

# Comparative Histological and Structural Analysis of Wound Healing with *Ehretia laevis* Roxb. Tincture Ointment versus Povidone-Iodine Ointment in Wistar Rats

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## ABSTRACT

**Background:** Delayed wound healing remains a significant clinical challenge, driving the search for effective and safe therapeutic agents. *Ehretia laevis* Roxb. is a medicinal plant traditionally used for various ailments, but its wound-healing properties, particularly in a tincture-ointment formulation, have not been systematically investigated. **Aim:** Structural analysis of wound healing with *Ehretia laevis* Roxb. Tincture ointment versus Povidone-iodine ointment in Wistar rats. **Methods:** An experimental preclinical study was conducted on 12 Wistar rats divided into two groups (n=6 each). A 10 mm diameter full-thickness excision wound was created on the dorsum. Group A received *E. laevis* tincture ointment, while Group B received povidone-iodine ointment, twice daily for 15 days. Wound contraction was assessed on days 7 and 15. Histopathological evaluation was performed on days 1, 7, and 15 using a semi-quantitative scoring system for granulation tissue, inflammatory infiltrate, collagen fiber orientation, and collagen deposition. Data were analyzed using unpaired t-tests, two-way ANOVA, and Mann-Whitney U tests. **Results:** The *E. laevis* group exhibited significantly higher mean wound contraction on day 7 ( $71.5 \pm 2.4\%$  vs.  $60.5 \pm 1.7\%$ ;  $p < 0.0001$ ) and day 15 ( $97.5 \pm 2.4\%$  vs.  $90.3 \pm 3.0\%$ ;  $p < 0.001$ ) compared to the control group. Histopathological analysis revealed that *E. laevis* significantly reduced inflammatory infiltrate ( $p = 0.045$ ) and promoted early collagen deposition ( $p = 0.035$ ) by day 7. By day 15, the test group showed significantly superior collagen maturation ( $p = 0.0037$ ) and orientation ( $p = 0.014$ ), resulting in significantly higher cumulative healing scores ( $p = 0.0076$ ). **Conclusion:** *Ehretia laevis* tincture ointment demonstrates superior and accelerated wound healing activity compared to povidone-iodine ointment. Its enhanced efficacy, supported by both macroscopic and histological evidence, positions it as a promising novel therapeutic option for wound management and warrants further investigation into its underlying molecular mechanisms.

**Keywords:** *Ehretia laevis*, Wound Healing, Histopathology, Povidone-Iodine, Phytotherapy, Excision Wound Model, Preclinical Study.

## INTRODUCTION

Wound healing is a complex, dynamic, and highly regulated biological process that restores the structural and functional integrity of injured tissue. This process involves a sequence of overlapping phases: hemostasis, inflammation, proliferation, and remodelling<sup>1</sup>. Any disruption in this coordinated cascade, often due to underlying pathologies such as diabetes, infection, or poor perfusion, can lead to chronic, non-healing wounds, imposing a substantial burden on healthcare systems and significantly impacting patients' quality of life<sup>2,3</sup>.

The standard of care for wound management often includes antiseptic agents like povidone-iodine, which is widely used for its broad-spectrum antimicrobial activity<sup>4</sup>. While effective in preventing infection, its potential to impair granulation tissue formation and delay healing in certain contexts has prompted a search for alternative agents that not only provide a conducive environment but also actively promote the proliferative and remodelling phases of healing<sup>5,6</sup>.

In recent years, there has been a resurgence of interest in medicinal plants as a source of

novel therapeutic agents, driven by their diverse bioactive compounds and traditional use in wound care<sup>7</sup>. *Ehretia laevis* Roxb. (Family: Boraginaceae), commonly known as 'Khandu Chakka', is a medium-sized tree native to the Indian subcontinent. In traditional Ayurvedic medicine, various parts of the plant, particularly the leaves, have been used for treating skin diseases, ulcers, and inflammation<sup>8</sup>. Phytochemical investigations of *E. laevis* leaves have revealed the presence of several bioactive constituents, including flavonoids, tannins, saponins, and alkaloids, which possess antioxidant, anti-inflammatory, and antimicrobial properties<sup>9,10</sup>. These pharmacological activities suggest strong potential for wound-healing applications. Previous studies have explored the wound-healing activity of crude extracts of *E. laevis* leaves<sup>11</sup>. Still, the efficacy of a tincture-based ointment formulation, a common and stable preparation in traditional medicine, has not been systematically evaluated or compared with a standard modern therapy.

Therefore, this study was designed to bridge this gap by scientifically evaluating the wound-healing potential of *Ehretia laevis* tincture ointment in a standardised rat excision wound model. The primary objective was to compare its efficacy with povidone-

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iodine ointment, the standard antiseptic, using both macroscopic (wound contraction) and microscopic (histopathological) parameters. We hypothesised that the bioactive phytoconstituents in *E. laevis* would accelerate healing, leading to superior outcomes compared with the standard treatment.

## MATERIALS AND METHODS

### Study Design and Ethical Approval

This was an experimental preclinical study. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Ref. No.: DMIHER/IAEC/2024-25/19), Datta Meghe Institute of Higher Education and Research, Wardha, India. The study was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines<sup>12</sup>.

### Sample Size Calculation

The sample size was determined using the resource equation method<sup>13</sup>. The formula  $E = \text{Total number of animals} - \text{Total number of groups}$  was used.

With two groups ( $n=6$  per group),  $E = (12) - 2 = 10$ .

As the E value fell within the recommended range of 10 to 20, the sample size of 12 animals was considered sufficient to obtain meaningful results. However, it is acknowledged that this sample size is relatively small, which may limit the statistical power to detect smaller effect sizes and affect the generalizability of the findings. The results of this study should therefore be interpreted as preliminary, and future studies with larger sample sizes are warranted to confirm these observations.

### Randomization and Blinding

Animals were randomly allocated to treatment groups using a computer-generated random number sequence (Microsoft Excel, version 2019). The allocation sequence was concealed in sealed opaque envelopes until the time of intervention. To minimize bias, the investigator responsible for measuring wound contraction and the pathologist performing histopathological evaluation were blinded to the treatment allocation. The ointments were coded as "Ointment A" and "Ointment B" by a third-party investigator who was not involved in the outcome assessments. The code was only broken after all data analysis was completed.

### Plant Material and Tincture Preparation

Fresh leaves of *Ehretia laevis* Roxb. were collected from the Bordharan Forest area in Wardha district, Maharashtra, India (Geographic coordinates: 20.7456° N, 78.6022° E) in January 2025. The plant was authenticated by the Department of Dravyaguna, Mahatma Gandhi Ayurved College, Wardha. A voucher specimen (Accession No.: MGAC/Herb/2024/EL-015) has been deposited at the institutional herbarium.

A tincture was prepared using a maceration process<sup>14</sup>. Briefly, 200 g of fresh, crushed leaves was immersed in 1000 mL of ethanol (95% v/v) in an airtight glass container and allowed to stand for seven days at room temperature ( $25 \pm 2^\circ\text{C}$ ) with intermittent shaking (twice daily). After maceration, the mixture was strained through a muslin cloth, and the marc was pressed to recover the extract. The combined liquid was then filtered through Whatman No. 1 filter paper to obtain a clear tincture. The extraction yield was calculated as 14.2% (w/w) on a dry weight basis.

For quality control, the tincture underwent preliminary phytochemical screening for the presence of flavonoids, tannins, saponins, and alkaloids using standard protocols<sup>15</sup>. Total phenolic content was

estimated using the Folin-Ciocalteu method ( $56.3 \pm 3.2$  mg gallic acid equivalent/g extract), and total flavonoid content was determined using the aluminium chloride colourimetric method ( $32.7 \pm 2.8$  mg quercetin equivalent/g extract)<sup>16</sup>. The reproducibility of the formulation was ensured by preparing three independent batches of the tincture and ointment, which were tested for consistency in physicochemical and organoleptic parameters, microbial contamination, and stability.

### Ointment Formulation

An ointment base was prepared by accurately weighing and melting white beeswax (1%), petroleum jelly (40%), stearic acid (1%), and sesame oil (8%) on a water bath. The molten base was allowed to cool slightly to a semi-solid consistency. The prepared *E. laevis* tincture (50%) was then incorporated into this base using an ointment slab and spatula, and the mixture was triturated to ensure homogeneity<sup>11</sup>. The prepared ointment was stored in airtight containers under refrigeration. Povidone-iodine ointment (5% w/w) was procured from a commercial source and served as the standard control.

### Experimental Animals

Healthy Albino Wistar rats of either sex, weighing between 150 and 200 gm, were used for the study. The animals were housed in polypropylene cages under standard laboratory conditions (a 12-hour light/dark cycle) with free access to a standard pellet diet and water ad libitum.

### Grouping and Interventions

After a one-week acclimatisation period, the animals were randomly divided into two groups ( $n=6$  per group):

- Group A (Interventional Group): *E. laevis* Tincture Ointment (ELTO) applied topically twice daily.
- Group B (Control Group): Povidone-Iodine Ointment (PIO) applied topically twice daily.

### Excision Wound Creation

The animals were anaesthetised by local anaesthesia infiltration (Lignocaine HCl 2%). The dorsal fur was shaved, and the site was cleaned with 70% ethanol. A full-thickness excision wound of approximately 10 mm in diameter ( $78.54 \text{ mm}^2$ ) was created on the dorsum of each rat using a sterile scalpel blade and pointed scissors<sup>17</sup>. Hemostasis was achieved with sterile gauze. The wounds were left open and dressed with the respective ointments twice daily for 15 days.

### Assessment of Wound Healing

#### Percentage Wound Contraction

Wound contraction was monitored on days 1, 7, and 15. The wound area was measured by tracing the wound margins on a transparent sheet, which was then transferred to graph paper. The area was calculated using the formula for an ellipse ( $\text{length} \times \text{width}$ ). The percentage wound contraction was calculated using the formula<sup>18</sup>:

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

(SAI = Surface area ( $L \times W$ ) on day 0, SAC = Surface area currently)

#### Histopathological Assessment

Full-thickness biopsy samples were collected from the wound edges on days 1, 7, and 15. The tissues were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. Sections of 5  $\mu\text{m}$  thickness were cut and stained with hematoxylin and eosin (H&E). Histological sections were evaluated under a light microscope and scored semi-quantitatively for the following parameters<sup>19</sup>:

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

A cumulative healing status score was calculated from these parameters, with a higher score indicating better healing. Healing status was graded as Good (16-19), Fair (12-15), or Poor (8-11).

To ensure reproducibility and minimise observer bias, all histopathological evaluations were performed by a single experienced pathologist who was blinded to the treatment allocation. Intra-observer reliability was assessed by re-evaluating 20% of randomly selected slides after a two-week interval. The kappa coefficient for intra-observer agreement was 0.85 (95% CI: 0.72-0.94), indicating substantial agreement.

## Statistical Analysis

All data were expressed as mean  $\pm$  standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test separately for each variable. For wound contraction data, which were normally distributed (Shapiro-Wilk  $p > 0.05$ ), an unpaired Student's t-test was used for intergroup comparisons, and two-way analysis of variance (ANOVA) was used to evaluate the effects of treatment and time. For the non-parametric histopathological scores, the Mann-Whitney U test was employed. To account for multiple comparisons across the six histopathological parameters, the Benjamini-Hochberg procedure was applied to control the false discovery rate at  $q < 0.05^{20}$ . A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA).

## RESULTS

### Wound Contraction Analysis

The effects of the treatments on wound contraction are presented in Table 1 and Table 2 and graphically in Figure 1. The *E. laevis* tincture ointment (ELTO) group showed significantly accelerated wound closure compared to the povidone-iodine ointment (PIO) group.

On day 7, the *E. laevis* tincture ointment (ELTO) group exhibited a mean wound contraction of  $71.5 \pm 2.4\%$  (95% CI: 69.0–74.0), which was significantly higher than the  $60.5 \pm 1.7\%$  (95% CI: 58.7–62.3) observed in the Povidone-iodine ointment (PIO) group ( $t = 9.1$ ,  $df = 10$ ,  $p < 0.0001$ ). By day 15, the ELTO group demonstrated near-complete healing with a mean contraction of  $97.5 \pm 2.4\%$  (95% CI: 95.0–100.0), which was again significantly higher than the  $90.3 \pm 3.0\%$  (95% CI: 87.1–93.5) seen in the control group ( $t = 4.6$ ,  $df = 10$ ,  $p < 0.001$ ). Three out of six animals in the *E. laevis* tincture ointment (ELTO) group achieved 100% wound closure by day 15, while none in the Povidone-iodine ointment (PIO) group did.

Error bars represent standard deviation (SD). The *E. laevis* group showed significantly higher wound contraction at both time points ( $p < 0.0001$  and  $p < 0.001$ ). An unpaired Student's t-test was used for intergroup comparisons.

Two-way ANOVA (Table 3) revealed a highly significant main effect of treatment ( $F = 85.2$ ,  $p < 0.0001$ ) and time ( $F = 197.3$ ,  $p < 0.0001$ ), confirming the superiority of *E. laevis* Tincture Ointment and the progression of healing over time. A significant interaction between

treatment and time was also observed ( $F = 5.9$ ,  $p < 0.05$ ), indicating that the *E. laevis* Tincture Ointment (ELTO) group had a greater increase in wound contraction rate over the study period than the control group.

### Histopathological Evaluation

Histopathological analysis provided a deeper understanding of the tissue-level changes during healing.

#### Baseline Characteristics (Day 1)

As expected, on day 1, both groups showed comparable histological features with a high degree of inflammation (Score 3), absence of granulation tissue (Score 4), and no significant differences across all parameters ( $p > 0.05$ ), confirming baseline homogeneity (Table 4).

#### Day 7 – Proliferative Phase

By day 7, the *E. laevis* Tincture Ointment-treated wounds exhibited accelerated progression into the proliferative phase. Significant differences were observed in the test group compared to the control (Table 5). The *E. laevis* Tincture Ointment (ELTO) group showed a moderate but significant reduction in inflammatory infiltrate (Score 2 vs. 1;  $p = 0.045$ ), indicating faster resolution of inflammation. Critically, early collagen deposition was significantly more profound in the ELTO group (Score 1 vs. 2;  $p = 0.035$ ), and the pattern of collagen had begun to shift from a reticular to a mixed pattern (Score 2 vs. 1;  $p = 0.020$ ). This was supported by a better fibre orientation (Score 2 vs. 1;  $p = 0.028$ ) and more mature collagen (Score 2 vs. 3;  $p = 0.021$ ).

#### Day 15 – Remodelling Phase

By day 15, the *E. laevis* Tincture Ointment (ELTO) group demonstrated a clear advantage in the remodelling phase (Table 6). Highly significant differences were observed in parameters related to collagen quality and maturation. The ELTO group showed a significantly better collagen pattern, now approaching a fascicle-like arrangement (Score 3 vs. 2;  $p = 0.009$ ), and early collagen levels were appropriately reduced (Score 3 vs. 1;  $p = 0.006$ ), indicating replacement with mature collagen. Most notably, the amount of mature collagen was profoundly higher in the ELTO group (Score 1 vs. 3;  $p = 0.0037$ ), and fibre orientation was predominantly horizontal (Score 3 vs. 2;  $p = 0.014$ ), suggesting superior tensile strength and organised tissue architecture.

#### Cumulative Healing Scores

The cumulative histopathological score, which provides an overall assessment of healing quality (Table 7, Figure 2), was significantly higher in the *E. laevis* Tincture Ointment group on day 7 (12 vs. 10;  $p = 0.035$ ) and highly significantly higher on day 15 (16 vs. 13;  $p = 0.0076$ ). This progression from a "Fair" healing grade on day 7 to a "Good" healing grade on day 15 in the ELTO group, compared to the "Poor" to "Fair" progression in the PIO group, further substantiates the superior efficacy of the test ointment.

The *E. laevis* tincture ointment group (ELTO) achieved significantly higher scores on both day 7 ( $p < 0.05$ ) and day 15 ( $p < 0.01$ ), indicating superior overall healing quality.

## DISCUSSION

The present study provides compelling evidence for the superior wound healing activity of a novel *Ehretia laevis* tincture ointment compared to the standard povidone-iodine ointment in a rat excision wound model. The findings demonstrate that the test formulation not only accelerates wound contraction but also significantly improves the histological quality of the healed tissue, highlighting its potential as a therapeutic agent for wound management. However, these findings should be interpreted with caution, given the study's preliminary nature and the limited sample size.

**Table 1. Percentage Wound Contraction in *E. laevis* Tincture Ointment (ELTO) Group (n=6)**

| Rats      | Day 1 Area (mm <sup>2</sup> ) | Day 7 Area (mm <sup>2</sup> ) | Day 15 Area (mm <sup>2</sup> ) | Day 7 % Contraction | Day 15 % Contraction |
|-----------|-------------------------------|-------------------------------|--------------------------------|---------------------|----------------------|
| Rat 1     | 78.54                         | 28                            | 0                              | 72%                 | 100%                 |
| Rat 2     | 78.54                         | 25                            | 0                              | 75%                 | 100%                 |
| Rat 3     | 78.54                         | 30                            | 0                              | 70%                 | 100%                 |
| Rat 4     | 78.54                         | 32                            | 5                              | 68%                 | 95%                  |
| Rat 5     | 78.54                         | 27                            | 4                              | 73%                 | 96%                  |
| Rat 6     | 78.54                         | 29                            | 6                              | 71%                 | 94%                  |
| Mean ± SD |                               |                               |                                | 71.5 ± 2.4          | 97.5 ± 2.4           |

**Table 2. Percentage Wound Contraction in Povidone-Iodine Ointment (PIO) Group (n=6)**

| Rats      | Day 1 Area (mm <sup>2</sup> ) | Day 7 Area (mm <sup>2</sup> ) | Day 15 Area (mm <sup>2</sup> ) | Day 7 % Contraction | Day 15 % Contraction |
|-----------|-------------------------------|-------------------------------|--------------------------------|---------------------|----------------------|
| Rat 1     | 78.54                         | 40                            | 14                             | 60%                 | 86%                  |
| Rat 2     | 78.54                         | 38                            | 10                             | 62%                 | 90%                  |
| Rat 3     | 78.54                         | 42                            | 12                             | 58%                 | 88%                  |
| Rat 4     | 78.54                         | 39                            | 9                              | 61%                 | 91%                  |
| Rat 5     | 78.54                         | 41                            | 8                              | 59%                 | 92%                  |
| Rat 6     | 78.54                         | 37                            | 5                              | 63%                 | 95%                  |
| Mean ± SD |                               |                               |                                | 60.5 ± 1.7          | 90.3 ± 3.0           |

**Table 3. Two-Way ANOVA for Treatment and Time Effects on Wound Contraction**

| Source of Variation            | SS     | df | MS     | F-value | p-value | Significance       |
|--------------------------------|--------|----|--------|---------|---------|--------------------|
| Treatment                      | 1058.4 | 1  | 1058.4 | ~85.2   | <0.0001 | Highly Significant |
| Time                           | 2450.6 | 1  | 2450.6 | ~197.3  | <0.0001 | Highly Significant |
| Interaction (Treatment × time) | 72.8   | 1  | 72.8   | ~5.9    | <0.05   | Significant        |
| Error                          | 248.4  | 20 | 12.42  |         |         |                    |

**Table 4. Baseline Histopathological Scores (Day 1)**

| Parameters              | <i>E.laevis</i> Tincture Ointment group | Povidone iodine Ointment group | p-value | Significance |
|-------------------------|-----------------------------------------|--------------------------------|---------|--------------|
| Granulation Tissue      | 4                                       | 4                              | 1.000   | NS           |
| Inflammatory Infiltrate | 3                                       | 3                              | 1.000   | NS           |
| Fibre Orientation       | 3                                       | 3                              | 1.000   | NS           |
| Pattern of Collagen     | 3                                       | 3                              | 1.000   | NS           |
| Early Collagen          | 4                                       | 4                              | 1.000   | NS           |
| Mature Collagen         | 1                                       | 1                              | 1.000   | NS           |

**Table 5. Parameter-wise Histopathological Comparison on Day 7**

| Parameters              | <i>E.laevis</i> Tincture Ointment group | Povidone iodine Ointment group | p-value | Significance |
|-------------------------|-----------------------------------------|--------------------------------|---------|--------------|
| Granulation Tissue      | 2                                       | 2                              | 1.000   | NS           |
| Inflammatory Infiltrate | 2                                       | 1                              | 0.045   | p < 0.05     |
| Fibre Orientation       | 2                                       | 1                              | 0.028   | p < 0.05     |
| Pattern of Collagen     | 2                                       | 1                              | 0.020   | p < 0.05     |
| Early Collagen          | 1                                       | 2                              | 0.035   | p < 0.05     |
| Mature Collagen         | 2                                       | 3                              | 0.021   | p < 0.05     |

**Table 6. Parameter-wise Histopathological Comparison on Day 15**

| Parameters              | <i>E.laevis</i> Tincture Ointment group | Povidone iodine Ointment group | p-value | Significance |
|-------------------------|-----------------------------------------|--------------------------------|---------|--------------|
| Granulation Tissue      | 3                                       | 2                              | 0.031   | p < 0.05     |
| Inflammatory Infiltrate | 3                                       | 2                              | 0.021   | p < 0.05     |
| Fibre Orientation       | 3                                       | 2                              | 0.014   | p < 0.05     |
| Pattern of Collagen     | 3                                       | 2                              | 0.009   | p < 0.01     |
| Early Collagen          | 3                                       | 1                              | 0.006   | p < 0.01     |
| Mature Collagen         | 1                                       | 3                              | 0.0037  | p < 0.01     |

**Table 7. Inter-group Comparison of Cumulative Histopathological Scores**

| Day    | ELTO | PIO | U Value | Z Value | p-value | Significance |
|--------|------|-----|---------|---------|---------|--------------|
| Day 1  | 4    | 4   | 18.0    | 0.00    | 1.000   | NS           |
| Day 7  | 2    | 2   | 18.0    | 0.00    | 1.000   | NS           |
| Day 15 | 3    | 2   | 7.0     | -2.15   | 0.031   | p < 0.05     |

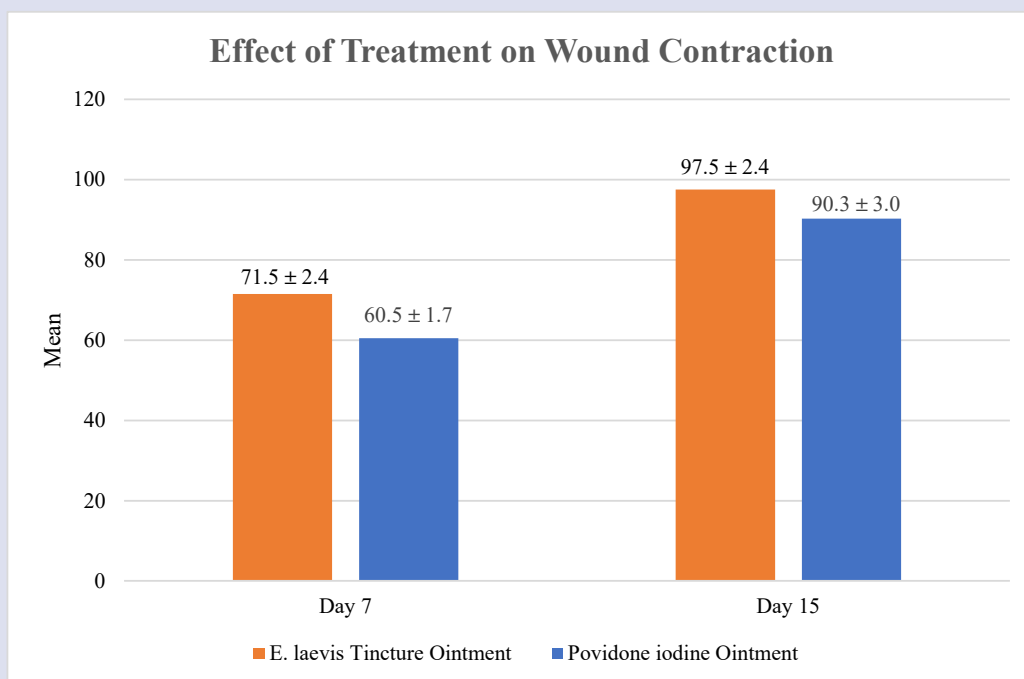


**Figure 1.** Comparison of Mean Percentage Wound Contraction between Groups

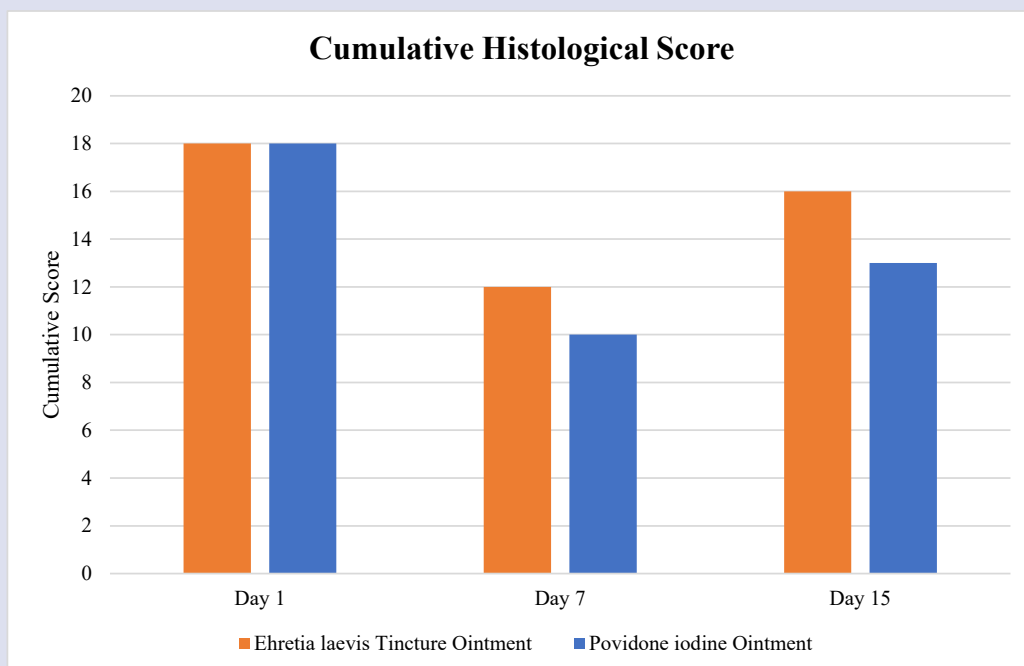


**Figure 2.** Comparison of Cumulative Histopathological Scores

Wound contraction, a critical parameter in the healing process, is driven by the action of myofibroblasts that pull the wound edges together<sup>1</sup>. The significantly higher wound contraction rates observed in the *E. laevis* group on days 7 (71.5%) and 15 (97.5%) indicate a marked acceleration of this process. This finding is consistent with previous studies on various plant extracts known to promote fibroblast proliferation and migration<sup>7</sup>. The superior performance of *E. laevis* over povidone-iodine is noteworthy. While povidone-iodine is an effective

antiseptic, some studies have reported its potential cytotoxic effects on fibroblasts and keratinocytes, which can paradoxically delay wound healing<sup>5,6</sup>. In contrast, the *E. laevis* ointment appears to promote the proliferative phase actively, leading to faster closure.

The histopathological analysis provided a mechanistic basis for the observed macroscopic healing. The ability of a wound to heal efficiently depends on a timely and orderly progression through the

inflammatory, proliferative, and remodelling phases<sup>19</sup>. In the *E. laevis* group, a significant reduction in the inflammatory infiltrate by day 7 suggests that the formulation possesses potent anti-inflammatory properties, which aligns with the plant's traditional use<sup>8</sup>. This early resolution of inflammation is crucial as it prevents the destructive effects of prolonged inflammation and allows for the timely onset of proliferation<sup>21</sup>.

The most striking differences between the two groups were observed in the parameters related to collagen. The significant increase in early collagen deposition in the test group by day 7 indicates that *E. laevis* effectively stimulates fibroblast synthetic activity. Early collagen (Type III) serves as a provisional scaffold essential for cell migration and tissue formation<sup>23</sup>. By day 15, the *E. laevis* group showed a highly significant reduction in early collagen and a correspondingly profound increase in mature collagen (Type I), along with superior fibre orientation (horizontal) and a more organised fascicle pattern. This demonstrates that the plant extract not only initiates collagen synthesis but also facilitates the crucial remodelling phase where the weak, disorganised Type III collagen is replaced by strong, well-aligned Type I collagen<sup>24</sup>. This process is key to restoring the tensile strength of the healed skin and preventing scar contractures<sup>25</sup>. The poor performance of the povidone-iodine group in these parameters, particularly the persistence of early collagen and lack of mature collagen by day 15, further supports the hypothesis that it may have a net negative effect on the quality of the healing tissue.

The observed pharmacological effects are likely attributable to the rich phytochemical profile of *E. laevis* leaves. Previous studies have documented the presence of flavonoids, tannins, saponins, and other phenolic compounds<sup>9,10</sup>. Flavonoids are well known for their antioxidant and anti-inflammatory properties, which can protect the wound microenvironment from oxidative stress, a key factor that impairs healing<sup>26, 27</sup>. Tannins are astringent and can help in wound contraction by forming a protective layer over the wound<sup>28</sup>. Furthermore, the saponins present may contribute to antimicrobial activity, providing an added benefit similar to that of povidone-iodine but potentially less cytotoxic<sup>29,30</sup>. The synergistic action of these compounds likely underpins the multifaceted healing-promoting effect observed in this study.

The significant interaction between treatment and time revealed by the two-way ANOVA ( $F = 5.9$ ,  $p < 0.05$ ) is an important finding. It indicates that the *E. laevis* ointment did not merely result in a constant, higher level of healing; rather, its effect amplified over time, leading to a greater increase in the rate of wound contraction between day 7 and day 15 than the standard. This suggests that the bioactive compounds in *E. laevis* may exert cumulative or sustained effects on the cellular processes underlying repair, further underscoring its potential as a therapeutic agent.

While these results are encouraging, it is important to acknowledge the limitations of this study. The relatively small sample size ( $n=6$  per group) may limit the generalizability of the findings and the statistical power to detect smaller but potentially clinically meaningful differences. The semi-quantitative histological scoring, while standard and validated with intra-observer reliability assessment, does not provide the same level of objectivity as quantitative techniques such as immunohistochemistry or morphometric analysis. Furthermore, this study only evaluated a single concentration of the tincture (50%) and did not include biochemical assays to measure markers of oxidative stress or specific inflammatory cytokines directly. The 15-day observation period, while covering the main phases of healing, did not allow for long-term assessment of scar quality and tensile strength.

Despite these limitations, this study represents a significant step in validating the traditional use of *Ehretia laevis* in wound care. The

superior efficacy of the tincture ointment, as demonstrated by both objective and subjective parameters, makes it a promising candidate for the development of a novel plant-based wound-healing formulation. However, it is premature to conclude that this formulation is clinically superior to povidone-iodine based solely on this preliminary animal study. The findings should be interpreted as proof of concept that warrants further investigation in larger preclinical studies and, eventually, well-designed clinical trials.

## LIMITATIONS AND FUTURE SCOPE

### Limitations:

- The semi-quantitative scoring, while standard, introduces a degree of subjectivity. Inter-observer bias was minimised by having a single, blinded pathologist evaluate all samples.
- The study used a fixed concentration of the tincture (50%). The optimal dose for maximum efficacy remains unknown.
- The study did not include biochemical assays to directly measure markers of oxidative stress (e.g., glutathione, malondialdehyde) or specific inflammatory cytokines (e.g., TNF- $\alpha$ , IL-6).
- The study was limited to 15 days, which, while covering the main phases of healing, did not allow for long-term assessment of scar quality and tensile strength.

### Future Scope:

- Conduct a study with multiple concentrations of the *E. laevis* tincture to establish the optimal therapeutic dose.
- Perform immunohistochemistry and biochemical assays to elucidate the molecular pathways involved, including the expression of growth factors (e.g., TGF- $\beta$ , VEGF), matrix metalloproteinases (MMPs), and inflammatory markers.
- Evaluate the long-term effects on scar tissue formation, including tensile strength measurement and histological analysis at later time points (e.g., 21, 30, 60 days).
- Assess the ointment's efficacy in a contaminated or infected wound model to evaluate its antimicrobial and wound-healing potential fully.
- Conduct a detailed phytochemical analysis of the tincture to identify the specific bioactive compounds responsible for the observed therapeutic effects.

## CONCLUSION

This study demonstrates that *Ehretia laevis* tincture ointment possesses superior wound healing activity compared to povidone-iodine ointment in a rat excision wound model under the conditions tested. The test formulation significantly accelerated wound contraction and led to superior histological outcomes, characterised by faster resolution of inflammation, enhanced early collagen deposition, and advanced maturation of collagen fibres with optimal orientation. These findings provide preliminary scientific validation for the traditional use of *E. laevis* in wound care and suggest that this novel formulation has potential as an alternative to standard antiseptic treatments. Assess the ointment's efficacy in a contaminated or infected wound model to evaluate its antimicrobial and wound-healing potential fully.

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