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Effects of Oral *Channa striata* and Topical *Centella asiatica* on Serum IL-6 and Wound Tissue TNF- α in a Diabetic Incisional Wound Model in Wistar Rats

Wistar Sıçanlarında Diyabetik İnsizyonel Yara Modelinde Oral *Channa striata* ve Topikal *Centella asiatica*'nın Serum IL-6 ve Yara Dokusu TNF- α Üzerindeki Etkileri

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ABSTRACT

Objective: To determine the effects of topical 10% *Centella asiatica* gel, oral *Channa striata* extract, and their combination on systemic interleukin (IL)-6 and wound tissue tumor necrosis factor (TNF)- α levels in a Wistar rat model of diabetic incision wound.

Methods: This *in vivo* experimental study used a post-test-only control group design. Diabetes was induced with nicotinamide followed by streptozotocin. Male Wistar rats aged 10 weeks and weighing 180-200 g were randomized into five groups (n = 5/group): Non-diabetic wound control, diabetic wound plus vehicle, diabetic wound plus oral *Channa striata* extract, diabetic wound plus topical 10% *Centella asiatica* gel, and diabetic wound plus combination therapy. Serum IL-6 and wound tissue TNF- α were measured by sandwich ELISA at study termination.

Results: Serum IL-6 differed significantly among groups (One-Way ANOVA, p = 0.008). Compared with the diabetic vehicle group, serum IL-6 was significantly lower in the oral *Channa striata* group (p = 0.004) and the combination group (p = 0.043). The oral *Channa striata* group did not differ significantly from the combination group (Tukey HSD, p = 0.796). Wound tissue TNF- α did not differ significantly among groups (Kruskal-Wallis, p = 0.100).

Conclusion: Oral *Channa striata*, either alone or combined with topical *Centella asiatica* gel, was associated with lower serum IL-6, whereas wound tissue TNF- α was not significantly changed. The combination did not show an additive serum IL-6 reduction beyond oral monotherapy in this model.

Öz

Amaç: Diyabetik insizyonel yara oluşturulan Wistar sıçanı modelinde, topikal %10 *Centella asiatica* jeli, oral *Channa striata* ekstresi ve bu iki uygulamanın kombinasyonunun sistemik interlökin (IL)-6 ve yara dokusu tümör nekroz faktörü (TNF)- α düzeyleri üzerindeki etkilerini belirlemek.

Yöntemler: Bu *in vivo* deneysel çalışmada, yalnızca son test ölçümünün yapıldığı kontrol gruplu deneysel tasarım kullanıldı. Diyabet, nikotinamid uygulamasını takiben streptozotosin verilerek induklendi. On haftalık, 180-200 g ağırlığındaki erkek Wistar sıçanları, her grupta beş hayvan olacak şekilde (n = 5/grup) rastgele beş gruba ayrıldı: Diyabetik olmayan yara kontrol grubu, taşıyıcı uygulanan diyabetik yara grubu, oral *Channa striata* ekstresi uygulanan diyabetik yara grubu, topikal %10 *Centella asiatica* jeli uygulanan diyabetik yara grubu ve kombinasyon tedavisi uygulanan diyabetik yara grubu. Çalışma sonunda serum IL-6 ve yara dokusu TNF- α düzeyleri sandviç ELISA yöntemiyle ölçüldü.

Bulgular: Serum IL-6 düzeyleri gruplar arasında istatistiksel olarak anlamlı farklılık gösterdi (tek-yönlü varyans analizi, p = 0,008). Diyabetik taşıyıcı grubuyla karşılaştırıldığında, serum IL-6 düzeyleri oral *Channa striata* ekstresi uygulanan grupta (p = 0,004) ve kombinasyon tedavisi uygulanan grupta (p = 0,043) anlamlı olarak daha düşüktü. Oral *Channa striata* ekstresi uygulanan grup ile kombinasyon tedavisi uygulanan grup arasında istatistiksel olarak anlamlı bir farklılık saptanmadı (Tukey HSD testi, p = 0,796). Yara dokusu TNF- α düzeyleri ise gruplar arasında

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ABSTRACT

Keywords: *Centella asiatica*, *Channa striata*, diabetic wound, interleukin-6, tumor necrosis factor-alpha, diabetes mellitus

Öz

istatistiksel olarak anlamlı farklılık göstermedi (Kruskal-Wallis testi, $p = 0,100$).

Sonuç: Oral *Channa striata* ekstresi, tek başına veya topikal *Centella asiatica* jeli ile birlikte uygulandığında daha düşük serum IL-6 düzeyleriyle ilişkili bulundu; buna karşılık yara dokusu TNF- α düzeylerinde istatistiksel olarak anlamlı bir değişiklik saptanmadı. Bu modelde, kombinasyon tedavisinin serum IL-6 düzeylerini oral monoterapinin sağladığı azalmanın ötesinde düşürdüğüne ilişkin bir bulgu elde edilmedi.

Anahtar Sözcükler: *Centella asiatica*, *Channa striata*, diyabetik yara, interlökin-6, tümör nekroz faktörü-alfa, diabetes mellitus

INTRODUCTION

Diabetes mellitus remains a major global health burden, with chronic wounds among its most serious complications. Persistent hyperglycemia disrupts normal wound healing by causing vascular dysfunction, impaired cellular responses, and prolonged inflammation. Diabetic wounds are characterized by excessive inflammatory activity, including dysregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6). This sustained inflammatory state increases proteolytic activity, impairs extracellular matrix formation, and delays progression to the proliferative and remodeling phases, resulting in poor wound healing and adverse clinical outcomes (1-3).

Recent evidence supports the use of botanical and nutraceutical adjuvants to optimize wound healing in diabetes. *Centella asiatica* has been widely investigated for topical wound care; contemporary reviews and experimental studies highlight triterpenoid-rich constituents, including asiaticoside and madecassoside, that may promote fibroblast activity, collagen synthesis, re-epithelialization, angiogenesis, and inflammatory regulation (4-7). In parallel, *Channa striata* supplementation has gained attention as a systemic nutritional therapy rich in albumin and essential amino acids; clinical and experimental reports suggest acceleration across inflammatory, proliferative, and maturation phases of wound repair, including in diabetic wound contexts (8-10). A recent systematic review and meta-analysis also indicates that topical and oral herbal products can support diabetic foot ulcer healing, strengthening the rationale for integrative approaches (11).

Despite this progress, the current state of the art remains fragmented. *Centella asiatica* is commonly evaluated as topical monotherapy or in topical combinations, whereas *Channa striata* is frequently studied as a standalone oral intervention with limited concurrent assessment of inflammatory biomarkers (6-8,10). Consequently, there is limited mechanistic evidence on whether a dual-route strategy, consisting of local topical therapy plus systemic oral support, can differentially affect systemic and local inflammatory biomarkers, including serum IL-6 and wound tissue TNF- α in diabetic incisional wounds. Therefore, this study aimed to evaluate the effects of topical *Centella asiatica* gel, oral *Channa striata* extract, and their combination on serum IL-6 and wound tissue TNF- α levels in a streptozotocin-nicotinamide-induced diabetic Wistar rat incisional wound model.

MATERIALS AND METHODS

Study Design and Animals

This *in vivo* study used a post-test-only control group design and was conducted at the IBL Laboratory, Universitas Islam Sultan Agung, Semarang, Indonesia, from November 2025 to January 2026. Wistar rats weighing 180-200 g were housed with a 12-hour light/dark cycle and chow and water *ad libitum*. After 7 days of acclimatization, animals were allocated into five groups: G1, non-diabetic wound with topical base gel and oral vehicle; G2, diabetic wound with topical base gel and oral vehicle; G3, diabetic wound with oral *Channa striata* extract and topical base gel; G4, diabetic wound with topical 10% *Centella asiatica* gel and oral vehicle; and G5, diabetic wound with combined oral *Channa striata* extract and topical 10% *Centella asiatica* gel.

After acclimatization, each rat was assigned a unique identification code. Rats assigned to the non-diabetic wound control group were allocated to G1. Rats intended for the diabetic groups underwent streptozotocin-nicotinamide induction, and random blood glucose was measured 72 hours later. Only rats with random blood glucose levels ≥ 200 mg/dL were considered eligible for diabetic group allocation. Eligible diabetic rats were then randomly allocated to G2, G3, G4, or G5 using a computer-generated random number list, with a final target of five rats per group.

As illustrated in Figure 1, several subjects could not be included in the final analysis during the study period. Four subjects did not meet the inclusion criteria based on post-induction random blood glucose levels, as their values did not reach the threshold specified in the protocol, and were therefore categorized as exclusions. In addition, one subject in Group 2 died during the study period and was categorized as a dropout. Therefore, the statistical analysis and reporting of results were conducted using an effective sample size of five rats in each treatment group.

The investigator responsible for outcome data collection, including ELISA-related outcome measurement and data recording, was blinded to group allocation to minimize assessment bias. Treatment administration was performed according to the assigned intervention groups, whereas outcome measurements were conducted using coded animal and sample identifiers without revealing group information to the data collector.

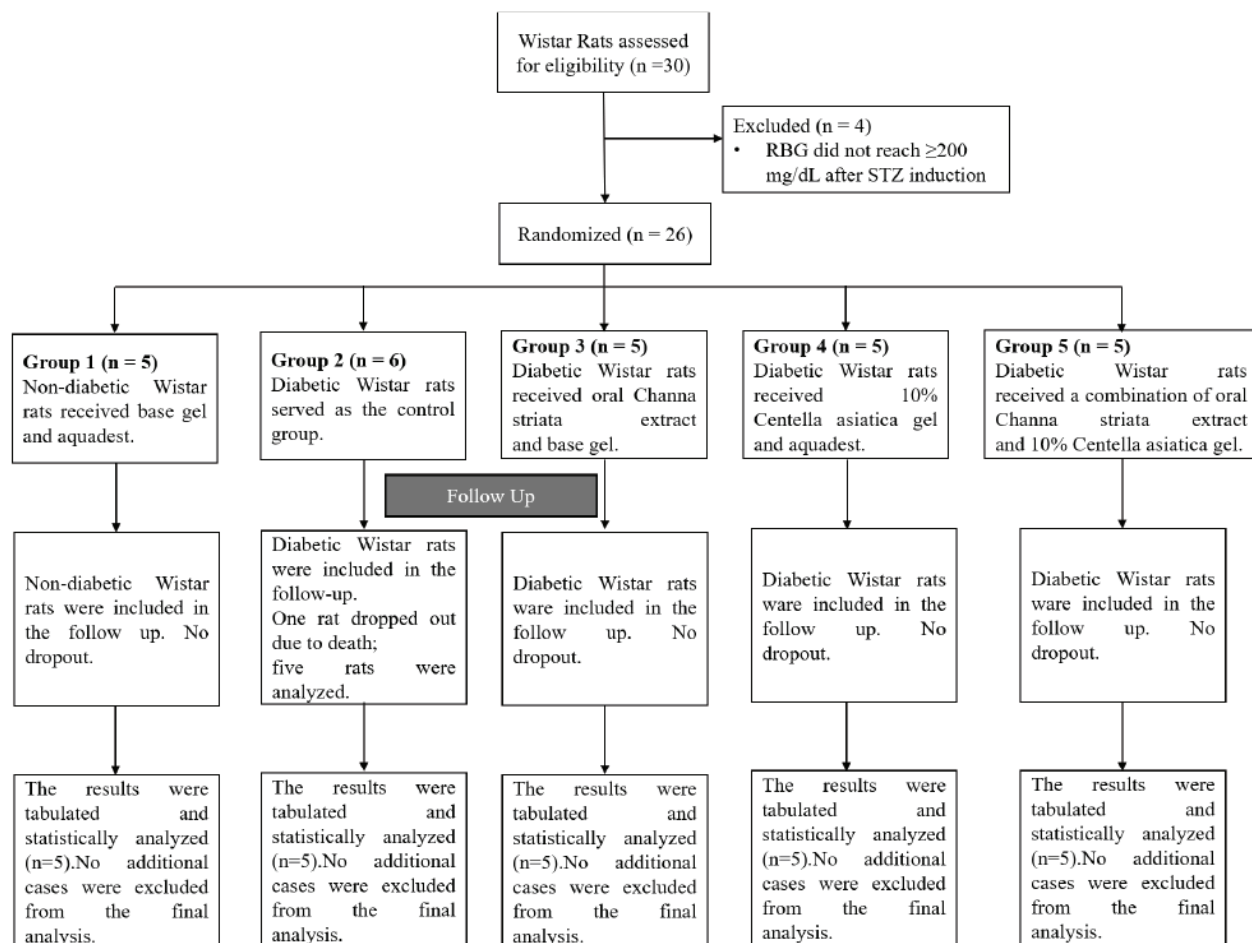


Figure 1. Animal flow diagram of the study. Thirty animals were initially prepared. Four animals were excluded because post-induction random blood glucose levels did not reach the diabetic threshold specified in the protocol. One animal in G2 died during the study period and was categorized as a dropout. The final analysis included 25 animals, with five rats in each group.

Sample size was estimated using the Federer formula, $(t-1)(n-1) \geq 15$, as a minimum sample-size approach for exploratory animal experiments involving multiple groups. This calculation yielded a minimum of five rats per group. A formal a priori power analysis based on predefined effect size, alpha level, and target power was not performed. Therefore, this study should be interpreted as an exploratory preclinical study, particularly for outcomes with potentially modest between-group differences.

Ethical Approval

Procedures were approved by the Bioethics Committee of Sultan Agung Islamic University, Semarang, Indonesia (no: 671/XII/2025/Komisi Bioetik, date: 15.12.2025). Patient consent was not applicable because this experimental study involved animals and did not include human participants.

Diabetes Induction and Wound Model

After overnight fasting, diabetes was induced by intraperitoneal nicotinamide at 120 mg/kg, followed 15 minutes later by streptozotocin at 65 mg/kg. Streptozotocin was freshly prepared immediately before injection by dissolving it in cold sodium citrate

buffer, pH 4.5. The streptozotocin solution was protected from light during preparation and administered shortly after dissolution to minimize degradation. The injection volume was calculated individually according to each rat's body weight and the prepared streptozotocin concentration to deliver the intended dose. Random blood glucose was measured from the tail vein 72 hours later, and rats with levels ≥ 200 mg/dL were considered diabetic. For wound creation, animals were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). The dorsal area was shaved, disinfected with povidone-iodine, and a 2-cm linear incision reaching the subcutaneous layer was created on the dorsal skin. The wound was left open.

An incisional wound model was selected because the primary objective of this study was to evaluate inflammatory biomarker responses in a standardized local wound environment, rather than to quantify macroscopic wound contraction or closure rate. Incisional wounds provide a reproducible linear injury with controlled length and depth, allowing wound-edge tissue sampling for local cytokine assessment. In contrast, excisional wound models are more commonly used when the main outcomes are wound area reduction,

contraction, granulation tissue formation, and closure dynamics. Therefore, the incisional model was considered appropriate for this exploratory biomarker-focused study, while the absence of wound contraction and closure-rate measurements was acknowledged as a limitation (12,13).

Interventions

Interventions were administered for 7 consecutive days. G1 and G2 received topical base gel and oral vehicle. G3 received oral *Channa striata* extract (100 mg/100 g body weight/day) with topical base gel. G4 received topical 10% *Centella asiatica* gel (200 µg/cm²) with oral vehicle. G5 received both interventions at the same doses. The *Channa striata* extract was reconstituted before use, and the *Centella asiatica* gel was prepared in a carbopol-based formulation containing 10% ethanolic extract.

Before formulation, the *Centella asiatica* ethanolic extract underwent qualitative triterpenoid screening and total flavonoid determination using a quercetin standard curve. Asiaticoside and madecassoside were not quantified. The *Channa striata* extract was not analyzed for albumin or total protein content.

Doses were selected as fixed exploratory preclinical doses based on prior wound-healing evidence for each component, rather than on formal dose-response optimization. The oral *Channa striata* dose of 100 mg/100 g body weight/day was selected to provide systemic nutritional support, because previous oral *Channa striata* studies reported improved wound-repair parameters, including tensile strength, fibroblast count, and hydroxyproline content, and systematic evidence suggests that *Channa striata* may exert wound-healing and anti-inflammatory effects through albumin, amino acids, fatty acids, and other bioactive constituents (8-10). The topical 10% *Centella asiatica* gel dose at 200 µg/cm² was selected to provide local wound-bed exposure, because topical *Centella asiatica* preparations and triterpenoid-rich formulations have been associated with improved wound repair through anti-inflammatory, antioxidant, fibroblast-stimulating, collagen-regulating, angiogenic, and re-epithelialization mechanisms (4-7,11). Because this study evaluated a dual-route combination rather than a dose-response model, one fixed dose of each intervention was used to assess preliminary biological plausibility.

Outcome Measures and Sample Collection

Random blood glucose was reassessed on day 8. At the end of treatment, blood was collected for IL-6, centrifuged, and stored until analysis. Wound-edge tissue was excised for TNF-α, rinsed with phosphate-buffered saline, and homogenized in lysis buffer.

Serum IL-6 was selected to represent systemic inflammatory activity, whereas wound tissue TNF-α was selected to represent the local inflammatory response at the wound site. Because these biomarkers were measured in different biological compartments, their responses were interpreted as complementary rather than directly equivalent indicators of inflammation.

ELISA Assay

Serum IL-6 and wound tissue TNF-α were quantified using commercially available sandwich ELISA kits specific for rat samples. Serum IL-6 was measured using a Rat IL-6 Quantikine ELISA Kit (R&D

Systems, USA; catalog no. R6000B; species reactivity: rat), whereas wound tissue TNF-α was measured using a Rat TNF-α Quantikine ELISA Kit (R&D Systems, USA; catalog no. RTA00; species reactivity: rat). For wound tissue TNF-α measurement, wound-edge tissue was weighed before homogenization, and tissue samples were homogenized using a standardized tissue-to-lysis buffer ratio. Specifically, 1 mL of fresh lysis buffer was added for every 200 mg of wound tissue. The homogenate was centrifuged, and the supernatant was used for ELISA analysis. TNF-α concentrations were calculated from the standard curve and expressed as pg/mL of tissue homogenate. The values were not normalized to total protein concentration because total protein quantification was not performed. Serum IL-6 concentrations were expressed as pg/mL. Absorbance was read at 450 nm with correction at 570/630 nm, and concentrations were calculated from standard curves according to the manufacturers' instructions.

Statistical Analysis

Data are presented as mean ± standard deviation. Normality was assessed using the Shapiro-Wilk test and homogeneity using Levene's test. Pre-treatment blood glucose levels were compared across groups using Welch's ANOVA followed by the Games-Howell post-hoc test. Post-treatment blood glucose levels and serum IL-6 levels were analyzed using One-Way ANOVA followed by Tukey's HSD post-hoc test. Within-group blood glucose changes were analyzed using the Wilcoxon signed-rank test. Wound tissue TNF-α levels were analyzed using the Kruskal-Wallis test because one group violated the normality assumption and the sample size was small, despite homogeneous variance based on Levene's test. This conservative non-parametric approach was selected to reduce reliance on normal-distribution assumptions. A p-value < 0.05 was considered statistically significant. Analyses were performed using IBM SPSS Statistics version 26.0 for Windows.

RESULTS

Phytochemical screening of the *Centella asiatica* ethanolic extract showed a positive triterpenoid result, indicated by the formation of an orange ring after the addition of acetic anhydride and H₂SO₄. Total flavonoid analysis using a quercetin standard curve (10–100 ppm; R² = 0.9988) revealed a mean absorbance of 0.64 and a total flavonoid content of 0.4006 mgQE/g extract.

As shown in Table 1, pre-treatment random blood glucose levels confirmed clear glycemic separation between the non-diabetic control group and the diabetic groups. G1 showed normoglycemic values, with a mean RBG of 104.2 ± 11.78 mg/dL and a range of 93–122 mg/dL. In contrast, the diabetic groups showed substantially higher pre-treatment RBG values, ranging from 344.6 ± 27.36 mg/dL in G5 to 396.2 ± 61.27 mg/dL in G4, confirming successful induction of diabetes before treatment allocation. After treatment, G1 remained normoglycemic, with a mean RBG of 78.4 ± 21.27 mg/dL. The diabetic groups continued to show higher RBG levels than G1, although descriptive variation was observed across groups. Among the diabetic groups, the highest post-treatment mean RBG was observed in G2 (355.0 ± 25.24 mg/dL), whereas the lowest was observed in G5 (290.6 ± 49.55 mg/dL).

As shown in Table 2 and Figure 2, serum IL-6 levels varied across groups, with the highest mean observed in G2 (6.478 ± 1.256 pg/

mL) and the lowest mean observed in G3 (4.064 ± 0.509 pg/mL). The combination group (G5) showed a lower IL-6 mean (4.708 ± 0.609) than G2. Assumption testing supported parametric comparison, with Shapiro-Wilk $p > 0.05$ for all groups and Levene's test $p = 0.512$. One-Way ANOVA confirmed a statistically significant difference in

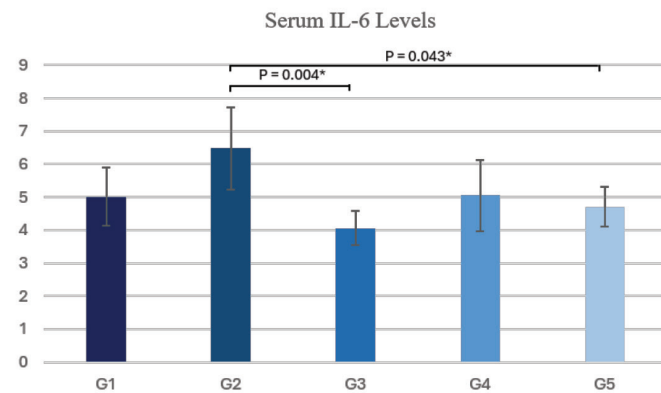


Figure 2. Serum IL-6 concentrations across experimental groups. Data are presented as mean $\pm$ SD (pg/mL), $n = 5$ per group. Statistical analysis was performed using One-Way ANOVA followed by Tukey HSD post-hoc test. * $p < 0.05$ for the indicated pairwise comparison.

Data are presented as mean $\pm$ SD. The asterisk (*) indicates statistically significant post-hoc differences between the indicated groups ($p < 0.05$). SD: Standard deviation, IL: Interleukin.

serum IL-6 among groups ($F = 4.703$; $p = 0.008$), with a large effect size (eta-squared = 0.485).

Post-hoc analysis using Tukey HSD showed that the statistically significant differences in serum IL-6 were primarily driven by comparisons involving G2. Serum IL-6 was significantly higher in G2 than in G3 (mean difference = 2.414, $p = 0.004$) and G5 (mean difference = 1.770, $p = 0.043$). Other relevant post-hoc comparisons were not statistically significant, including G1 vs. G2 ($p = 0.121$), G2 vs. G4 ($p = 0.139$), G3 vs. G4 ($p = 0.445$), G3 vs. G5 ($p = 0.796$), and G4 vs. G5 ($p = 0.973$). These findings indicate that the combination group did not differ significantly from either oral *Channa striata* monotherapy or topical *Centella asiatica* monotherapy and did not demonstrate an additional systemic IL-6 reduction beyond oral *Channa striata* alone.

Table 3 and Figure 3 summarize wound tissue TNF- α concentrations expressed as pg/mL of tissue homogenate, together with assumption testing and the between-group comparison. Mean TNF- α was 230.82 ± 16.41 in G1, 234.12 ± 19.71 in G2, 245.44 ± 4.26 in G3, 239.91 ± 15.11 in G4, and 252.83 ± 5.83 in G5. Shapiro-Wilk testing identified a deviation from normality in G2 ($p = 0.042$), whereas the remaining groups satisfied the normality assumption ($p > 0.05$). Variance homogeneity was supported by Levene's test ($p = 0.112$). Although variance homogeneity was satisfied, the Kruskal-Wallis test was selected because one group violated the normality assumption and the sample size was small. This conservative non-parametric approach was used to reduce reliance on normal-distribution

Table 1. Pre - and post-treatment random blood glucose levels across experimental groups.

Group	n	Pre-treatment RBG, mean $\pm$ SD (mg/dL) (min-max)	Post-treatment RBG, mean $\pm$ SD (mg/dL) (min-max)
G1	5	104.2 $\pm$ 11.78 (93-122)	78.4 $\pm$ 21.27 (58-112)
G2	5	381.6 $\pm$ 50.76 (329-445)	355.0 $\pm$ 25.24 (326-392)
G3	5	393.8 $\pm$ 31.29 (361-425)	327.0 $\pm$ 35.27 (292-364)
G4	5	396.2 $\pm$ 61.27 (314-465)	336.0 $\pm$ 30.09 (298-374)
G5	5	344.6 $\pm$ 27.36 (310-382)	290.6 $\pm$ 49.55 (216-339)

RBG: Random blood glucose, SD: Standard deviation, G1: Non-diabetic wound control, G2: Diabetic wound plus vehicle, G3: Diabetic wound plus oral *Channa striata* extract, G4: Diabetic wound plus topical 10% *Centella asiatica* gel, G5: Diabetic wound plus combination therapy.

Table 2. Serum IL-6 concentrations (pg/mL): Descriptive statistics, assumption tests, and One-Way ANOVA.

Parameter	G1	G2	G3	G4	G5
Mean $\pm$ SD (pg/mL)	5.012 $\pm$ 0.883	6.478 $\pm$ 1.256	4.064 $\pm$ 0.509	5.056 $\pm$ 1.081	4.708 $\pm$ 0.609
Shapiro-Wilk p-value	0.975	0.357	0.806	0.893	0.954
Levene p-value (based on mean)	0.512				
One-Way ANOVA p-value	0.008*				

Shapiro-Wilk $p > 0.05$ indicates normal distribution. Levene $p > 0.05$ indicates homogeneous variances, *: Indicates $p < 0.05$ (statistically significant), SD: Standard deviation, G1: Non-diabetic wound control, G2: Diabetic wound plus vehicle, G3: Diabetic wound plus oral *Channa striata* extract, G4: Diabetic wound plus topical 10% *Centella asiatica* gel, G5: Diabetic wound plus combination therapy, IL: Interleukin.

assumptions. The Kruskal-Wallis test did not demonstrate a statistically significant difference in wound tissue TNF- α across groups ($\chi^2 = 7.769$, $df = 4$, $p = 0.100$).

Individual animal-level values for pre-treatment random blood glucose, post-treatment random blood glucose, serum IL-6, and wound tissue TNF- α are provided in Supplementary Table S1.

DISCUSSION

This study showed a clearer effect on systemic IL-6 than on wound tissue TNF- α . Oral *Channa striata* (G3) and the combined intervention of topical *Centella asiatica* plus oral *Channa striata* (G5) were associated with significantly lower serum IL-6 compared with the diabetic vehicle group (G2), whereas wound tissue TNF- α did not differ significantly among groups. This pattern suggests that the tested interventions modulated systemic inflammatory tone more detectably than local TNF- α expression at the selected endpoint. Because blood glucose changes among diabetic groups were not significant, the IL-6 reduction was more likely related to inflammatory modulation than to short-term glycemic improvement.

The key negative finding of this study is the absence of a clear combination advantage. Although both oral *Channa striata* monotherapy and the combination treatment showed lower serum IL-6 than the diabetic vehicle group, the combination group was not significantly different from oral *Channa striata* alone (G3 vs. G5,

$p = 0.796$). Therefore, the present data do not support superiority of the combined intervention over oral *Channa striata* monotherapy, and the findings should not be interpreted as evidence of additive or synergistic anti-inflammatory effects.

The diabetic vehicle group showed the highest serum IL-6, which was consistent with the inflammatory biology of diabetic wounds. Hyperglycemia can sustain oxidative stress, immune-cell dysfunction, macrophage dysregulation, and delayed inflammatory resolution, thereby maintaining a pro-inflammatory cytokine milieu that includes IL-6 and TNF- α (14-17). However, the difference in serum IL-6 between the non-diabetic wound control group (G1) and the diabetic vehicle group (G2) was not statistically significant. This finding does not invalidate the diabetic wound model, because successful diabetes induction was supported by clear post-induction hyperglycemia and sustained elevation of random blood glucose in the diabetic groups (18). Nevertheless, it indicates that serum IL-6 alone may not function as a robust systemic inflammatory discriminator between non-diabetic and diabetic wounded rats in this experimental setting, since circulating IL-6 is generally interpreted as a marker of peripheral or systemic inflammatory activity (19). Several factors may explain this pattern, including the acute incisional wound stimulus in both G1 and G2, the short observation window, biological variability, and the limited sample size, as wound healing involves dynamic and phase-dependent inflammatory responses mediated by immune cells and pro-inflammatory cytokines (20). Therefore, the IL-6 results should be interpreted primarily as treatment-associated systemic inflammatory modulation within this model, rather than as definitive evidence that serum IL-6 independently distinguished diabetic from non-diabetic wound inflammation. In this context, the lower IL-6 levels observed in G3 and G5 should be interpreted as attenuation of systemic inflammatory activity, not as complete suppression of the inflammatory phase required for tissue repair.

The most important interpretive point is that the serum IL-6 effect appeared to be driven primarily by the oral *Channa striata* component. This interpretation was supported by the post-hoc pattern: G3 had the lowest mean IL-6, G5 was also lower than G2, and G3 did not differ significantly from G5 ($p = 0.796$). Oral *Channa striata* may influence systemic inflammatory status through albumin-rich nutritional support, amino acid availability, and bioactive lipid or protein-related components that participate in tissue repair and immune regulation (8-10). Therefore, the dual-route design remains theoretically plausible, but the systemic cytokine signal observed in this study was not additive when topical *Centella asiatica* was combined with oral *Channa striata*.

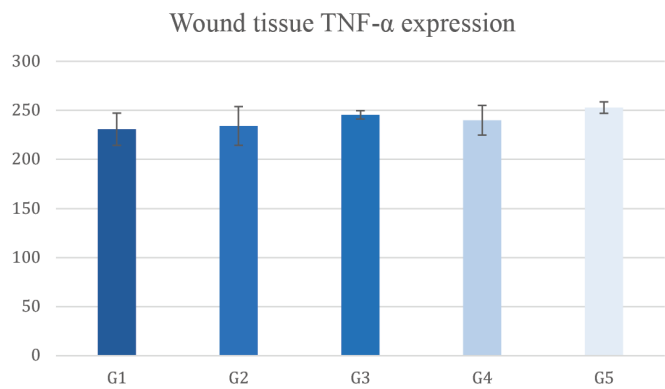


Figure 3. Wound tissue TNF- α concentrations across experimental groups. Data are presented as mean $\pm$ SD (pg/mL tissue homogenate), $n = 5$ per group. Statistical analysis was performed using the Kruskal-Wallis test. No statistically significant difference was observed among groups ($p = 0.100$). SD: Standard deviation, TNF: Tissue tumor necrosis factor.

Table 3. Wound tissue TNF- α concentrations (pg/mL tissue homogenate): Descriptive statistics and assumption tests (Shapiro-Wilk, Levene) with Kruskal-Wallis.

Parameter	G1	G2	G3	G4	G5
Mean $\pm$ SD	230.82 $\pm$ 16.41	234.12 $\pm$ 19.71	245.44 $\pm$ 4.26	239.91 $\pm$ 15.11	252.83 $\pm$ 5.83
Shapiro-Wilk p-value	0.878	0.042	0.901	0.267	0.440
Levene p-value (based on mean)	0.112				
Kruskal-Wallis p-value	0.100				

Shapiro-Wilk $p > 0.05$ indicates normal distribution. Levene $p > 0.05$ indicates homogeneous variances, SD: Standard deviation, G1: Non-diabetic wound control, G2: Diabetic wound plus vehicle, G3: Diabetic wound plus oral *Channa striata* extract, G4: Diabetic wound plus topical 10% *Centella asiatica* gel, G5: Diabetic wound plus combination therapy, TNF: Tissue tumor necrosis factor.

The absence of additive IL-6 reduction in the combination group may have several biological explanations. First, oral *Channa striata* may have produced a near-maximal systemic IL-6 response within the short treatment window, leaving limited measurable room for further reduction by the topical component. Second, topical *Centella asiatica* may act predominantly at the wound microenvironment rather than in the circulation, through effects on fibroblast activity, extracellular matrix formation, re-epithelialization, angiogenesis, oxidative stress, and local inflammatory signaling (4-7). Third, a single serum cytokine at one endpoint may not capture local reparative benefits of topical therapy. Thus, the present findings do not negate the rationale for combination therapy, but they indicate that systemic IL-6 alone is insufficient to prove an added contribution of topical *Centella asiatica*.

The TNF- α findings require careful interpretation. Although mean wound tissue TNF- α was numerically highest in G5, the overall between-group difference was not statistically significant ($p = 0.100$). This directional pattern warrants discussion because the combination group showed a higher mean TNF- α value than both the non-diabetic wound control group and the diabetic vehicle group. One possible interpretation is that the combined intervention may have been associated with transiently greater local inflammatory signaling at day 7, which could reflect ongoing wound-edge remodeling rather than treatment failure (21,22). However, this interpretation remains speculative because wound inflammation is dynamic and phase-dependent, particularly in diabetic wounds, and the present study did not include serial cytokine measurements, histological assessment, wound-closure analysis, collagen deposition, or angiogenesis endpoints to distinguish adaptive inflammatory activity from persistent maladaptive inflammation (14,21,22). We therefore interpret this pattern primarily as biological variability and limited statistical resolution rather than evidence that the combination therapy increased local inflammation.

Serum IL-6 and wound tissue TNF- α were measured in different biological compartments; therefore, serum IL-6 was interpreted as a marker of systemic or peripheral inflammatory activity, whereas wound tissue TNF- α was interpreted as a marker of local wound-site inflammation. These outcomes were considered complementary and were not directly compared as equivalent inflammatory readouts (19,21,22).

TNF- α expression in wounds is dynamic and phase-dependent; at approximately one week after injury, local inflammatory signaling may still coexist with active repair processes. Consequently, local TNF- α values should not be assumed to parallel serum IL-6, and a single endpoint cannot determine whether the observed TNF- α pattern represented adaptive inflammation, delayed resolution, or random variation.

Overall, the findings refine rather than fully confirm the original dual-route premise. The study supports a systemic anti-inflammatory contribution of oral *Channa striata*, while the incremental systemic benefit of adding topical *Centella asiatica* was not demonstrated by serum IL-6 measurement. Importantly, this study should be

interpreted as an inflammatory biomarker study in a diabetic incisional wound model rather than as direct evidence of improved wound repair. No direct wound-healing endpoints were assessed, such as wound closure rate, wound area reduction, epithelialization, granulation tissue formation, collagen deposition, angiogenesis, or histopathological scoring. Therefore, the present findings indicate changes in selected inflammatory biomarkers, particularly systemic IL-6, but do not demonstrate that the interventions improved structural or functional wound healing. Future combination studies should not rely solely on circulating cytokines but should integrate serial cytokine profiling with local structural and functional endpoints, including wound contraction, re-epithelialization, collagen deposition, angiogenesis, histopathology, and tissue-level inflammatory markers. Such endpoints are needed to determine whether biomarker modulation translates into improved wound repair (20-22).

Study Limitations

This study has several limitations. The sample size was determined using the Federer formula rather than a formal power analysis, which may have limited the ability to detect smaller between-group differences, particularly for wound tissue TNF- α . Outcome measurement was performed with coded samples and blinded assessment, but treatment administration was not blinded because the interventions differed in route and formulation. Extract characterization was limited because specific *Centella asiatica* triterpenoids and *Channa striata* protein components were not quantified. Cytokines were measured at a single endpoint, and serum IL-6 did not differ significantly between G1 and G2, limiting its discriminatory value as a systemic inflammatory marker in this model. No direct structural or functional wound-healing endpoints, such as wound contraction, epithelialization, collagen deposition, angiogenesis, or histopathological scoring, were assessed. Therefore, the observed IL-6 reduction cannot be interpreted as direct evidence of improved tissue repair. Additionally, only male Wistar rats and an incisional wound model were used, limiting generalizability to chronic diabetic ulcers in humans.

CONCLUSION

Oral *Channa striata*, either alone or combined with topical 10% *Centella asiatica* gel, was associated with lower serum IL-6 compared with diabetic vehicle treatment in this Wistar rat diabetic incisional wound model. Wound tissue TNF- α did not differ significantly among groups, and the combination did not show an additive systemic IL-6 reduction beyond oral *Channa striata* monotherapy.

Ethics

Ethics Committee Approval: Procedures were approved by the Bioethics Committee of Sultan Agung Islamic University, Semarang, Indonesia (no: 671/XII/2025/Komisi Bioetik, date: 15.12.2025).

Informed Consent: Patient consent was not applicable because this experimental study involved animals and did not include human participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.I.N., E.K., Concept: D.I.N., E.K., J.W.W., Design: D.I.N., E.K., J.W.W., Data Collection or Processing: D.I.N., Analysis or Interpretation: D.I.N., E.K., J.W.W., Literature Search: D.I.N., E.K., Writing: D.I.N.

Conflict of Interest: No conflict of interest was declared by the authors.

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Supplementary Table S1: <https://d2v96fxpocvxx.cloudfront.net/ae7a798f-afa1-42f4-9efd-6a2ed974293f/content-images/e1aca7eb-de59-4997-9834-9e23cf063882.pdf>

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