



RESEARCH ARTICLE



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Wound Healing Activity of *Tribulus Terrestris* Linn. Fruits in Swiss Albino Rats

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Abstract

The main objective of this study was to evaluate the wound healing activity of *Tribulus Terrestris* Linn. (TT) fruits in swiss albino rats. The fruit body of *Tribulus Terrestris* rich in tannins and phenolic content. Dried fruits of *Tribulus Terrestris* powder were used for ointment preparation. Healthy adult swiss albino rats (18-22)g were subjected to acute toxicity studies as per OECD guidelines 423. The selected swiss albino rats were used for wound healing model experiments. Excision wounds were made by cutting a skin from the dorsal abdominal region. The prepared ointment were applied to the area where the excision wounds were made and the wound healing parameters like wound contraction rate were assessed and monitored every day. Rapid regeneration of tissues at the wound site indicates that the *Tribulus Terrestris* fruit showed the higher healing process by increasing the wound contraction level and decrease in epithelization period were also observed. The histopathological examination showed much advance phase of healing process, it was concluded that the ointment preparation of *Tribulus Terrestris* fruit showing higher wound healing activity compared to control in swiss albino rats.

Keywords: *Tribulus Terrestris* Linn. , Ointment, Excision wound, wound healing, Swiss albino rats.

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INTRODUCTION

Tribulus Terrestris Linn. is belongs to the family zygophyllaceae [1]. It is originated from southern Europe, Africa, Temperate and Tropical Asia [2]. *Tribulus Terrestris* grows on sand dunes in the desert regions and also on sandy loam soils [3]. *Tribulus Terrestris* have some medicinal property such as antimicrobial, antihypertension, diuretic, antiacetylcholine, haemolytic activity, to stimulate spermatogenesis, libido and antitumor activity and effects on cardiovascular system [4] and *Tribulus Terrestris* exhibits high antioxidant activity [5]. The leaves of *Tribulus terrestris* are widely used for the treatment of various kinds of wounds [6]. Ethanolic extract of the fruit and leaves of *Tribulus Terrestris* shows antibacterial activity against *E.coli*, and *S. aureus* [7]. Methanolic extracts of the plant active against bacteria like *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus Vulgaris* and *Staphylococcus aureus* [8]. *Tribulus Terrestris* Linn. having an antifungal activity against fungi such as *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei* and *Cryptococcus neoformans* [9]. Based on these observations, the present study was conducted to evaluate the wound healing activity of *Tribulus Terrestris* fruits.

Commonly the wounds are open wounds, closed wounds, acute and chronic wounds. Wound healing process is a complex series of inter related events [10]. Wounds are the physical injuries that result in opening or breaking of the skin and appropriate method for healing of wound is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [11]. Wound healing is a process that is fundamentally a connective tissue response [12]. Traditionally the leaves of *Tribulus Terrestris* were used for the treatment of various kinds of wounds [6]. The methanolic extract of *Tribulus Terrestris* fruits were possessed wound healing activity was reported by Javed Akhar Ansari et al 2012 [13]. The aim of the present study was to investigate the influence of fruit region of *Tribulus Terrestris* on excision wounds in swiss albino rats.

MATERIAL AND METHODS

Plant material collection:

The fruits of *Tribulus Terrestris* were collected from Velachery, Chennai, Tamil Nadu, India. Botanically identified and confirmed by Dr.G.Kathiravan, Associate Professor, Department of Biotechnology, Vels University, Chennai. Specimen Number VUCC0001 (Vels University Culture Collection). The fruits were

washed with water and then dried in shade, powdered. The powder (10 g) was taken to prepare ointment.

Ointment preparation (100gm):

Wool fat – 5gm: Hard paraffin – 5gm: Cetostearyl alcohol – 5gm: Yellow soft paraffin – 75gm and *Tribulus Terrestris* fruit powder : 10gm were taken in china dish and heated on water bath until they are melted. Contents were stirred continuously until became semisolid. Then it was cooled and transferred into wide mouth container [14,15].

Experimental animals:

Healthy adult swiss albino rats were obtained from Vels University animal house. They have been provided food and water *ad libitum* during the whole period of the experiment. An acute toxicity study was conducted according to the OECD guidelines 423 in swiss albino rats. The study was undertaken after obtaining the approval of institutional ethical committee clearance. Registration no: (XIII/VELS/PCOL/68(b)/2000/CPCSEA/IAEC/8/08/2012). Male swiss albino rats weighing 25-30 gm were used for wound healing model experiments; they were physically active and consuming food, water in a regular way.

Excision wound model:

The dorsal skins of the swiss albino rats were shaved. They were divided into three groups. The rats were depilated on the cutaneous circular wound of 8mm diameter were created on the pre-shaved sterile dorsal surface of the rats by cutting under ether anesthesia. The wound was left undressed to the open environment and the animals were kept individually in separate cages [16]. Following treatment was given.

Group I: Control (treated with vaseline)

Group II: Standard (treated with povidone iodine, 5% w/w ointment)

Group III: Test (treated with *Tribulus Terrestris* ointment, % strength).

The drug was applied once a day after cleaning the wound with surgical cotton wool.

Measurement of wound area:

The progressive changes in wound area were measured on a millimeter scale graph paper at every 5 days interval. Progressive decrease in the wound size was monitored periodically.

Histopathological examination and study:

At day 16th the experiment was terminated and the wound area was removed from the surviving animals for histological examination. At the insisted area, in every skin section area just beneath the epidermis and

also deep dermis randomly selected. Histopathological studies were performed and examined as described by Sourav Kanti Royi *et al* [17]. The different animal groups were assessed by the pathologist and result was compared with the control groups.

RESULTS

In excision wound model, the wound was created and examined on different days (1,5,10,15 days) as shown in Fig.1. The percentage of wound contraction on 1st, 5th, 10th and 15th days was observed and tabulated (Table 1) (Fig. 2). The rats treated with *Tribulus Terrestris* ointment was found to be significantly higher margin contraction compared to control and standard. This activity was maximum on the day 15th, in histopathological study the wound skin treated with *Tribulus Terrestris* showed excellent tissue regeneration compared with standard groups (Fig. 3). In the present study, the wound healing potential in swiss albino rats was evident on 5th day onwards. This potential was further confirmed in the histological evaluation on 15th day, which was highlighted by the full thickness coverage of the wound area by an organized epidermis in the presence of mature scar tissue in the dermis. In the microscopical examination following findings were observed such as collagenization, Epithelisation was early and complete. Fibroblast proliferation was maximum with the test group compared to standard and control.



Figure 1: Excision wound model of control, standard, test albino rats (1st and 14th day)

Wound contraction mean \pm SE day (mm) ²					
Groups	1 st day	5 th day	10 th day	15 th day	Epithelisation period mean \pm SE (Days)
Control	112.12 \pm 5.8 2 (1)	84.59 \pm 3.74 (5)	60.2 \pm 2.9 (37)	37.76 \pm 3.1 4 (55)	21.84 \pm 0.30
Standard	100.43 \pm 6.3 1 (1)	52.33 \pm 3.82 a (23)	29.12 \pm 1.90 b (53)	13.74 \pm 2.2 4 (74)	19.46 \pm 0.30
Test	87.5 \pm 3.76 _a (1)	50.45 \pm 2.09 b (45)	26.16 \pm 1.39 (72)	8.63 \pm 1.32 _b (88)	19.00 \pm 0.32

Table 1: Evaluation of *Tribulus Terrestris* Fruits on wound healing by excision wound method in swiss albino rats.

Each values are expressed as mean \pm SEM, One way ANOVA followed by Dunnett's t-test. t - value denotes significance at a ; P<0.05, b; P<0.01. % Wound contraction is given within parantheses.

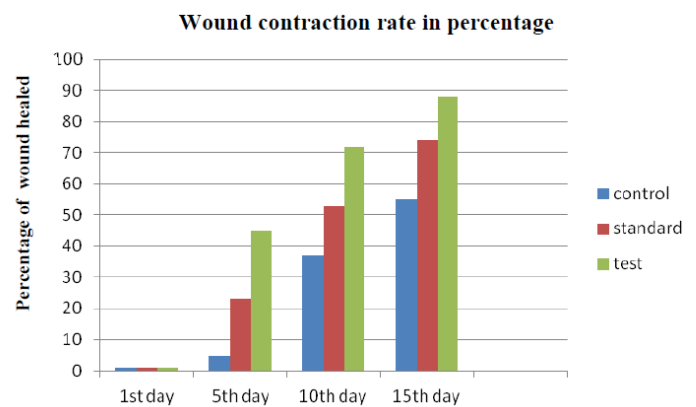


Figure 2: Graph showing contraction of wound in percentage

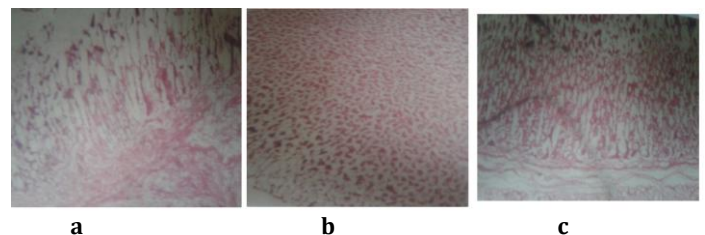


Figure 3: Histopathological study- Skin of swiss albino rat
a) Collagen deposition in the control (40X Magnification)
b) Collagen deposition in the standard (40X Magnification)
c) Collagen deposition in the test (40X Magnification)

DISCUSSION

In excision wound healing model, the prepared ointment of *Tribulus Terrestris* showed significant increase in percentage of closure by enhanced the epithelization. It may be due to the effect of *Tribulus Terrestris* to enhanced collagen synthesis. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair [18]. The observation revealed that tissue regeneration was much quicker in the treated group compared to the control group. The treated rats showed new blood vessels formation, extracellular matrix synthesis. Collagen synthesis and significant increase in percentage closure by enhanced epithelialization. It

may be due to the effect of *Tribulus terrestris*. During the wound healing process migration of fibroblast and epithelial cells to the wound site was observed in the treated group. The result reveals that the tissue regeneration was much quicker in the *Tribulus Terrestris* treated group compared to standard and control. The ointment of *Tribulus Terrestris* shows significant wound healing activity on cutaneous wound in swiss albino rats.

CONCLUSION

The results obtained in the present study clearly indicate that the fruits of *Tribulus Terrestris* are having significant wound healing activity in swiss albino rats, it may be due to the presence of its active principles, which accelerate the healing process and confer tensile strength to the healed wound.

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