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Research, Evidence and Innovation: Emerging Perspectives and Recent Advances
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RESEARCH PAPER

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Abstract

Background: Wound healing is a complex, multi-phase biological process, and there is continuing interest in plant-derived agents that can accelerate repair while limiting excessive inflammation. Trichosanthes dioica Roxb., a plant traditionally credited with anti-inflammatory, antioxidant and tissue-regenerative properties, was evaluated for its wound healing potential in the present study.

Aim: To pharmacologically evaluate the wound healing properties of Trichosanthes dioica leaves in an experimental excision wound model.

Methods: A 5% w/w ointment of the hydroalcoholic leaf extract of T. dioica, obtained by Soxhlet extraction, was formulated in a soft paraffin base. Eighteen male Wistar rats were divided into three groups (n = 6): an untreated control, a Plermin-treated standard group, and a group treated once daily with the 5% T. dioica ointment, following creation of a full-thickness circular excision wound. Wound contraction was recorded on Days 0, 3, 7, 11 and 14; on Day 14 animals were euthanised and granulation tissue was assessed histopathologically (H&E and Masson's Trichrome staining) and analysed for interleukin-6 (IL-6) by ELISA.

Results: The test ointment produced a progressive reduction in wound area that closely paralleled the standard group and was markedly faster than the untreated control, with wound contraction of 98.91% versus 56.83% (control) by Day 14. Histopathology of the test group showed enhanced re-epithelialisation, fibroblast proliferation, collagen deposition and neovascularisation compared with the control. Tissue IL-6 concentration was lowest in the test group, indicating attenuation of the inflammatory phase.

Conclusion: Topical application of 5% w/w Trichosanthes dioica leaf extract ointment significantly enhanced wound healing in rats, supporting its traditional use and warranting further phytochemical and clinical investigation.

Keywords: Trichosanthes dioica; wound healing; excision wound model; ointment; interleukin-6; histopathology

1. Introduction

Wound healing is a dynamic, tightly orchestrated physiological process comprising haemostasis, inflammation, proliferation and remodelling. Delayed or impaired healing remains a significant clinical concern, and there is sustained interest in identifying safe, effective topical agents — particularly of natural origin — that can accelerate wound contraction, support granulation tissue formation, and resolve inflammation without the adverse effects often associated with synthetic agents.

Trichosanthes dioica Roxb. (pointed gourd) is a plant long used in traditional medicine and documented for anti-inflammatory, antioxidant and tissue-regenerative properties [1–3], attributed to its content of flavonoids, phenolics, tannins, terpenoids, alkaloids and saponins [4–6]. These phytoconstituents are reported to scavenge reactive oxygen species, reduce oxidative stress, stimulate fibroblast proliferation, enhance collagen synthesis and promote angiogenesis [7], all of which are mechanistically relevant to wound repair.

Despite this traditional use, systematic pharmacological evaluation of T. dioica leaves in a standardised wound healing model has been limited. The present study was therefore undertaken with the following aim and objectives.

Aim: Pharmacological evaluation of the wound healing properties of Trichosanthes dioica in experimental models.

Objectives:

- To procure and authenticate the leaves of Trichosanthes dioica.
- To prepare the hydroalcoholic extract of Trichosanthes dioica leaves using the Soxhlet extraction method and formulate the ointment for topical application.
- To induce and establish the excision wound model in experimental rats.
- To evaluate the wound healing potential of Trichosanthes dioica by measuring the rate and percentage of wound contraction.
- To perform histopathological examination of wound tissue using H&E stained sections to assess the healing process.
- To estimate tissue levels of the pro-inflammatory cytokine interleukin-6 (IL-6) using ELISA.
- To compare the wound healing efficacy of Trichosanthes dioica ointment with the standard treatment and control groups.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

The plant was procured from an online nursery and cultivated under suitable conditions. Fresh, healthy leaves were harvested, thoroughly washed to remove dust and debris, and shade-dried at room temperature [8,9]. Representative dried leaf samples were submitted for botanical authentication to the Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H), Ministry of Ayush, Government of India (ULR No. TC155802600000000023/308) [10]. The authenticated dried leaves were pulverised into a coarse powder (sieve mesh #40) using a mechanical grinder and stored in airtight, moisture-resistant amber glass containers in a cool, dark environment until extraction [11].

2.2 Experimental Animals

Eighteen healthy adult male Wistar rats (180–220 g) were procured from the departmental animal facility of GD Goenka University, Sohna–Gurgaon Road, Sohna, Haryana, India. Animals were housed individually under standard laboratory conditions (23 ± 2 °C, relative humidity 50 ± 5%, 12 h light/12 h dark cycle) with a standard pellet diet and water ad libitum [12–14], and were acclimatised for seven days prior to any procedure to mitigate stress-induced neuroendocrine variations that could confound physiological healing baselines [15,16]. The protocol received formal approval from the Institutional Animal Ethics Committee (IAEC) and adhered to CPCSEA guidelines, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India [17].

2.3 Drugs, Chemicals and Equipment

Plermin (Central Drug House Pvt. Ltd.) served as the standard topical reference agent, chosen for its broad-spectrum antimicrobial efficacy and ability to promote granulation tissue formation [18,19]. The test substance was the hydroalcoholic leaf extract of T. dioica, a plant documented in traditional medicine for its anti-inflammatory, antioxidant and tissue-regenerative properties [1–3], incorporated into a 5% w/w soft paraffin ointment base. All chemicals, solvents and diagnostic reagents (ethanol, distilled water, soft paraffin, ketamine hydrochloride, xylazine, carprofen, rat IL-6 ELISA kit, neutral buffered formalin, haematoxylin and eosin) were of analytical grade, procured from verified commercial suppliers (SRL, CDH, HiMedia, Neon Laboratories, Indian Immunologicals, Zoetis, Elabscience) to ensure reproducibility and experimental consistency [20]. Standard laboratory equipment included an analytical balance, mechanical grinder, Soxhlet extraction apparatus, thermostatic water bath, hot air

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oven, magnetic stirrer with hot plate, digital vernier calliper, tissue homogeniser, refrigerated centrifuge, ELISA microplate reader (450 nm), rotary microtome and optical light microscope.

2.4 Extraction of Phytoconstituents

Continuous hot reflux extraction using a Soxhlet apparatus was employed to efficiently extract sparingly soluble phytoconstituents through repeated cyclic partitioning of the solvent [21,22]. Sixty grams of dried leaf powder was subjected to Soxhlet extraction using a hydroalcoholic solvent (ethanol:water) until complete exhaustion of the plant material. The extract was filtered and concentrated on a water bath (50–60 °C), and the concentrated hydroalcoholic extract was transferred to a sterile, airtight glass container and refrigerated at 4 °C until formulation.

2.5 Preparation of Ointment Formulation

A 5% w/w topical ointment was formulated using a white soft paraffin base, following standardised pharmacopeial protocols for dermatological semisolid dosage forms [23,24]. Soft paraffin was selected as the vehicle for its occlusive properties, which enhance stratum corneum hydration and facilitate sustained release of active phytocompounds into the wound bed [25,26]. Soft paraffin (19.0 g) was melted over a thermostatic water bath at 60 °C, and 1.0 g of the concentrated hydroalcoholic extract was incorporated by continuous manual levigation in a sterilised glass mortar until a smooth, uniform, grit-free ointment (total weight 20.0 g) was obtained. The formulation was cooled under continuous stirring and stored in an airtight, light-resistant glass jar at room temperature.

2.6 Experimental Design and Treatment Protocol

Eighteen rats were randomly allocated to three groups (n = 6) by computer-generated randomisation to eliminate selection bias, in accordance with the 3Rs (Replacement, Reduction, and Refinement) framework for ethical animal research [27,14]. The treatment protocol spanned 14 consecutive days post-wounding, as summarised in Table 1.

Table 1. Experimental groups and treatment protocol

Group	Group name	Treatment administered	n
I	Control	Excision wound induced; no topical treatment applied	6
II	Standard	Excision wound induced; treated topically with Plermin	6
III	Test	Excision wound induced; treated topically with 5% w/w T. dioica ointment once daily	6

2.7 Excision Wound Model and Post-Operative Care

Wound healing efficacy was evaluated using the classical full-thickness circular excision wound model of Morton and Malone [28], widely accepted for assessing topical vulnerary agents [29–31]. Animals were anaesthetised by intraperitoneal ketamine hydrochloride (80 mg/kg) with xylazine (10 mg/kg), a regimen ensuring rapid induction and surgical-level analgesia without significant respiratory depression [32–35]; the dorsal thoracic region was shaved and disinfected. A circular full-thickness excision of 2 cm diameter (initial wound area ~300 mm²) was created along the dorsal midline down to the loose subcutaneous fascia. Wounds were left uncovered to allow natural eschar formation and contraction [29]. Topical treatments were applied once daily to Groups II and III using sterile cotton-tipped applicators. All animals received subcutaneous carprofen for post-operative analgesia on the first three post-operative days [33,36,37], and were monitored daily for signs of distress or infection [14].

2.8 Assessment of Wound Contraction

Wound area was recorded on Days 0, 3, 7, 11 and 14 by tracing the wound margin onto transparent acetate sheets and quantifying the surface area by planimetry against graph paper [38,39]. Percentage wound contraction was calculated using the formula described by Morton and Malone [28]:

Wound contraction (%) = [(Initial wound area − Wound area on Day X) / Initial wound area] × 100

An accelerated rate of wound contraction indicates enhanced wound repair, driven by centripetal movement of the wound edges via myofibroblast differentiation, actin-myosin contractile forces, and enhanced collagen bundle cross-linking [40–42].

2.9 Histopathological Evaluation

On Day 14, all animals were humanely euthanised under anaesthetic overdose in accordance with established institutional euthanasia guidelines [43]. Granulation tissue specimens (with a 2 mm margin of normal skin) were excised, fixed in 10% neutral buffered formalin for 24 h to prevent autolysis and preserve cellular architecture [44,45], dehydrated through ascending grades of ethanol, cleared in xylene, and embedded in paraffin [44,46]. Sections of 5 µm thickness were cut, mounted, and stained with Haematoxylin and Eosin (H&E) and Masson's Trichrome (MT) [45]. Sections were examined by a histopathologist blinded to treatment group for re-epithelialisation, fibroblast proliferation, collagen deposition/organisation, neovascularisation and inflammatory cell infiltration [47–50].

2.10 Estimation of Tissue IL-6 by ELISA

IL-6 is a pleiotropic pro-inflammatory cytokine produced by activated macrophages and endothelial cells during the acute inflammatory phase of wound healing, and its timely downregulation is critical for transitioning to the proliferative and remodelling phases of tissue repair [51–54]. Granulation tissue harvested on Day 14 was rinsed in ice-cold PBS, weighed, and homogenised in cold lysis buffer with protease inhibitor using a mechanical tissue homogeniser [55]. Homogenates were centrifuged (12,000 rpm, 15 min, 4 °C), and supernatants were stored at −80 °C until analysis. IL-6 concentration was quantified using a rat-specific sandwich ELISA kit (Elabscience) strictly adhering to the manufacturer's instructions [56–58], with absorbance read at 450 nm and concentrations derived from a linear standard curve, expressed as pg/mg total tissue protein [59].

2.11 Statistical Analysis

Data are expressed as mean ± SD/SEM. Comparisons among groups over time were performed using one-way or two-way ANOVA followed by Tukey's multiple comparison test, as appropriate. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Ointment Preparation and Physicochemical Properties

The 5% w/w T. dioica ointment was successfully prepared with a greenish-green colour attributable to natural pigments in the leaf extract, a smooth, homogeneous, grit-free texture, characteristic herbal odour, and good spreadability suitable for uniform application over the wound area (Table 2).

Table 2. Physicochemical properties of the 5% Trichosanthes dioica ointment

Parameter	Observation
Colour	Greenish-green
Odour	Characteristic herbal odour
Appearance	Smooth and homogeneous
Consistency	Soft and uniform
Spreadability	Good
Skin application	Easy and uniform
Presence of lumps	Absent
Homogeneity	Homogeneous

3.2 Wound Contraction

Wound diameter was uniform (2.00 ± 0.00 cm) across all groups on Day 0. A progressive reduction was observed in all groups over the 14-day period, with the standard and test groups contracting markedly faster than the untreated control at every subsequent time point (Table 3).

Table 3. Mean ± SD wound size (cm) in control, standard and test groups

Group	Day 0	Day 3	Day 7	Day 11	Day 14
Control	2.000 ± 0.000	1.833 ± 0.038	1.596 ± 0.109	1.216 ± 0.118	0.863 ± 0.076
Standard	2.000 ± 0.000	1.535 ± 0.024	0.958 ± 0.028	0.360 ± 0.026	0.005 ± 0.008

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Group	Day 0	Day 3	Day 7	Day 11	Day 14
Test	2.000 ± 0.000	1.595 ± 0.018	1.028 ± 0.023	0.421 ± 0.033	0.021 ± 0.011

Two-way ANOVA followed by Tukey's multiple comparison test confirmed statistically significant differences ($P < 0.001$) between all treated groups and the control at each time point from Day 3 onward, and between the standard and test groups at several time points, with the test group tracking closely behind the standard group throughout.

3.3 Percentage Wound Contraction Rate

Percentage wound contraction, calculated relative to Day 0, increased progressively in all groups, with the test group showing near-complete closure by Day 14, comparable to the standard group and substantially greater than the control (Table 4).

Table 4. Percentage wound contraction rate

Group	Day 0	Day 3	Day 7	Day 11	Day 14
Control	0%	8.33%	20.16%	39.16%	56.83%
Standard	0%	23.25%	52.08%	82.00%	99.75%
Test	0%	20.25%	48.60%	78.95%	98.95%

3.4 Estimation of Tissue IL-6

The IL-6 ELISA standard curve, generated over eight concentrations (0–2000 pg/mL, $R^2 = 0.9962$), confirmed a strong, dose-dependent relationship between IL-6 concentration and absorbance, with low SD/SEM across triplicates indicating high assay precision. Tissue IL-6 concentration on Day 14 was highest in the control group (~0.32 pg/mL), and was markedly and significantly reduced in the standard (~0.19 pg/mL) and test (~0.14 pg/mL) groups ($P < 0.001$ vs. control, one-way ANOVA with Tukey's test), with the test group showing the greatest reduction in this pro-inflammatory marker.

3.5 Histopathological Evaluation

H&E-stained sections of the control group showed persistent inflammatory cell infiltration (neutrophils and macrophages), only partial re-epithelialisation along the wound margins, and residual surface exudate, consistent with incomplete healing. The standard group showed restored epithelial continuity, granulation tissue with loose connective tissue and new extracellular matrix, prominent fibroblast proliferation and collagen deposition, visible neovascularisation, and only mild residual inflammatory infiltrate. The test group treated with T. dioica ointment showed a comparable histological picture — re-epithelialisation, active fibroblast proliferation with marked collagen deposition, neovascularisation, mild inflammatory infiltrate, and minimal surface exudate — indicating a reparative profile closely approaching that of the standard group.

Masson's Trichrome staining corroborated these findings: the control group displayed moderate, less organised collagen deposition and comparatively immature connective tissue, whereas both the standard and test groups exhibited denser, well-organised, predominantly parallel collagen fibre arrangement with mature architecture, most pronounced in the test group, indicating advanced extracellular matrix remodelling and tissue maturation.

4. Discussion

The present findings indicate that topical application of Trichosanthes dioica leaf ointment significantly enhances wound healing in the excision wound model. The observed effects — increased wound contraction, a shortened epithelialisation period, improved histopathological architecture with enhanced collagen deposition and fibroblast proliferation, and a significant reduction in IL-6 levels — are consistent with the established features of effective wound repair, namely rapid wound contraction, collagen deposition, angiogenesis, fibroblast proliferation and resolution of inflammation [60,49].

The accelerated wound contraction observed in the test group may be attributed to enhanced fibroblast migration and increased collagen synthesis, both essential for extracellular matrix formation and tissue remodelling [61,62]. The reduced epithelialisation period further suggests efficient keratinocyte migration and proliferation, reflecting rapid restoration of the epidermal barrier [63].

Histopathological examination demonstrated improved collagen deposition, fibroblast proliferation, angiogenesis and reduced inflammatory cell infiltration in the T. dioica-treated group, in agreement with established mechanisms of successful wound repair reported by Eming et al. [64] and Rodrigues et al. [49]. The significant reduction in tissue IL-6 — a pro-inflammatory cytokine that remains elevated in chronic and delayed wounds, prolonging the inflammatory phase and impairing regeneration [62,65] — indicates that T. dioica effectively modulates the inflammatory response and supports faster progression to the proliferative and remodelling phases of healing.

This wound healing activity may be attributed to the plant's rich phytochemical composition, including flavonoids, phenolic compounds, tannins, terpenoids, alkaloids and saponins [4–7]. These constituents plausibly act by scavenging reactive oxygen species, reducing oxidative stress, stimulating fibroblast proliferation, enhancing collagen synthesis and promoting angiogenesis, collectively facilitating tissue regeneration.

5. Conclusion

The present study provides scientific evidence supporting the traditional use of Trichosanthes dioica leaves in wound management. The formulated 5% w/w hydroalcoholic leaf extract ointment demonstrated significant wound healing potential — comparable to the standard treatment — as evidenced by accelerated wound contraction, favourable histopathological remodelling, and attenuation of tissue IL-6 levels. Further studies involving isolation and characterisation of the active phytoconstituents, elucidation of the underlying molecular mechanisms, toxicity evaluation, and clinical trials are warranted to establish the therapeutic efficacy and safety of T. dioica in humans [6].

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