EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF LANTANA CAMARA IN STREPTOTOTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT

Background: Lantana camara (Verbanacea) is a commonly available medicinal plant throughout India. Wound healing property of the plant in various wound models has been studied. Thorough literature survey revealed that the wound healing property of Lantana camara in diabetic wound was not studied. This study was aimed to evaluate the wound healing property of Lantana camara in diabetic rats.

Methods: Group-1 rats served as normal control in which excision wound was created in normal, non-diabetic rats and wound was topically applied with vehicle. To induce diabetes mellitus in group 2-5, a single injection of streptozotocin (45 mg/kg, i.p.) prepared by dissolving in 0.9% ice cold citrate buffer was given. Excision wound was inflicted in the back of the rats. Group-2 was the diabetic control in which diabetic rats received vehicle ointment topically. Group 3, 4 and 5 were the test drug groups in which diabetic rats were topically applied ethanolic extract of Lantana camara in three doses 10%, 15% and 20% respectively. Wound healing parameters such as percentage of wound contraction rate and epithelialization period were observed. Data was analyzed using SPSS software by one way ANOVA and the statistical significance was fixed as p < 0.005.

Results: There was a delay in wound healing in diabetic rats compared to non-diabetic rats. The extract showed dose dependent increase in wound contraction rate and hastened the epithelialization period. Extracts enhanced contraction rate only during later phase of wound healing process. High dose (20%) extract showed maximum healing effect.

Conclusion: Topical application of ethanolic extract of Lantana camara showed dose dependent wound healing activity in diabetic rats.

Keywords: Lantana camara, Diabetic ulcer, Excision wound, Ayurveda, Siddha.

INTRODUCTION

Diabetes affects approximately 170 million people worldwide and by 2030 these numbers are projected to double. The diabetic foot ulcer is estimated to occur in 15% of diabetics, which is the major morbidity associated with diabetes, often leading to pain and poor quality of life for patients. The moment a person with diabetes suffers a break in the skin of their foot, they become at danger of amputation. Currently, very few FDA approved therapies such as growth factor and antispasmodic properties comparable to those of quinin activity.

Indian traditional medical systems such as Ayurveda and Siddha literature describe diabetic ulcers as Mega Katti or Pilavai. Siddhar Agastyar of Siddha system has written three manuscripts regarding wound management including diabetic wound. Lantana camara (Verbanacea) is a commonly available medicinal plant throughout India. In Central America, the leaves were used for the management of sores, chicken pox and measles. In India, the leaves are used to treat cuts, ulcers, swelling, cough and intestinal worms. Scientific studies showed that lancamamine, a steroid component of the plant has cardiac tonic property and lantamide, an alkaloid for the plant has antipyretic ad antispasmodic properties comparable to those of quinin. This plant was proved for its antimicrobial, antifungal, antihelmintic and antiviral activity. Antioxidant, anti-hyperglycemic, anti-ulcerogenic and antipyretic properties of this plant were proved in animal models [2]. It showed the wound healing property in excision wound and burn wound in rats in normal rats [3-5]. Thorough literature survey revealed that the wound healing property of Lantana camara in diabetic rat was not studied. Hence, this study was aimed to evaluate the wound healing property of Lantana camara in diabetic rats.

MATERIALS AND METHODS

Preparation of ethanolic extract and ointment[6]

The fresh Lantana camara leaves were collected surrounding Manipal area, shade dried and ground to powder. The powder was loaded in Soxhlet extractor in 8 batches of 200 g each and was subjected to extraction for 20 hours with 200ml of 95% ethanol. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to a syrupy consistency. Then it was dried in a desicator. For this study (topical application), ointment of ethanolic extract was prepared using simple ointment base in three strengths i.e 10%, 15% and 20%.

Preliminary phytochemical analysis

The dried extract was subjected to quantitative analysis to find out the presence of saponins, tannins, triterpenes, alkaloids and flavonoids by standard procedure [7].

Experimental Animals

The experimental protocol was approved by Institutional Animal Ethics Committee of Kasturba Medical College, Manipal University, India and animals were maintained under standard conditions in animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy, 30 male albino Wistar rats weighing 250-300 g were used. Rats were housed under controlled conditions of temperature 23 ± 2°C, humidity 50 ± 5% and 10-14 hours light and dark cycle respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk as bedding after making burn wound till completion of wound healing. Animals were maintained on normal diet (Amrut lab animal feed, Pranav agro industries Ltd., Sangli, Maharashtra, India) and water ad libitum.

Induction of diabetes mellitus in rats [8]

After overnight fasting, 24 rates were induced diabetes mellitus by giving a single injection of streptozotocin (45 mg/kg, i.p.) prepared by dissolving in 0.9% ice cold citrate buffer (pH 4.5). Fasting blood glucose level was estimated using Glucometer (Ames, Bayer Diagnostic) 24 h of the injection by taking blood from tail vein.
Normal control group was not induced diabetes mellitus. Rats showing blood glucose level of 250-300mg/dl were included for the wound healing study.

Excision wound and treatment

Under anesthesia, the hair was shaved from the dorsal thoracic central region. One excision wound was inflicted by cutting away a 300 mm² full thickness of skin from a predetermined area. Excision wound was induced in diabetic as well as normal rats. Each animal was maintained in a separate gauge till the end of study. The ointments were applied topically once daily on every day from the day of creation of excision wound, till the wound was completely healed. Normal control and diabetic control groups received vehicle (simple ointment base) topically. Three treatment groups received the topical extract ointment in the dose 10%, 15% and 20%.

Animals were divided into 5 groups of 6 in each. The normal control group A was left with out any treatment, normal treated group B was treated with Lantana Camara Ethanolic extract twice a day. Diabetic control group C was also left with any treatment. Diabetic experimental rats group D was treated with Lantana Camara ethanolic extract twice a day and the positive control group E were treated with the standard drug which is available in market (bacitracin ointment). The wound areas were measured on 0th, 7th and 11th day for the groups using butter paper.

Measuring wound healing activity

Wound healing activity was measure by assessing two parameters such as % wound contraction rate and epithelization period. Wound area was traced using butter paper and was retracted on a millimeter scale graph paper. Percent wound contraction was calculated using the following formula;

\[
\text{% wound contraction} = \frac{\text{initial wound size - specific day wound size}}{\text{initial wound size}} \times 100
\]

Epithelization period was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind[9].

Statistical analysis

Results were analyzed using SPSS 11.5 version and expressed in mean ± SD. The difference between experimental groups were compared using one way Analysis of Variance (ANOVA) followed by Posthoc Test viz Tukey Alpha (0.05). The results were considered statistically significant at p < 0.05.

RESULTS AND DISCUSSION

Phytochemical analysis

Preliminary qualitative phytochemical analysis showed the absence of saponin, tannin and alkaloids, whereas tests showed positive for the presence of triterpenes and flavonoids.

Wound contraction rate [Table 1, Figure 1]

Wound contraction rate was delayed in diabetic rats compared to normal (non-diabetic) rats throughout the study. In fact, Lantana camara ethanolic extract further delayed the wound contraction during initial seven days. After seven days, all three doses of extract showed a statistically significant wound contraction than diabetic control. Day 11 onwards, all three doses of extracts showed more wound contraction when compared to non-diabetic rats too. It was also observed that the extract produced dose dependent wound contraction rate. Among three doses, high dose (20% extract) showed faster wound contraction rate.

Table 1: Effect of topical Lantana camara ethanolic extract in wound contraction rate in excision wound

<table>
<thead>
<tr>
<th>Groups</th>
<th>% wound contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 3</td>
</tr>
<tr>
<td>Normal control</td>
<td>32.41 ± 1.366*</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>18.43 ± 1.000*</td>
</tr>
<tr>
<td>diabetic + 10% extract</td>
<td>-4.36 ± 0.684*</td>
</tr>
<tr>
<td>diabetic + 15% extract</td>
<td>-7.07 ± 0.684*</td>
</tr>
<tr>
<td>diabetic + 20% extract</td>
<td>-19.35 ± 0.684*</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SD, n = 6.

*p < 0.001 vs. diabetic control *p = 0.005 vs. diabetic + 10% extract , *p = 0.010 vs. diabetic control *p = 0.002 vs. diabetic + 10% extract

*p = 0.001 vs. diabetic control ‡*p = 0.007 vs. diabetic control , ‡p = 0.005 vs. diabetic control ‡p = 0.034 vs. diabetic + 10% extract

‡ p = 0.003 vs. diabetic control ‡p = 0.011 vs. diabetic + 15% extract, ‡p = 0.006 vs. normal control ‡p = 0.010 vs. normal control
Diabetic rats (E) showed delayed wound contraction compared to non-diabetic rats (D). All three doses of extract showed higher wound contraction rate than diabetic control (E) as well as normal control (D). High dose (A) exhibited faster wound contraction rate compared to other two low doses.

**Period of epithelialization [Figure 2]**

Diabetic rats showed delayed (p = 0.001) epithelialization period compared to non-diabetic rats. All three doses of extract hastened the epithelialization period (p < 0.001) compared to diabetic control as well as non-diabetic control rats. The extract exhibited dose dependent effect in hastening wound healing. Among the three doses, high dose (20% extract) produced faster healing effect.

Initial inflammatory phase, proliferation & migration of epithelial cells, granulation tissue formation and wound contraction are the major phases of wound healing mechanism. A study was conducted by using Ethanol Phragmites vallatoria leaves extract applied topically increased healing of wound contraction in STZ induced diabetic rats where healing is delayed[10]. In diabetic rats, ethanolic extract of Lantana camara delayed wound healing during initial inflammatory phases, which was evident by negative wound contraction rate. But, extract has wound healing only during later phases, possibly by enhancing proliferation & migration of epithelial cells, improving granulation tissue formation and by enhancing wound contraction.

**CONCLUSION**

In diabetic rats, ethanolic extract of Lantana camara increased the wound contraction activity as well as epithelialization period only during the later phase of wound healing. Further studies are required to isolate the compound responsible for the wound healing activity of Lantana camara and to evaluate the mechanism of wound healing activity.

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**REFERENCES**

2. Kazmi, L, et al., Received by Qing Received on 2012-3-23 ID No. B 192 Revised on 2012-5-2 Anticonvulsant and depressant-like activity of ursoic acid stearoyl glucoside isolated from Lantana camara L.(verbanaceae).
6. Shanbhag, T., A. Amuthan, and S. Shenoy, Effect of< i> 

**Fig. 1: Wound contraction rate (%) in different groups of rats**

A – Diabetic + 20% extract
B – Diabetic + 15% extract
C – Diabetic + 10% extract
D – Non diabetic (Normal control)
E – Diabetic + vehicle control