Research Article

Topical Transfersome Gel Containing Clitoria Ternatea. L Extract Modulates Interleukin-6 Expression and Enhances Re-Epithelialization in A Rat Model of Second-Degree Burns

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ABSTRACT

Background: Burn injuries remain a clinical challenge because excessive inflammation can delay tissue repair. Interleukin-6 (IL-6) is a key inflammatory cytokine, while re-epithelialization is essential for skin restoration. Clitoria ternatea L. contains bioactive compounds with potential anti-inflammatory and wound-healing properties, which may be enhanced by transfersome delivery. **Objective:** This study aimed to evaluate the effects of topical transfersome gel containing Clitoria ternatea L. extract on serum IL-6 levels, re-epithelialization, and wound-healing rate in Wistar rats with second-degree burns. **Methods:** A randomized post-test-only control group design involved 60 male Wistar rats divided into two experimental sets, each comprising six groups (n = 5). Treatments included transfersome gel base, silver sulfadiazine (SSD), 2.5%, 5%, or 10% extract-loaded transfersome gel, and 10% extract-loaded gel combined with SSD. Serum IL-6 was measured by enzyme-linked immunosorbent assay on day 3, re-epithelialization was assessed histologically on day 7, and wound-healing rates were evaluated on days 3 and 7. **Results:** Treatment significantly affected IL-6 levels ($p = 0.006$) and re-epithelialization thickness ($p < 0.001$). IL-6 levels were significantly lower in the SSD and combination groups than in the negative control. The 10% formulation produced the greatest epithelial thickness among extract treatment groups. Wound-healing rates did not differ significantly on day 3 ($p = 0.220$) but differed on day 7 ($p = 0.003$), with the combination group achieving the highest mean healing rate. **Conclusion:** Clitoria ternatea L. transfersome gel showed potential to modulate inflammation and support re-epithelialization. The 10% formulation enhanced epithelial thickness, while combination therapy with SSD achieved the highest wound-healing rate on day 7.

Keywords: Burns; Interleukin-6; Wound Healing; Drug Delivery Systems; Phytotherapy; Re-epithelialization.

INTRODUCTION

Burn injury is one of the leading causes of trauma-related morbidity worldwide, accounting for approximately 180,000 deaths each year, with the greatest burden occurring in low- and middle-income countries [1]. In Indonesia, the 2018 National Health Survey reported a burn prevalence of 1.3% [2].

Besides increasing mortality, burn injuries frequently result in prolonged hospitalization, functional impairment, psychological distress, and substantial healthcare costs [3]. Among the different types of burns, second-degree burns are particularly challenging because damage extends through the epidermis into the dermis, increasing the risk of infection, delayed tissue repair, hypertrophic scarring, and functional disability [4].

The healing of second-degree burns is a dynamic process involving overlapping inflammatory, proliferative, and remodeling phases. A tightly regulated inflammatory response is essential for removing damaged tissue and initiating repair; however, excessive or persistent inflammation can disrupt tissue regeneration and delay wound closure [5]. Interleukin-6 (IL-6) is a major pro-inflammatory cytokine released shortly after burn injury and plays an important role in leukocyte recruitment, angiogenesis, and immune regulation [5,6]. Sustained elevation of IL-6 has been associated with prolonged inflammation, impaired re-epithelialization, and increased fibrosis, making it a useful biomarker for evaluating burn wound healing [5,7].

Silver sulfadiazine (SSD) remains the standard topical treatment for burn wounds because of its broad-spectrum antimicrobial activity. Nevertheless, accumulating evidence indicates that prolonged SSD application may delay epithelial regeneration and interfere with the normal healing process despite its effectiveness in preventing wound

infection [8,9]. These limitations have encouraged the exploration of alternative therapies capable of controlling inflammation while simultaneously promoting tissue repair.

Clitoria ternatea L., commonly known as butterfly pea flower, is rich in flavonoids, anthocyanins, phenolic compounds, tannins, saponins, and alkaloids with reported antioxidant and anti-inflammatory activities [10,11]. Experimental studies have shown that its bioactive constituents can suppress inflammatory mediators, reduce oxidative stress, stimulate fibroblast proliferation, enhance collagen synthesis, and support epithelial regeneration during wound healing [10–12]. Despite these promising pharmacological properties, the clinical application of herbal extracts is often limited by poor penetration through the skin barrier, reducing the amount of active compounds reaching the target tissue.

Transfersomes are ultra-deformable phospholipid vesicles designed to enhance transdermal drug delivery by improving penetration through the stratum corneum while maintaining drug stability and local bioavailability [13]. Incorporating Clitoria ternatea L. extract into a transfersome gel may increase the delivery of its bioactive compounds to burn tissue and enhance its therapeutic effects. Although the anti-inflammatory and wound-healing potential of Clitoria ternatea L. has been reported, studies evaluating a transfersome-based formulation using biomolecular and histological indicators of second-degree burn healing remain scarce. In addition, evidence regarding its effects on serum IL-6 levels and re-epithelialization is still limited.

Therefore, this study aimed to evaluate the effects of topical transfersome gel containing Clitoria ternatea L. extract on serum IL-6 levels, re-epithelialization, and healing rate in a Wistar rat model of second-degree burns. We hypothesized that the transfersome-based formulation would attenuate inflammation, promote epithelial regeneration, and improve wound healing compared with untreated burn wounds.

MATERIALS AND METHODS

This randomized post-test only control group laboratory experiment was conducted between October 2025 and January 2026 to evaluate the effects of topical transfersome gel containing Clitoria ternatea L. extract on second-degree burn wound healing in Wistar rats. The sample size was calculated using G*Power version 3.1.9.7 [14]. A one-way analysis of variance (ANOVA) fixed-effects omnibus test was selected because the study compared multiple independent groups using identical outcome variables, namely serum interleukin-6 (IL-6) levels and re-epithelialization. The calculation was based on a significance level (α) of 0.05 [14], a statistical power (1−β) of 0.80 [15], and a large effect size (Cohen's f = 0.80). The selected effect size was supported by previous studies demonstrating marked improvements in burn wound healing following treatment with Clitoria ternatea L. extract [16]. The minimum required sample size was five animals per group. Two independent experimental sets were conducted, one for serum IL-6 analysis and the other for histopathological evaluation of re-epithelialization, resulting in a total of 60 rats included in the study.

Preparation of Clitoria ternatea L. extract, transfersome formulation, gel preparation, and phytochemical screening were carried out at the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. Animal maintenance, burn wound induction, topical treatment, and serum IL-6 analysis were performed at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Ciputra, Surabaya, Indonesia. Histopathological processing and re-epithelialization assessment were conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Surabaya, Surabaya, Indonesia. Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Ciputra, Surabaya, Indonesia (No. 291/EC/KEPK-FKUC/X/2025).

Sixty healthy male Wistar rats (Rattus norvegicus), aged 8–12 weeks and weighing 150–200 g [17], were acclimatized for seven days under standard laboratory conditions with free access to food and water [18,19]. Following acclimatization, the animals were randomly allocated into two experimental sets using a simple lottery method. Each set consisted of six groups (n = 5 per group): a negative control treated with transfersome gel base, a positive control treated with silver sulfadiazine (SSD) cream, P1 treated with 2.5% transfersome gel containing Clitoria ternatea L. extract, P2 treated with 5% transfersome gel, P3 treated with 10% transfersome gel, and P4 treated with a combination of 10% transfersome gel and SSD cream. In the combination group, the transfersome gel was applied in the morning and SSD cream in the afternoon [20,21]. Blinding was not performed because treatment administration and outcome assessment were conducted by the same investigator.

Dried Clitoria ternatea L. flowers were extracted by maceration using 70% ethanol following a previously published method with minor modifications [22–24]. The concentrated extract was subsequently incorporated into transfersomes using the thin-film hydration technique. Soy phosphatidylcholine and Tween 80 were mixed at a ratio

of 9:1 and dissolved in a chloroform-methanol mixture (2:1, v/v). The organic solvent was evaporated using a rotary evaporator at 37°C and 150 rpm for approximately 70 min to form a thin lipid film, which was hydrated with phosphate-buffered saline (PBS, pH 7.4) to obtain transfersome vesicles. The transfersome suspension was then incorporated into a Carbopol 940 gel base containing propylene glycol, triethanolamine, and purified water to prepare formulations containing 2.5%, 5%, and 10% Clitoria ternatea L. extract [25].

General anesthesia was induced by intraperitoneal administration of a combination of ketamine (40–100 mg/kg body weight) and xylazine (5–13 mg/kg body weight) [26]. After shaving the dorsal skin, a standardized second-degree burn wound was created using a stainless-steel coin (2.4 cm diameter) heated to 98°C and applied to the skin for 10s [27]. The first topical treatment was administered 6 h after burn induction and continued once daily according to the assigned treatment [20]. Macroscopic wound healing was documented on days 0, 3, and 7 using standardized digital photography. Wound area was measured using ImageJ software [28], and the healing rate was calculated from the reduction in wound area relative to the initial wound size [29].

For serum IL-6 analysis, blood samples were collected by cardiac puncture on day 3 under general anesthesia [30]. Serum was separated by centrifugation and stored until analysis. IL-6 concentrations were measured using a Rat IL-6 ELISA Kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader, and cytokine concentrations were determined using a four-parameter logistic standard curve [31,32]. For histopathological evaluation, skin tissue specimens were harvested on day 7, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) [33–35]. Re-epithelialization was assessed by measuring epithelial thickness from the stratum basale to the stratum corneum using an Olympus BX53 light microscope equipped with an Optilab imaging system and Raster Image software at 400× magnification [26].

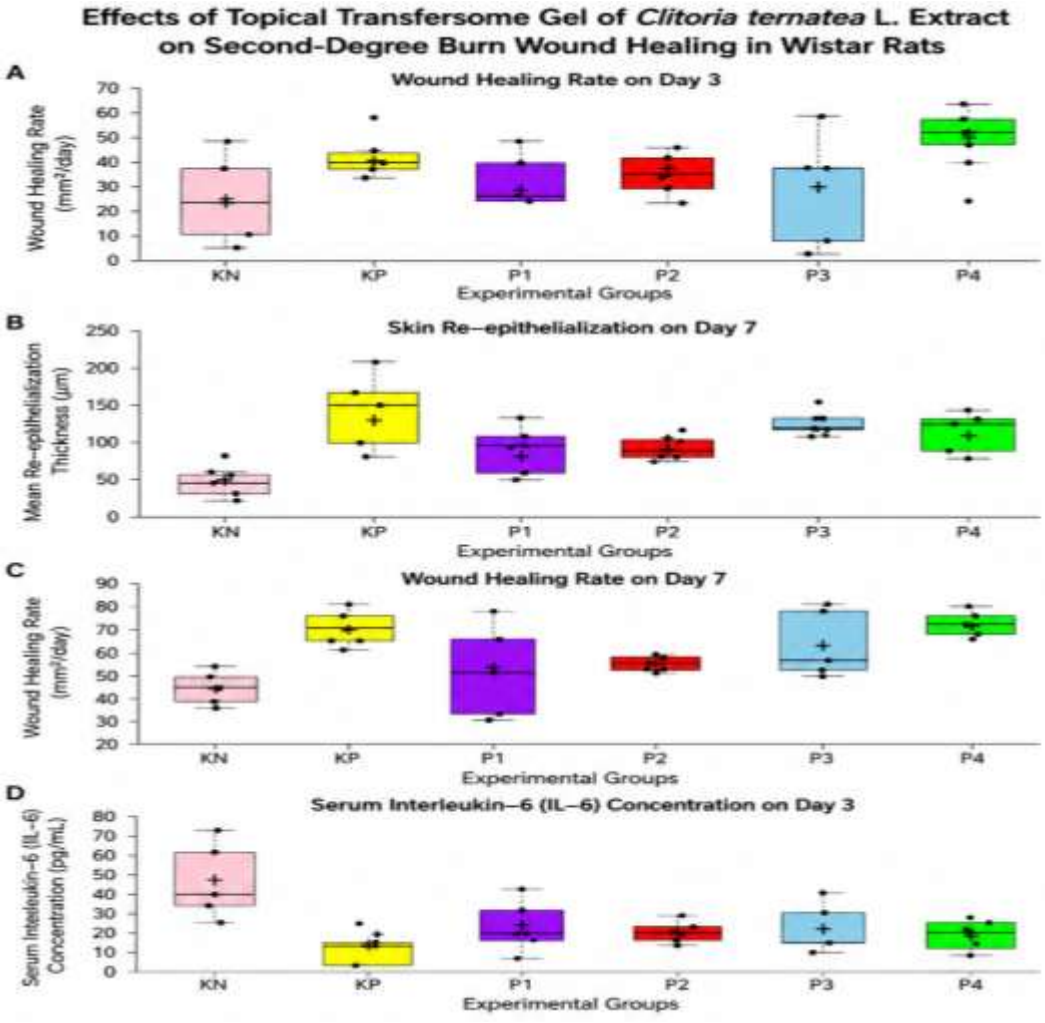


Figure 1. Effects of topical transfersome gel containing Clitoria ternatea L. extract on wound healing in a second-degree burn model in Wistar rats.

(A) Wound healing rate on Day 3 (mm²/day); (B) Skin re-epithelialization thickness on Day 7 (μm); (C) Wound healing rate on Day 7 (mm²/day); and (D) Serum interleukin-6 (IL-6) concentration on Day 3 (pg/mL). Experimental groups consisted of a negative control (KN), positive control treated with silver sulfadiazine (KP), P1 (2.5% transfersome gel), P2 (5% transfersome gel), P3 (10% transfersome gel), and P4 (10% transfersome gel combined with silver sulfadiazine). Box plots represent the interquartile range (IQR), the horizontal line within each box indicates the median, Whiskers represent the minimum and maximum values, individual dots represent each animal, and the plus sign (+) indicates the mean. Data are presented for n = 5 animals per group.

At the end of each experimental period, animals were deeply anesthetized with ketamine and xylazine administered intraperitoneally before euthanasia by cervical dislocation magnification [26]. Statistical analyses were performed using IBM SPSS Statistics for Windows version 31.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation (SD). Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. One-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test was used for normally distributed data with homogeneous variances. Welch's ANOVA followed by the Games–Howell post hoc test was applied when the assumption of homogeneity was violated. Effect size was expressed as eta-squared (η^2), and a p-value <0.05 was considered statistically significant [36].

RESULTS

A total of 60 male Wistar rats completed the study without mortality or exclusion from analysis. Serum interleukin-6 (IL-6) levels and histopathological re-epithelialization were evaluated in two independent experimental sets, each comprising 30 animals randomly allocated into six groups (n = 5 per group). Wound healing progression was assessed by calculating the healing rate on days 3 and 7 after burn induction.

Serum IL-6 levels differed significantly among the experimental groups (one-way ANOVA, $F = 4.355$, $p = 0.006$, $\eta^2 = 0.476$). The highest mean IL-6 concentration was observed in the negative control group (44.63 ± 17.28 pg/mL), whereas the positive control group had the lowest concentration (12.30 ± 12.18 pg/mL). The treatment groups showed lower mean IL-6 concentrations than the negative control, with values of 21.28 ± 14.53 pg/mL in P1, 18.36 ± 6.00 pg/mL in P2, 19.75 ± 12.30 pg/mL in P3, and 16.00 ± 8.09 pg/mL in P4. Tukey's post hoc analysis demonstrated that IL-6 levels in the negative control group were significantly higher than those in the positive control ($p = 0.004$), P2 ($p = 0.027$), P3 ($p = 0.040$), and P4 ($p = 0.013$). No significant difference was found between the negative control and P1 ($p = 0.061$), and no significant differences were observed among the treatment groups.

Re-epithelialization thickness measured on day 7 also differed significantly among the experimental groups (Welch's ANOVA, Welch = 11.294, $p < 0.001$, $\eta^2 = 0.603$). The negative control group exhibited the thinnest epithelial layer (66.93 ± 16.19 μm), while the positive control group showed the greatest epithelial thickness (149.37 ± 40.24 μm). Mean epithelial thicknesses in the treatment groups were 101.88 ± 29.60 μm in P1, 111.78 ± 16.59 μm in P2, 138.40 ± 11.91 μm in P3, and 128.30 ± 20.99 μm in P4. Games–Howell post hoc analysis revealed that re- epithelialization thickness was significantly lower in the negative control group than in the positive control ($p = 0.047$), P2 ($p = 0.021$), P3 ($p < 0.001$), and P4 ($p = 0.009$). No significant difference was identified between the negative control and P1 ($p = 0.310$), and no significant differences were observed among the treatment groups or between the treatment groups and the positive control.

Healing rate was subsequently evaluated on days 3 and 7 after burn induction. On day 3, no significant difference was detected among the experimental groups (one-way ANOVA, $F = 1.524$, $p = 0.220$, $\eta^2 = 0.241$). The highest mean healing rate was recorded in P4 (50.08 ± 13.19%), followed by the positive control (42.60 ± 16.50%), P2 (39.01 ± 11.36%), P3 (31.05 ± 20.61%), P1 (29.82 ± 15.07%), and the negative control (28.12 ± 15.40%).

By day 7, healing rate differed significantly among the experimental groups (Welch's ANOVA, Welch = 7.326, $p = 0.003$, $\eta^2 = 0.431$). The highest mean healing rate was observed in P4 (72.15 ± 5.71%), followed by the positive control (70.07 ± 5.89%), P3 (64.14 ± 12.54%), P2 (59.94 ± 8.94%), P1 (56.44 ± 18.13%), and the negative control (47.67 ± 6.89%). Games–Howell post hoc analysis showed that the healing rate in the negative control group was significantly

lower than that in the positive control (p = 0.005) and P4 (p = 0.003). No significant differences were observed between the negative control and P1, P2, or P3, or among the treatment groups and the positive control.

DISCUSSION

This study demonstrated that topical transfersome gel containing Clitoria ternatea L. extract improved the healing response of second-degree burn wounds through complementary anti-inflammatory and regenerative effects. The combination of 10% transfersome gel with silver sulfadiazine produced the greatest reduction in serum IL-6 levels and the highest healing rate on day 7, whereas the 10% transfersome gel alone resulted in the greatest re-epithelialization among the treatment groups. These findings indicate that controlling inflammation and promoting tissue regeneration are closely related but not necessarily identical processes during burn wound healing.

Table 1. Biological outcomes of the experimental groups following topical treatment of second-degree burn wounds

Group	IL-6 (pg/mL) Mean ± SD	Re-epithelialization (µm)Mean ± SD	Healing rate Day 3 (%)Mean ± SD	Healing rate Day 7 (%)Mean ± SD
K–	44.63 ± 17.28^{a}	66.93 ± 16.19^{a}	28.12 ± 15.40	47.67 ± 6.89^{a}
K+	12.30 ± 12.18^{b}	149.37 ± 40.24^{b}	42.60 ± 16.50	70.07 ± 5.89^{b}
P1	21.28 ± 14.53^{ab}	101.88 ± 29.60^{ab}	29.82 ± 15.07	56.44 ± 18.13^{ab}
P2	18.36 ± 6.00^{b}	111.78 ± 16.59^{b}	39.01 ± 11.36	59.94 ± 8.94^{ab}
P3	19.75 ± 12.30^{b}	138.40 ± 11.91^{b}	31.05 ± 20.61	64.14 ± 12.54^{ab}
P4	16.00 ± 8.09^{b}	128.30 ± 20.99^{b}	50.08 ± 13.19	72.15 ± 5.71^{b}
Statistical test	One-way ANOVA	Welch's ANOVA	One-way ANOVA	Welch's ANOVA
Test statistic	F = 4.355	Welch = 11.294	F = 1.524	Welch = 7.326
p-value	0.006	<0.001	0.220	0.003
Effect size (η^2)	0.476	0.603	0.241	0.431

Values are presented as mean ± standard deviation (SD). Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$) based on post hoc analysis. Serum IL-6 concentrations were analyzed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) test. Re-epithelialization thickness and healing rate on day 7 were analyzed using Welch's ANOVA followed by Games–Howell post hoc test because the assumption of homogeneity of variance was not met. Healing rate on day 3 showed no significant differences among the experimental groups; therefore, no post hoc comparisons were performed.

Inflammation is an essential component of burn wound healing because it initiates the removal of damaged tissue and prepares the wound for subsequent repair. However, excessive or prolonged inflammation may delay the transition from the inflammatory phase to the proliferative phase, thereby impairing tissue regeneration. The lower serum IL-6 concentrations observed in the treatment groups suggest that topical administration of Clitoria ternatea L. extract may help regulate this early inflammatory response. IL-6 is released rapidly following burn injury and functions in leukocyte recruitment, acute inflammatory signaling, and activation of immune cells. Although these responses are necessary during the initial phase of healing, sustained IL-6 production has been associated with persistent inflammation, delayed epithelial repair, and increased scar formation [5,7].

The anti-inflammatory activity observed in this study is likely related to the phytochemical constituents of Clitoria ternatea L. Butterfly pea flowers contain anthocyanins, particularly ternatins, together with flavonoids, phenolic compounds, and tannins, which have been reported to modulate inflammatory signaling pathways. Anthocyanins and flavonoids suppress activation of nuclear factor-kappa B (NF-κB), thereby reducing the production of pro-inflammatory mediators, including IL-6, while simultaneously limiting oxidative stress through their antioxidant activity [37–39]. By reducing excessive inflammatory signaling, these bioactive compounds may create a microenvironment that is more favorable for tissue repair without completely interrupting the physiological inflammatory response required for normal wound healing.

Histopathological findings further supported the anti-inflammatory effects by demonstrating enhanced re-epithelialization in the treatment groups. Re-establishment of the epidermal layer is a critical event during the

proliferative phase because it restores the skin barrier and reduces the risk of secondary infection. The 10% transfersome gel (P3) produced the greatest epithelial thickness among all treatment groups and approached the response observed in the positive control. This finding suggests that the higher concentration of Clitoria ternatea L. extract provided sufficient bioactive compounds to support keratinocyte migration and proliferation. Flavonoids and anthocyanins have previously been shown to stimulate epithelial regeneration by reducing oxidative stress, preserving cell viability, and facilitating cellular proliferation within the wound bed [40,41].

An interesting observation in the present study was that the treatment producing the lowest IL-6 concentration was not the same treatment that resulted in the greatest re-epithelialization. Although P4 showed the strongest suppression of IL-6, epithelial thickness was slightly greater in P3. This finding suggests that maximal inhibition of inflammation may not necessarily produce the most favorable regenerative response. A certain degree of inflammatory activity remains necessary for macrophage polarization, growth factor release, angiogenesis, and intercellular signaling that coordinate tissue regeneration [42]. Therefore, the 10% transfersome gel alone may have achieved a more balanced inflammatory environment that was sufficient to support epithelial regeneration, whereas the addition of silver sulfadiazine appeared to enhance anti-inflammatory activity without providing additional benefit to epithelial thickening.

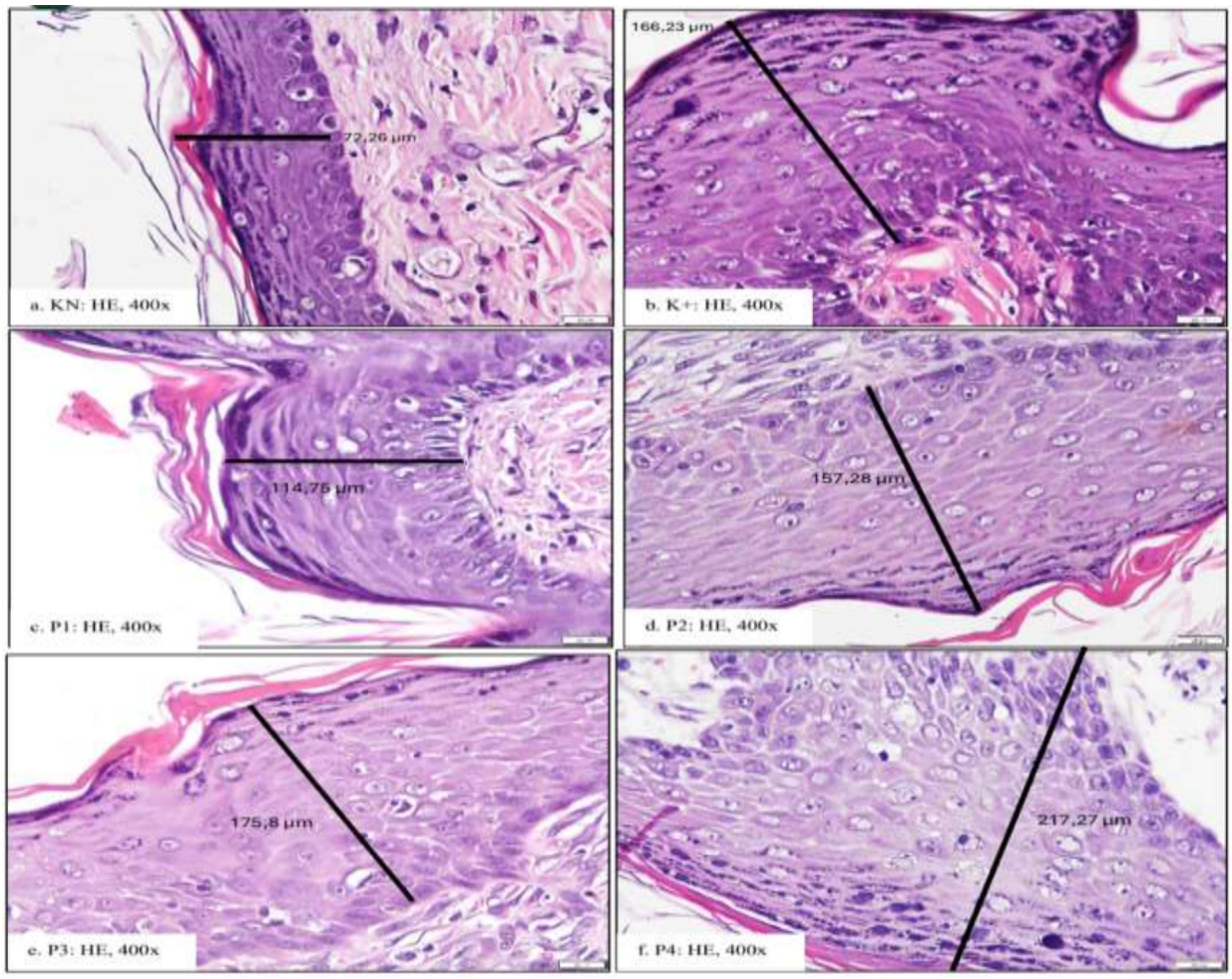


Figure 2. Representative histopathological sections showing re-epithelialization on day 7 following treatment with Clitoria ternatea L. extract transfersome gel (Hematoxylin–Eosin staining, 400× magnification).
Representative sections from K−, K+, P1, P2, P3, and P4 are shown. Black lines indicate epithelial thickness measurements. The P3 and P4 groups exhibited a thicker and more continuous epithelial layer than the negative control group, indicating enhanced re-epithelialization.

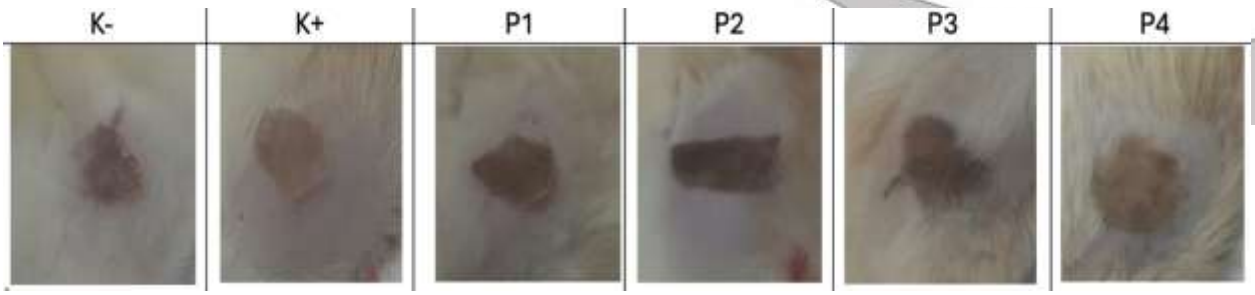


Figure 3. Macroscopic change in the skinof the rat's over 7 days.

Macroscopic wound healing demonstrated a similar pattern. Although no significant difference in healing rate was detected on day 3, all treatment groups showed numerically greater wound closure than the untreated control. The absence of statistical significance during the early observation period is not unexpected because visible wound contraction generally occurs after molecular and histological changes have already begun. Regulation of inflammatory mediators and initiation of epithelial regeneration often precede measurable reduction in wound area [43,44]. This sequence may explain why differences in healing rate became evident only on day 7, when the combination treatment achieved the highest percentage of wound closure.

The improved healing response observed in this study may also be attributed to the transfersome delivery system. Unlike conventional topical formulations, transfersomes are ultra-deformable phospholipid vesicles capable of penetrating the stratum corneum through narrow intercellular pathways while protecting encapsulated bioactive compounds from degradation. This property enhances drug retention within the skin and increases local bioavailability at the wound site [13]. Improved penetration of Clitoria ternatea L. phytochemicals may therefore contribute to more effective modulation of inflammation and stimulation of tissue regeneration than would be expected from a conventional herbal gel.

Several limitations should be considered when interpreting these findings. The inflammatory response was evaluated using only serum IL-6, whereas other cytokines and growth factors involved in burn wound healing, such as tumor necrosis factor-α, interleukin-10, transforming growth factor-β, and vascular endothelial growth factor, were not assessed. Furthermore, the observation period was limited to seven days and therefore did not include the remodeling phase or scar maturation. Characterization of transfersome physicochemical properties was also beyond the scope of this study. Future studies incorporating additional molecular markers, longer observation periods, and comprehensive characterization of the transfersome formulation are warranted to further clarify the mechanisms underlying its therapeutic effects.

The present findings indicate that topical transfersome gel containing Clitoria ternatea L. extract represents a promising approach for second-degree burn wound management. The 10% transfersome formulation effectively promoted epithelial regeneration, while its combination with silver sulfadiazine provided superior control of inflammation and improved wound closure during the early stages of healing. These findings support the potential of transfersome-based delivery systems to enhance the therapeutic efficacy of herbal bioactive compounds in burn wound treatment.

Strength and limitations

This study has several strengths. Burn wound healing was evaluated using complementary outcome measures, including serum interleukin-6 (IL-6) as a biomarker of inflammation, histopathological assessment of re-epithelialization, and macroscopic wound healing rate. The integration of these parameters provided a more comprehensive understanding of both the biological response and structural tissue changes throughout the wound healing process. Furthermore, the transfersome gel formulation underwent physicochemical evaluation, including organoleptic properties, pH, and viscosity, as well as phytochemical screening for tannins, saponins, and flavonoids before biological testing. These evaluations support the quality of the formulation used in this study. The application of a transfersome-based delivery system also represents a promising approach to enhance the topical delivery of Clitoria ternatea L. extract, supporting its potential as an alternative therapeutic option for second-degree burn wounds.

Nevertheless, several limitations should be acknowledged. The inflammatory response was evaluated solely by measuring serum IL-6 levels, which does not fully represent the complex network of inflammatory cytokines, growth

factors, and oxidative stress markers involved in wound healing. In addition, the seven-day observation period was insufficient to evaluate the remodeling phase and scar maturation. Although physicochemical and phytochemical analyses of the formulation were performed, further characterization of the transfersome delivery system, such as vesicle size, polydispersity index, zeta potential, and entrapment efficiency, was not conducted.
Future studies should include a longer observation period, evaluate additional molecular biomarkers involved in wound healing, and perform comprehensive characterization of the transfersome formulation to better elucidate its mechanism of action and support its potential clinical application.

CONCLUSION

The findings of this study indicate that formulating Clitoria ternatea L. extract into a transfersome gel can improve the healing process of second-degree burn wounds. While the 10% formulation showed the greatest effect on re-epithelialization, combining it with silver sulfadiazine provided better inflammatory control and wound closure. These results highlight the potential of transfersome technology to improve the topical delivery of herbal bioactive compounds and support the development of Clitoria ternatea L. extract as an alternative approach for burn wound treatment. Further studies with longer observation periods and clinical evaluation are needed before its application in clinical practice.

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Conflict of interest
None.

Ethical approval
This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Ciputra, Surabaya, Indonesia (Approval No. 291/EC/KEPK-FKUC/X/2025, 31 October 2025). All animal experiments were conducted in accordance with institutional ethical standards and internationally accepted guidelines for the care and use of laboratory animals. Appropriate measures were implemented throughout the study to ensure animal welfare, minimize pain and distress, and reduce the number of animals used while maintaining scientific validity.

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N/A.

Author contribution
DAPA (Guarantor) contributed to the conception and design of the study, methodology development, investigation and data collection, statistical analysis, data analysis and interpretation, drafting of the manuscript, and final approval of the article. As the guarantor, DAPA accepts full responsibility for the integrity of the work from its inception to publication. **AM** contributed to the conception and study design, methodology development, drafting of the manuscript, critical revision for important intellectual content, supervision, administrative and technical support, and final approval of the article. **MAE** contributed to the study design, statistical analysis, critical revision of the manuscript for important intellectual content, supervision, and final approval of the article. **LS** contributed to the conception and study design, investigation and data collection, drafting of the manuscript, critical revision for important intellectual content, and final approval of the article. **SSB** contributed to the conception of the study, methodology development, critical revision of the manuscript for important intellectual content, and final approval of the article.

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