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Research Protocol

Ayurveda Section

Structural Analysis of Wound Healing with Ehretia Laevis Roxb. Tincture Ointment versus Povidone-iodine Ointment in Wistar Rats: A Research Protocol

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ABSTRACT

Introduction: A wound is an opening in the skin's epidermis that can lead to infection and sepsis. Individuals who experience injuries often report negative social, psychological, and physical effects as a result of their wounds and the treatment involved. Several factors can influence wound healing, including the site of the wound, medications, nutritional status, and bacterial infections. Ehretia laevis Roxb., a medicinal plant, is particularly beneficial for wound healing. It is commonly used by tribal communities for wound care, and they have reported positive outcomes. Additionally, this plant is also utilised to treat joint pain and minor fractures.

Need of the study: Wound infections are frequently treated with potent antibiotics, which often remain inaccessible to rural populations. Furthermore, human resistance to these stronger drugs is increasing at an alarming rate, and patients are compelled to endure the adverse effects associated with such treatments. While povidone-iodine presents non allergic side effects, these cannot be overlooked. Notably, Ehretia laevis tincture ointment has not previously been formulated or employed in Ayurveda for wound healing. This study aims to develop a tincture-based ointment, providing a more standardised and clinically applicable form. Such an innovation may enhance both the practical usability and therapeutic efficacy of this natural remedy in wound care.

Aim: To conduct a structural analysis of wound healing with *Ehretia laevis Roxb*. tincture ointment versus povidone-iodine ointment in Wistar rats, based on the percentage of wound reduction and histological parameters.

Materials and Methods: An experimental preclinical study will be conducted in the central preclinical research facility of Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from April 2025 to September 2025. The study will employ 12 albino Wistar rats of either sex, weighing between 150 and 200 grams. They will be divided into two groups, with six rats in each group. Each group will be kept in a separate enclosure with a 12-hour dark and light cycle in an animal house. The study will span a treatment period of 15 days, with follow-up assessments conducted on the 7th and 15th days. The outcomes will focus on wound contraction rates, histological evidence of tissue regeneration, and the overall healing timeline. A two-sample t-test will be employed to analyse quantitative data, while comparisons for qualitative data will be made using the Chi-square test. A p-value of less than 0.05 will be considered statistically significant.

Keywords: Amputation, Antibiotics, Infection, Wound contraction

INTRODUCTION

Healthy, intact skin naturally regulates microbial populations to prevent pathogen colonisation and deeper tissue invasion. A wound, defined as a breach of the skin's epidermis, can lead to infection and sepsis. Wounds may result from surgery, accidents, external factors such as pressure or shear, or underlying conditions like diabetes and vascular disease [1,2]. They can significantly impact quality of life, leading to pain, discomfort, social isolation, anxiety, prolonged hospital stays, chronic illness, or even death [3]. Certain wounds, termed "hard-to-heal" wounds, fail to heal as expected due to factors such as age and chronic comorbidities, despite standard therapies [4]. Factors influencing wound healing include wound location, medications, nutrition, and bacterial infections [5-7]. In a study conducted in the Indian population, wound incidence was reported as 15.03 per 1,000 individuals, with acute and chronic wound prevalence at 4.48 and 10.55 per 1,000 people, respectively [8]. The lower extremities are most commonly injured, with rural patients experiencing higher amputation rates and more frequent foot infection recurrences than metropolitan patients [9]. Some factors that can hinder the healing of wounds include infections, ageing, anxiety, diabetes, chemotherapy medications, obesity, alcohol use, smoking, and malnourishment [10].

Wound healing consists of four interrelated and overlapping phases: haemostasis, inflammation, proliferation, and tissue remodelling [11]. Haemostasis is achieved within a certain period after injury. The events that occur during the inflammation stage include vasoconstriction, vasodilatation, platelet activation, followed by clot formation and infiltration of immune cells [10]. Several species within the *Ehretia* genus are known for their antimicrobial, anti-inflammatory, antidiabetic, and antibacterial properties [12]. They can also be used to alleviate joint pain and minor fractures [13]. Furthermore, components of the *Ehretia laevis* plant are used as an antidote to vegetable poisons and for a variety of other therapeutic applications [14].

Ehretia laevis contains compounds that promote vasoconstriction, platelet aggregation, and reduce inflammation. It stimulates fibroblast proliferation for collagen synthesis and enhances collagen deposition, leading to stronger, functional scars with proper alignment. Wound infections are treated with various high-cost antibiotics, which are often unaffordable for people in rural areas [15]. Furthermore, people are becoming increasingly resistant to stronger antibiotics every day [16]. The *Ehretia laevis* tincture ointment has not been previously prepared or utilised in *Ayurveda* for wound healing. This study will be the first to apply *Ehretia laevis* tincture ointment for wound healing in rats.

REVIEW OF LITERATURE

The origin of the term "*Vrana*" comes from the Sanskrit word "*Vru Dhatu*," which means "*vrunoti*" or "*vrunute*." There are two main types of *Vrana*: *Nija* and *Agantuja*. *Agantuj Vrana* is caused by trauma, while *Nija Vrana* is caused by dosha vitiation. The *Vrana* that leads to "*gatravichurnana*" (where "*Gatra*" means body tissue and "*Vichurnane*" refers to the destruction or discontinuity of the body or tissue) and performs "*shareera vivarnata*" (the structural breakdown of the body or tissue due to injury or trauma) is considered healed if the injured or inflamed tissue returns to its normal state [17].

The three major *Ayurvedic* treatises, known as the *Bruhatraye—Charaka Samhita, Sushruta Samhita*, and *Ashtanga Sangraha*—provide extensively elaborated formal descriptions of the treatment of *Vrana* within the framework of *Ayurveda* [7]. A study conducted by Harne K et al., concluded that the chloroform fraction of the ethanol extract of *Ehretia laevis* exhibits wound healing properties similar to those of povidone-iodine ointment in an animal model. This finding further reinforces the therapeutic potential of *E. laevis* as an effective natural alternative to conventional treatments, such as povidone-iodine, which is commonly used in clinical practice [18]. Similarly, Borkar AV et al., conducted a review to assess the role of Ehretia Roxb in wound healing and concluded that *Ehretia laevis* Roxb. is highly effective in wound healing, providing a promising alternative to more invasive and expensive treatments, such as surgical management [16].

The study by Rushikesh T et al., demonstrated notable healing properties of the paste made from *Ehretia laevis* Roxb. leaves in both infected and non-infected wounds, as well as in chronic and fresh wounds [9]. The tincture ointment is more suitable for storage and widespread use compared to fresh leaf paste or volatile extracts. Additionally, the tincture ointment is easier to apply than raw paste or liquid extracts. The ointment form provides better coverage, protects the wound from external contaminants, and offers moisturising effects that support the healing process [17].

This study aims to conduct a structural analysis of wound healing by comparing the effectiveness of *Ehretia laevis* Roxb. tincture ointment versus povidone-iodine ointment in Wistar rats.

Primary objectives

1. To assess the efficacy of the tincture ointment of *Ehretia laevis* Roxb. in wound healing in albino Wistar rats.
2. To assess the efficacy of povidone-iodine ointment in wound healing in albino Wistar rats.
3. To compare the efficacy of *Ehretia laevis* tincture ointment and povidone-iodine ointment in wound healing in albino Wistar rats.

Null hypothesis: The application of *Ehretia laevis* Roxb. tincture ointment will be equally or less efficacious than povidone-iodine in wound healing.

Alternate hypothesis: The application of *Ehretia laevis* Roxb. tincture ointment will be more efficacious than povidone-iodine in wound healing.

MATERIALS AND METHODS

An experimental preclinical study will be conducted at the Central Preclinical Research Facility of Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi (Meghe), Wardha, Maharashtra, India, from April 2025 to September 2025. Ethical approval has been obtained from the Institutional Animal Ethics Committee (IAEC) (DMIHER/IAEC/24-25/19).

Inclusion criteria:

1. Healthy albino Wistar rats of either sex will be selected.
2. The rats will weigh between 150 grams and 200 grams.

Exclusion criteria:

1. Gravid and unhealthy rats;
2. Other experimental trial rats.

Sample size calculation: The sample size in an animal study may be estimated using this method. The degree of freedom for the analysis of variance is quantified as a value "E." The value of E should be in the range of 10 to 20. In cases where E is less than 10, the probability of obtaining meaningful findings will increase with the addition of more animals; however, if E is greater than 20, the likelihood of obtaining meaningful findings will not improve. Any sample size that maintains E between 10 and 20 should be regarded as suitable [19].

The following formula can be used to calculate:

E= Total number of animals - Total number of groups

In the present study, there are two groups (one group receiving *E. laevis* tincture ointment and one standard group). Therefore, E will be calculated as follows:

Therefore, 'E' will be:

$$E=(2x6)-2$$

$$E=12-2=10$$

The value of E is 10, indicating that the sample size is sufficient or appropriate. Consequently, the sample size is 12, with 6 rats in each group.

Intervention Description

The fresh leaves of the identified plant, *Ehretia laevis*, will be collected from the Wardha district, specifically from the Bordharan Forest area, and authenticated by the Department of *Dravyaguna*. The ointment will be prepared at *Dattatreya Ayurved Rasa Shala*.

The tincture will be made by placing *Ehretia laevis* leaves along with the entirety of the menstruum in a closed vessel to prevent the evaporation of the menstruum for seven days, allowing the soluble matter to dissolve. During this period, the mixture will be shaken occasionally to replace the saturated layers around the drug with fresh menstruum. Afterward, the mixture will be strained using sieves and nets; the marc will be pressed, and the combined liquids will be filtered for clarification [20].

The wax will be heated until it liquefies, after which the tincture will be added to the liquefied wax. Continuous stirring will be performed until a homogeneous mixture is formed, resulting in a semisolid ointment with a consistent texture. The ointment will then be filled into appropriate tubes. The ingredients of *Ehretia laevis* tincture ointment are detailed in [Table/Fig-1].

S. No.	Ingredients	Quantity
1.	White beewax	2%
2.	Hard paraffin	3%
3.	White soft paraffin	90%
4.	*E.laevis* tincture	5%

[Table/Fig-1]: Ingredients of *Ehretia laevis* Roxb. tincture ointment.

Properties of the Drug

Overall, a thorough understanding of drug properties is fundamental for optimising therapeutic outcomes while minimising risks to patient health. The properties of *Ehretia laevis* Roxb. are detailed in [Table/Fig-2] [21].

The study will employ healthy albino Wistar rats of either sex, weighing between 150 and 200 grams, and will divide them into two groups: Group ELTO (Intervention group) and group PIO (Control group), with six rats in each group. Group ELTO will be treated with the application of *Ehretia laevis* Roxb. tincture ointment, while group PIO will receive the application of povidone-iodine ointment over the wound twice a day for 15 days. Each group will be housed in a separate enclosure with a 12-hour dark and light cycle in the animal

S. No.	Drug	Taste (Rasa)	Properties (Guna)	Potency (Virya)	Post digestive effect (Vipaka)	Therapeutic uses (Dosha Karma)
1.	*Charmavriksha* (*Ehretia laevis*. Roxb)	Astringent (Kashaya) Pungent (*Katu*)	Light (*Laghu*) Piercing (*Tikshna*)	Hot (*Ushna*)	Pungent (*Katu*)	Antihelmintic, demulcent, expectorant, diuretic. Leaves useful for headaches and ulcers. Ringworm can be cured by combining oil and powdered kernel [21].

[Table/Fig-2]: Properties of *Ehretia laevis* Roxb [21].

house. The animals will be provided with a regular pellet meal and complete access to water. The study will span a treatment period of 15 days, with follow-up assessments conducted on the 7th and 15th days [Table/Fig-3].

Group	Sample size	Intervention	Frequency	Duration	Follow-up
Group ELTO	06	*E.laevis* tincture ointment	Application of ointment will be done daily twice a day	15 days	On 7th, and 15th day
Group PIO	06	Povidone iodine ointment	Application of ointment will be done daily twice a day	15 days	On 7th, and 15th day

[Table/Fig-3]: Group posology.

Primary outcomes

Assessment criteria

Percentage of Wound Reduction [22] or Wound Healing Rate [23].

The following formula will be used to measure the wound on the 7th and 15th days:

$$\frac{SAI-SAC}{SAI} \times 100 = \_\_\_\_\_ \% \text{ of reduction}$$

(SAI=surface area (L×W) on day 0, SAC=surface area currently)

- WHR equal to 1 or 100% indicates complete re-epithelialisation.
- WHR equal to 0 or 0% indicates no signs of re-epithelialisation.
- WHR >0 or 0% indicates a decrease in area.
- WHR <0 or 0% indicates increase in area [23].

Subjective criteria: Biopsies for evaluating healing and repair will be collected on Days 0, 7, and 15 from the edge of the wound. The biopsy samples will be assessed using the following histological parameters to calculate the healing score [24]:

1. Amount of granulation tissue (Profound-1, Moderate-2, Scanty-3, Absent-4)
2. Inflammatory infiltrate (Plenty-1, Moderate-2, A few-3)
3. Collagen fibre orientation (Vertical-1, Mixed-2, horizontal-3)
4. Pattern of collagen (Reticular-1, Mixed-2, Fascicle-3)
5. Amount of early collagen (Profound-1, Moderate-2, Minimal-3, Absent-4)
6. Amount of mature collagen (Profound-1, Moderate-2, Minimal-3)

➢ Healing status grades:

- Good: 16-19
- Fair: 12-15
- Poor: 8-11 [24].

The evaluation of healing in the study group and control group will be based on the final cumulative score.

STATISTICAL ANALYSIS

The analysis of all results will be conducted using SPSS software version 17. For qualitative measurements, percentages will be calculated, while for quantitative measurements, the mean and standard deviation will be computed. Both parametric and non parametric tests will be utilised for quantitative and qualitative data, respectively. A two-sample t-test will be employed to analyse quantitative data, while comparisons for qualitative data will be made using the Chi-square test. A p-value of <0.05 will be considered statistically significant.

Intervention modification: Any adverse effects observed during therapy shall be documented and promptly reported to the ethics committee.

Discontinuation criteria: Rats will be excluded from the study if any adverse drug reaction is observed that significantly impacts their quality of life or health, such as an allergic reaction to the ointment.

Modification criteria: Interventions may be modified based on the response of rats to the ointment. For instance, if rats experience mild adverse effects, the frequency or duration of the ointment application may be adjusted.

The flow of interventions that will be conducted in the study is outlined in [Table/Fig-3].

Data Collection Plan: Retention

Plans to promote retention and ensure complete follow-up will include a separate enclosure with a 12-hour dark and light cycle in the animal house. The rats will be provided with a regular pellet meal and complete access to water. Each day, aseptic precautions will be maintained while applying the ointment over the wound. The ointment will be applied until the wound is healed, with regular monitoring of the wound site for any swelling, redness, or oozing.

Data management: The data management plan will ensure rigorous procedures for data entry, coding, security, and storage to maintain integrity throughout the trial. Standardised data entry protocols will be implemented to minimise errors, potentially including double data entry for critical information. Variables will be coded according to established guidelines to facilitate analysis. Security measures will include restricted electronic access and encryption of sensitive data, with physical documents stored securely. Data will be centralised on secure servers, with regular backups planned to prevent loss. Range checks during data entry will be conducted to promptly identify outliers or errors. Detailed data management procedures will be outlined in the protocol documentation for transparency and adherence to guidelines.

Protocol amendments: There are no plans to notify relevant parties (e.g., investigators, journals, and regulators) of significant protocol changes (e.g., changes to eligibility criteria, outcomes, or analysis).

Declaration of Interests

Principal investigators overseeing the entire trial and at each study site will transparently disclose any financial or other competing interests that could potentially influence the study's integrity or interpretation of results.

Data access: Access to the final trial dataset will be restricted to authorised personnel directly involved in the study. Any existing contractual agreements that limit access for investigators will be openly disclosed.

Dissemination: This protocol will be published as a thesis to share our research on excision wounds. The thesis will provide a thorough overview of the study's design, methodology, data gathering methods, data analysis plan, and ethical considerations. We hope that by making this protocol public, we will contribute to expanding knowledge in the field and promoting future research efforts.

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