of the results obtained in the present study, it is possible to conclude that Dalbergia sissoo leaves ointment has significant wound healing activity in rats with diabetes.

IndexTerms - Dalbergia Sisso, Wound, Diabetes, Alloxan.

1. INTRODUCTION
Wound healing is a complex process of recovering the forms and functions of injured tissues. The process is tightly regulated by multiple growth factors and cytokines released at the wound site. Any alterations that disrupt the healing processes would worsen the tissue damage and prolong repair process. Various conditions may contribute to impaired wound healing, including infections, underlying diseases and medications. Numerous studies on the potential of natural products with anti-inflammatory, antioxidant, antibacterial and pro-collagen synthesis properties as wound healing agents have been performed. Their medicinal properties can be contributed by the content of bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, saponins, and phenolic compounds in the natural products. This review highlights the in vitro, in vivo and clinical studies on wound healing promotions by the selected natural products and the mechanisms involved.

Wound healing in diabetes patient take longer time than healing wound in patient without diabetes. A non-healing wound face complication which slow-up the healing process & these complication are - Difficulty in walking, Infection including, Cellulitis Abscess, Vascular changes in diabetic patient are some of the problem with healing of wound. Diabetic patient blood supply is decrease to the wound which affect speed of the healing process. At the site of injury leucocyte reaching speed is slow down due to blood vessels are thicker than normal, this is because of alteration of inflammation phase. Foot ulcer is a long term complication of diabetes mellitus foot problem are associated with both diabetes i.e. type-1 & type-2. Injury is a main reason of foot ulcer in existence of peripheral neuritis (nerve damage). 50% of amputation can be prevent by the awareness & identify at the right time. Foot ulceration occur as a consequences of injury in the existence of neuropathy.

2. MATERIAL AND METHODS
2.1. Plant collection
The leaves of medicinal plant Dalbergia Sisso was collected locally from Bhopal, M.P
2.2. EXTRACTION OF PLANT

The air dried medicinal leaves of plant of *Dalbergia sisso* has been extracted with ethanol by continuous hot extraction method by using soxhlet apparatus at 60°C for 8 hrs, filtered and dried using rotatory evaporator at 40°C.

2.3. Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

\[
\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}}
\]

2.4. Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.5. In vivo wound healing activity of *Dalbergia sisso*

2.5.1. Formulation of ointment

(a) Preparation of 20g simple ointment (B.P.) base: Wool fat (1g), hard paraffin (1g), cetostearyl alcohol (1g) and white soft paraffin (17g) was mixed properly and heated gently with continuous stirring then cooled.

(b) 1 gm 50% methanolic extract of *Dalbergia sisso* was added separately to 20gm of base (5% ointment).

(c) 2 gm 50% methanolic extract of *Dalbergia sisso* was added separately to 20gm of base (10% ointment).

2.5.2 Animal Care and Handling

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal. *Wistar* Rats (100±150 gm), either sex were housed in a group of six in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly.

2.5.3 Induction of diabetes in rats

After 15 h fasting, rats were intraperitoneally treated daily with 125 mg/kg of alloxan monohydrate (Sigma Chemicals) freshly dissolved in distilled water (5%) for two consecutive days. After the 24 hr of injection, blood was drawn from the orbital plexus and the glucose level was determine. Wounds were made on the rats showing elevated blood glucose (> 250 mg/dL).

2.5.4 Incision wound model

Wounds were created after the induction of diabetes. Incision wound healing method was used to calculate the wound healing potential of different extracts in experimental rats. Rats in each group were anesthetized and longitudinal paravertebral-long incisions (6 cm in length) were made through the skin and cutaneous muscles at a distance of about 1 cm from the midline on each side of the depilated back of the rat. After the incision was made, the parted skin was held together and stitched with black silk thread at 0.5 cm intervals. The wound was left undressed. The ointments prepared from the extract (5%, and 10% w/w) and the standard ointment were administered once daily for 20 day.

2.5.5 Grouping of Animals

The animals were randomly divided into following 8 groups; each group consists of six animals. Animal grouping and their treatment is as follows:


Group-V: Alloxan-induced diabetic rats with wound given extract 10% ointment

Group-VI: Alloxan-induced diabetic rats with wound treated with standard drug nitrofurazon

All the above groups received respective treatments at a single daily dose for a period of 20 days.

3. RESULT

3.1. Percentage yield

The plant material was extracted by hot extraction and the percentage yield calculated by the following formula was found to be 0.35 % (by petroleum ether) and 8.47 % (by ethanol).
3.2. Phytochemical analysis

Table no. 1

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
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</tr>
<tr>
<td></td>
<td>Mayer’s reagent test</td>
<td>Fail</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent test</td>
<td>Fail</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent test</td>
<td>Fail</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>Present</td>
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<tr>
<td></td>
<td>Molish’s test</td>
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</tr>
<tr>
<td></td>
<td>Fehling’s test</td>
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</tr>
<tr>
<td></td>
<td>Benedict’s test</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Barfoed’s test</td>
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</tr>
<tr>
<td>3</td>
<td>Protein and amino acids</td>
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<td>Biuret test</td>
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<tr>
<td></td>
<td>Million’s test</td>
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<td>4</td>
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<td></td>
<td>Shinoda test</td>
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<td></td>
<td>Lead acetate test</td>
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<td>5</td>
<td>Glycoside</td>
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<tr>
<td></td>
<td>Borntrager</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Legal’s test</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Killer-killiani test</td>
<td>Pass</td>
</tr>
<tr>
<td>6</td>
<td>Tannin and phenolic compound</td>
<td>Present</td>
</tr>
</tbody>
</table>
Ferric chloride test | Pass  
|----------------------|------  
Lead acetate test | Pass  
|----------------------|------  
Dilute iodine solution | Pass  

7  **Saponin**  

Foam test | Fail  

8  **Test for triterpenoids and steroids**  

Salwonki test | Pass  

Libberman and burchard’s test | Pass  

**Percentage yield**  
The plant material was extracted by hot extraction and the percentage yield calculated by the following formula was found to be 0.35 % (by petroleum ether) and 8.47 % (by ethanol).
4. DISCUSSION AND CONCLUSION
Wound healing (WH) is a physiological function in which a balanced inflammatory response is required for tissue regeneration and successful wound closure. Diabetes-impaired wound healing and other tissue abnormalities are considered to be a major concern for clinicians. The biochemical mechanisms involved in the healing process are mainly associated with disorders in collagen production that consequently delay re-epithelialization in wounds and compromise migration and proliferation of keratinocytes and fibroblasts.

The prevalence of diabetes has become a major clinical problem and a serious issue for public health; impaired wound healing in patients with diabetes is a complication. Lack of cellular and molecular signals required for the normal wound repair process, including angiogenesis, granulation tissue formation, epithelialization, and remodeling, are encountered in these patients, which contribute to poor wound healing. The normal healing process in healthy individuals occurs at an optimal rate; however, this is usually delayed or even completely compromised in patients with diabetes.

There is an increasing interest in the potential of traditional and complementary medicines in wound care that has led to studies investigating a range of plant extracts and other products as traditional wound healing agents. These agents usually influence one or more phases of the healing process, and they are involved in disinfection and provide a moist area to encourage the establishment of a suitable environment for wound healing.

Plant extracts with wound healing properties have the potential for antioxidant, chelation, and antimicrobial activities and may act by one or more of these mechanisms. Natural antioxidants have been reported to play a major role in blocking the oxidative stress induced by free radicals. Therefore, it is important to explore other sources of safe antioxidants or natural agents. Recently, researchers have shown interest in edible and medicinal plants for their phenolic contents and related total antioxidant activities.

Wound healing in patients with diabetes is impaired and delayed due to high blood glucose levels, which hampers cell proliferation and decreases collagen production, resulting in decreased chemotaxis and phagocytosis. Elevated blood glucose levels, a reduction in the levels of growth factors, and the inhibition of fibroblast proliferation all have been suggested to contribute to the observed impairment in wound healing. Alloxan-induced diabetes in animals is one of the most extensively studied models of diabetes. In this study, therefore, rats with alloxan-induced diabetes were used as the model to study diabetic wound healing.

Collagen, the major component that strengthens and supports extracellular tissue, is composed of the amino acid hydroxyproline, which has been used as a biochemical marker for tissue collagen. Antioxidants have been reported to play a significant role in improving the wound healing process and protecting the tissues from oxidative damage. Wound healing mechanisms may contribute to stimulating the production of antioxidants in the wound site and to providing a favorable environment for tissue healing.
Antioxidants were also detected for their presence in the extracts using ascorbic acid as standard through superoxide scavenging assay and reducing power assay. The superoxide scavenging assay is performed by making dilutions ranging from concentrations 20, 40, 60, 80 and 100 µg/ml. % inhibition was calculated in order to evaluate the reducing ability of the extract. The ethanolic extract was tested and the results were obtained showing % inhibition was 38.19.

In the present study, the extract of *Dalbergia sissoo* leaves had properties that render it capable of promoting accelerated wound healing activity compared with the controls. On the basis of the results obtained in the present study, it is possible to conclude that *Dalbergia sissoo* leaves ointment has significant wound healing activity in rats with diabetes. Dose-response studies should be conducted on the *Dalbergia sissoo* leaves to determine maximal efficacy on diabetic wound healing.

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