ABSTRACT

The present study was conducted to evaluate the wound healing effect of *Melia azedarach* L. leaf extract in alloxan induced diabetic rats. The methanolic leaf extract of *Melia azedarach* L (2% and 5%) were topically applied and the wound healing potential was evaluated in alloxan induced diabetic rats. The wound healing efficacy was studied by using excision wound model, which was inflicted by cutting away 500 mm² of the skin on the anterio–dorsal side of the alloxan induced diabetic rats. Povidone iodine ointment was used as reference standard. Wound healing property, based on wound contraction & percentage of wound contraction was measured and assessed upto 18 days. The topical application of methanolic leaf extract of *Melia azedarach* L (2% and 5%) had promoted wound healing in diabetic rats. There was significant contraction of wound observed with topical application of methanolic leaf extract of *Melia azedarach* L in alloxan induced diabetic rats. Further phytochemical studies are required to isolate the active compounds responsible for these pharmacological actions.

**Keywords:** *Melia azedarach* L, Diabetic mellitus, wound healing
INTRODUCTION

Diabetes mellitus is one of the major contributors to chronic wound healing problems. When diabetic patients develop an ulcer, they are at high risk to develop major complications, including infection and amputation.

Diabetes mellitus is a condition which is known to be associated with a variety of connective tissue abnormalities (Abdollah et al., 2010). The collagen content of the skin is decreased as a result of reduced biosynthesis and accelerated degradation of newly synthesized collagen. These abnormalities contribute to impaired wound healing in diabetics. One of the most outward and debilitating complications of diabetes is the development of chronic non-healing foot ulcerations, occurring in 15% of diabetics (Goodson and Hunt., 1977). Many Ayurvedic medicinal plants play a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way.

*Melia azedarach* L (Photo slides I) belonging to the family Meliaceae is one of the most useful traditional medicinal plants like *Azadirachta indica*. *M. azedarach* L is native to tropical Asia. It is widespread and naturalized in most of the tropics and subtropical countries. It was introduced and naturalized in Philippines, United States of America, Brazil, Argentina, many African and Arabian countries (Adnan and AL-Rubae., 2009).

Photo Slides I: (a,b,c) displaying different parts of *Melia Azedarach* L

a. Branch of *M. azedarach* L  
b. Leaves of *M. azedarach* L  
c. Fruits of *M. azedarach* L

Leaves are used in leprosy, scrofula etc. It is also a known anthelmintic, antilithiatic, diuretic, deobstructant and resolvent. Seed oil is the most active medicinal product of the plant and used as an antiseptic for sores and ulcers that show no tendency to heal. Fresh leaf extract is applied externally for burns. Fresh leaf extract is used as a mouth wash in gingivitis. 5 ml of Leaf extract is administered orally thrice a day for treating piles (Abdul Viqar Khan et al., 2011). Seeds of *Melia azedarach* L have been scientifically reported to exert antimalarial (Nathan et al., 2006), antifungal (Carpinella et al., 2005), ovicidal (Maciel et al., 2006) insecticidal (Akthar et al., 2008), antifeedant (Charleston et al., 2005), rodenticidal (Roop et al., 2005) activities in-vitro and in-vivo studies.

Studies have been reported that *Azadirachta indica* leaf which is closely related species of *Melia azedarach* L possess hypoglycemic (Murty et al., 1978) and wound healing activity (Chaw et al., 1994). It was also reported that *Azadirachta indica* leaf exhibited wound healing effect in alloxan induced diabetic rats (Sengottuvelu et al., 2007).

In view of the ethno-botanical uses and medicinal properties of *M. azedarach* L, it is surmise that this plant might possess wound healing properties. Therefore, the present study was aimed to evaluate the wound healing activity of the leaf extract of *M. azedarach* L in alloxan induced diabetic rats.
MATERIAL AND METHODS

Chemicals and Reagents

Alloxan procured from LOBA Chemie (Mumbai). All other chemicals and reagents used in this study were of analytical grade.

Plant material

The leaves of *Melia azedarach* L were collected from outskirts of Erode, in the month of May. The leaves of *M. azedarach* L were identified and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore. The (voucher no:45/212) specimen had been deposited in the herbarium for future reference.

Preparation of extract

100 g of powdered drug was soaked in 250 ml of 95% methanol solution for 24 h followed by cold maceration for further 48 h with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by means of rotatory evaporator. The dried extract was used for the study.

Preparation of formulation

2 g and 5 g of dried extract was admixed to 99 g of simple ointment base to obtain 2% and 5% of *M. azedarach* L ointment respectively.

Animals

Male Wistar Albino rats weighing between 150–220 g were used for the study. The animals were obtained from animal house, IRT Perundurai medical college, Erode, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30–70 %. A 12:12 light: dark cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chow (Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Induction of diabetes

Alloxan monohydrate (SD Fine chemicals Ltd, Boisar.) was used to induce diabetes in rats. The base line plasma glucose levels were determined prior to alloxan administration. Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) in sterile saline. After 72 h of alloxan injection, the diabetic rats (blood glucose > 250 mg/dl) were used for the study (Ravi *et al.*, 2003).

Excision wound model

An excision wound was inflicted by cutting away approximately 500 mm² full thickness of shaved skin at a predetermined area on the antero dorsal side of the alloxan induced diabetic rats under pentobarbitone (30 mg/kg., i.p) anesthesia. The entire wound was left open. Animals were closely observed for any infection and those which showed signs of infection were separated, excluded from the study and replaced (Udupa *et al.*, 1994).

Experimental protocol

Totally 30 animals were used in this study. The rats were divided into 5 groups of 6 animals each. Excision wound was inflicted in all the rats of 5 groups. Group I, non diabetic animals were treated with simple ointment base. Group II, diabetic control animals were treated with simple ointment base. Group III and IV, diabetic animals were treated with 2% and 5% of *M. azedarach* L methanolic extract in simple ointment base respectively. Group V, diabetic animals were treated with standard povidone iodine ointment. All the test drugs were applied topically on the wound, twice daily for 18 days.

Assessment of wound contraction

Wound contraction was monitored by metric measurement of the wound area once in 3 days upto 18th day. This was studied by
tracing the raw wound area on a transparent polythene paper and the traced area was measured by using a graph paper. The wound contraction was measured as a percentage decrease of original wound size of 500 mm² for each animal of a group.

Statistical analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet’s t test. P values < 0.05 were considered as significant.

RESULTS

The results of wound healing activity and % (percentage) of wound contraction of Melia azedarach L leaf extract in alloxan induced diabetic rats are showed in Table I & Figure I respectively. Topical application of both concentrations 2% and 5% of Melia azedarach L leaf extract promotes the contraction of wound in diabetic rats, when compared to diabetic control. The percentage of wound contraction after topical application of test drugs on 6th day of observation shows that in diabetic control it was 3.29%. In 2% and 5% of M. azedarach L leaf extract it was 9.29% and 10.79% respectively, whereas in standard drug povidone iodine ointment it was 9.69%. Observation shows that on 9th day onwards, the topical applications of both the concentrations M. azedarach L leaf extract promoted the wound contraction faster than diabetic control. Wound contraction progressed much faster in M. azedarach L leaf extract from 12th day than the normal control and diabetic control. There was significant (p < 0.001) contraction of wound, observed with M. azedarach L leaf extract and standard povidone iodine ointment on 12th to 18th day when compared with diabetic control. Topical applications of M. azedarach L leaf extracts promoted wound contraction better than povidone iodine treated groups and non diabetic control groups.

Table I: Effect of Melia azedarach L leaf extract on excision wound in alloxan induced diabetic rats.

<table>
<thead>
<tr>
<th>Post Wounding Days</th>
<th>Wound Area (mm²)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group I Non-Diabetic control</td>
</tr>
<tr>
<td>3</td>
<td>486.15 ± 23.12</td>
</tr>
<tr>
<td>6</td>
<td>460.53 ± 26.25*</td>
</tr>
<tr>
<td>9</td>
<td>303.22 ± 24.50**</td>
</tr>
<tr>
<td>12</td>
<td>206.20 ± 18.21***</td>
</tr>
<tr>
<td>15</td>
<td>113.36 ± 8.61***</td>
</tr>
<tr>
<td>18</td>
<td>17.94 ± 1.12***</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM; (n = 6)
*P < 0.05, **P < 0.01, *** P < 0.001 Vs Diabetic Control
DISCUSSION

The present study was undertaken to evaluate the wound healing activity of *Melia azedarach* L leaf extract in alloxan induced diabetic rats. Wound healing deficits with Diabetes mellitus believed to be largely caused by some basic mechanisms (Sidhu et al., 1999). (i) Increased blood sugar that impairs blood flow and the release of oxygen. (ii) Impaired local immune and cell defenses and (iii) Microbial infections. *M. azedarach* L leaves possess hypoglycemic activity, immunomodulatory activity, anti fertility activity and anti microbial activity (Kausik Biswas et al., 2002). In this study it has been proven that the topical application of *M. azedarach* L leaf extract promotes wound healing in diabetic rats and its effect was comparable with standard povidone iodine. The delay in the healing of wound in diabetic rats may be due to enhanced blood glucose, which favors the microbial growth. The *Melia azedarach* L leaf extract enhanced the wound healing in diabetic rats in our study which may be due to its antimicrobial activity.

CONCLUSION

From the result it could be concluded that the topical application of methanolic leaf extract of *Melia azedarach* L shows significant wound healing activity in alloxan induced diabetic rats. Further phyto-chemical studies are required to isolate the active compounds responsible for wound healing activity which could be a major contribution to prove the claims in Indian systems of medicine.
REFERENCES


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Conflict of Interest: None Declared