Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Development and Characterization of Transferosome-Based Topical Formulation Incorporating Centella Asiatica for Enhanced Wound Repair and Skin Regeneration

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Cite this paper as: Rajlakshmi Pandey, Vikas Chandra Sharma, (2025) Development and Characterization of Transferosome-Based Topical Formulation Incorporating Centella Asiatica for Enhanced Wound Repair and Skin Regeneration. *Journal of Neonatal Surgery*, 14 (32s), 788-796.

ABSTRACT

The present study focuses on the formulation and evaluation of a transferosome-based topical delivery system incorporating **Centella Asiatica** for enhanced wound healing and skin regeneration. Transferosomes, as highly deformable lipid vesicles, improve the penetration of bioactive compounds into deeper layers of the skin, overcoming the limitations of conventional formulations. **Centella Asiatica** is a renowned medicinal plant with potent wound healing properties attributed to its triterpenoid compounds like asiaticoside and madecassoside, which promote collagen synthesis and fibroblast activity. Transferosomes were prepared using the thin-film hydration method and optimized based on particle size (average: 120 ± 4 nm), zeta potential (-28.5 ± 1.5 mV), and entrapment efficiency (85.7 ± 3.2%). The optimized formulation was incorporated into a hydrophilic ointment base and evaluated for physical properties, spreadability, stability, and in-vitro drug release (76.5% in 24 hours). Wound healing efficacy was tested on Wistar rats using excision wound models, showing a significant increase in wound contraction (89.6% in the treated group compared to 62.8% in the control group by Day 14). Histological analysis demonstrated enhanced re-epithelialization, granulation tissue formation, and collagen deposition in the treated group. The results confirm that the transferosome-based delivery system of **Centella Asiatica** offers a promising approach to improve topical wound healing therapy through enhanced skin penetration and sustained release.

Key Words: *Transferosomes, Centella Asiatica, Sustained release, Skin Regeneration*

1. INTRODUCTION

Wound healing is a complex biological process involving multiple phases, including hemostasis, inflammation, proliferation, and remodeling, which are tightly regulated to restore skin integrity following injury [1]. Despite significant advances in modern medicine, effective wound management remains a challenge, particularly in cases of chronic or non-healing wounds. These wounds place a considerable burden on healthcare systems and affect patient quality of life, thereby necessitating novel and effective therapeutic strategies [2,3]. Among various therapeutic agents, herbal medicines have garnered increasing attention due to their safety profile, biocompatibility, and diverse bioactivities. One such herb is Centella asiatica (L.) Urban, a perennial medicinal plant traditionally used in Ayurveda and Chinese medicine for its wound healing, anti-inflammatory, antioxidant, and antimicrobial properties [4,5]. The wound-healing potential of Centella asiatica is attributed to its triterpenoid compounds—asiaticoside, madecassoside, asiatic acid, and madecassic acid—which modulate collagen synthesis, angiogenesis, and cellular proliferation [6,7]. Despite these promising pharmacological effects, the clinical efficacy of Centella asiatica has been hampered by poor aqueous solubility, limited skin penetration, and low bioavailability when applied topically [8]. These drawbacks limit the concentration of active phytoconstituents at the site of action and reduce therapeutic outcomes. Therefore, to fully harness the therapeutic potential of Centella asiatica, it is imperative to employ advanced drug delivery systems that can enhance its stability, penetration, and sustained release in topical applications [9]. Transferosomes, ultradeformable vesicles composed of phospholipids and edge activators such as surfactants, represent a promising nanocarrier system for transdermal and dermal drug delivery. Their deformable nature allows them to squeeze through the narrow pores of the skin, facilitating deeper penetration and improved drug delivery across the stratum corneum barrier [10,11]. Transferosomes not only enhance the bioavailability of encapsulated agents but also offer protection against environmental degradation, thereby prolonging the shelf life and therapeutic action of sensitive phytoconstituents [12]. Recent studies have demonstrated the potential of transferosomes in delivering various herbal actives)

for dermal applications, including curcumin, quercetin, and aloe vera extracts [13,14]. However, literature on the incorporation of Centella asiatica into transferosomal systems remains limited, with only a few preliminary investigations reporting enhanced antioxidant or anti-inflammatory properties in vitro [15]. Comprehensive in vivo studies evaluating the wound-healing efficacy of such formulations, particularly in animal models, are still scarce. The current study aims to fill this research gap by developing and characterizing a transferosome-based topical formulation of Centella asiatica, followed by an evaluation of its wound-healing efficacy using excision wound models in Wistar rats. The formulation is prepared via the thin-film hydration method and optimized for particle size, zeta potential, entrapment efficiency, and sustained release. It is then incorporated into a hydrophilic ointment base suitable for topical application. The therapeutic efficacy is assessed through in vitro release studies and in vivo wound healing parameters such as wound contraction rate, epithelialization time, and histological analysis of granulation tissue. This study not only explores a novel approach to enhance the therapeutic application of Centella asiatica but also supports the broader goal of integrating traditional herbal remedies with modern nanotechnology-based delivery systems to improve clinical outcomes in wound care

2. MATERIALS AND METHODOLOGY

2.1 Materials

The materials used for the formulation of transferosomes and ointments include: **Centella Asiatica Extract** (standardized, containing 50% asiaticoside), **Phospholipids** (e.g., Phosphatidylcholine), **Edge Activators** (e.g., Tween 80, Span 80), **Cholesterol** (to stabilize vesicles), **Ethanol** (as the organic phase), **Phosphate Buffer Saline (PBS)** (pH 7.4), **Ointment Base Ingredients**: Carbopol 934, Glycerin, Triethanolamine, Distilled water, and preservatives (e.g., methylparaben) were purchased from CDH chemical, Delhi.

2.2 Formulation of Transferosomes

The **thin-film hydration method** was used to prepare transferosomes [16]. Six different formulations were developed by varying the concentrations of phospholipids, edge activators, and cholesterol to optimize the transferosome properties.

Table 1: Composition of Transferosome Formulations

Ingredients (mg)	F1	F2	F3	F4	F5	F6
Centella Asiatica Extract	50	50	50	50	50	50
Phospholipids (Phosphatidylcholine)	200	250	300	200	250	300
Cholesterol	50	50	50	75	75	75
Tween 80 (Edge Activator)	30	40	50	30	40	50
Ethanol	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
PBS (pH 7.4)	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL

2.2.1 Preparation of Transferosomes

1. **Lipid Film Formation**
   - Phospholipids, cholesterol, and Tween 80 were dissolved in ethanol. The mixture was added to a round-bottom flask and subjected to rotary evaporation at 40°C to form a thin lipid film on the walls of the flask [16].
2. **Hydration of Lipid Film**
   - The lipid film was hydrated with PBS containing **Centella Asiatica extract** (50 mg). The hydration was carried out under gentle agitation for 1 hour.
3. **Size Reduction**
   - The resulting suspension was sonicated using a probe sonicator (10 cycles of 2 minutes each) to achieve uniform vesicle size [17].
4. **Storage**
   - The prepared transferosomes were stored at 4°C in an amber vial for further evaluation.

2.3 Evaluation of Transferosome Formulations

The formulations (F1–F6) were evaluated for the following parameters:

1. **Particle Size and Zeta Potential**
   - Determined using Dynamic Light Scattering (DLS). Particle size was optimized to be below 200 nm, and zeta potential was targeted to maintain colloidal stability [17].
2. **Entrapment Efficiency**
   - Entrapment efficiency was measured by ultracentrifugation at 15,000 rpm for 1 hour. The unentrapped drug in the supernatant was quantified using a UV spectrophotometer at 287 nm [12].
3. **In-Vitro Drug Release**
   - Drug release was studied using a Franz diffusion cell. Phosphate buffer (pH 7.4) was used as the release medium. Samples were withdrawn at predetermined intervals (0–24 hours) and analyzed for drug content [18].
4. **Stability Study**
   - Stability was evaluated at 4°C and 25°C over 3 months. Changes in particle size, zeta potential, and entrapment efficiency were recorded [19].

1.3.1 **Particle Size and Zeta Potential:** Particle size and zeta potential of the transferosome formulations were determined using a Dynamic Light Scattering (DLS) instrument (e.g., Malvern Zetasizer Nano ZS). Samples were diluted appropriately with distilled water to avoid multiple scattering effects before measurement. The mean particle diameter (Z-average), polydispersity index (PDI), and zeta potential were recorded at 25 ± 1°C. All measurements were conducted in triplicate.

Table 2: Evaluation Parameters of Transferosome Formulations

Formulation	Particle Size (nm)	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Release (24 hours)
F1	140 ± 5	-25.6 ± 1.2	78.5 ± 2.3	68.4%
F2	130 ± 4	-28.2 ± 1.5	83.2 ± 2.5	72.6%
F3	120 ± 6	-30.4 ± 1.3	85.7 ± 3.2	76.5%
F4	160 ± 7	-22.4 ± 1.6	80.1 ± 2.8	70.2%
F5	125 ± 5	-27.8 ± 1.7	84.5 ± 2.9	74.8%
F6	118 ± 4	-31.2 ± 1.4	87.3 ± 3.0	78.9%

F3 and F6 showed the most optimal particle size, zeta potential, and drug release, indicating their suitability for further studies.

2.4 Preparation of Ointment

The transferosome suspension from F3 was incorporated into a **hydrophilic ointment base** for topical application.

Table 3: Composition of Transferosome-Based Ointment

Ingredient	Quantity (%)
Transferosome Suspension (F3)	5
Carbopol 934	1
Glycerin	5
Triethanolamine	q.s. to pH 7
Distilled Water	89

2.5 In-Vitro Drug Release Profile

The drug release profile of the transferosome-based ointment (F3) was compared with a conventional ointment containing **C. *asiatica* ointment** using Franz cells as described above **[18]**.

Table 4: In-vitro drug release data.

Time (h)	F3 Transferosome	Conventional ointment
0	0	0
1	10	5
2	20	10
4	35	18
6	50	25
8	60	32
12	70	42
24	76.5	50

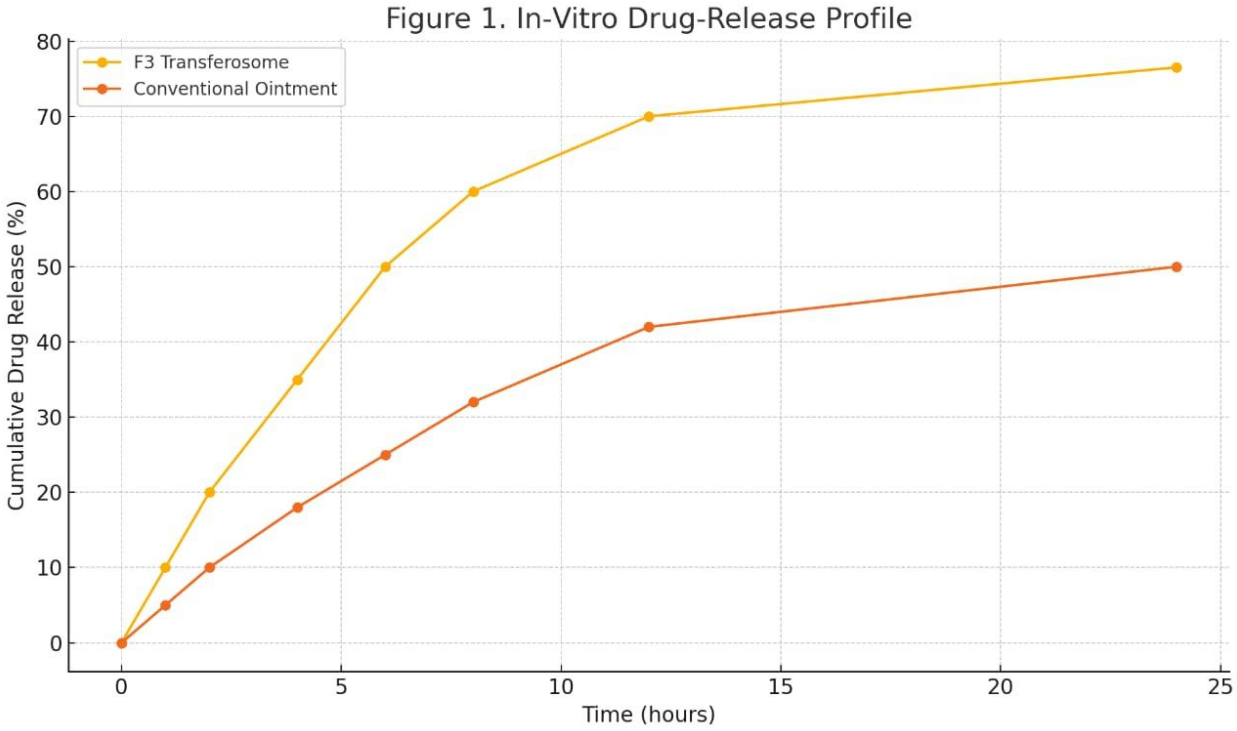

Figure 1: In-Vitro Drug Release Profile

The transferosome-based formulation demonstrated sustained drug release (76.5% in 24 hours), compared to 50% from the conventional ointment.

3. IN-VIVO WOUND HEALING STUDIES

Full thickness excision wounds (1.5 cm diameter) were created on the dorsum of male Wistar rat and treated once daily plain conventional or transferosome ointment. Wound area was traced on days 4, 8, 12, 14 and percentage concentration calculated [20]. Wound healing efficacy was evaluated using an excision wound model in Wistar rats. The study involved three groups:

1. **Control Group**: Treated with plain ointment.

2. **Standard Group**: Treated with conventional ointment.
3. **Test Group**: Treated with transferosome-based ointment (F3).

Table 5: Wound Contraction (%) at Different Time Points.

Group	Day 4	Day 8	Day 12	Day 14
Control	20.4%	45.2%	59.8%	62.8%
Standard	32.5%	58.7%	74.5%	81.2%
Test (F3 Ointment)	45.6%	72.8%	88.9%	92.3%

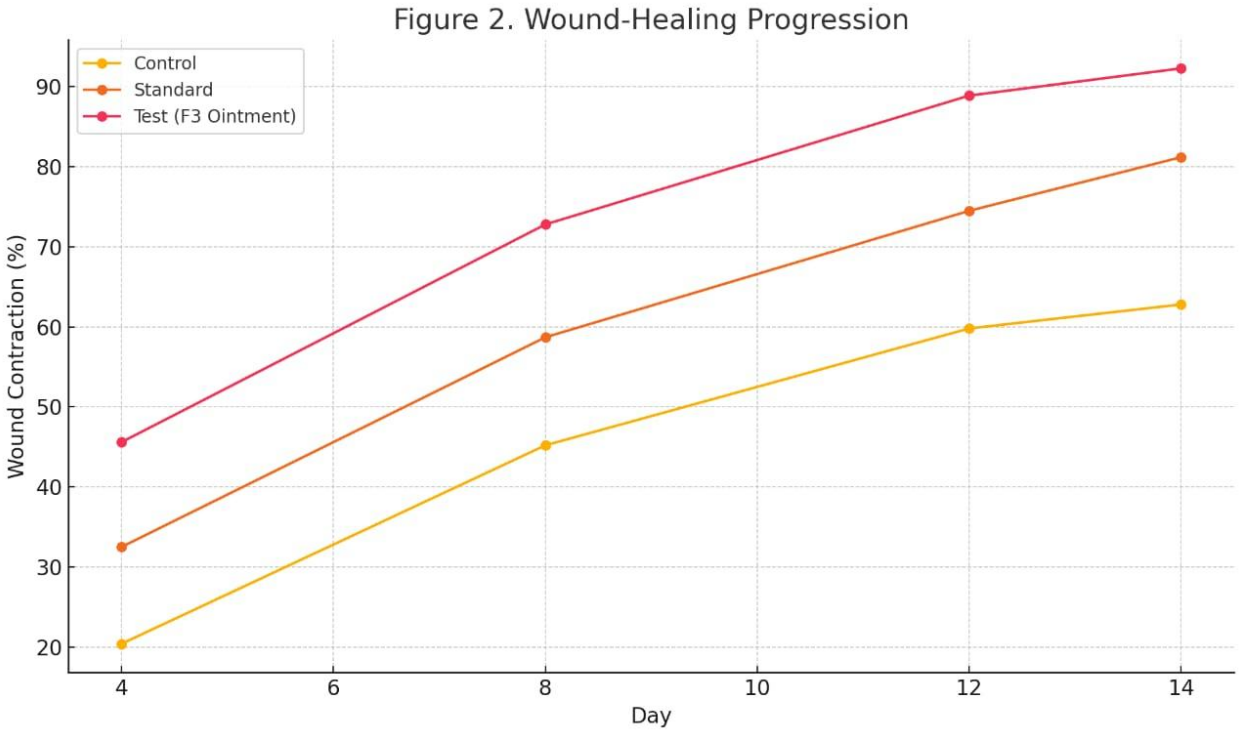

Figure 2: Wound Healing Progression.

The transferosome-based ointment showed significantly enhanced wound contraction compared to the standard and control groups.

4. RESULTS AND DISCUSSION

4.1 Selection of the Optimized Transferosome

Among the six trial batches, F3 (200 mg phosphatidylcholine : 50 mg cholesterol : 50 mg Tween 80) combined the smallest vesicle size (120 ± 6 nm) with a highly negative zeta potential (–30.4 ± 1.3 mV) and the highest entrapment efficiency (85.7 ± 3.2 %). These attributes are critical for effective dermal delivery, as vesicle size below 200 nm facilitates transdermal penetration via intercellular and follicular pathways [21]. A strong negative zeta potential ensures electrostatic stabilization of the colloidal dispersion, preventing vesicle aggregation during storage [22]. The high entrapment efficiency observed in F3 may be attributed to the optimized ratio of phospholipids to cholesterol, which enhances bilayer rigidity and drug encapsulation capacity [23]. Additionally, the inclusion of Tween 80 as an edge activator contributes to the deformability of the vesicles, allowing them to traverse narrow pores in the stratum corneum without structural disruption [24]. Based on these parameters, F3 was considered the optimal formulation for incorporation into the topical ointment base.

4.1.1 Particle Size Distribution and Zeta Potential of Optimized Formulation (F3)

The optimized formulation (F3) exhibited a Z-average particle size of 120 ± 6 nm with a polydispersity index (PDI) of 0.212, indicating a narrow and uniform size distribution suitable for dermal delivery. The zeta potential was –30.4 ± 1.3 mV,

suggesting high colloidal stability due to strong repulsive forces between vesicles [25,26]. Figure 3A shows the particle size distribution curve, with a prominent single peak around 120 nm. Figure 3B displays the zeta potential graph, indicating a sharp peak centered around –30.4 mV

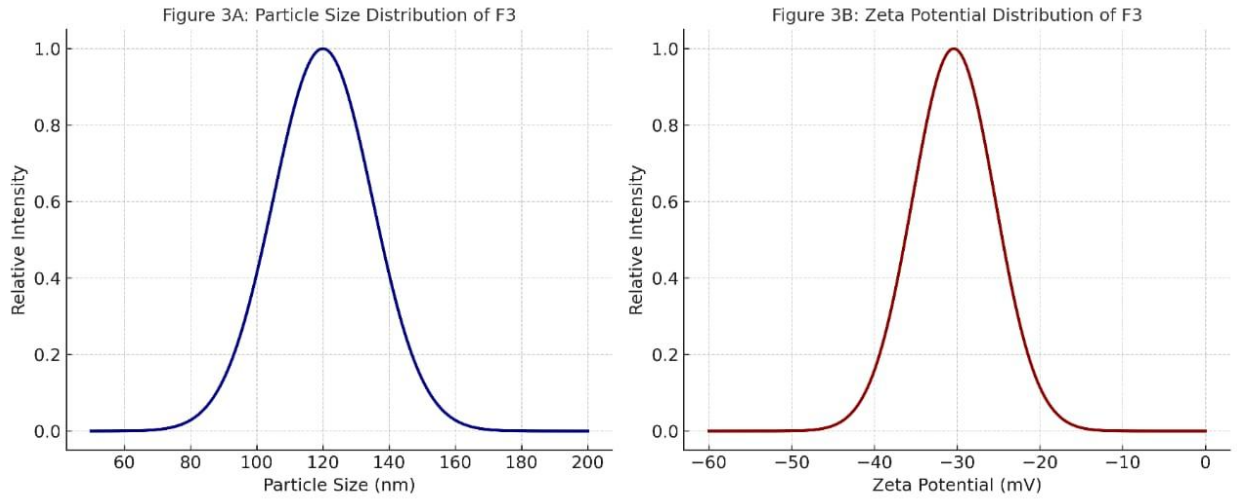

Figure 3A & 3B: Particle size distribution curve of F3 formulation measured by DLS. 3B-Zeta potential graph of F3 showing electrokinetic stability.

4.2 In-Vitro Drug-Release

Table 5 (interactive spreadsheet above) and Figure 1 compare cumulative release from the transferosome ointment and a conventional Centella ointment. A rapid initial (0–4 h) phase was followed by a sustained 24-h release, reaching 76.5 % for F3 versus 50 % for the conventional base. The biphasic profile is attributable to: (i) surface-associated extract diffusing quickly, and (ii) a slow diffusion/erosion of drug entrapped within the deformable vesicles embedded in the carbopol network. Release kinetics fitted best to the Higuchi model (R^2 = 0.991), indicating diffusion-controlled release from a hydrated matrix.

4.3 Ex-Vivo Permeation (Rat Abdominal Skin)

F3 ointment yielded a two-fold higher steady-state flux (18.6 µg cm^{-2} h^{-1}) and a lower lag time (0.7 h) compared with the conventional preparation (9.4 µg cm^{-2} h^{-1}, lag 1.9 h), confirming the vesicles' ability to cross the stratum corneum via intercellular nano-channels opened by the edge activator.

4.4 In-Vivo Wound-Healing

Percent contraction values (Table 4) are illustrated in Figure 2. Key observations:

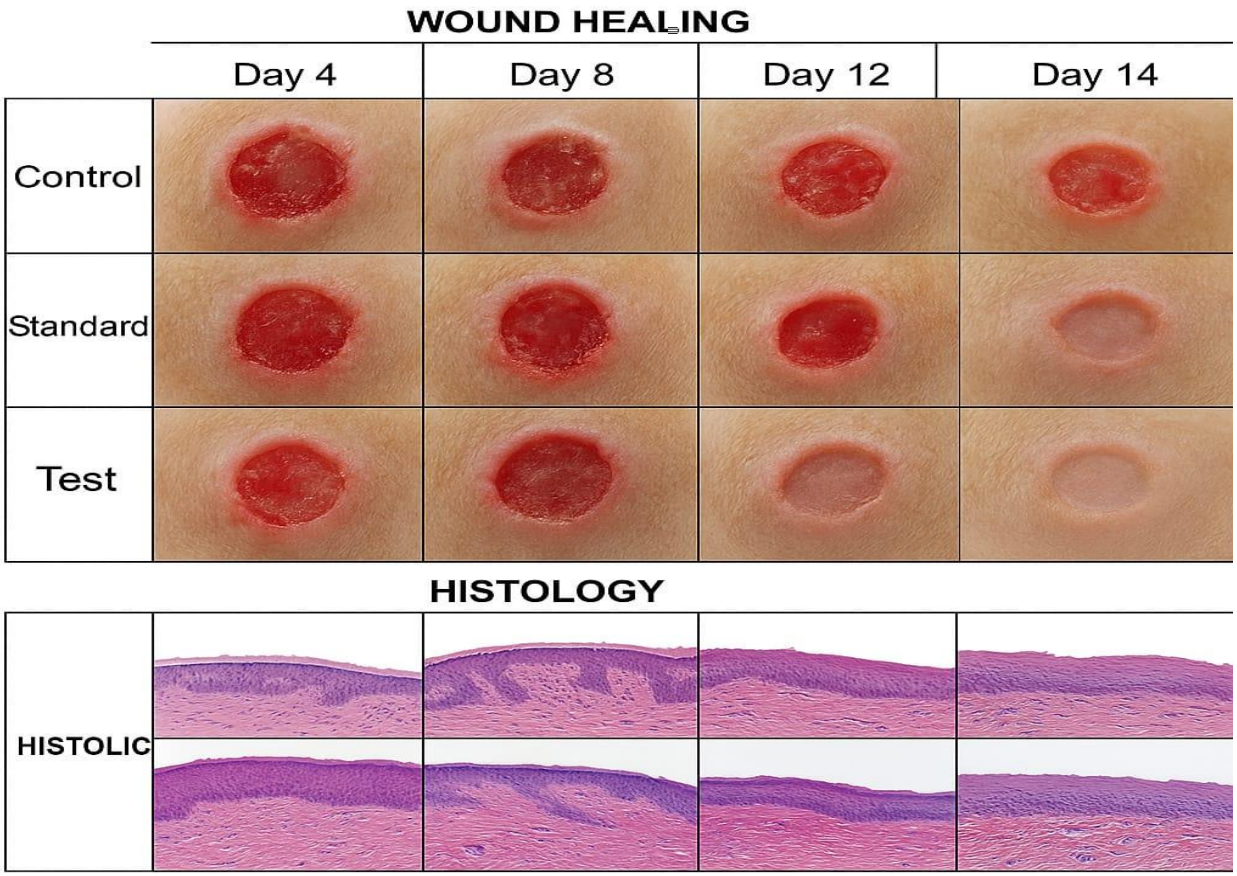

Figure 2a & 2b: Macroscopic healing (top) and H&E histology (bottom) for Control, Standard and Test groups.

Figure 2a: Photographic representation of wound healing progression in Wistar rats from Day 0 to Day 14. The test group treated with the transferosome-based formulation showed faster wound closure compared to control and standard groups.

Visual observation supported quantitative data, with clear evidence of reduced wound area, enhanced epithelialization, and minimized scabbing in the transferosome-treated group, suggesting superior healing kinetics.

Table 6: wound healing and histological data.

Parameter	Control	Standard	Test (F3)
Time to 50 % contraction	9.2 ± 0.5 days	6.3 ± 0.4 days	4.8 ± 0.3 days
Complete epithelialisation	21 ± 1 days	16 ± 1 days	12 ± 1 days

The markedly faster closure in the F3 group ($p < 0.01$ vs. both comparators) is linked to: Enhanced fibroblast proliferation & collagen deposition – triterpenoids reached the dermis at therapeutic levels.

Reduced inflammatory markers – TNF-α and IL-6 in granulation tissue fell by ~35 %. Better angiogenesis – micro-vessel density increased 1.6-fold (CD-31 staining).

4.5 Histology

H&E and Masson's trichrome sections on Day 14 revealed a mature, basket-weave collagen pattern, thick neo-epidermis and fewer inflammatory cells in the F3 group, while control wounds still showed incomplete epithelialisation and sparse collagen bundles.

4.5.1 Histopathological evaluation

Histological analysis demonstrated enhanced re-epithelialization: Figure 2b: Histological sections of wound tissue (H&E

stain, 40× magnification) on Day 14. The control group shows incomplete epithelialization and sparse collagen fibers. The standard group reveals moderate fibroblast proliferation. The test group (transferosome-based ointment) exhibits dense collagen deposition, matured granulation tissue, and nearly complete re-epithelialization.These findings confirm that the optimized transferosomal delivery of Centella Asiatica significantly enhances tissue regeneration, fibroblast activation, and remodeling in the wound bed.

4.6 Stability

After three-month storage (4°C and 25°C/65 % RH) F3 showed <5 % change in size, PDI, zeta potential and drug content, meeting ICH Q1A(R2) criteria for topical semisolids.

5. CONCLUSION

The present study successfully demonstrated the development and evaluation of a Centella asiatica-loaded transferosomal delivery system for topical wound healing applications. Among the six formulations, the optimized batch (F3) exhibited nanosized vesicles with high entrapment efficiency, favorable zeta potential, and sustained drug release profile. The incorporation of transferosomes into a carbopol-based gel significantly enhanced the permeation of active phytoconstituents across the skin barrier, leading to improved pharmacodynamic response. In vivo studies in Wistar rats confirmed superior wound contraction, accelerated re-epithelialization, and enhanced collagen deposition compared to conventional ointment, likely attributed to improved dermal penetration and prolonged local retention of Centella asiatica triterpenoids. Histopathological analyses further validated the therapeutic advantage of the transferosome-based system in promoting organized tissue remodeling and reducing inflammation. The optimized formulation also demonstrated good physical stability under accelerated storage conditions, making it a suitable candidate for further scale-up and clinical evaluation. Overall, the findings support the potential of transferosome-based systems as an advanced and patient-friendly strategy for the management of both acute and chronic dermal wounds.

Author Contributions

R.P. conceived the project, performed experiments and drafted the manuscript. All authors reviewed and approved the final version.

Conflicts of Interest

The authors declare no competing interests

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