EVALUATION OF FLAVONOID FRACTION OF *TRIGONELLA FOENUM-GRAECUM* FOR ITS WOUND HEALING POTENTIAL

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Summary

Wound healing potential of *Trigonella foenum- graecum* (TFG) was evaluated in the present study. The study was done on rodents using incision, excision and dead space wound models. The flavonoid fraction of TFG seed enhanced the breaking strength of incision wounds significantly at 200mg/kg and 500mg/kg ($p < 0.01$) as compared to control. At both the dose levels, TFG fraction showed significant reduction in the wound area in the excision wound model. Dry granuloma weight and granuloma tissue breaking strength were increased significantly by TFG. Histopathological reports of the granulation tissue of the treated groups showed better healing when compared to control. These results indicated the pro-healing effect of flavonoid fraction of TFG seed

Keywords: *Trigonella foenum-graecum*, wound, tensile strength, excision model, granuloma, Hydroxyproline

Introduction

Wound healing is a complex phenomenon driven by processes, viz., induction of an acute inflammatory process, regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, synthesis of extra cellular matrix proteins, remodeling of connective tissue, and acquisition of wound strength [1]. When tissue is disrupted following injury, collagen is needed to repair the defect and restore anatomic structure and function. The healing process requires a sophisticated interaction among inflammatory cells, biochemical mediators, extra cellular matrix molecules and micro-environmental cell population [2]. Wound healing disorders present a serious clinical problem and are likely to increase since they are associated with diseases such as diabetes, hypertension and obesity. Wound healing is a complex and

complicated process. It runs through a number of phases, such as coagulation, inflammation, granulation, fibroplasia, collagenation, wound contraction and epithelialization etc., that occur between injury and healing. These phases either run concurrently or intimately inter-linked through some chemical, biochemical and cellular pathways. An intervention into any of these phases could influence the healing. Wound healing promoters are essential in the management of the delayed wound healing. Scientific evaluation of existing herbal medicine can be a good strategy to identify potential healing promoters. The seeds of Trigonella foenum-graecum (Family: Leguminosae) has long been known as a folk medicine for the treatment of inflammation [3]. However, there is no scientific evidence to substantiate the folklore claims of Trigonella foenum- graecum against healing disorders. Thus the present study has been undertaken.

Materials and Methods

The dried fenugreek seeds (edible) were procured from local market of Anavatti, Shimoga district, Karnataka and were authenticated by Professor V S Salimath, Dept of Botany, R L S Institute, Belgaum, Karnataka. A voucher specimen (#KLECOPH/2/2003) was deposited at the department's drug archive. Methanol, diethyl ether, ethyl acetate were procured from Loba chemicals, Mumbai. The standard pellets for rat feed were procured from Amruth Feeds, Belgaum

Extraction and preparation of flavonoid rich fraction

150 grams of powdered seeds were packed in the thimble of a soxhlet extractor and subjected to 95% ethanol which was continued for about 20 cycles. The temperature was maintained at 60°C. The extract obtained was distilled and later evaporated to get a thick mass.

The so obtained extract was concentrated to dryness. The dried extract was dissolved in minimal amount of ethyl acetate. This was fractionated with cold diethyl ether to get a precipitate. The standard phytochemical tests were performed to ensure the presence of flavonoids in the fraction. [4, 5]

Animals

Animal care and handling was done according to the statutory guidelines issued by the CPCSEA, Govt. of India. Approval was obtained from the Institutional Animal Ethics Committee for the conduct of the study. Healthy inbred albino rats of either sex (180g - 280g) were used for the wound models. They were maintained under controlled conditions of temperature (23±20C), humidity (50±5º %) and light (14 and 10 h of light and dark, respectively) at the animal house. The animals were provided with food and water *ad libitum*. One animal was housed in each polypropylene cage containing paddy husk as bedding. The surgical intervention was carried out under ketamine anesthesia (10mg/kg)

Acute Toxicity Study

The overnight fasted mice were administered orally with aqueous, alcoholic extract and butanol fraction of SS suspended in 2% gum acacia at doses of 500, 1000, 2000 and 5000 mg/kg body weight. The animals were observed continuously for 2 hours then, frequently up to the next 6 hours post dose. The number of survivors was recorded at 72 hours [6,7].

Drug Administration

200mg/kg and 500mg/kg were the doses selected for the flavonoid fraction. Control group was administered distilled water orally. The test groups received the respective doses dissolved in distilled water orally.The extracts were administered orally for 0-9 days in dead space and incision wound models. In excision model they were administered for 21 days or till the complete healing of wound, whichever was early.

Incision wound model [8]

Two para-vertebral straight incisions of 4 cm each were made of entire thickness on either side, at least one cm lateral to the vertebral column. The interrupted sutures were placed at equidistant points of 1 cm each. Animals were treated with the extracts and dexa. Sutures were removed on 7th post wounding day. The wound breaking strength was estimated on 10th post wounding day by constant water flow technique [9]

Excision wound model [10]

Using Accupunch of 10 mm diameter, a full thickness skin was excised to get wound size of 500 mm2 in the dorsal thoracic central region and 5 cm away from ears of anaesthetized rats. After haemostasis, animals were placed in the cages and were administered extracts. The wound contraction was calculated as the percentage of the original wound size (500 mm^2) for each animal of group on day 4,8,12 and14.Falling of scab leaving no raw wound behind was taken as end point of complete epithelialization and the days required for this were taken as period of epithelialization

Dead space wound model

The dead space wound was created by implanting subcutaneously 2.5×0.5cm polypropylene tube in the lumbar region on the dorsal side [11]. Animals received treatment as per the grouping. Granulation tissue grown on the implanted tube was carefully harvested along with the tube. The tubular granulation tissue was cut along its length to obtain the sheet of the granulation tissue which was further cut into two equal pieces. A piece of granulation tissue was fixed between two Babcock forceps and its breaking strength was measured by constant water flow technique [12]. The average of the two readings was taken for group mean. Both pieces of granulation tissue were dried at 60^oC for 24h and dry weight was taken. The dried granulation tissues from each group were digested using 6N HCl and neutralized with sodium hydroxide. The content of hydroxyproline was estimated [13.]

Statistical analysis
Results were analyzed by One-Way ANOVA followed by Post- Hoc Tukey's test using the Graphpad Prism 5.0. The results were expresses as mean ± SE.

Results

Effect on the tensile strength (incision model)
The mean breaking strength in control was 200 ± 14.49 g while it was 347 ± 24.37 g and 386 ± 23.51 g for 200 mg/kg and 500 mg/kg treated groups respectively.

Table1: Effect of flavonoid fraction of TFG on the breaking strength of incision wound

Group	**n**	**Breaking strength (g) Mean ± SE**
Control	10	200 ± 14.49
TFG 200mg/kg	10	347 ± 24.37*
TFG 500 mg/kg	10	386 ± 23.51*

* Dunnet's t test, $p < 0.01$ vs control

Effect on wound contraction (excision model)
On 8th post wounding day, the % wound closure in drug treated groups were little more than that of the control, although statistically not significant. A statistically significant promotion of wound closure was seen by both the treated groups on 12th, 16th and 20th post wounding day.

Table 2: Effect of flavonoid fraction of TFG on the excision wound

Group	**N**	**% closure**				**Epithelization (days) Mean ± SE**	**Scar area (mm^2) Mean ± SE**
		8^{th} day Mean ± SE	**12^{th} day Mean ± SE**	**16^{th} day Mean ± SE**	**20^{th} day Mean ± SE**		
Control	10	61.23 ±11.77	77.11 ± 2.92	81.55 ± 2.31	89.81 ± 1.76	24.50 ± 0.42	44.17 ± 1.99
TFG 200mg/kg	10	64.23 ± 2.67	84.40 ± 2.25	91.41 ± 1.43*	97.95 ± 0.50*	21.00 ± 0.52*	33.17 ± 1.35*
TFG 500 mg/kg	10	71.88 ± 1.93	89.89 ± 2.41*	97.07 ± 0.70*	100*	18.50 ± 0.22*	29.83 ± 1.94*

* Dunnet's t test, $p < 0.01$ vs control

Effects on granulation tissue breaking strength and dry granulation weight

Mean value of granulation breaking strength in both the treated groups were 367.5 ± 11.95 g and 408.5 ± 7.18 g respectively as compared to 284 ± 8.58 g of control. The treated groups significantly enhanced the dry granulation weight when compared to the control.

Effects on collagen content of granulation tissue (dead space model)

Hydroxyproline content of granulation tissue was significantly increased in the treated groups at both the dose levels.

Table 3: Effect of flavonoid fraction of TFG on the dead space wound

Group	**n**	**Granulation Tissue Parameters (Mean ± SE)**		
		Breaking strength	**Dry weight (mg %)**	**Collagen content (µg/g)**
Control	10	284.13 ± 8.58	9.19 ± 0.22	0.82 ± 0.01
TFG 200 mg/kg	10	367.5 ± 11.95*	11.65 ± 0.71*	1.29 ± 0.01*
TFG 500 mg/kg	10	408.5 ± 7.18*	14.33 ± 1.24*	1.64 ± 0.01*

* Dunnet's t test, $p < 0.01$ vs control

Histopathological evaluation

The wound sections from the animals in control group exhibited healed granulation tissue with frequent fibroblasts and good collagenation. The features of the sections of the treated groups were similar to that of the control.

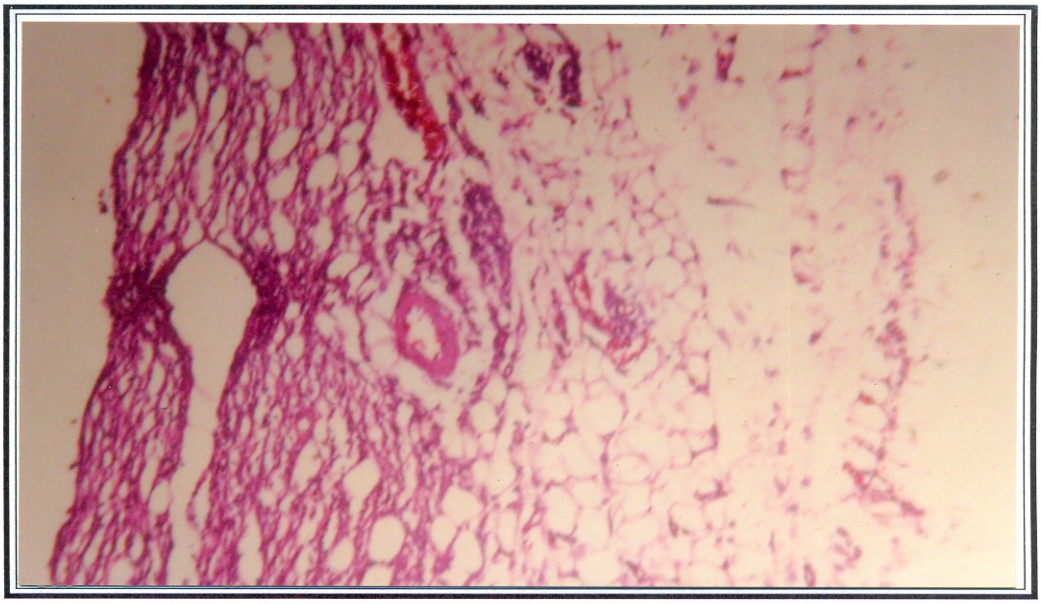

Figure 1: Photomicrograph of granuloma tissue of control group

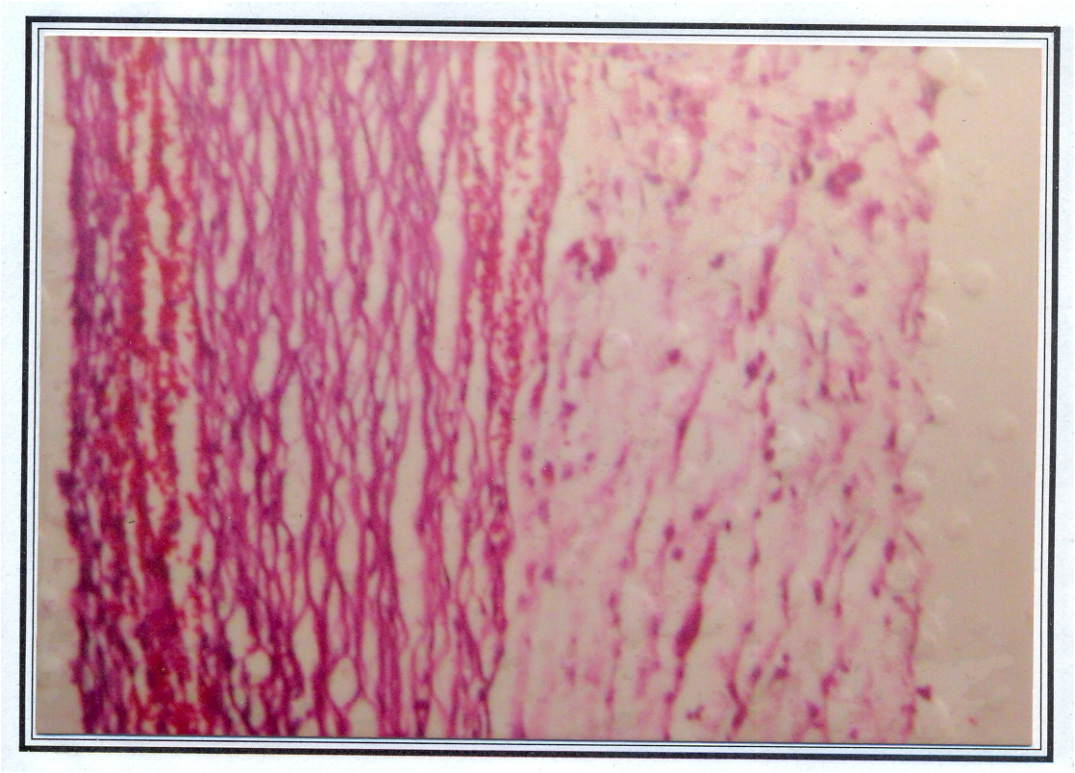

Figure 2: Photomicrograph of granuloma tissue of TFG seeds [200mg/kg] group

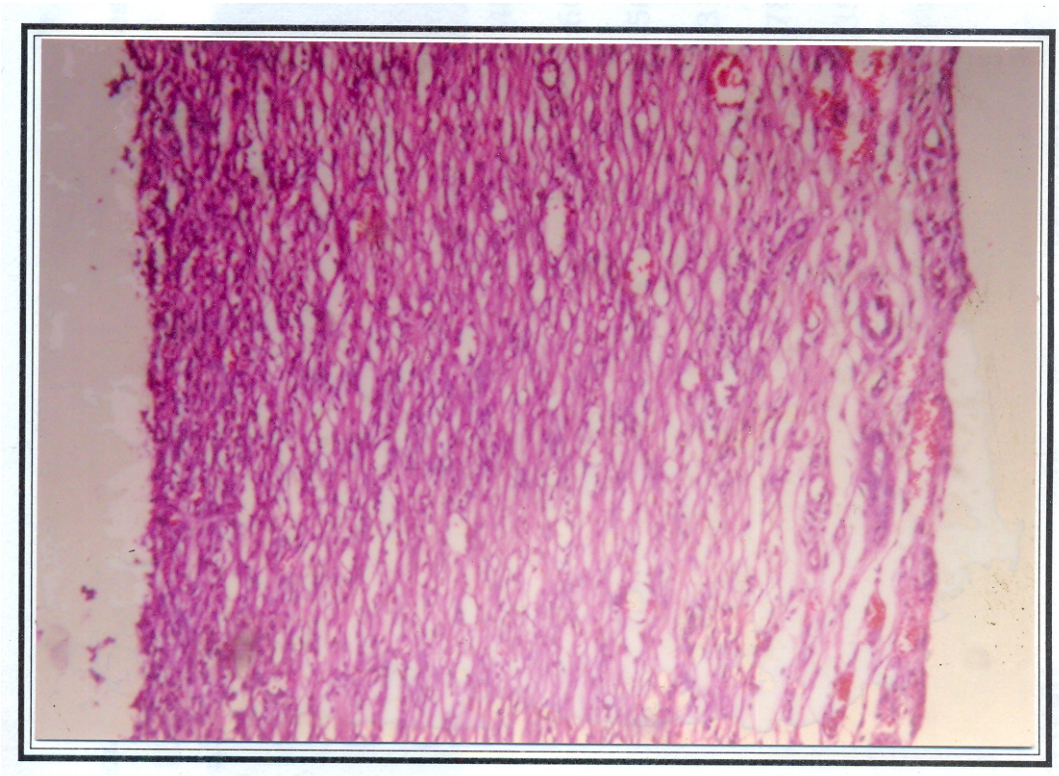

Figure 3: Photomicrograph of granuloma tissue of TFG seeds [500mg/kg] group

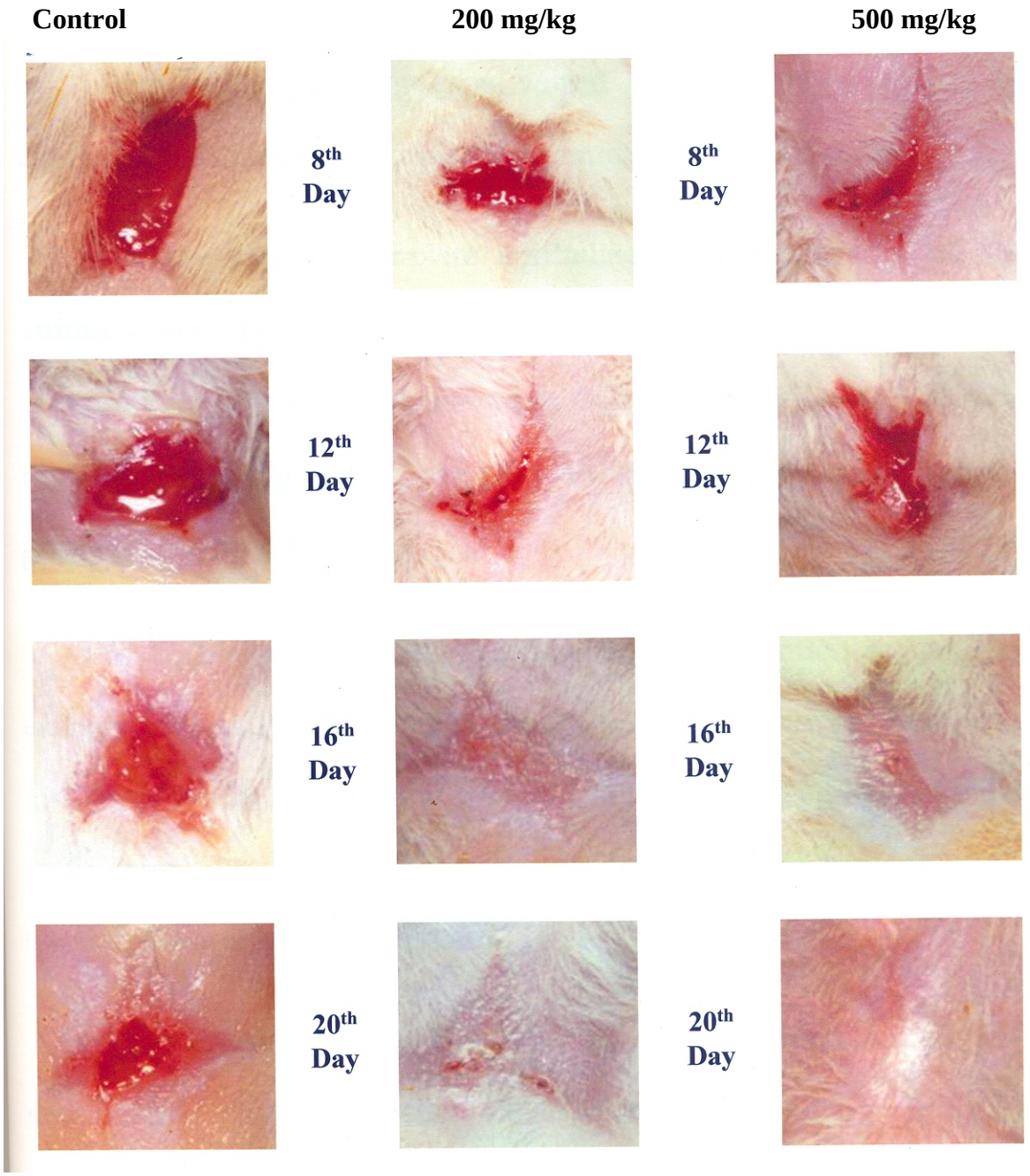

Figure 4: Photomicrographs showing excision wound

Discussion

Wound is a disruption in the continuity of the living tissues. Restitution of the continuity in a damaged area is achieved by a repair process. Tissue repair involves regeneration or replacement or at times both, leading to wound healing. Wound healing is a perplexed action which involves a number of phases from injury to healing. An interference into any of these phases can modulate the healing process, viz oxidative stress. Oxidative stress is triggered by the injury itself. . Molecular oxygen plays a central role in the pathogenesis and therapy of chronic wounds. When reactive oxygen species (ROS) are overproduced, oxidative stress results, with detrimental cytotoxic effects, causing delayed wound healing. Therefore, elimination of ROS could be an important strategy to improve healing of chronic wounds [14]. Fenugreek (TFG) seeds are found to be a potential source of natural antioxidants [15].But the antioxidant properties have not been experimentally tested for their influence on healing. Hence the present study was undertaken to see if TFG could influence healing. Accordingly, the flavonoid rich fraction of TFG was tested in the three wound models. Not only did it increase the breaking strength of the wounds, but also increased the amount of collagen present in it. The results have shown that flavonoid fractions of alcoholic extract of *Trigonella foenum- graecum* seeds have shown good wound healing action. Further it was observed that the activity increased in a dose dependent manner.

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