WOUND HEALING POTENTIAL OF ETHANOLIC LEAF EXTRACT OF *HELIOTROPIUM INDICUM* ON EXCISION WOUND MODEL IN DIABETIC RATS

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ABSTRACT

The objective of the study is to evaluate the wound healing effect of ethanolic leaf extract of *Heliotropium indicum* on excision wound model in Streptozotocin (STZ) induced diabetic rats. The ethanolic leaf extract of 5% and 10% *Heliotropium indicum* was formulated as ointment for topical application and applied on the excision wound induced in STZ induced diabetic rats. The wound healing efficacy of *Heliotropium indicum* was studied by using excision wound, which was inflicted by cutting away 500 mm² of the skin on the anterio – dorsal side of the STZ induced diabetic rats. Mupirocin was used as reference control. All the test drugs were topically applied once daily for 14 days. The wound contraction and percentage wound contraction were observed at various time intervals (4th, 8th and 14th day). Both 5% and 10% of ethanolic leaf extract of *Heliotropium indicum* promotes significant (P<0.001) wound contraction and the effects were equipotent as that of Mupirocin. From the result it was concluded that, *Heliotropium indicum* exhibits wound healing property in STZ induced diabetic Wistar rats.

Key words: *Heliotropium indicum*, Streptozotocin, Wound healing, Mupirocin.

INTRODUCTION

*Heliotropium indicum* Linn., (Family: Boraginaceae) commonly known as ‘Indian heliotrope’ is very common in India with a long history of traditional medicinal uses in many countries in the world. It is a coarse foetid herb, up to 2 ft. high, with ascending hirsuit branches found throughout India in sunny localities, on waste lands and anthropogenic habitats in periodically desiccating pools and ditches and anthropogenic habitats, generally below 800 m altitude, widely considered as a weed of fields. The leaves are simple, alternate or subopposite, 4.5 to 10 cm/2.5 to 5 cm, ovate or ovate oblong, margin undulate, sparsely strigose along nerves on either side, serulate or undulate with cordate, minutely pilose beneath nerves and veins conspicuous on the lower side.

An ethnopharma-cological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use *Heliotropium indicum* to cure skin diseases, poison bites, stomachache and nervous disorders (Chellaiah et al., 2006). *Heliotropium indicum* is believed to be useful in treating malaria, abdominal pain and dermatitis (Togola et al., 2005). The decoction of the entire plant is taken orally for treatment of intractable fever, ulcers, venereal diseases and sore throat and used externally in vaginal cavity to induce abortion in pregnant females and administered rectally to treat local sores in the rectum and orally as diuretic and for the treatment of kidney stone (Quisumbing, 1951; Berhault, 1974). The sap of the stem is used orally by females for treating dysmenorrhea (Gurib Fakim et al., 2000). The leaf paste is applied externally to cure rheumatism (Nagaraju and Rao, 1990), while the seeds are used to treat cholera, malaria, and for wound-healing (Wiart, 2006). *Heliotropium indicum* is very rich in pyrrolizidine alkaloids and apart from alkaloids, several triterpenes and steroids (Pandey et al., 1996), were also isolated from the plant. The *Heliotropium indicum* reported to have antibacterial, antifungal activity (Singh et al., 1994; Das,
2011). Researcher have evaluated its anti-fertility (Andhiwal et al., 1985), anti-tumor, gastroprotective (Adelaja et al., 2006), anti-diabetic, anti-inflammatory, wound healing potential, muscle relaxant and uterine stimulant property (Dodehe et al., 2011; Shalini et al., 2010).

Diabetic wound are slow, non – healing wound that can persist for weeks despite adequate appropriate care. Though the exact pathogenesis of poor wound healing in diabetic wounds is not clearly understood, evidence from studies involving both human and animal models reveal abnormalities in the various phases of the wound healing process. The present study was under taken to evaluate the wound healing potential of Heliotropium indicum in diabetic animal.

MATERIAL AND METHODS

Plant Material

The leaves of Heliotropium indicum were collected from outskirts of Tirunelveli District, in the month of October. The leaves of Heliotropium indicum were identified and authenticated by Scientist ‘F’ Botanical Survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The Voucher specimen (BSI/SRC/5/48/14-15/Tech - 529) has been deposited in department for further references.

Preparation of Extract

100 g of shade dried, coarse powdered leaves were soaked in 250 ml of 95% ethanol 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 stored in desiccators and used for the study.

Preparation of Formulation

5 g and 10 g of dried leaf extract of Heliotropium indicum was admixed with simple ointment base to obtain 5% and 10% of Heliotropium indicum ointment respectively.

Animals

Healthy adult male albino rats of Wistar strain, weighing about 180-220 g were obtained from the animal house of Chengalpattu Medical College, Chengalpattu. The rats were isolated and housed in separate cages during the course of experimental period and kept them at room temperature (24± 2°C) with a 12 : 12 h light/dark cycle. The animals were fed with standard pellet diet and provided water ad libitum. All the procedures and protocols were reviewed and approved by the Institutional Animal Ethics Committee (ECR/774/Inst/TN/2015) of Chengalpattu Medical College, Chengalpattu.

Wound Healing Activity in STZ induced Diabetic Rats Induction of Diabetes

Streptozotocin (STZ) obtained from Sigma was used to induce diabetic in rats. The base line plasma glucose levels were determined prior to STZ administration. Diabetes was induced in rats by a single dose of STZ (50mg/kg) in cold 0.1M citrate buffer, pH 4.5 (freshly prepared) in tail vein to induce diabetes. Three days after STZ administration, the blood sugar levels more than 250mg/dl, were confirmed as diabetes and used in the study (Shivananda Nayak et al., 2007).

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 antro dorsl side of STZ induced diabetic rats under Pentobarbitone (45 mg/kg, ip) anesthesia.

Experimental Protocol

Totally 36 animals were used in this study. The rats were divided in to 6 groups of 6 animals each. Excision wound was inflicted in all the rats of 6 groups. Group I contain non diabetic rats and groups II to V contain STZ induced diabetic rats. Group I, served as non diabetic control, the wound was treated with topical application ointment base. Group II, served as diabetic control, the wound was treated with topical application ointment base. Group III, served as reference control treated with topical application of Mupirocin ointment. Group IV, treated with topical application of 5% Heliotropium indicum leaf extract and group V, treated with topical application of 10% Heliotropium indicum leaf extract. All the test drugs were applied once daily for 14 days.

Assessment of Wound Contraction

Wound contraction was measured on days 4, 8 and 15. This was studied by tracing the raw wound area on a transparent sheet and the area assessed by using a graph paper. The wound contraction was measured as a percentage decrease of original wound size 500 mm² for each animal of a group.

Statistical Analysis

Results are expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet’s t test. P values < 0.01 were considered as significant.

RESULT

The results of wound healing activity and Percentage wound contraction of 5 and 10 % Heliotropium indicum leaf extract on Streptozotocin induced diabetic rats were shown in table 1 and figure I.
Topical application of *Heliotropium indicum* leaf extract promotes the contraction of wound in diabetic rat, when compared to diabetic control. The observation shows that on 4th day, there was significant (P<0.01 and P<0.001) increase in wound contraction with topical application of 5 and 10 % *Heliotropium indicum* extract respectively. The % wound contraction of Mupirocin, 5 and 10 % *Heliotropium indicum* extract were 17.30%, 12.02% and 14.25 % respectively. On 8th and 15 day of observation both concentrations of *Heliotropium indicum* showed significant (P<0.001) increase in wound contraction. The reference control Mupirocin promotes significant (P<0.001) wound contraction from the 4th day onwards. The % wound contraction of Mupirocin, 5 and 10 % *Heliotropium indicum* extract, on 15th day was 98.36%, 81.15% and 90.93% as compared to diabetic control with 5.30%.

Table 1. Effect of *Heliotropium indicum* leaf extract on excision wound model in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug Treatment</th>
<th>Wound Contraction (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>38.90±1.98</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>18.42 ± 1.64</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic Control Mupirocin Ointment</td>
<td>86.52 ±5.71***</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic Control <em>Heliotropium indicum</em> 5%</td>
<td>60.44 ±5.32**</td>
</tr>
<tr>
<td>V</td>
<td>Diabetic Control <em>Heliotropium indicum</em> 5%</td>
<td>71.26±4.66***</td>
</tr>
</tbody>
</table>

Values are in Mean ± SEM; (n=6)

*P < 0.05, **P < 0.01, *** P < 0.001 Vs Control.

Figure 1. Percentage Wound Contraction of *Heliotropium indicum* leaf extract on excision wound model in diabetic rats.

CONCLUSION
The wound healing effect of ethnaolic leaf extract of *Heliotropium indicum* was studied on excision wound model in Streptozotocin induced diabetic rats. The result showed that, 5 and 10% of *Heliotropium indicum* exhibited wound healing activity on excision wound model in STZ induced diabetic rats. Further study may be required to isolate the active principal from the plant responsible for wound healing activity will bring a new potential medicinal plant for diabetic wound.

REFERENCES


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