Skin irritation & healing activity of *Trichosanthes dioica* Roxb on burn wounds

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**ARTICLE INFORMATION**

**ABSTRACT**

Literature survey revealed that plants having flavonoids and tannins showed wound healing activity. Preliminary phytochemical screening of *Trichosanthes dioica* stated the presence of these chemical constituents so it was hypothesized to screen the plant for this activity. This study was aimed at investigating the healing efficiency of petroleum ether extract of *Trichosanthes dioica* Roxb fruits formulated as 5% ointment. Burns were induced in albino rats divided into three groups as following; Group-I (control) received ointment base. Group-II was treated with standard drug 0.01% silver sulphadiazine. Groups-III was treated with 5% petroleum ether extract ointment. The efficacy of treatment was evaluated based on the wound contraction, epithelialization period, hydroxyproline content and histopathological studies. The effect produced by the extract ointment showed significant (P < 0.01) healing when compared with control group. All parameters such as wound contraction, epithelialization period, hydroxyproline content and histopathological studies showed significant changes when compared to control. The present documented findings may suggest the use of petroleum ether extract of *Trichosanthes dioica* to treat and management of burn wounds.

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1. **Introduction**

Wounds are substantial injuries that consequence in an opening or fracture of the skin. Appropriate healing of wounds is necessary for the restitution of disrupted anatomical continuity and disturbed functional position of the skin (Begum et al., 2000). Burn wound healing is a multifaceted course and does not require much help but still cause distress and are prone to infection and other complications (Mohandas et al., 1998). Burns constitute a chief fitness crisis because the incidence of severe complications is high and financial resources are limited.

Medicinal plants are an indispensible part of conventional medicine. A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns. *Trichosanthes dioica* Roxb (Pointed gourd) is a perennial, dioecious cucurbit that grows as a creeper and it is cultivated in subtropical and tropical regions around the globe. The Bengal and Assam region of India is the primary centre of its origin (Singh et al., 1992). It is grown almost in every districts of Bangladesh, especially in Rajshahi, Bogra, Pabna, Jessore and Kushtia (Rashid, 1993). The fruit is the edible part of the plant, which is cooked in various ways either alone or in combinations with other vegetables or meats. The fruits of *Trichosanthes dioica* find an important place in indigenous medicine as a febrifuge, cathartic, hydrogogue and tonic (Chaterjee et al., 1994). The fresh juice of the unripe fruit is often
used as a cooling and laxative to some alternative medicine (Kirtikar and Basu, 1956). Extract of this plant has shown antihyperglycemic (Rai, 2008) and hypolipidemic (Sharma and pant., 1988) potential. A survey of the published literatures revealed that no systematic approach has been made to study burn wound healing potential of this plant, hence the present study was undertaken to evaluate the burn wound-healing activity of petroleum ether extract of *Trichosanthes dioica*.

2. Materials And Methods

2.1 Plant material

The fruits of the plant *Trichosanthes dioica* were procured from the local market of Bhopal (India). The plant was authenticated by Department of Botany, MVM College, Bhopal, where a voucher specimen (Bot/Herbarium/0532) has been deposited. The fruits were air dried in shade, powdered by a mechanical grinder, passed through a 40-mesh sieve and stored in well closed container for further use.

2.2 Preparation of extract

About 125 g of dried powder of *Trichosanthes dioica* fruits were extracted with petroleum ether using soxhlet apparatus. The extract was filtered and concentrated under reduced pressure to a semi-solid mass (yield 8 g w/w).

2.3 Qualitative test analysis of plant drug

Petroleum ether extract of *Trichosanthes dioica* was subjected to various qualitative tests for the identification of various plant constituents present in the plant drug (Kokate, 1994; Harbone, 1998).

2.4 Preparation of plant drug formulation and standard used

A 5% (w/w) simple ointment containing the petroleum ether extract of *Trichosanthes dioica* was prepared by triturating method in a ceramic mortar and pestle using white soft paraffin base, obtained from S.d. fine chemical, India (Cooper, 1987). For this, 5 g of petroleum ether extract was incorporated in 100 g of the base. Silver sulfadiazine (0.01%) obtained from Rexin Pharmaceutical Pvt. Ltd was used as standard drug for comparing the wound healing activity of petroleum ether extract of *Trichosanthes dioica* in burn wound model.

2.5 Experimental animals

Healthy albino rats of either sex, weighing 150-200 g, housed in standard condition of temperature, humidity and light were used for present in vivo studies. They were fed with standard rodent diet and water ad libitum. The experiment was conducted in division of pharmacology, VNS Institute of Pharmacy, Bhopal. Institutional animal ethical committee (Registration No. 778/03/c/CPCSEA) permitted the study.

2.6 Grouping of animals

Three groups of animals containing six in each were used. The animals of group I, II and III were considered as the control (ointment base), reference standard (0.01% silver sulphadiazine) and treated (5% ointment of petroleum ether extract) respectively.

2.7 Skin irritation study

Rabbits were used to assess the skin irritation of prepared formulation. The formulation base and formulation containing petroleum ether of *Trichosanthes dioica* were applied on shaved portion of the dorsal side. After 4 hours, the skin was observed for signs of inflammation and edema (Gfeller et al., 1985).

2.8 Burn wound model and drug treatment

In burn wound model (Priya, 2002) three groups of animals containing six in each group were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved one day prior to the experiment. Burn wounds were created on dorsal part of shaved rats using a metal rod (1.5 cm diameter) heated to 80-85°C and exposed for 20 seconds. After 24 h, dead tissues were excised using sterile surgical blade. Control rats were dressed with ointment base alone, while experimental rats were dressed with the 5% ointment formulated with petroleum ether extract of *Trichosanthes dioica*. All the rats were given regular dressing changes at every alternative day.

2.9 Wound healing evaluation parameters

The parameters observed in the study were as follows:

*Evaluation of rate of wound contraction*

Wound contraction is important parameter for the assessment of healing process. The measurements of the wound areas were taken in each 2 days interval, until complete wound healing, by using transparent paper and a permanent marker. The wound areas were recorded and measured on graph paper (Sadaf, 1993). Wound contraction was expressed as reduction in percentage of the original wound size by using the following formula:

\[ WC = \frac{(W_{Si} - W_{Sd})}{W_{Si}} \times 100 \]

where

- WC = Wound contraction (%)
- W_{Si} = Initial wound size
- W_{Sd} = Specific day wound size
Epithelialization time

Epithelialization time was evaluated by noting the number of days required for the escher to fall off from the wound surface exclusive of departure a raw wound behind (Srivastava et al., 2008).

Assessment of hydroxyproline content

Hydroxyproline is an unusual amino acid present in the collagen fibers of granulation tissues. Its evaluation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70°C to constant weight and were hydrolysed in 6N HCl at 130°C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid and color was developed with the help of Ehrlich reagent at 60°C. The absorbance was measured at 557 nm using a spectrophotometer. The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure L-hydroxyproline at the same time (Woessner, 1961)

Histopathological studies

A sample of skin tissues from control, standard and treated groups were excised from the healed wound and preserved in 10% buffered formalin. Thin sections were cut and stained with haematoxylin and eosin (McManus, 1965) and observed under microscope for the histological changes.

3. Statistical Analysis

Experimental data are expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Dunnet vs. control test followed by one-way ANOVA to identify the differences between pair of groups. Data were considered significant at p < 0.01.

4. Results

4.1 Qualitative test analysis of plant drug

The qualitative test analysis of the petroleum ether extract of *Trichosanthes dioica* showed the presence of triterpenoids and steroids.

4.2 Skin irritation study

The skin irritation study on the rabbit skin proved that drug in 5% concentration of petroleum ether extract does not show any type of irritation and inflammation.

4.3 Rate of wound contraction

The maximum area of the wound injury was decreased by treatment with petroleum ether extract of *Trichosanthes dioica* as compared to control group. An improved healing pattern with absolute wound closure was observed in standard and treated group within 12 and 14 days respectively while it was about 16 days in control rats as shown in Fig. 1.

4.4 Epithelialization time

The epithelialization time was calculated from initial day. The results indicate that petroleum ether extract of *Trichosanthes dioica* has shown the complete epithelialization on an average 13.00 ± 0.408 days when compared to control 23.00± 0.577 days and it was found to be significantly reduced (p < 0.01) as depicted in Fig. 2.

4.5 Hydroxyproline content

In the present study, the petroleum ether extract treated animals showed significantly increased levels of hydroxyproline content (p < 0.01) as compared with the control group of animals as shown in Fig. 3.

4.6. Histopathological results

On the histopathological examination of the burn wound sections, control group exhibited in poor collagen fibers (FC), fibroblasts cells (CF) and blood vessels (BV) which indicates the healing was not completed (Fig 1) while histopathological examinations of the tissue samples from petroleum ether extract of *Trichosanthes dioica* treated group demonstrated significant healing as in fibroblasts cells, collagen fibers and new blood vesicles (Fig. 4, 5 and 6).

5. Discussion

Numerous studies indicate that plant products are potential agents for wound healing activity and basically favored because of the absence of unwanted side effects and their effectiveness. In the present investigation, it was reported that the topical application of petroleum ether extract of *Trichosanthes dioica* promoted wound healing activity in burn wound. This wound model was used in our study to evaluate the wound healing effect of petroleum ether extract of *Trichosanthes dioica* on various phases of wound healing, which run concomitantly, but independent of each other. The standard drug 0.01% silver sulphadiazine is used as a standard reference to assess the healing potency of the plant extract against the control.

The mechanism of wound repair occurs by four basic processes such as inflammation, wound contraction, epithelialization and granulation tissue formation. The progression of wound healing can be judged by the periodic assessment of the contraction of excision wounds. Topical application of petroleum ether extract of *Trichosanthes dioica* markedly improved wound contraction and closure,
and the effects were distinctly visible from day 2 post-wounding. The epithelialization of the wound mainly occurs by proliferation and migration of the marginal basal cells lying close to the wound margin. The measurement of hydroxyproline can be used as an index for collagen revenue (Nayak & Pinto, 2006). Increase in hydroxyproline content indicates increased collagen synthesis which in turn leads to improved wound healing process. Histopathological evaluation of the wound site provides evidence of a more desirable histological organization of the tissue in response to petroleum ether extract treatment. Treatment of rats with petroleum ether extract resulted in an enhancement of wound healing, as evidenced by increased collagen fibers, fibroblast cells and the formation of new blood vessels. The healing process depends to a large extent, on the regulated biosynthesis and deposition of new collagens and their subsequent maturation (Gao, 2006). Collagen is produced by fibroblasts and helps the wound gain tensile strength during repair (McFarlin, 2006). The fibroblasts are responsible for production of the mucopolysaccharide ground substance. This histopathological observation provided additional evidence for the experimental wound healing studies based on the contraction value of wound areas.

Preliminary phytochemical screening of the petroleum ether extract of *Trichosanthes dioica* showed the presence of triterpenoids and steroids. Any one of the observed phytochemical constituents present in *Trichosanthes dioica* may be responsible for the wound healing activity. Recent studies have shown that phytochemical constituents like triterpenoids (Scortichini and Pia, 1991) and flavonoids (Nayak et al., 2009) are known to promote the wound-healing process, which appears to be responsible for wound contraction and increased rate of epithelialization. Qualitative test analysis showed the presence of triterpenoids which were responsible for the effective wound healing activity of petroleum ether extract of *Trichosanthes dioica*. The wound-healing property of petroleum ether extract of *Trichosanthes dioica* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents.

6. Conclusion

The present study suggested that the topical administration of petroleum ether extract of *Trichosanthes dioica* plays a major role in burn wound healing. It may therefore be concluded that petroleum ether extract of *Trichosanthes dioica* possesses burn-healing properties as depicted by the increment in the hydroxyproline content, faster wound contraction and shortening of the epithelialization time. The present documented findings may suggest the use of *Trichosanthes dioica* to treat and management of burn wounds.

7. Acknowledgement

Yogesh Shivhare, the author, sincerely thankful to Mr. Rakesh Punekar, Head and Vice Principal of RKDF College of Pharmacy, Bhopal (M.P.), for the help rendered in all academic aspects and constant encouragement to carry out this research work successfully.

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