Med (Zenica) Glas

Medicinski Glasnik

ISSN 1840-2445 (Online); ISSN 1840-0132 (Print)

Medical Association of Zenica-Doboj Canton

Included by: PubMed (MEDLINE), SCOPUS, EMBASE, CNKI and DOAJ.



| | |
|---|---|
| **Type of the paper:** | **ORIGINAL ARTICLE** |
| **Running title:** | **Setiawan et al.** *Centella asiatica* gel in diabetic wounds |
| **Title:** | **Topical *Centella asiatica* gel enhances tissue repair and attenuates inflammation in diabetic wounds: an experimental study in male Wistar rats** |
| **Authors:** | Eko Setiawan[1,2,3,*], Dimas Irfan Nabih[3], Farhan Arifendy Rahman[4], Hasan Syarief[4] |
| **Affiliations:** | [1]Department of Doctoral Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [2]Department of Surgery, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [3]Department of Master of Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [4]Department of Medicine, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; Indonesia |
| **Corresponding author:** | Eko Setiawan, Department of Doctoral Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia, Jl. Raya Kaligawe Km. 4, Terboyo Kulon, Genuk, Semarang 50112, Central Java, Indonesia<br>Phone: +62 812-2550-5591<br>E-mail: drekosetiawan@unissula.ac.id<br>ORCID ID: 0000-0002-7194-2359 |
| **DOI:** | https://doi.org/10.17392/medglas-2181-23-2 |

**Received**: 03 June 2026
**Revised:** 10 July 2026
**Accepted:** 14 July 2026

ORIGINAL ARTICLE

# Topical *Centella asiatica* gel enhances tissue repair and attenuates inflammation in diabetic wounds: an experimental study in male Wistar rats

Eko Setiawan[1,2,3,*], Dimas Irfan Nabih[3], Farhan Arifendy Rahman[4], Hasan Syarief[4]

[1]Department of Doctoral Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [2]Department of Surgery, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [3]Department of Master of Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; [4]Department of Medicine, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang; Indonesia

**Running title: Setiawan et al.** *Centella asiatica* gel in diabetic wounds

*** Correspondence:**

Eko Setiawan

Department of Doctoral Biomedical Science, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia

Jl. Raya Kaligawe Km. 4, Terboyo Kulon, Genuk, Semarang 50112, Central Java, Indonesia

Phone: +62 812-2550-5591

E-mail: drekosetiawan@unissula.ac.id

ORCID ID: 0000-0002-7194-2359

## ABSTRACT

**Aim:** To evaluate the effects of topical *Centella asiatica* extract gel on early healing of diabetic excision wounds.

**Methods:** A randomised, post-test-only in vivo experiment included 28 male Wistar rats with nicotinamide/streptozotocin-induced diabetes and dorsal excision wounds. Rats were assigned to base gel, 0.1% gentamicin, 10% *C. asiatica* gel, or 20% *C. asiatica* gel (n = 7 per group) for seven days. Epithelial thickness, fibroblast counts, collagen deposition, angiogenesis, inflammation score, and serum C-reactive protein (CRP) were assessed on day eight. Data were analysed using one-way analysis of variance with post hoc testing and Holm adjustment.

**Results:** Between-group differences were significant for epithelial thickness ($p = 0.044$), fibroblast counts ($p = 0.020$), CRP levels ($p = 0.002$), collagen deposition ($p = 0.007$), angiogenesis ($p < 0.001$), and inflammation score ($p = 0.009$), and all remained significant after Holm adjustment. The 10% *C. asiatica* gel group showed the highest epithelial thickness, fibroblast count, and collagen deposition, whereas the 20% *C. asiatica* gel group showed the lowest CRP level and inflammation score and the highest angiogenesis level.

**Conclusion:** Topical *C. asiatica* gel was associated with favourable changes in selected early histological repair and inflammatory parameters in diabetic excision wounds. The different patterns observed with the 10% and 20% formulations should be interpreted descriptively rather than as evidence of dose-response superiority.

**Keywords:** angiogenesis; C-reactive protein; *Centella asiatica*; fibroblasts; inflammation

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterised by hyperglycaemia caused by impaired insulin secretion, insulin action, or both, and is associated with substantial mortality (1). According to the International Diabetes Federation, at least 537 million people worldwide live with diabetes (2). A serious complication is the development of chronic diabetic foot ulcers, which affect approximately 15% of patients with diabetes and cause substantial morbidity and mortality (3). These wounds may fail to heal because a prolonged inflammatory phase and growth-factor dysregulation impair the proliferation and migration of keratinocytes and fibroblasts, ultimately preventing the formation of a functional epithelial layer (4,5).

Plant-derived treatments such as *Centella asiatica* have therapeutic potential because they contain bioactive triterpenoids and flavonoids with anti-inflammatory, antioxidant, and regenerative properties (6,7). Previous studies have shown that *C. asiatica* preparations may accelerate diabetic wound healing, reduce scar formation, increase fibroblast activity, stimulate collagen synthesis, support angiogenesis, and promote epithelial repair (8,9). Triterpenoid components, including asiaticoside and madecassoside, may also promote angiogenesis and stimulate type I collagen production while suppressing inflammatory reactions through inhibition of the NF-κB pathway (10,11). However, previous studies have often focused on isolated outcomes, such as wound closure or fibroblast counts, leaving uncertainty about how topical *C. asiatica* simultaneously affects epithelial repair and systemic inflammatory markers in diabetic models (12,13).

This study aimed to evaluate the effects of topical *C. asiatica* gel on epithelial thickness, fibroblast counts, C-reactive protein (CRP) levels, collagen deposition, angiogenesis, and inflammation score in male Wistar rats with diabetic excision wounds.

## MATERIALS AND METHODS

### Subjects and study design

A prospective, post-test-only controlled animal study was conducted from 23 May to 10 June 2025 at the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, Indonesia. The study included 28 male Wistar rats (*Rattus norvegicus*), aged 2–3 months and weighing 150–200 g, obtained and maintained at the Universitas Islam Sultan Agung animal laboratory.

The sample size was determined using the Federer formula, $(t - 1)(n - 1) \geq 15$, where t represents the number of treatment groups and n the number of animals per group. With four groups, at least six rats per group were required; seven rats were included in each group, resulting in a total sample of 28 animals.

Eligible rats were healthy, active, and free from anatomical abnormalities. Animals that became ill or died during the seven-day acclimatisation period were excluded.

Animals were randomised into four groups (n = 7 per group): negative control/base gel (G1), positive control/0.1% gentamicin (G2), 10% *C. asiatica* gel (G3), and 20% *C. asiatica* gel (G4).

### Methods

All animals underwent seven days of acclimatisation with standard feed and water ad libitum. Diabetes was induced after 8–20 h of fasting by intraperitoneal nicotinamide (120 mg/kg body weight; Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.9% NaCl, followed 15 min later by streptozotocin (60–65 mg/kg body weight; Sigma-Aldrich, St Louis, MO, USA) dissolved in 0.01 M citrate buffer (pH 4.5). The doses were selected with reference to an experimental model of type 2 diabetes (14). Blood glucose was measured on day seven using an Accu-Chek glucometer (Roche Diabetes Care GmbH, Mannheim, Germany); rats with

glucose levels > 200 mg/dL were considered diabetic. Before wound creation, the animals were anaesthetised with intraperitoneal ketamine (Ketalar®, Pfizer Inc., New York, NY, USA; 60 mg/kg body weight) and xylazine (Bayer AG, Leverkusen, Germany; 20 mg/kg body weight). The dorsal hair was shaved, and the skin was disinfected with 70% ethanol. Under anaesthesia, a 6-mm dorsal excision wound extending through the dermis into the subcutaneous tissue to a depth of approximately 2 mm was created.

*C. asiatica* leaf extract was prepared by macerating dried powdered leaves in 96% ethanol at a 1:10 ratio for three days. The filtrate was concentrated under reduced pressure and further evaporated until a thick extract was obtained. The 10% and 20% gels were formulated by incorporating the extract into a hydroxypropyl methylcellulose base containing liquid paraffin, Span 80, Tween 80, methylparaben, and propylparaben. Treatments were applied topically once daily for seven days according to group allocation. On day eight, following completion of treatment, the animals were euthanised by intraperitoneal anaesthetic overdose followed by cervical dislocation as a secondary method to confirm death. Wound-tissue samples were collected and fixed in 10% neutral buffered formalin for 24 h.

Samples were dehydrated in graded alcohol, cleared in xylene, embedded in paraffin, sectioned at 5–6 µm, and stained with haematoxylin and eosin. Histological evaluation was performed using a Nikon Eclipse Ei microscope (Nikon Corporation, Tokyo, Japan) fitted with an Optilab SIGMA MTN020 digital camera (PT Miconos Transdata Nusantara, Yogyakarta, Indonesia). Three non-overlapping fields within the wound area were evaluated for each animal, and their mean was used as the animal-level value. A single veterinary examiner performed the assessments. Formal blinding to treatment allocation was not documented, and interobserver reliability was not evaluated.

Epithelial thickness was measured in micrometres across three fields at 400× magnification. Fibroblasts were counted manually in the dermis at 400× magnification and expressed as cells per high-power field. Measurements and counts were performed using Image Raster version 4.05 (PT Miconos Transdata Nusantara, Yogyakarta, Indonesia).

Collagen deposition was estimated semi-quantitatively as the percentage of eosinophilic collagen-containing dermal area on haematoxylin and eosin-stained sections. For each animal, three non-overlapping digital images of the dermal wound area were acquired as 24-bit RGB images at a resolution of 5496 × 3694 pixels using a 4× objective. Before thresholding, images were converted to 8-bit greyscale. The dermal region of interest was delineated, and image analysis was performed using ImageJ version 1.54j (National Institutes of Health, Bethesda, MD, USA) with the Auto Threshold plugin version 1.17. The same default thresholding procedure was applied to all images to segment the selected dermal signal. Collagen deposition was calculated as the number of segmented pixels divided by the total number of pixels within the dermal region of interest, multiplied by 100. The mean percentage from the three images was used as the animal-level collagen value.

Angiogenesis was assessed by manually counting newly formed blood vessels in three non-overlapping fields within the wound granulation tissue at 400× magnification. A newly formed blood vessel was defined as a discrete vascular lumen lined by endothelial cells and containing intraluminal erythrocytes. Each separate vascular lumen was counted as one vessel, and the mean vessel count across the three fields was used as the animal-level angiogenesis value.

Inflammatory-cell infiltration was assessed in three non-overlapping fields at 400× magnification using a four-point ordinal scale: 0, no inflammatory-cell infiltration, including polymorphonuclear leukocytes and macrophages; 1, occasional or sparse infiltration

involving < 30% of the wound area; 2, clearly visible, moderately distributed infiltration involving 30–60% of the wound area; and 3, dense or diffuse infiltration or microabscess formation, with inflammatory cells predominating in > 60% of the wound area. The mean score across the three fields was used as the animal-level inflammation value.

Serum CRP concentrations were measured using a rat-specific sandwich enzyme-linked immunosorbent assay kit (Rat C-Reactive Protein/CRP ELISA Kit, catalogue no. RK00195; ABclonal Technology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Serum samples were diluted 1:200. A calibration curve was prepared using CRP standards at 0.312, 0.625, 1.25, 2.5, 5, 10, and 20 ng/mL. Optical density was measured at 450 nm. Values in the certified laboratory report were treated as the final reportable concentrations and expressed in ng/mL.

### Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance using Levene's test. Continuous data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare normally distributed outcomes among groups. Pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test when variances were homogeneous and Tamhane's T2 test when variances were heterogeneous. Because six outcome domains were evaluated, omnibus ANOVA p values were also adjusted across outcomes using the Holm method to reduce the risk of type I error. Omnibus effect sizes are reported as eta squared ($\eta^2$) with 95% confidence intervals (CIs). A p value < 0.05 was considered statistically significant.

## RESULTS

Representative histological images of epithelial thickness are shown in Figure 1. Epithelial thickness differed among the four groups (ANOVA $p = 0.044$; Holm-adjusted $p = 0.044$; $\eta^2 = 0.327$, 95% CI 0.131–0.737) (Table 1, Figure 2). The 10% *C. asiatica* gel group (G3) had the highest mean epithelial thickness, and the negative control/base gel group (G1) the lowest. Tamhane's T2 test showed significantly lower epithelial thickness in G1 than in G2 ($p = 0.008$), G3 ($p = 0.035$), and G4 ($p = 0.001$), with no significant differences among the treated groups.

Fibroblast counts differed among groups (ANOVA $p = 0.020$; Holm-adjusted $p = 0.040$; $\eta^2 = 0.381$, 95% CI 0.202–0.727) (Table 1, Figure 2). The highest mean fibroblast count was observed in G3 and the lowest in G1. Tukey HSD testing showed a significant difference only between G1 and G3 ($p = 0.019$); the remaining pairwise comparisons were not statistically significant.

Serum CRP levels were quantified using a standard calibration curve generated from serial CRP standards (Figure 3). The curve covered concentrations from 0.312 to 20 ng/mL and was used to convert sample absorbance values into serum CRP concentrations. The coefficient of determination was $R^2 = 0.9678$.

Serum CRP levels differed among groups (ANOVA $p = 0.002$; Holm-adjusted $p = 0.011$; $\eta^2 = 0.511$, 95% CI 0.313–0.810) (Table 1, Figure 2). G4 had the lowest mean CRP level, whereas G1 had the highest. Tukey HSD testing showed significantly lower CRP levels in G2 ($p = 0.013$) and G4 ($p = 0.002$) than in G1; the other pairwise comparisons were not statistically significant.

Collagen deposition differed among groups (ANOVA $p = 0.007$; Holm-adjusted $p = 0.027$; $\eta^2 = 0.449$, 95% CI 0.212–0.784) (Table 1, Figure 2). The highest mean collagen deposition was

observed in G3, followed by G4. Tukey HSD testing showed significantly higher collagen deposition in G3 than in G1 ($p = 0.005$); the G1–G4 comparison approached but did not reach statistical significance ($p = 0.053$). Representative histological images of tissue biomarkers are shown in Figure 4.

Angiogenesis differed among groups (ANOVA $p<0.001$; Holm-adjusted $p = 0.002$; $\eta^2 = 0.603$, 95% CI 0.503–0.805) (Table 1, Figure 2). G4 had the highest mean vessel count. Tukey HSD testing showed significantly higher angiogenesis in G3 ($p = 0.005$) and G4 ($p < 0.001$) than in G1, and in G4 than in G2 ($p = 0.029$); the G1–G2 and G3–G4 comparisons were not statistically significant.

Inflammation scores differed among groups (ANOVA $p = 0.009$; Holm-adjusted $p = 0.028$; $\eta^2 = 0.430$, 95% CI 0.207–0.750) (Table 1, Figure 2). G4 had the lowest mean inflammation score, whereas G1 had the highest. Tukey HSD testing showed significantly lower inflammation scores in G4 than in G1 ($p = 0.007$) and G3 ($p = 0.045$); the G4–G2 comparison was not statistically significant ($p = 0.136$).

## DISCUSSION

Topical *C. asiatica* gel was associated with favourable changes in selected early repair and inflammatory parameters in diabetic excision wounds. All six outcomes showed between-group differences that remained significant after Holm adjustment. However, pairwise findings varied across outcomes, and the active treatment groups were not consistently different from each other. The 10% and 20% formulations should therefore be viewed as showing different descriptive response patterns rather than a confirmed dose-response relationship. Neither formulation consistently differed from gentamicin, so superiority to the positive control was not established.

These findings are biologically plausible because diabetic wound healing is impaired by persistent inflammation, oxidative stress, reduced fibroblast activity, delayed epithelial repair, and abnormal extracellular-matrix formation (4,15). *C. asiatica* contains triterpenoid saponins and flavonoids with reported antioxidant, anti-inflammatory, fibroblast-stimulating, collagen-promoting, and pro-angiogenic properties (6,7,10,12,16,17). Fibroblasts are central to extracellular-matrix production and wound repair (18), while angiogenesis supports oxygen and nutrient delivery to regenerating tissue (19). These mechanisms may account for the observed changes, although molecular pathways were not measured directly.

The 10% *C. asiatica* gel group had the highest mean epithelial thickness, fibroblast count, and collagen deposition, whereas the 20% group had the lowest CRP level and inflammation score and the highest vessel count. This pattern may reflect differences in proliferative, vascular, and anti-inflammatory responses at day eight. Because the study was not designed or powered as a formal dose-response experiment, these concentration-related patterns remain descriptive.

Previous clinical and experimental studies have also reported improved wound healing with *C. asiatica* preparations (8,9). The anti-inflammatory findings are consistent with reports that asiaticoside and madecassoside can attenuate inflammatory signalling, including NF-κB-related pathways, and that antioxidant compounds in *C. asiatica* may reduce oxidative stress (7,10,11). Reduced inflammation may facilitate progression from the inflammatory to the proliferative phase and thereby support epithelial repair, fibroblast activity, collagen deposition, and angiogenesis. However, CRP was a systemic marker, whereas inflammatory scoring was histological; additional cytokine and molecular markers are needed to define the mechanism more precisely.

This study has several limitations. The post-test-only design did not permit assessment of baseline wound characteristics. The seven-day observation period captured early inflammatory and proliferative changes but not complete wound closure, remodelling, scar quality, or tensile strength. The small sample size limited detection of modest differences between active treatment groups. The extract was not standardised by quantitative phytochemical analysis, and the botanical source and voucher identification were not reported, so the active-compound concentrations and botanical traceability are uncertain. The method of random-sequence generation, allocation concealment, outcome-assessment blinding, and interobserver reliability were not documented. A single examiner assessed histological outcomes, and collagen deposition was estimated from haematoxylin and eosin-stained sections rather than a collagen-specific stain. The amount of topical preparation applied per wound, wound-dressing conditions, and the perioperative analgesic regimen were not documented in the source records. Potential confounders such as glucose variability, wound-depth consistency, gel distribution, and local infection may also have influenced the results. Future studies should use standardised and botanically authenticated extracts, longer follow-up, broader dose ranges, blinded assessment, collagen-specific staining, wound-closure measurements, and molecular markers such as VEGF, CD31, von Willebrand factor, and inflammatory cytokines.

## CONCLUSION

Topical *C. asiatica* gel was associated with favourable changes in selected early wound-repair and inflammatory parameters in male Wistar rats with diabetic excision wounds. The 10% and 20% formulations showed different descriptive response patterns, but the study did not establish dose-response superiority or consistent superiority to gentamicin. Further preclinical studies using standardised extracts, longer follow-up, blinded assessment, and

designs powered for dose-response comparisons are needed before clinical application can be considered.

**Funding:** No specific funding was received for this study.

**Conflicts of interest:** None to declare.

**Author contributions (CRediT):** Conceptualization—E.S. and D.I.N.; Methodology—E.S. and D.I.N.; Formal analysis—E.S. and D.I.N.; Investigation—F.A.R. and H.S.; Data curation—F.A.R. and H.S.; Writing—original draft—E.S., D.I.N., F.A.R., and H.S.; Writing—review and editing—E.S. and D.I.N.; Supervision—E.S.; Project administration—E.S. All authors reviewed and approved the final manuscript.

**Ethics statement:** Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Islam Sultan Agung, Semarang, before commencement of the study (No. 151/III/2025/Komisi Bioetik; 25 March 2025).

**Data availability statement:** The datasets generated and analysed during the study are not publicly available because public deposition was not included in the approved animal-research protocol, and the data remain subject to institutional ethical oversight.

## REFERENCES


1. Baena-Díez JM, Peñafiel J, Subirana I, Ramos R, Elosua R, Marín-Ibañez A, et al. Risk of cause-specific death in individuals with diabetes: a competing risks analysis. Diabetes Care 2016;39:1987-95. doi: 10.2337/dc16-0614.
2. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels: International Diabetes Federation; 2021. Available from: https://diabetesatlas.org/resources/previous-editions/.
3. Akkus G, Sert M. Diabetic foot ulcers: a devastating complication of diabetes mellitus continues non-stop in spite of new medical treatment modalities. World J Diabetes 2022;13:1106-21. doi: 10.4239/wjd.v13.i12.1106.
4. Bártolo I, Reis RL, Marques AP, Cerqueira MT. Keratinocyte growth factor-based strategies for wound re-epithelialization. Tissue Eng Part B Rev 2022;28:665-76. doi: 10.1089/ten.teb.2021.0030.
5. Čoma M, Fröhlichová L, Urban L, Zajíček R, Urban T, Szabo P, et al. Molecular changes underlying hypertrophic scarring following burns involve specific deregulations at all wound-healing stages (inflammation, proliferation, and maturation). Int J Mol Sci 2021;22:897. doi: 10.3390/ijms22020897.
6. Bylka W, Znajdek-Awiżeń P, Studzińska-Sroka E, Brzezińska M. *Centella asiatica* in cosmetology. Postepy Dermatol Alergol 2013;30:46-9. doi: 10.5114/pdia.2013.33378.
7. Sabaragamuwa R, Perera CO. Total triterpenes, polyphenols, flavonoids, and antioxidant activity of bioactive phytochemicals of *Centella asiatica* by different extraction techniques. Foods 2023;12:3972. doi: 10.3390/foods12213972.

8. Paocharoen V. The efficacy and side effects of oral Centella asiatica extract for wound healing promotion in patients with diabetic wounds. J Med Assoc Thai 2010;93 Suppl 7:S166-70.
9. Poernomo H, Setiawan DS. The effect of Pegagan gel (*Centella asiatica* (L.) Urban) on wound healing process in mice (*Mus musculus*) in vivo. ODONTO Dent J 2022;9:137-46. doi: 10.30659/odj.9.0.137-146.
10. Bandopadhyay S, Mandal S, Ghorai M, Jha NK, Kumar M, Radha, et al. Therapeutic properties and pharmacological activities of asiaticoside and madecassoside: a review. J Cell Mol Med 2023;27:593-608. doi: 10.1111/jcmm.17635.
11. Shen X, Guo M, Yu H, Liu D, Lu Z, Lu Y. *Propionibacterium acnes-related* anti-inflammation and skin hydration activities of madecassoside, a pentacyclic triterpene saponin from *Centella asiatica*. Biosci Biotechnol Biochem 2019;83:561-8. doi: 10.1080/09168451.2018.1547627.
12. Diniz LRL, Calado LL, Duarte ABS, de Sousa DP. *Centella asiatica* and its metabolite asiatic acid: wound-healing effects and therapeutic potential. Metabolites 2023;13:276. doi: 10.3390/metabo13020276.
13. Min DH, Yu YB, Kim TH, Kim H, Lee S. Pharmacological effects of pentacyclic triterpenoids isolated from *Centella asiatica*. Hortic Environ Biotechnol 2024;65:189-97. doi: 10.1007/s13580-023-00561-8.
14. Nayak Y, Hillemane V, Daroji VK, Jayashree BS, Unnikrishnan MK. Antidiabetic activity of benzopyrone analogues in nicotinamide-streptozotocin-induced type 2 diabetes in rats. ScientificWorldJournal 2014;2014:854267. doi: 10.1155/2014/854267.

15. Patel S, Srivastava S, Singh MR, Singh D. Mechanistic insight into diabetic wounds: pathogenesis, molecular targets, and treatment strategies to pace wound healing. Biomed Pharmacother 2019;112:108615. doi: 10.1016/j.biopha.2019.108615.
16. Park KS. Pharmacological effects of *Centella asiatica* on skin diseases: evidence and possible mechanisms. Evid Based Complement Alternat Med 2021;2021:5462633. doi: 10.1155/2021/5462633.
17. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, et al. Therapeutic potential of *Centella asiatica* and its triterpenes: a review. Front Pharmacol 2020;11:568032. doi: 10.3389/fphar.2020.568032.
18. Dick MK, Miao JH, Limaiem F. Histology, fibroblast. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. PMID: 31082109.
19. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004;56:549-80. doi: 10.1124/pr.56.4.3.

## TABLES AND FIGURES

**Table 1. Effects of topical *C. asiatica* gel on wound-healing parameters in diabetic excision wounds.**

| Variable | G1: base gel | G2: 0.1% gentamicin | G3: 10% *C. asiatica* gel | G4: 20% *C. asiatica* gel | ANOVA p | Holm-adjusted p | $\eta^2$ (95% CI) |
|---|---|---|---|---|---|---|---|
| | Mean ± SD | | | | | | |
| Epithelial thickness, µm | 35.27 ± 11.32 | 54.20 ± 9.82 | 59.07 ± 22.81 | 45.72 ± 7.85 | 0.044 | 0.044 | 0.327 (0.131–0.737) |
| Fibroblast count, cells/HPF | 38.06 ± 4.91 | 48.39 ± 4.49 | 50.33 ± 6.70 | 46.50 ± 7.31 | 0.020 | 0.040 | 0.381 (0.202–0.727) |
| CRP, ng/mL | 15.54 ± 0.15 | 14.76 ± 0.18 | 15.16 ± 0.12 | 14.60 ± 0.18 | 0.002 | 0.011 | 0.511 (0.313–0.810) |
| Collagen deposition, % | 22.90 ± 2.16 | 28.28 ± 1.23 | 34.53 ± 2.77 | 31.20 ± 2.01 | 0.007 | 0.027 | 0.449 (0.212–0.784) |
| Angiogenesis, vessels/field | 11.27 ± 1.23 | 17.17 ± 2.24 | 21.72 ± 2.39 | 25.50 ± 1.59 | <0.001 | 0.002 | 0.603 (0.503–0.805) |
| Inflammation score | 2.12 ± 0.14 | 1.83 ± 0.14 | 1.95 ± 0.09 | 1.38 ± 0.16 | 0.009 | 0.028 | 0.430 (0.207–0.750) |

Pairwise comparisons were performed using Tamhane's T2 for epithelial thickness and Tukey HSD for all other outcomes. Significant post hoc differences were as follows: epithelial thickness, G1 < G2 ($p = 0.008$), G1 < G3 ($p = 0.035$), and G1 < G4 ($p = 0.001$); fibroblast count, G1 < G3 ($p = 0.019$); CRP, G1 > G2 ($p = 0.013$) and G1 > G4 ($p = 0.002$); collagen deposition, G1 < G3 ($p = 0.005$); angiogenesis, G1 < G3 ($p = 0.005$), G1 < G4 ($p < 0.001$), and G2 < G4 ($p = 0.029$); inflammation score, G4 < G1 ($p = 0.007$) and G4 < G3 ($p = 0.045$).

CI, confidence interval; CRP, C-reactive protein; G1, negative control/base gel; G2, positive control/0.1% gentamicin; G3, 10% *C. asiatica* gel; G4, 20% *C. asiatica* gel; HPF, high-power field; SD, standard deviation.

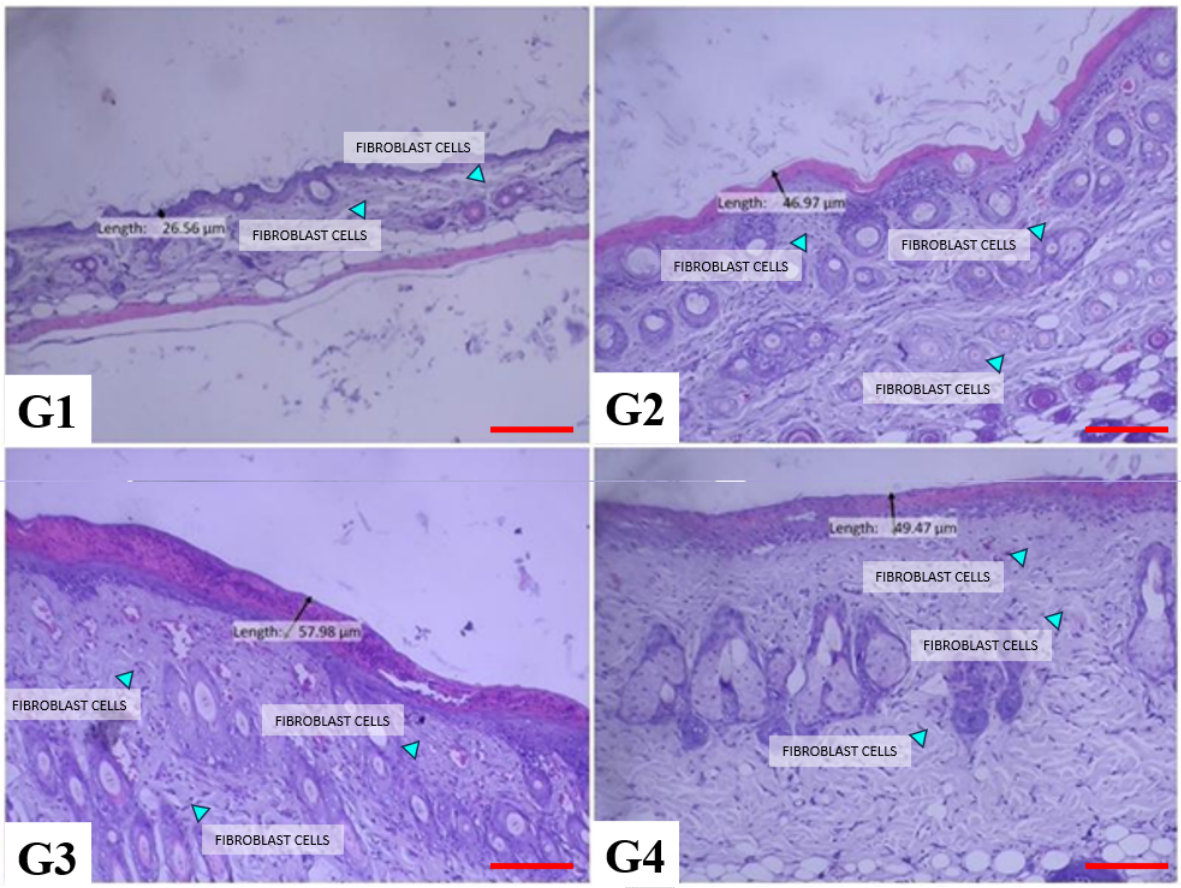


**Figure 1. Representative haematoxylin and eosin-stained sections showing epithelial thickness measurements and fibroblast distribution in diabetic excision wounds of male Wistar rats after seven days of topical treatment.**

G1, base gel; G2, 0.1% gentamicin; G3, 10% *C. asiatica* gel; and G4, 20% *C. asiatica* gel. Images were assessed at 400× magnification. The red scale bar indicates 100 µm.

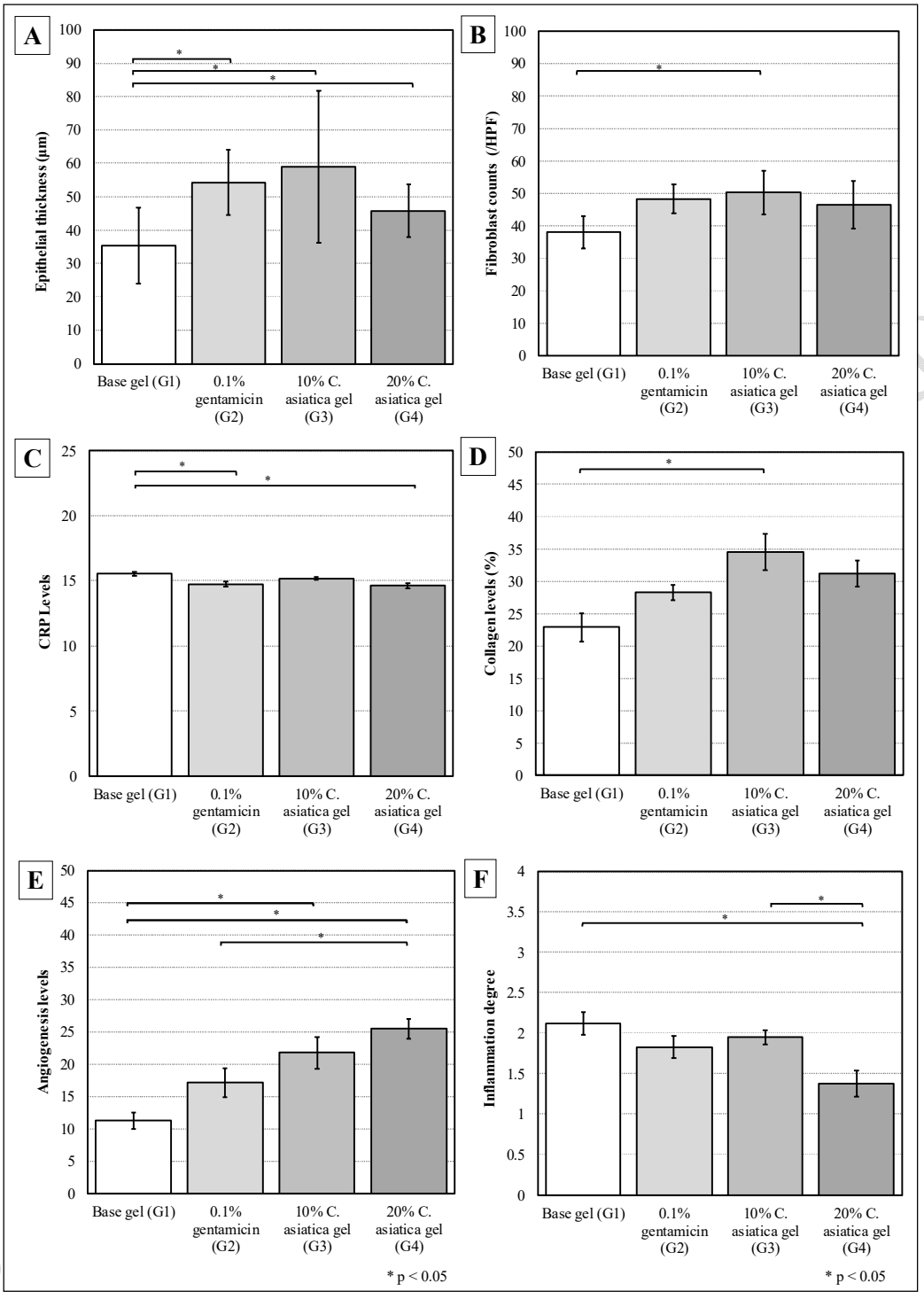


**Figure 2. Effects of topical *C. asiatica* gel on wound-healing parameters in diabetic excision wounds of male Wistar rats after seven days of treatment: (A) epithelial thickness, (B) fibroblast count, (C) C-reactive protein level, (D) collagen deposition, (E) angiogenesis, and (F) inflammation score.**

Bars represent mean ± SD. G1, base gel negative control; G2, 0.1% gentamicin positive control; G3, 10% *C. asiatica* gel; G4, 20% *C. asiatica* gel. *p < 0.05 indicates a statistically significant pairwise difference.

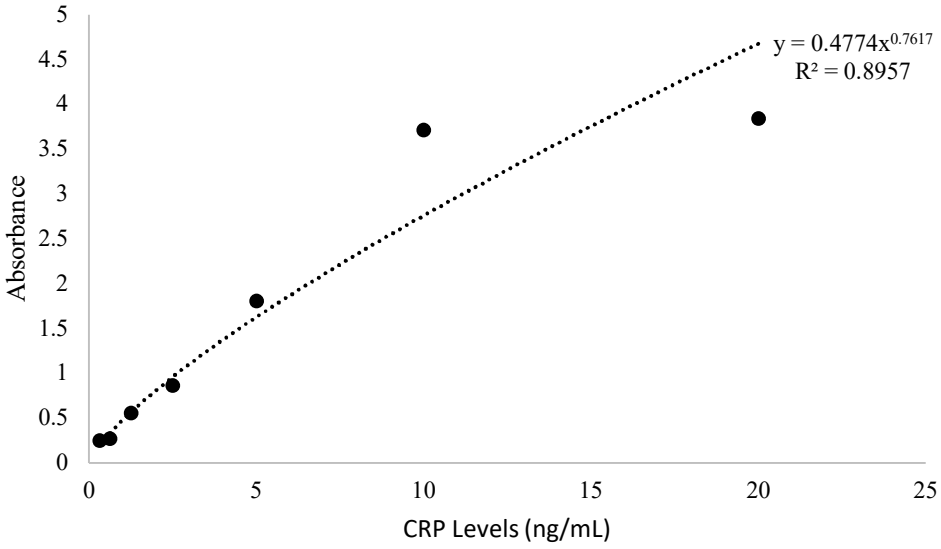


**Figure 3. Standard calibration curve for serum C-reactive protein (CRP) measurement ($R^2 = 0.9678$).**

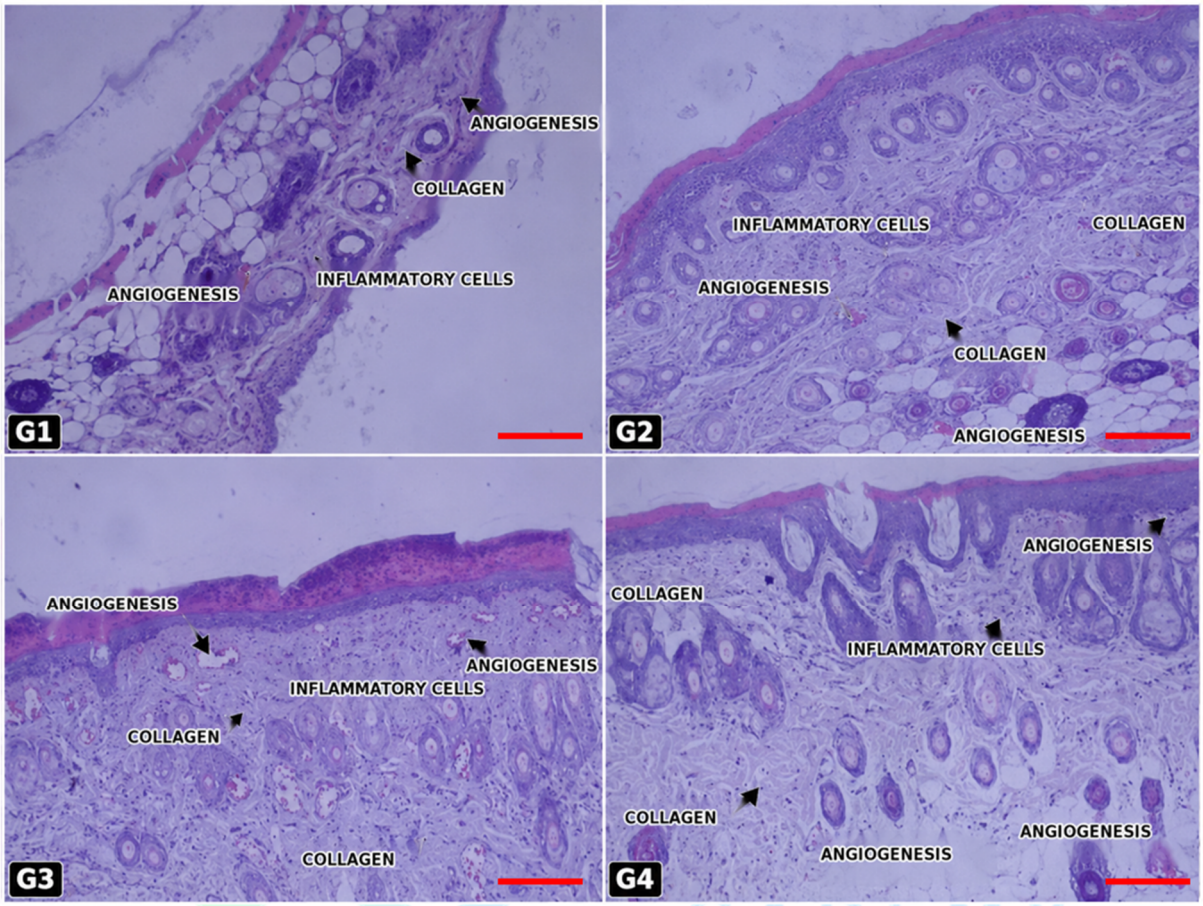


**Figure 4. Histopathological features of diabetic wound tissue in each experimental group.**

G1, base gel; G2, 0.1% gentamicin; G3, 10% *C. asiatica* gel; G4, 20% *C. asiatica* gel. The red scale bar indicates 100 μm.