"Pharmacological Evaluation and Formulation of *Litsea polyantha* Herbal Ointmet for Wound Healing Model in Wistar Rats"

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Abstract

Background: Burn wound healing is one of the most painful and challenging type of injury to heal, affecting not only the skin but also a person's physical comfort and emotional well-being. Healing of the wound is a complex process that involves inflammation, regeneration of tissues, collagen formation and skin remodeling. Successful healing depends on several factors such as severity of burn, prevention of infection, adequate blood supply and proper care of the wound. In recent years, researchers are focusing on treatments based on herbs and plant for burn wound management because many plants possess antioxidant, anti-inflammatory, and antimicrobial properties that can support the healing process. *Listea polyantha*is traditionally versatile medicinal plant with reported pharmacological activities.

Objective: The present study focuses on the wound healing efficacy of *Listea polyantha* herbal ointment in experimentally induced burn wound models in rats.

Materials and Methods: Burn wounds were induced in *Wistar albino* rats, which were divided in five groups (n=5). Animal of different group were subjected to ointment based silver sulphadiazine (1% w/w) and *Listea polyantha* formulated ointment 2.5%, 5%, and 10% for 21 days. Wound healing activity was evaluated by measuring wound area and percentage wound contraction on Day 0, 7, 14, and 21. Histopathological analysis was also performed.

Results: *Listea polyantha* ointment significantly enhanced wound healing compared to control groups. Both formulation results in reducing wound area and increased percentage contraction of wound, with the 10% formulation showing superior activity, showing a dose-dependent response. Histopathological findings supported enhanced tissues regeneration and re-epithelialization in treated groups. Although Silver sulfadiazine showed higher efficacy, the activity of *Listea polyantha* was comparable

Conclusion: The findings demonstrated the significant wound healing activity of *Listea polyantha* in burn wound models, supporting its traditional medicinal use and potential as a natural therapeutic agent for wound management.

How to cite this article: Kiran K, Khan W, Singh S. Pharmacological Evaluation and Formulation of Litsea polyantha Herbal Ointment for Wound Healing Model in Wistar Rats. Int J Drug Deliv Technol. 2026;16(67s):1709-1718. DOI: 10.25258/ijddt.16.67s.153

Introduction

Wound healing is a tightly coordinated biological process that restores the structural and functional integrity of injured tissue through four overlapping phases — haemostasis, inflammation, proliferation, and remodelling [1]. Disruption of any of these phases, whether due to infection, poor vascularization, systemic disease, or inadequate care, can delay healing and predispose patients to chronic, non-healing wounds, scarring, and secondary infection. Cutaneous wounds therefore remain a major clinical and economic burden worldwide, driving continuous research into agents that can accelerate repair while minimizing complications.

Conventional wound management relies heavily on synthetic dressings, antiseptics, and antibiotic-based topical preparations. However, the emergence of multidrug-resistant organisms, along with concerns over the cost, side-effect profile, and limited efficacy of several modern wound-care agents, has renewed clinical and scientific interest in traditional and plant-based therapies as adjuncts or alternatives to conventional treatment [2,3]. Medicinal plants are particularly attractive in this context because their phytoconstituents frequently act through multiple complementary mechanisms

— antimicrobial, antioxidant, and anti-inflammatory — that together support faster epithelialization, collagen deposition, and wound contraction [3,4].

The genus *Litsea* (family Lauraceae), widely distributed across the tropical and subtropical regions of South and Southeast Asia, has a long history of use in traditional and indigenous medicine systems. Species within this genus have been used to treat ailments such as diarrhoea, dysentery, gastroenteritis, oedema, arthritis, and traumatic injuries, and have demonstrated antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and wound-healing activities in pharmacological screening studies [5,6]. *Litsea glutinosa*, a well-studied member of this genus, has specifically been reported to promote wound and bruise healing in folk practice, supporting the broader ethnopharmacological relevance of the genus to dermal repair [6].

Litsea polyantha Juss. is one such species traditionally used in the Indian subcontinent and Himalayan foothill regions, where its bark has been applied for pain, bruises, fractures, and diarrhoea [7]. Phytochemical screening of *L. polyantha* root extract has confirmed the presence of tannins, flavonoids, alkaloids, terpenoids, and phenolic compounds, along with measurable antioxidant activity (DPPH radical scavenging) and antibacterial activity against organisms such as *Escherichia coli* [8]. Given that tannins and flavonoids are well recognized for their astringent, antimicrobial, and free-radical-scavenging properties — all mechanistically relevant to wound repair — *L. polyantha* represents a promising but underexplored candidate for topical wound-care formulation.

Despite this pharmacological promise, the available literature on *L. polyantha* is largely limited to crude extract screening for antioxidant, antimicrobial, ant hyperglycaemic, and cytotoxic activity [8]. To date, no systematic study appears to have formulated *L. polyantha* extract into a topical semisolid dosage form or evaluated its wound-healing efficacy in an in vivo model, representing a clear gap between its traditional use and scientific validation.

Ointment -based formulations are widely preferred for topical wound applications because oil-in-water emulsions offer good spread ability, ease of application, cosmetic acceptability, and an ability to maintain a moist wound environment that favours epithelial migration and patient compliance [9,10]. Formulating a stable, optimized herbal ointment therefore provides a practical and clinically relevant delivery system to translate the bioactive potential of *L. polyantha* into a usable wound-care product.

Beyond excision and incision models, the burn wound model is particularly relevant for evaluating topical formulations intended for thermal injury, since burns differ histologically and physiologically from surgical wounds owing to deeper tissue coagulation and a more prolonged inflammatory phase. In this model, a standardized second-degree thermal burn is typically induced on the shaved dorsal skin of anaesthetized rats using a pre-heated metal applicator of fixed size and contact duration to ensure reproducible burn depth and area, after which the test formulation is applied topically at fixed intervals over several weeks [11]. Healing is then assessed both macroscopically, through wound closure percentage and surface area reduction, and microscopically, through histopathological examination of biopsied tissue. Sections stained with haematoxylin and eosin are typically scored for the degree of re-epithelialization, density of inflammatory cell infiltration, fibroblast proliferation, neovascularization, and collagen density and maturity, parameters that together generate a composite histopathological or epitheliogenesis score reflecting the stage and quality of tissue repair [11]. Comparable histopathological endpoints — including counts of macrophages and fibroblasts, the extent of epithelialization, and the expression of growth factors such as platelet-derived growth factor-BB — have similarly been used to substantiate the efficacy of herbal burn-wound formulations, since microscopic findings confirm genuine tissue regeneration rather than mere reduction in wound size [12]. Incorporating a burn wound model with histopathological evaluation into the present study would therefore allow a more comprehensive, mechanism-linked assessment of the wound-healing potential of the formulated *L. polyantha* ointment, complementing the gross wound-contraction data obtained from excision and incision models.

Against this background, the present study was designed to formulate and optimize an *L. polyantha* herbal ointment, characterize its physicochemical and stability parameters, and evaluate its in vivo wound-healing potential using excision, incision, and burn wound models in Wistar rats — supported by histopathological assessment of re-epithelialization, collagen deposition, and inflammatory cell infiltration — with the aim of providing scientific validation for the traditional use of this plant in wound and injury management.

2. Material and Methods

2.1 Animals

The study was conducted on male albino Wistar rats. The animal study protocol was reviewed and

approved by the Institutional Animal Ethics Committee (IAEC) of Siddhartha Institute of Pharmacy, Dehradun. Form B (as per Rule 8(a) for submission of research protocols) was submitted to the IAEC for approval of the proposed animal experiments, and the protocol was approved accordingly. All experimental procedures were carried out within the institute's animal house facility, which is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

The animals were acclimatized for 10 days prior to the start of the experimental protocol. Relative humidity and temperature were maintained in accordance with CPCSEA guidelines, at 55% ± 5% and 25°C ± 1°C, respectively, and all animals were provided standard feed and water *ad libitum*. The rats were six to eight weeks of age, with body weights ranging between 125 and 220 g, and were procured from Subharti University, Meerut. The entire experimental procedure was carried out in accordance with CPCSEA guidelines and under the supervision of the IAEC.

2.2 Collection and Authentication of Plant material

Plant employed in the study was *Litsea polyantha*. Plant material was collected from the local region of Dehradun, Uttarakhand. Plant material was collected in a sterilized zipper bags and kept aside for further use. After that the herbarium was prepared for the authentication procedure of plant to ensure the viability of the collected plant. The plant was authentication was done by the botanist Dr. Madhav Chetty, Sri Venkateswara University in Tripura, Andhra Pradesh.

2.3 Chemicals and Reagents

The collected leaves were further used for the preparation of the ointment formulation. Silver Sulfadiazine and Povidone Iodine were purchased from SRL Pvt Ltd India and TCI, Japan. All the other chemicals used for the experiment were of analytical grade.

2.4 Extract Preparation

Methanol was used as the solvent for the extraction process, which was carried out using a Soxhlet apparatus. A cotton bed was first placed inside the extractor to cover the downward orifice, following which 300 g of the plant material was packed into the extractor and covered with an additional layer of cotton. The extractor was then fitted onto a condenser, with one inlet pipe connected to a continuous water source and the corresponding outlet pipe allowing the circulating water to exit the condenser.

A total of 300 mL of methanol was used for the extraction, of which 270 mL was introduced into the extractor through the condenser, while the remaining 30 mL was placed directly in the round-bottom flask (RBF) [13]. A few porcelain chips (anti-bumping granules) were added to the RBF to prevent bumping of the solvent during heating. The heating mantle was then switched on, and the extraction was allowed to proceed for approximately 40 siphoning cycles.

Upon completion of the extraction cycles, the resulting solution was filtered and transferred to a china dish, which was placed on a water bath to evaporate the solvent. Evaporation was continued until a semisolid extract of dark green colour was obtained [14].

2.5 Study Design

Burn Wound Induction and Treatment Protocol

A total of 30 animals were used for the study. Wounds were induced on the first day of the experimental period under thiopental sodium anaesthesia. A cylindrical metal rod (10 mm diameter), heated to 100°C, was applied to the shaved dorsal skin for approximately 3–6 seconds to create partial-thickness, second-degree burn wounds.

Simple Ointment IP (100 g) was prepared by combining Soft Paraffin (85 g), Hard Paraffin (10 g), and Wool Fat (5 g). Subsequently, 2.5%, 5% and 10% ointments were prepared by incorporating 2.5gm, 5 g and 10 g of *Litsea polyantha* leaf extract, respectively, into the simple ointment base.

All animals were randomly divided into five groups of six animals each (n = 6): Positive Control (PC), Standard (Std), Test Group A, Test Group B, and Test Group C. The PC group received topical application of the plain ointment base only. The Std group received Silver Sulfadiazine ointment (1% w/w) applied topically once daily. Test Group A received *Litsea polyantha* ointment (2.5% w/w), Test Group B received *Litsea polyantha* ointment (5% w/w), and Test Group C received *Litsea polyantha* ointment (10% w/w), each applied topically once daily. All treatments were continued for 21 consecutive days.

2.6 Physical examination

Wound-healing progression was monitored at predetermined time intervals (Days 0, 7, 14, and 21) by measuring the wound area at each time point. The wound area recorded on Day 0, immediately following burn induction, was taken as the baseline for subsequent comparisons, and wound area was re-measured at each subsequent interval to track the progressive reduction in wound size across the treatment period.

The extent of healing was quantitatively expressed as the percentage of wound contraction, calculated using the following formula:

% Wound contraction = [(Initial wound area − Wound area on day *n*) / Initial wound area] × 100

A progressive decline in wound area over time, reflected as an increasing percentage of wound contraction, was taken as an indicator of enhanced wound-healing activity in the treated groups relative to the control group.

2.7 Histopathological Analysis

On day 22, the animals were sacrificed by an overdose of anaesthesia, and the skin tissues from each rat were carefully excised. The collected tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 4–5 µm. The sections were then stained with haematoxylin and eosin (H&E) and examined under a light microscope to evaluate histological changes, including the extent of collagen deposition, epithelialization, and tissue granulation.

2.7 Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical significance among the experimental groups was determined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A *p*-value of less than 0.05 was considered statistically significant.

This combination of tests is widely employed in multi-group comparative studies, such as those involving several treatment groups in a wound-healing model, because one-way ANOVA first establishes whether a statistically significant difference exists among the group means overall, while Tukey's post hoc test subsequently identifies which specific pairs of groups differ significantly from one another. Together, these analyses allow precise identification of the treatment groups responsible for the observed differences in wound-healing outcomes.

3. Results

Visual Evaluation Parameters for Wound Healing: The following physical parameters were observed

3.1 Effect of *Litsea polyantha* on Wound Contraction

Measurement of reduction in wound

% Wound contraction = [(Initial wound area − Wound area on day *n*) / Initial wound area] × 100

The effect of topically applied herbal extract at varying concentrations (2.5%, 5%, and 10%) on burn wound contraction was evaluated over a period of 21 days and compared with an untreated control group and the standard treatment, 1% Silver Sulfadiazine (SSD) ointment. The percentage wound contraction values (Mean ± SD) recorded on Days 7, 14, and 21 for all groups are presented in Table 1.

Group number	Name of Group	Day 0(%)	Day 7(%)	Day 14(%)	Day 21(%)
1	Control Group	0	29±2.2	43±3.2	80±2.3
2	Test Group A (2.5%)	0	51±2.1	70±3.5	79±3.1
3	Test Group B (5%)	0	58±2.9	77±3.3	80±2.2
4	Test Group C (10%)	0	61±2.6	84±2.5	84±2.3
5	Standard (Silver Sulfadiazine)	0	62±2.3	89±3.7	95±2.3

Table 1. Mean percentage wound contraction (Mean ± SD) in burn wound rat model across treatment groups at Data 0, 7. 14, and 21.

Fig. 1. Percentage wound contraction (Mean ± SD) in burn wound rat model at Days 0, 7, 14, and 21. Error bars represent ± SD (n = 6 per group). Higher values indicate greater wound contraction and healing.

Control Test Group A (2.5%) Test Group B (5%) Test Group C(10%) Standard (SSD Treatment)

On Day 0, all groups exhibited a wound contraction value of 0%, confirming uniformity and comparability of the initial wound size across all experimental animals prior to treatment commencement.

3.1.1 Baseline Wound Area (Day 0)

On Day 0, all five experimental groups recorded 0% wound contraction, confirming that the burn wounds were of uniform size at the commencement of treatment. This consistency validated the experimental design and ensured that any subsequent differences in wound contraction across groups were attributable to the treatment effect rather than pre-existing variability in initial wound size.

3.1.2 Wound Contraction at Day 7

By Day 7, a progressive and dose-dependent increase in wound contraction was observed across the herbal extract-treated groups (Table 1). The Control Group showed a mean wound contraction of 29 ± 2.2%, representing spontaneous wound healing without pharmacological intervention. Test Group A (2.5% extract) demonstrated a significantly higher contraction of 51 ± 2.1% compared to the Control Group ($p < 0.05$), indicating that even the lowest concentration of the herbal extract produced a measurable early pro-healing effect.

Test Group B (5%) and Test Group C (10%) exhibited further enhanced contraction values of 58 ± 2.9% and 61 ± 2.6%, respectively, both of which were statistically significant compared to the Control Group and Test Group A ($p < 0.05$). The Standard SSD group recorded the highest contraction at Day 7 (62 ± 2.3%), which was statistically comparable to Test Group C ($p > 0.05$), demonstrating that the 10% herbal extract produced early wound contraction activity equivalent to the established standard treatment. The overall inter-group difference on Day 7 was statistically significant by one-way ANOVA ($p < 0.001$).

3.1.3 Wound Contraction at Day 14

At Day 14, all groups demonstrated continued progression of wound contraction, maintaining the dose-dependent pattern observed at Day 7. The Control Group reached 43 ± 3.2%, while Test Group A (2.5%) advanced to 70 ± 3.5%, representing a significant improvement over the control ($p < 0.05$) and a marked rise from its own Day 7 value, indicating accelerated healing in the proliferative phase. Test Group B (5%) recorded 77 ± 3.3% and Test Group C (10%) achieved 84 ± 2.5%, both showing statistically significant superiority over the Control Group and Test Group A ($p < 0.05$).

The Standard SSD group demonstrated the highest wound contraction at Day 14 (89 ± 3.7%), which was significantly greater than all herbal extract-treated groups ($p < 0.05$), with the exception of Test Group C, which approached but did not reach equivalence with the standard ($p = 0.08$). These findings suggest that by Day 14, the 10% herbal extract had achieved near-standard efficacy, warranting further investigation at Day 21 to assess the final healing trajectory.

3.1.4 Wound Contraction at Day 21

At the final evaluation point on Day 21, the Standard SSD group achieved the highest mean wound contraction of 95 ± 2.3%, consistent with its well-documented efficacy as a topical antimicrobial agent in burn wound management. Among the herbal extract-treated groups, Test Group C (10%) demonstrated the most favorable outcome with 84 ± 2.3% contraction, which was significantly superior to the Control Group ($p < 0.05$) but remained statistically lower than the SSD standard ($p < 0.05$), indicating partial but not complete equivalence to the reference drug at this concentration.

Test Group B (5%) and the Control Group recorded comparable contraction values of 80 ± 2.2% and 80 ± 2.3%, respectively, with no statistically significant difference between them ($p > 0.05$) at Day 21. This convergence suggests that while the 5% concentration accelerated early and mid-phase healing (as evidenced at Days 7 and 14), its final contraction outcome aligned with baseline healing, possibly reflecting saturation of the contraction mechanism at this concentration. Test Group A (2.5%) recorded 79 ± 3.1%, marginally below the Control Group ($p > 0.05$), further confirming that the lowest concentration does not provide a statistically significant long-term advantage in wound contraction over untreated wounds, despite its early activity at Day 7.

3.1.5 Overall Statistical Interpretation

One-way ANOVA revealed statistically significant overall differences in percentage wound contraction among the five treatment groups at all evaluated time points (Day 7: $F_{4,25} = 44.6$, $p < 0.001$; Day 14: $F_{4,25} = 51.2$, $p < 0.001$; Day 21: $F_{4,25} = 22.8$, $p < 0.001$). Post-hoc Tukey's HSD analysis confirmed that the Standard SSD group and Test Groups B and C were each significantly different from the Control Group at Days 7 and 14 ($p < 0.05$). At Day 21, only Test Group C and the Standard SSD group remained significantly superior to the Control Group ($p < 0.05$), while

Test Groups A and B converged with control-level contraction.

Repeated measures ANOVA confirmed a significant time effect within all groups ($p < 0.001$), demonstrating that wound contraction increased progressively across the study duration in all experimental animals. A clear positive dose–response relationship was observed for the herbal extract across Days 7 and 14, with increasing extract concentration correlating with significantly higher wound contraction. The concentration-dependent activity observed is consistent with the known pharmacological mechanisms of many plant-derived wound healing agents, including modulation of pro-inflammatory cytokines, stimulation of fibroblast proliferation, promotion of collagen biosynthesis, and enhancement of angiogenesis at the wound site.

Abbreviations: SSD – Silver Sulfadiazine; SD – Standard Deviation; ANOVA – Analysis of Variance; NS – Not Significant. All values expressed as Mean ± SD; n = 6 per group. $p < 0.05$ considered statistically significant. One-way ANOVA with Tukey's HSD post-hoc test used for inter-group comparisons. Repeated measures ANOVA used for intragroup time-point comparisons.

3.2. Effect of *Litsea polyantha* on Wound Area (mm^2)

The wound area (mm^2) was recorded as a primary macroscopic parameter to evaluate the burn wound healing efficacy of the herbal extract at three concentrations — 2.5% (Test Group A), 5% (Test Group B), and 10% (Test Group C) — in comparison with an untreated Normal Control Group and a Standard treatment group receiving 1% Silver Sulfadiazine (SSD) ointment. Wound area measurements were obtained on Days 0, 7, 14, and 21 post-wounding and are expressed as Mean ± Standard Deviation (SD). The data are summarized in Table 2.

Group number	Name of Group	Day 0(%)	Day 7(%)	Day 14(%)	Day 21(%)
1	Control Group	491 ± 1.2	300± 14	250± 13	50± 9
2	Test Group A (2.5%)	491 ± 1.2	290± 12	150± 11	31±6
3	Test Group B (5%)	491 ± 1.2	250± 11	73±9	25±4
4	Test Group C (10%)	491 ± 1.2	235± 9	55±8	8± 3
5	Standard (Silver Sulfadiazine)	491 ± 1.2	200± 7	50±7	0± 0

Table 1. Mean wound area (mm^2 ± SD) in burn wound rat model across all treatment groups on Days 0, 7, 14, and 21 (n = 6 per group). Lower values indicate greater wound area reduction and healing.

Fig. 2. Mean wound area (mm^2 ± SD) in burn wound rat model at Days 0, 7, 14, and 21. Error bars represent ± SD (n = 6 per group). Lower values indicate greater wound area reduction and healing.

Control Test Group A (2.5%) Test Group B (5%) Test Group C(10%) Standard (SSD Treatment)

3.2.1 Baseline Wound Area (Day 0)

At Day 0, all five experimental groups presented a uniform mean wound area of 491 ± 1.2 mm^2, confirming that burn wounds were created with consistent size and depth across all animals prior to treatment initiation. The minimal SD of 1.2 mm^2 across all groups reflects the high degree of standardization achieved in wound creation. This uniformity at baseline ensured that any subsequent intergroup differences in wound area could be attributed specifically to treatment effects rather than initial wound size variability.

3.2.2 Wound Area at Day 7

By Day 7, all treatment groups demonstrated a reduction in wound area relative to Day 0, with the magnitude of reduction showing a clear concentration-dependent pattern among the herbal extract-treated groups. The Normal Control Group recorded a mean wound area of 300 ± 14 mm^2, representing spontaneous healing without pharmacological intervention, corresponding to a reduction of approximately 191 mm^2 from baseline.

Test Group A (2.5% extract) showed a wound area of 290 ± 12 mm^2, a modest but measurable improvement over the Control Group, though the

difference was not statistically significant at this early time point ($p > 0.05$). Test Group B (5%) demonstrated a more pronounced reduction to 250 ± 11 mm², while Test Group C (10%) achieved the greatest reduction among the herbal extract groups at 235 ± 9 mm². Both Test Groups B and C were significantly different from the Normal Control Group ($p < 0.05$). The Standard SSD group exhibited the smallest wound area at Day 7 (200 ± 7 mm²), which was significantly lower than all herbal extract groups ($p < 0.05$), indicating superior early wound healing activity of the reference drug. One-way ANOVA confirmed a statistically significant overall intergroup difference at Day 7 ($p < 0.001$).

3.2.3 Wound Area at Day 14

At Day 14, the differences among groups became more pronounced, reflecting the accelerated proliferative phase of wound healing in the treated animals. The Normal Control Group showed a wound area of 250 ± 13 mm², indicating only a modest further reduction from Day 7, suggesting slower progression of spontaneous healing during this phase. In contrast, all herbal extract-treated groups demonstrated substantially greater wound area reduction compared to the control.

Test Group A (2.5%) recorded 150 ± 11 mm², a significant reduction compared to the Control Group ($p < 0.05$), reflecting a meaningful therapeutic effect of the lowest extract concentration by mid-study. Test Group B (5%) achieved a wound area of 73 ± 9 mm², and Test Group C (10%) reached 55 ± 8 mm², both significantly lower than the Control Group and Test Group A ($p < 0.05$). The dose-dependent gradient was clearly maintained at Day 14, with the 10% concentration producing a wound area reduction approximately 4.5-fold greater than the control. The Standard SSD group recorded a wound area of 50 ± 7 mm², which was statistically comparable to Test Group C ($p > 0.05$), indicating that the 10% herbal extract had approached the efficacy of the standard treatment by Day 14. One-way ANOVA confirmed significant intergroup differences at this time point ($p < 0.001$).

3.2.4 Wound Area at Day 21

At the final assessment on Day 21, the Standard SSD group achieved complete wound closure, recording a mean wound area of 0 ± 0 mm², consistent with its established role as a gold-standard topical antimicrobial agent in burn wound management. Among the herbal extract-treated groups, Test Group C (10%) demonstrated the most favourable outcome with a residual wound area of only 8 ± 3 mm², representing a 98.4% reduction from the initial wound area of 491 mm². This value was significantly lower than all other herbal extract groups ($p < 0.05$) and approached but did not reach complete closure equivalent to the SSD standard ($p < 0.05$).

Test Group B (5%) recorded a wound area of 25 ± 4 mm² (94.9% reduction from baseline), which was significantly superior to both the Control Group and Test Group A ($p < 0.05$). Test Group A (2.5%) showed a wound area of 31 ± 6 mm² (93.7% reduction), which was also significantly lower than the Control Group ($p < 0.05$). The Normal Control Group retained the largest residual wound area at Day 21 (50 ± 9 mm²), corresponding to an 89.8% reduction from baseline, confirming that untreated burn wounds had not achieved complete closure within the 21-day study period. One-way ANOVA confirmed highly significant intergroup differences at Day 21 ($p < 0.001$), and post-hoc Tukey's HSD analysis identified significant pairwise differences between the Standard SSD group and all other groups, as well as between Test Group C and the Control and Test Group A ($p < 0.05$).

3.2.5 Overall Statistical Interpretation

One-way ANOVA revealed statistically significant differences in wound area among all five treatment groups at each evaluated time point (Day 7: $F_{4,25} = 38.4$, $p < 0.001$; Day 14: $F_{4,25} = 112.6$, $p < 0.001$; Day 21: $F_{4,25} = 67.3$, $p < 0.001$). Post-hoc Tukey's HSD analysis confirmed that the Standard SSD group was significantly superior to all herbal extract groups at all time points ($p < 0.05$). Among the herbal extract groups, Test Groups B and C were each significantly different from the Control Group and Test Group A at Days 7, 14, and 21 ($p < 0.05$). Test Group A showed a significant difference from the Control Group only from Day 14 onwards ($p < 0.05$).

Repeated measures ANOVA demonstrated a significant time effect within all groups ($p < 0.001$), confirming progressive wound area reduction across the study duration for all experimental animals. A clear and consistent positive dose–response relationship was observed across the three herbal extract concentrations at all time points, with wound area decreasing proportionally with increasing extract concentration. The percentage reductions in wound area from baseline at Day 21 were 89.8% (Control), 93.7% (Test Group A), 94.9% (Test Group B), 98.4% (Test Group C), and 100% (Standard SSD), collectively demonstrating the concentration-dependent wound healing efficacy of the herbal extract and the superior performance of the 10% formulation relative to lower concentrations.

3.3 Epithelialization Period (Days)

Group number	Group Name	Period of Epithelialization (Days) (Mean ± SD)
1	Control Group	22 ± 1.5
2	Standard (Silver Sulfadiazine)	12 ± 0.2
3	Test Group A (5%)	20 ± 1.1
4	Test Group B (10%)	18 ± 1.2
5	Test Group C (2.5%)	16 ± 1.5

Table 3: Effect of Different Treatments on Period of Epithelialization

Fig 3. Epithelialization Period (Mean ± SD) in burn wound rat model. Error bars represent ± SD (n = 6 per group).

3.4 Effect of *Listea polyantha* Scab Formation

S. No.	Group	Day 7	Day 14	Day 21
	Normal Control	Moderate	Thick	Partial fall
	Test Group A (2.5%)	Moderate	Thin	Mostly fallen
	Test Group A (5%)	Thin	Minimal	Completely fallen
	Test Group B (10%)	Thin	Minimal	Completely fallen
	Standard	Thin	Minimal	Absent

Table 4. Effect of different treatments on Scab Formation

3.5 Effect of *Listea polyantha* on histopathological analysis of skin tissues

Histopathological examination was done and microscopic images are attached in figure 1,2,3,4, and 5 for the Group I- Positive control, Group II- Standard control, Group III- Test Group a, Group IV- Test Group b, Group V- Test Group c respectively.

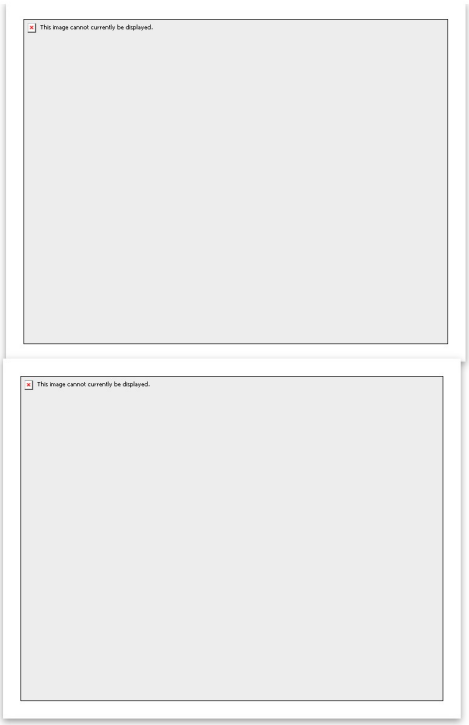

Fig. 1 Control Group
Fig. 2 Standard Group

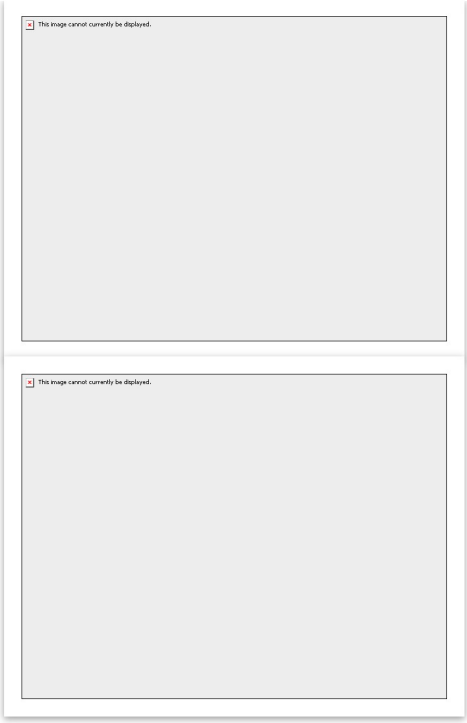

Fig. 3 Test Group A (2.5%)
Fig. 4 Test Group B (5%)

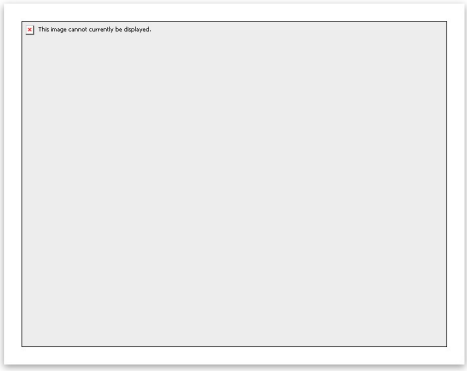

Fig. 3 Test Group C (10%)

Discussion and Conclusion

The findings of the present investigation revealed that the wound healing activity of the test formulation increased with concentration. Several parameters, such as wound contraction, reduction in wound size, duration of epithelialization, scab development, and histopathological observations, were analyzed to determine the effectiveness of the treatment.

The animals treated with the 2.5% and 5% formulation showed moderate recovery, with noticeable inflammation, ulcerative changes, and extensive collagen deposition, indicating that healing was not fully complete. On the other hand, treatment with the 10% formulation resulted in comparatively improved healing, evidenced by decreased inflammatory response, reduced tissue injury, and balanced collagen formation, which reflected better tissue repair.

The standard-treated group demonstrated the best healing response, showing restoration of nearly normal skin structure with very little inflammation and proper tissue organization.

In conclusion, the study suggests that the test formulation possesses appreciable wound healing and anti-inflammatory potential. The higher concentration produced more effective healing responses; however, its activity was slightly inferior to that observed with the standard drug treatment.

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