
Wound Healing Activity of *Desmodium triquetrum* Leaves

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The ethanol extract of the dried leaves of *Desmodium triquetrum* was studied for wound healing effect using incision, excision and dead space wound models in rats. Significant increase in wound contraction rate, skin breaking strength, granuloma strength and dry granuloma weight and significant decrease in epithelization period was observed. The prohealing actions seemed to be due to increased collagen deposition as well as better alignment and maturation.

Desmodium triquetrum DC. (Leguminosae) is an erect or sub-erect under shrub distributed from the central to the eastern Himalayas, ascending to 4000 ft. It is distributed in Kumaon, Sikkim, Khasi hills, Southern India and in Sri Lanka. The leaves are used as a substitute for tea. The leaf extract or the pills made therefrom is used for the treatment of piles¹. The plant is reportedly used by some native villagers in Southern India for the rapid healing of the wounds. A review of literature however did not reveal any information on the wound healing properties of the plant. The present study is an attempt to assess the efficacy of this indigenous herb on various parameters related to wound healing in rats.

MATERIALS AND METHODS

The leaves of *Desmodium triquetrum* were collected from the Indrali temple area, Udupi, Karnataka in the month of October 1999 and was authenticated by the Department of Botany, Poorna Prajna College, Udupi. A voucher specimen (PP 501) has been deposited at the College of Pharmaceutical Sciences, Manipal.

Preparation of ethanolic extract:

The shade-dried powdered leaves (750 g) were exhaustively extracted with 95% ethanol using soxhlet apparatus. The total ethanolic extract was concentrated *in*

vacuo to a syrupy consistency (yield- 361 g).

Phytochemical screening:

The coarse powder of the leaves (100 g) was subjected to successive extraction with different solvents in increasing order of polarity from petroleum ether (60-80), benzene, chloroform, acetone, alcohol and finally chloroform-water. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents^{2,3}.

Acute toxicity studies:

Healthy adult Wistar rats of either sex, weighing around 200-220 g were used for the study. They were housed individually under standard environmental conditions, fed with pellet rodent diet and water *ad libitum*. The experimental protocol was subjected to the scrutinization of the Institutional Animal Ethics Committee and was cleared by the same before starting the experiments (No. IAEC/KMC/07/2001, CPCSEA Regd No. 94/1999). The animals, starved overnight were divided into 6 groups (n=2) and were fed with increasing doses (10, 30, 100, 300, 1000 and 2000 mg/kg) of the ethanolic extract of *D. triquetrum*. The animals were continuously observed for mortality and behavioral responses for 48 h and thereafter once daily for 14 d after administration.

Wound healing studies:

Studies were carried out using ether anaesthetised rats. Animals were divided into two groups (control and test) of

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TABLE 1: EFFECT OF THE ETHANOLIC EXTRACT ON WOUND HEALING.

| Treatment | Incision Breaking strength | Dead Space | | |
|--|-------------------------------|----------------------------|-----------------------|----------------------|
| | | Granuloma weight (g/100 g) | Breaking strength (g) | Hydroxyproline (g/g) |
| Control | 304±7.98 | 0.017±0.0035 | 255±8.64 | 721±109 |
| Ethanollic extract of <i>D. triquetrum</i> | 464±7.30 | 0.055±0.0036 | 450±13.49 | 863±119 |

Values are mean±S.E. (n=8); p≤0.005 vs control; control group was administered 1% acacia emulsion and test group was administered ethanollic extract (200 mg/kg) for 10 consecutive days.

eight animals each. They were orally administered with emulsion of the ethanollic extract *D. triquetrum* in 1% acacia (200 mg/kg) daily for 10 consecutive days in the incision and dead space wound model and for 21 d the excision wound model. The control group was treated with 1% acacia emulsion.

Incision wounds:

Two 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat⁴. Wounds were closed with interrupted sutures, 1 cm apart. The sutures were removed on the d 7. Wound breaking strength was measured on the d 10 by the method of Lee⁵.

Excision wounds:

A circular piece of full thickness (approximately 500 mm²) was cut off from a predetermined area on the back of the rat⁶. The wounds were traced on mm² graph paper on the day of wounding and subsequently on alternate days until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. The number of days required for falling of the eschar without any residual raw wound gave the period of epithelization.

Dead space wounds:

Dead space wounds were obtained by implantation of polypropylene tube (0.5 cm x 2.5 cm) one on either side on the dorsal paravertebral surface of rat. On the d 10 the granuloma tissue formed on the surface of the polypropylene tube was excised. Tensile strength was determined⁶. The granuloma tissue was dried in an oven at 60° and the dry weight was noted. Acid hydrolysate of one part of the dry tissue was used for the determination of hydroxyproline⁷. The other part of the tissue was kept in 10% formalin solution for histopathological studies to evaluate the effect of the

extract on collagen formation.

Statistical analysis:

Results, expressed as mean±S.E., were evaluated by Student's t-test. Values of p≤0.05 were considered statistically significant.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT ON EXICISION WOUND MODEL.

| Epithelization period (d) | % of wound contraction | |
|---------------------------|------------------------|--|
| | Control | Ethanollic extract of <i>D. triquetrum</i> |
| | 21.8±0.25 | 15.7±0.29 |
| 2 | 3.50±3.8 | 26.7±4.35 |
| 4 | 4.00±3.7 | 48.8±3.15 |
| 6 | 11.0±4.6 | 59.3±4.43 |
| 8 | 24.2±6.4 | 79.1±1.68 |
| 10 | 43.1±3.4 | 90.3±0.86 |
| 12 | 59.6±2.5 | 95.7±0.68 |
| 14 | 68.5±6.78 | 97.5±0.56 |
| 16 | 75.6±5.22 | |
| 18 | 79.2±8.13 | |
| 20 | 83.5±5.81 | |
| 22 | 99.8±1.26 | |

Values are mean±S.E. (n=8); p=0.005 vs control; control group was administered 1% acacia emulsion and test group was administered ethanollic extract (200 mg/kg) for 21 consecutive days.

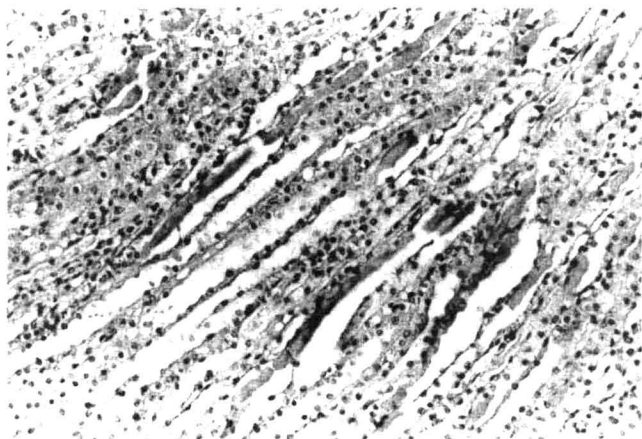


Fig. 1: Photomicrograph of granuloma tissue (Control)
Figure shows granulomatous tissues with interspersed collagen in control animals. Histological slides are stained with haematoxylin and eosin $\times 200$.

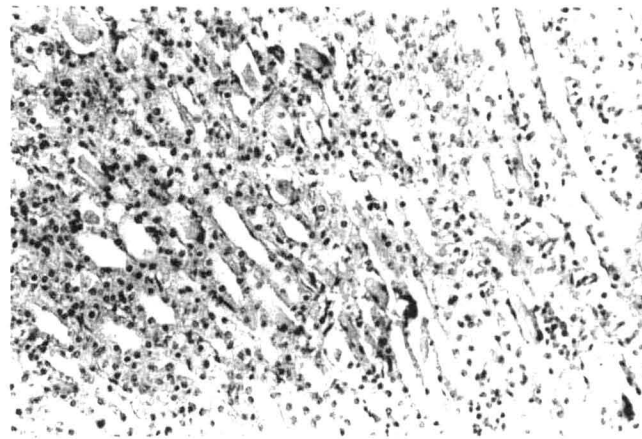


Fig. 2: Photomicrograph of granuloma tissue (Treated)
Figure shows granulomatous tissue obtained from rats that were treated with ethanolic extract of *D. triquetrum*. Granulomatous tissue with interspersed collagen. Haematoxylin and Eosin $\times 200$.

RESULTS

Preliminary phytochemical screening revealed the presence of flavanoid glycosides, steroids, saponins, phenolic compounds, amino acids and fixed oils. Acute toxicity studies of the ethanolic extract of the leaves of *D. triquetrum* did not exhibit any signs of toxicity upto a maximum of 2 g/kg body weight. The effect of the ethanolic extract of the leaves of *D. triquetrum* on various wound healing parameters using different wound models is given in Tables 1 and 2. In the incision wound model, significant increase was observed in the tensile strength of ethanol extract treated group (463 ± 7.9) on d 10 after wounding. The drug-treated animals of the dead space wound model showed significant increase in granuloma weight and its breaking strength (Table 1). Increase in the hydroxyproline content however was not statistically significant. Histopathological study revealed increased collagen deposition in the drug treated group (figs. 1 and 2).

Studies using the excision wound model (Table 2) showed significant decrease in the epithelization period. Epithelization was found to be enhanced significantly ($p \leq 0.05$) by the ethanolic extract of *D. triquetrum* as evidenced by the shorter period for the fall of eschar (15.7 ± 0.29 d) as compared to the control (21.8 ± 0.25 d). The drug extract also facilitated wound contraction significantly.

DISCUSSION

Wound healing involves various phases viz granulation,

collagenation, collagen maturation and scar maturation. The use of a single model is inadequate and there is no recommended reference standard which can collectively represent the various components of wound healing. Hence three different models have been used in our study to assess the effect of the various phases which run concurrently but independent of each other. Drugs which influence one phase may not necessarily influence another.

The results of our study show that the ethanol extract of the leaves of *D. triquetrum* possess a definite prohealing action. This is demonstrated by a significant increase in the rate of wound contraction by enhanced epithelization, increase in skin breaking strength, increase in hydroxyproline content which is a reflection of increased collagen levels⁸ and which is further supported by histopathological evidence, gain in granuloma breaking strength which indicates increased collagen maturation by increased cross-linking and an increase in dry granuloma weight indicating higher protein content.

From the study carried out, it may be concluded that the plant *D. triquetrum* is endowed with significant wound healing activity, thereby justifying its use in the indigenous system of medicine.

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