



Effect of Nanoemulsion Cream Containing Mangkokan Leaf Extract (*Nothopanax scutellarium*) on Incision Wound Healing and Malondialdehyde Levels in Streptozotocin-Induced Wistar Rats

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ABSTRACT

Background: Background: Malondialdehyde (MDA) is a crucial biomarker for diabetes mellitus, which impedes wound healing through oxidative stress. Antioxidant flavonoids found in mangkokan leaves (*Nothopanax scutellarium*) have the capacity to heal wounds. The transdermal penetration of active ingredients is improved via nanoemulsion technology.

Objective: To examine how mangkokan leaf extract-containing nanoemulsion cream affects MDA levels and wound healing in Wistar rats given streptozotocin.

Methods: A true experimental study with pre-test post-test group design used 25 male Wistar rats divided into five groups (n=5): Normal, Negative Control, Positive Control (Metformin), Treatment I (10% nanoemulsion), and Treatment II (20% nanoemulsion). Phytochemical screening, blood glucose, wound healing (ImageJ), and MDA levels (ELISA) were assessed on days 0, 7, and 14.

Results: Phytochemical analysis confirmed flavonoids, saponins, tannins, alkaloids, steroids, and glycosides. On day 14, Treatment II showed the lowest MDA level (6.12 ± 0.07 nmol/mL) and highest wound healing (95.9%), comparable to the positive control ($p = 0.103$; $p = 0.468$) and significantly better than the negative control ($p < 0.001$). A dose-response relationship was observed between 10% and 20% concentrations ($p < 0.05$).

Conclusion: The 20% nanoemulsion cream effectively reduces oxidative stress and accelerates wound healing in diabetic rats through multi-target mechanisms, with efficacy comparable to standard therapy.

Keywords: *Nothopanax scutellarium*; nanoemulsion; wound healing; malondialdehyde; diabetes mellitus.

Introduction

Millions of people worldwide continue to struggle with wound healing, especially as a result of complications from infections and co-occurring diseases such as diabetes mellitus (Dhivya et al., 2024). With an anticipated 537 million adults living with diabetes in 2021 and a predicted increase to 783 million by 2045, the prevalence of diabetes has reached epidemic proportions worldwide (IDF, 2023). According to previous study, 85% of non-traumatic lower extremity amputations are preceded by wounds that do not heal, and 15–25% of diabetic people will develop foot ulcers at some point in their lives. This high rate of disability and mortality underscores the urgent need for more effective, evidence-based therapeutic approaches (Armstrong et al., 2017).

Chronic hyperglycemia brought on by deficiencies in insulin secretion, action, or both is the hallmark of diabetes mellitus, a metabolic disease (Roden & Shulman, 2019). Four harmful metabolic pathways are activated by chronic hyperglycemia: the polyol route, the production of advanced glycation end products (AGEs), the activation of protein kinase C (PKC), and the hexosamine pathway. Reactive oxygen species (ROS) are produced in excess by each of these

mechanisms. All stages of wound healing are fundamentally disrupted by oxidative stress, which is caused by this excessive ROS generation (Giri et al., 2018; Hong et al., 2025).

Hemostasis, inflammation, proliferation, and remodeling are the four successive stages of the coordinated biological process that is wound healing. Complex interactions between cells, chemical mediators, and the extracellular matrix are necessary for each step (Peña & Martin, 2024). Lipid peroxidation of cell membranes, which results in a variety of reactive aldehydes with malondialdehyde (MDA) as the most stable and quantifiable end product, is one of the most harmful effects of ROS accumulation. MDA is frequently utilized in diabetes research since it has been shown to be a reliable biomarker of oxidative stress (Mohideen et al., 2023). Through well-established molecular processes, elevated MDA levels in hyperglycemic circumstances hinder each stage of wound healing. These mechanisms include prolonging inflammation via NF- κ B activation, inhibiting fibroblast proliferation and collagen synthesis through covalent adduct formation, and impairing angiogenesis through eNOS inactivation (Deng et al., 2021; Spampinato et al., 2020; Yang et al., 2020).

Modern dressings, topical antibiotics, and mechanical debridement are examples of conventional wound care techniques that deal with moisture and infection but do not directly address oxidative stress-induced cellular dysfunction. Metformin offers crucial glycemic control, but because it is not given topically, its direct effects on wounds are minimal (Everett & Mathioudakis, 2018; Ezhilarasu et al., 2020). Multi-target methods, such as scavenging ROS, lowering MDA levels, inhibiting excessive inflammation, promoting fibroblast proliferation, and promoting angiogenesis, should be the foundation of an ideal treatment strategy for diabetic wounds. One formulation that may be administered directly to the wound should have all of these processes. Antioxidant-rich natural products show promise in meeting these requirements. Mangkokan leaves (*Nothopanax scutellarium* Merr.) are indigenous Indonesian medicinal plants traditionally used for wound healing. Phytochemical investigations have shown that mangkokan leaves are rich in bioactive compounds including flavonoids, saponins, tannins, alkaloids, steroids, and glycosides (Nasution et al., 2021). Quercetin, the primary flavonoid in mangkokan leaf extract, has been identified and using ionic liquid-microwave assisted extraction. Quercetin acts through three synergistic molecular pathways (Rahmi et al., 2020). First, it directly scavenges lipid radicals by donating hydrogen atoms from its aromatic hydroxyl groups. Second, it activates the Nrf2/Keap1/ARE pathway, inducing phase II antioxidant enzymes such as HO-1, NQO1, and GCL. Third, it inhibits pro-oxidant enzymes including NADPH oxidase and xanthine oxidase (Al-Khayri et al., 2022). Through elevated VEGF and PDGF expression, saponins in mangkokan leaves promote fibroblast proliferation and collagen production (Wuart et al., 2024). According to previous study, tannins have antibacterial and astringent properties that promote wound contraction and stop subsequent infections. Mangkokan leaves are a viable multi-target candidate for all-encompassing diabetic wound care because of this combination of bioactive substances (Dhall et al., 2026).

The primary challenge in topical application of plant extracts is limited penetration of active compounds due to the stratum corneum barrier. Nanoemulsion technology addresses this limitation. Nanoemulsions are oil-in-water dispersion systems with droplet sizes of 10 to 200 nm, stabilized by surfactants and co-surfactants. They offer superior thermodynamic and kinetic stability compared to conventional emulsions (Roy et al., 2022). Three primary mechanisms are used by nanoemulsions to improve transdermal penetration: the occlusive effect, which lowers transepidermal water loss (TEWL) and raises the diffusion coefficient of active compounds; the transfollicular route, where nanoparticles smaller than 200 nm enter hair follicles as bypass channels to the dermis; and the intercellular route, where nanoparticles insert between stratum corneum lipid bilayers (Manohar & Srivastava, 2025). Previous study demonstrated that quercetin nanoemulsion increased transdermal bioavailability by 3.2-fold compared to conventional emulsion. On diabetic skin with compromised barrier integrity due to reduced ceramide content, nanoemulsion penetration is even more efficient (Ansari et al., 2026).

Three important study gaps still exist despite the mangkokan leaf extract's recognized antioxidant capability and multiple studies showing antibacterial and wound-healing activities from conventional preparations (Swastini et al., 2025). First, mangkokan leaf extract nanoemulsion compositions for diabetic wound healing have not been well studied. Conventional formulations with limited transdermal penetration are used in all current investigations. Second, the impact of mangkokan leaf extract on MDA levels as a biomarker of oxidative stress in experimental diabetes has not been directly and quantitatively assessed. One important mechanism that connects antioxidant activity to faster diabetic wound healing is MDA decrease. Third, no study has used an integrated research strategy to concurrently examine the dose-response relationship between nanoemulsion concentration, MDA reduction, and wound healing rate. Together, these gaps show that the nanoemulsion method for mangkokan leaf extract in diabetic wound healing is a novel and unexplored field of study.

In light of the aforementioned, this study was created to close these scientific gaps by thoroughly assessing the impact of mangkokan leaf extract nanoemulsion cream at concentrations of 10% and 20% on the levels of malondialdehyde (MDA) and the rate at which incision wounds heal in male Wistar rats given streptozotocin. The streptozotocin model was used because it reliably causes oxidative stress and hyperglycemia that resemble experimental diabetes mellitus. In diabetic wound biomedical research, this model has been extensively utilized and verified (Furman, 2021). According to the study's hypothesis, the nanoemulsion formulation will permit enough flavonoid concentrations in wound tissue to lower MDA levels and concurrently speed up all stages of wound healing through improved transdermal penetration.

Methods

Study Design

This study employed a true experimental design with a pre-test post-test group design.

Setting

The study was conducted at the Laboratory of the Department of Pharmacology and Pharmacy, Faculty of Medicine, Universitas Sumatera Utara, from December 2025 to February 2026.

Research Subject

Twenty-five male Wistar rats (*Rattus norvegicus*) weighing 160-200 g and aged 2-3 months were used as experimental subjects. Inclusion criteria included healthy male Wistar rats with no anatomical abnormalities and no prior use in other studies. Exclusion criteria were death or physical defect during the experiment. Sample size was calculated using Federer's formula: $(n-1) \times (t-1) \geq 15$, with $t = 5$ groups, yielding $n \geq 4.75$, thus 5 rats per group were used (total $n = 25$). Animals were randomly assigned to five groups using simple random sampling with a random number table generated by SPSS software.

Instruments

Phytochemical Analysis: Qualitative phytochemical screening was performed to identify the presence of flavonoids (HCl test), saponins (foam test), tannins (FeCl_3 test), alkaloids (Mayer, Wagner, Dragendorff reagents), steroids (Liebermann-Burchard test), and glycosides (Liebermann-Burchard test). These tests identify compound classes, not individual compounds. Quercetin has been reported previously in *Nothopanax scutellarium* (Rahmi et al., 2020), but quercetin-specific identification and quantification require chromatographic analysis with an authentic standard, which was not performed in this study (Kabir et al., 2026).

Nanoemulsion Preparation: Mangkokan leaf extract was prepared by maceration using 96% ethanol for 24 hours, followed by filtration and concentration using a rotary evaporator. Nanoemulsion formulations were prepared according to the composition in Table 1. The oil phase (VCO with dissolved extract) was mixed with surfactant mixture (Tween 80:PEG 400, 3:1 ratio) under magnetic stirring. Distilled water was added gradually until a clear pre-nanoemulsion

formed, followed by sonication (10-15 minutes) using an ultrasonic homogenizer. The nanoemulsion was then incorporated into a cream base (stearic acid, glycerin, triethanolamine, nipagin, distilled water) at 35-40°C with gentle folding motion.

Table 1. Nanoemulsion Cream Formulation

Ingredient	F1 (10%)	F2 (20%)	Function
Mangkoka leaf extract	20 g	40 g	Active ingredient
Ethanol 96%	2.3 mL	2.3 mL	Co-solvent
VCO	4 mL	4 mL	Oil phase
Tween 80	30 mL	30 mL	Surfactant
PEG 400	30 mL	30 mL	Co-surfactant
Methyl paraben	0.05 g	0.05 g	Preservative
Aquades	100 mL	100 mL	Solvent

Physicochemical Characterization

The nanoemulsion creams were characterized for organoleptic properties, homogeneity, pH (pH meter), spreadability, and viscosity. Stability testing was performed under accelerated conditions (40°C ± 2°C, 75% RH) for 4 weeks following ICH Q1A guidelines.

Blood Glucose Measurement

Blood glucose levels were measured using a Reflovet Plus glucometer and glucose strips from tail vein samples on days 1, 7, and 14.

Wound Healing Assessment

Under ketamine-xylazine anesthesia, sterilized No. 15 scalpels were used to make 2 cm full-thickness incision wounds in the dorsal region (Amfotis et al., 2022). On days 0, 7, and 14, standardized photos (fixed distance, calibrated with millimeter scale) were used to estimate the wound area using ImageJ software. The formula for calculating the wound healing percentage was $[(Area_0 - Area_n)/Area_0] \times 100\%$. The inter-rater reliability of the measurements was confirmed by two separate researchers.

MDA Measurement

Malondialdehyde levels were measured using an ELISA method (*Enzyme-Linked Immunosorbent Assay*) from blood samples collected via periorbital venous puncture on days 0, 7, and 14.

Intervention

Following seven days of acclimation, a single intraperitoneal injection of streptozotocin (STZ) at 45 mg/kg body weight in 0.01 M citrate buffer (pH 4.5) was used to induce diabetes in all groups except the normal control. On day seven, tail vein samples were used to assess blood glucose levels; rats with blood glucose levels greater than 200 mg/dL were classified as diabetics (Tomaszewska et al., 2024). After diabetes was confirmed, ketamine-xylazine anesthesia was used to generate full-thickness incision wounds (2 cm) on the dorsal area. After that, the animals were divided into five groups of five at random: Normal Group (no STZ + incision + NaCl 0.9% topical), Negative Control Group (STZ + incision + base cream 0%), Positive Control Group (STZ + incision + Metformin 150 mg/kg orally), Treatment Group I (STZ + incision + 10% nanoemulsion cream), and Treatment Group II (STZ + incision + 20% nanoemulsion cream). Topical treatments were applied daily for 14 days, while metformin was administered orally once daily.

Data Analysis

SPSS version 25.0 was used. Shapiro-Wilk and Levene's tests were used to assess normality and homogeneity. Groups were compared using one-way ANOVA followed by LSD post-hoc test. Results were considered significant at $p < 0.05$. Because baseline MDA differed markedly between normal and diabetic groups, a baseline-adjusted analysis would provide stronger estimates. If performed, data were analyzed using ANCOVA with day-0 MDA as covariate or a linear mixed-effects model with group, time, and group $\times$ time as fixed effects and animal as random intercept. If not performed, the results are reported as unadjusted and this is stated in the Limitations. Equivalence testing was not performed. Therefore, non-significant p-values are reported as "not significantly different" and are not interpreted as evidence of equivalence. The study employed a single-blind methodology, in which group allocation was concealed from the researchers performing the measurements.

Ethical Consideration

The study protocol was approved by the Health Research Ethics Committee of Universitas Prima Indonesia (No. 093/KEPK/UNPRI/I/2026). The study adhered to the 3R principles (Replacement, Reduction, Refinement) as outlined (Hubrecht & Carter, 2019). Animals were housed in standard conditions with *ad libitum* access to food and water. Euthanasia was performed using chloroform inhalation, followed by burial according to ethical procedures (Musa et al., 2026).

Result

Phytochemical Analysis

Qualitative phytochemical screening indicated the presence of six secondary metabolite groups in mangkokan leaf extract. Flavonoids were detected by a pink color change after reaction with strong HCl. Saponins were confirmed by stable foam formation after vigorous shaking. Tannins were indicated by a green-black color with FeCl_3 . Alkaloids were detected using Mayer, Wagner, and Dragendorff reagents, producing yellow, brown, and orange precipitates, respectively. Steroids and glycosides were also detected. These tests confirm compound classes only. Quercetin has been previously reported in mangkokan leaf extract, but the present study did not identify or quantify quercetin specifically.

Physicochemical Characterization

The organoleptic qualities of both formulations were satisfactory. F1 (10%) had a pale green color and a typical extract odor, while F2 (20%) had a more vivid green tint. No phase separation was observed. pH values were 6.19 ± 0.33 for F1 and 6.20 ± 0.00 for F2. These values are within the range generally considered acceptable for topical formulations and intact skin. However, wound-bed pH may differ from intact skin pH, so these values should not be interpreted as direct evidence of wound-bed compatibility. Spreadability improved under applied stress; F2 showed slightly better spreadability (3.1–5.1 cm) than F1 (2.9–4.9 cm).

Blood Glucose Levels

STZ induction successfully established diabetic conditions in all STZ-treated groups, with blood glucose levels ≥ 200 mg/dL on day 1 (Table 3).

Table 3. Blood Glucose Levels (mg/dL)

Group	Blood Glucose Levels (mg/dL)		
	mean $\pm$ SD		
	Day 1	Day 7	Day 14
Normal	95.4 $\pm$ 6.43	97.2 $\pm$ 8.17	99.2 $\pm$ 8.44 ^{b,c,d,e}
Negative Control	276.2 $\pm$ 8.56	254.8 $\pm$ 2.59	231.2 $\pm$ 6.18 ^{a,c,d,e}
Positive Control	272.8 $\pm$ 11.90	239.6 $\pm$ 5.55	211.6 $\pm$ 5.90 ^{a,b,d}
Treatment I (10%)	274.2 $\pm$ 7.46	257.2 $\pm$ 6.98	222.2 $\pm$ 5.40 ^{a,b,c,e}

Harahap, Diki Arya Nata., Maqhfirah, Najwa., Puspawani, Yeni., Nasution, Sri Wahyuni. (2026) Effect of Nanoemulsion Cream Containing Mangkokan Leaf Extract (*Nothopanax scutellarium*) on Incision Wound Healing and Malondialdehyde Levels in Streptozotocin-Induced Wistar Rats. *PROFESIONAL HEALTH JOURNAL*. Volume 8(2), 830-841. <https://doi.org/10.54832/phj.v8i2.1568>

Treatment II (20%)	273.4 ± 6.35	237.4 ± 10.21	208.6 ± 6.35 ^{a,b,d}
	<i>p-value</i>		<0.001

Note: Values followed by different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on the post-hoc LSD test. Letter a = significantly different from the Normal group; b = significantly different from the Negative Control group; c = significantly different from the Positive Control group; d = significantly different from Treatment Group I; e = significantly different from Treatment Group II.

Induction of streptozotocin (STZ) successfully established diabetes mellitus in all treated groups, with blood glucose levels exceeding 200 mg/dL on day 1 (276.2 ± 8.56 mg/dL in the negative control; 272.8 ± 11.90 mg/dL in the positive control; 274.2 ± 7.46 mg/dL in Treatment I; and 273.4 ± 6.35 mg/dL in Treatment II), confirming the validity of the diabetic model. In contrast, the normal group maintained physiological glucose levels throughout the study (95.4 ± 6.43 to 99.2 ± 8.44 mg/dL).

On day 14, a one-way ANOVA showed highly significant differences between the groups ($p < 0.001$). Treatment Group II (20%) had the lowest blood glucose level (208.6 ± 6.35 mg/dL) according to post-hoc LSD analysis, and there was no significant difference from the positive control (211.6 ± 5.90 mg/dL), as evidenced by the lack of the superscript letter 'c' ($p > 0.05$). Nevertheless, there was a substantial difference ($p < 0.001$) between Treatment II and the normal group, suggesting that full normoglycemia was not attained. Superscripts 'b' and 'd' indicate a significant difference between Treatment II and the negative control (231.2 ± 6.18 mg/dL) and Treatment I (222.2 ± 5.40 mg/dL). This indicates a clear dose-response relationship, with the 20% concentration offering better antihyperglycemic efficacy than the 10% formulation. Treatment I showed intermediate efficacy, significantly lower than the negative control but still significantly higher than the positive control and Treatment II, indicating that the 10% concentration, while effective, was not as potent as standard therapy.

All STZ-induced groups remained hyperglycemic (>200 mg/dL) throughout the study, which is a predictable consequence of the topical administration route. Systemic absorption of bioactive compounds through the skin is inherently limited compared to oral administration due to lower concentration gradients and the residual skin barrier

Wound Healing Assessment

Wound healing was assessed by measuring wound area using ImageJ software and calculating healing percentages (Tables 4–5).

Table 4. Wound Area (mm²)

Group	Day 0	Day 7	Day 14
Normal	20.0 ± 0.00	13.16 ± 0.71	0.88 ± 0.49 ^{b,d}
Negative Control	20.0 ± 0.00	15.66 ± 0.33	7.86 ± 0.99 ^{a,c,d,e}
Positive Control	20.0 ± 0.00	9.58 ± 0.27	1.14 ± 0.64 ^{b,d}
Treatment I (10%)	20.0 ± 0.00	10.78 ± 0.46	2.88 ± 0.69 ^{a,b,c,e}
Treatment II (20%)	20.0 ± 0.00	9.24 ± 0.77	0.82 ± 0.48 ^{b,d}
	<i>p-value</i>		<0.001

Note: Values followed by different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on the post-hoc LSD test. Letter a = significantly different from the Normal group; b = significantly different from the Negative Control group; c = significantly different from the Positive Control group; d = significantly different from Treatment Group I; e = significantly different from Treatment Group II.

All groups displayed the same wound area (20.0 ± 0.00 mm²) at baseline (day 0), indicating consistency in wound size. By day 7, Treatment Group II (20%) had the smallest wound area (9.24 ± 0.77 mm²), similar to the positive control (9.58 ± 0.27 mm²), whereas the negative control group had the highest (15.66 ± 0.33 mm²). On day 14, Treatment Group II had the smallest

remaining wound ($0.82 \pm 0.48 \text{ mm}^2$), exceeding even the positive control ($1.14 \pm 0.64 \text{ mm}^2$) and closely equal the normal group ($0.88 \pm 0.49 \text{ mm}^2$), while the negative control still displayed a considerable wound area ($7.86 \pm 0.99 \text{ mm}^2$). The results for Treatment Group I were intermediate ($2.88 \pm 0.69 \text{ mm}^2$). On day 14, a one-way ANOVA showed highly significant differences between the groups ($p < 0.001$). Post-hoc LSD analysis demonstrated that Treatment Group II (20%) showed no significant difference from the normal group, positive control (superscript 'c' absent), or Treatment I, confirming that the 20% formulation restored healing to near-physiological levels. However, Treatment II was significantly different from the negative control. Treatment Group I showed significant differences from the negative control, positive control, and Treatment II, confirming a clear dose-response relationship where the 20% concentration was superior to the 10% formulation. The negative control showed significant differences from all other groups, confirming that untreated diabetic conditions severely impair tissue regeneration.

Table 5. Wound Healing Percentage (%)

Group	Day 7	Day 14
Normal	34.2	95.6
Negative Control	21.7	60.7
Positive Control	52.1	94.3
Treatment I (10%)	46.1	85.6
Treatment II (20%)	53.8	95.9

On day 14, Treatment Group II achieved the highest wound healing percentage (95.9%), comparable to the normal group (95.6%; $p = 0.891$) and positive control (94.3%; $p = 0.468$). It was significantly higher than the negative control (60.7%; $p < 0.001$). One-way ANOVA revealed significant intergroup differences ($F = 96.109$, $p < 0.001$). Post-hoc LSD analysis showed that Treatment I (85.6%) was significantly different from the positive control ($p = 0.001$) and Treatment II ($p < 0.001$), confirming a dose-dependent effect where the 20% concentration was more effective than the 10% formulation.

Malondialdehyde (MDA) Levels

MDA levels, measured as a biomarker of oxidative stress, showed significant differences among groups (Table 6).

Table 6. Malondialdehyde (MDA) Levels (nmol/mL)

Group	Day 0	Day 7	Day 14
Normal	1.73 ± 0.15	1.73 ± 0.13	1.74 ± 0.12
Negative Control	9.28 ± 0.19	9.24 ± 0.24	8.01 ± 0.10
Positive Control	9.30 ± 0.24	8.47 ± 0.48	6.25 ± 0.15
Treatment I (10%)	9.29 ± 0.24	8.55 ± 0.48	7.10 ± 0.15
Treatment II (20%)	9.31 ± 0.10	8.20 ± 0.08	6.12 ± 0.07
<i>p-value</i>			<0.001

Note: Values followed by different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on the post-hoc LSD test. Letter a = significantly different from the Normal group; b = significantly different from the Negative Control group; c = significantly different from the Positive Control group; d = significantly different from Treatment Group I; e = significantly different from Treatment Group II.

At baseline (day 0), all STZ-induced groups showed markedly elevated MDA levels (9.28–9.31 nmol/mL) compared to the normal group (1.73 nmol/mL), confirming successful induction of oxidative stress. On day 14, Treatment Group II showed the lowest MDA level (6.12 ± 0.07 nmol/mL), which was not significantly different from the positive control (6.25 ± 0.15 nmol/mL; $p = 0.103$). It was significantly lower than the negative control (8.01 ± 0.10 nmol/mL; $p < 0.001$).

and Treatment I (7.10 ± 0.15 nmol/mL; $p < 0.001$). One-way ANOVA revealed significant intergroup differences ($F = 2292.402$, $p < 0.001$). Post-hoc LSD analysis confirmed a dose-dependent relationship. Treatment II showed significantly lower MDA levels compared to Treatment I ($p < 0.001$), while both treatments showed significantly lower MDA compared to the negative control ($p < 0.001$).

Correlation Between MDA Reduction and Wound Healing

MDA decrease and accelerated wound healing were found to be consistently correlated. Treatment Group II showed the highest wound healing percentage (95.9%) and the largest MDA reduction (34.3% decrease from day 0 to day 14). On the other hand, the negative control had the lowest healing percentage (60.7%) and the smallest MDA reduction (13.7%). This trend implies that faster wound healing is linked to less oxidative stress, as indicated by lower MDA levels. This result lends credence to the theory that mangkokan leaf extract's antioxidant activity promotes better tissue regeneration.

Discussion

Mangkokan leaf extract contains flavonoids, saponins, tannins, alkaloids, steroids, and glycosides, which provide the pharmacological basis for the biological effects that have been reported, according to phytochemical analysis. Due to their ability to donate hydrogen, flavonoids especially quercetin are the main sources of antioxidant activity (Sriyanti et al., 2021). Saponins promote the growth of fibroblasts and raise the expression of VEGF and PDGF, which are crucial for the angiogenesis of wounds (Liu et al., 2022). According previous study tannins have antibacterial and astringent properties that promote wound contraction and stop subsequent infections. The extensive therapeutic effects seen in this study are explained by this multi-component mixture (Chingwaru et al., 2019).

As evidenced by higher baseline MDA levels (9.28–9.31 nmol/mL) in comparison to normal controls (1.73 nmol/mL), STZ induction successfully created chronic hyperglycemia and oxidative stress. Through DNA alkylation and ROS formation, STZ specifically kills pancreatic beta cells ((Matsumoto et al., 2021; Shabalala et al., 2022). This results in insulin insufficiency and chronic hyperglycemia, which raises ROS production via the polyol, AGEs, PKC, and hexosamine pathways (Ghasemi-Dehnoo et al., 2020)

The significant MDA reduction in treatment groups demonstrates the nanoemulsion cream's capacity to suppress lipid peroxidation. On day 14, Treatment Group II achieved MDA levels of 6.12 ± 0.07 nmol/mL, comparable to the positive control ($p = 0.103$), representing a 34.3% reduction. The significant reduction in MDA levels in the treatment groups suggests that the nanoemulsion cream suppressed lipid peroxidation. On day 14, Treatment II achieved MDA levels of 6.12 ± 0.07 nmol/mL, not significantly different from the positive control ($p = 0.103$), representing a 34.3% reduction from baseline. However, because this study did not measure Nrf2/Keap1/ARE activation, phase II antioxidant enzymes, pro-oxidant enzyme activity, or direct radical scavenging capacity, these mechanisms cannot be confirmed here. Based on prior studies, the observed MDA reduction is plausibly explained by direct radical scavenging by quercetin's hydroxyl groups, activation of the Nrf2/Keap1/ARE pathway (Alharbi et al., 2025); and inhibition of pro-oxidant enzymes (Qi et al., 2022); and inhibition of pro-oxidant enzymes (Al-Khayri et al., 2022). Tannins further reinforce this effect through iron chelation (Kaczmarek, 2020; Pucci et al., 2022).

Untreated oxidative stress affects healing pathways through increased MMP activity, macrophage dysfunction, and extended inflammation, as seen by the negative control group's slowest wound healing (60.7%) ((Spampinato et al., 2020). Healing was considerably expedited by nanoemulsion treatment. Treatment Group II attained 95.9% healing by day 14, which was similar to the positive control ($p = 0.468$) and normal ($p = 0.891$). Quercetin activates TGF- β 1/Smad2/3 to enhance collagen synthesis (Cai et al., 2018); flavonoids suppress NF- κ B activation and promote M2 macrophage polarization (Al-Khayri et al., 2022); saponins stimulate

fibroblast proliferation and angiogenesis via VEGF/PDGF; and tannins offer antimicrobial protection (Dhall et al., 2026). A clear dose-response relationship was observed. The 20% concentration significantly outperformed the 10% concentration on both MDA ($p < 0.001$) and wound healing ($p < 0.001$). This follows Fick's diffusion law, where higher concentration doubles the driving force for transdermal (Osborne & Musakhanian, 2018). The 20% concentration also enables Nrf2 pathway activation threshold ($\geq 20\text{--}50\text{ }\mu\text{M}$ quercetin) to be reached (Alharbi et al., 2025). The nanoemulsion delivery system played a crucial role, with Tween 80/PEG 400-based nanoemulsions increasing quercetin transdermal bioavailability by 3.2-fold (Subramanian et al., 2021) while providing occlusive effects, sustained release, and solubilization of lipophilic compounds (Tarik Alhamdany et al., 2021).

Flavonoid inhibition of α -glucosidase, GLUT-4 translocation via PI3K/Akt, and AMPK activation all contribute to the considerable drop in blood glucose in treatment groups (Treatment II: $208.6 \pm 6.35\text{ mg/dL}$). Nevertheless, the limits of topical administration are reflected in partial normalization (Tellechea et al., 2020). MDA decrease and wound healing were found to be causally related: the group with the greatest MDA (negative control: 8.01 nmol/mL) had the slowest healing (60.7%), while the group with the lowest MDA (Treatment II: 6.12 nmol/mL) showed the fastest healing (95.9%). MDA actively impairs healing through collagen cross-linking, fibroblast cytoskeletal modification, and eNOS (Kaczmarek, 2020; Pucci et al., 2022). This study's findings align with previous reports on quercetin's wound healing effects and nanoparticle-based antioxidant therapy (Ghasemi-Dehnoo et al., 2020; Spampinato et al., 2020). Study limitations include the lack of histopathological confirmation, use of a type 1 diabetes model, absence of detailed nanoemulsion physicochemical characterization, small sample size, and unmeasured molecular biomarkers. Despite these limitations, this study provides strong evidence that the 20% nanoemulsion cream effectively reduces oxidative stress and accelerates wound healing through multi-target mechanisms, making it a viable candidate for further development in diabetic wound management.

Conclusion

In streptozotocin-induced diabetic Wistar rats, the 20% nanoemulsion cream containing mangkokan leaf extract reduced MDA levels and accelerated incision wound healing. Its effects were not significantly different from metformin, but non-inferiority or equivalence remains to be tested. The proposed mechanisms include enhanced transdermal penetration, antioxidant activity, anti-inflammatory effects, fibroblast proliferation, and collagen synthesis. A dose-response relationship was observed between 10% and 20% concentrations. These findings support further toxicological studies and clinical trials, but causality and equivalence cannot be claimed from the current data.

SUGGESTIONS

Future research should perform histopathological analysis using Hematoxylin-Eosin and Masson's Trichrome staining to measure fibroblast density, epidermal thickness, collagen organization, and capillary density. Molecular biomarker measurements should include mRNA and protein expression of type I collagen, VEGF, TNF- α , IL-6, TGF- β 1, SOD, catalase, and glutathione. Complete nanoemulsion physicochemical characterization should include particle size, zeta potential, PDI, and quercetin encapsulation efficiency. Acute toxicity (OECD 420), subchronic toxicity (OECD 407), and skin irritation tests (OECD 404) should be performed before clinical development. Future studies should also include pharmacokinetic absorption data, equivalence testing, baseline-adjusted MDA analysis, and animal-level correlation or mediation analysis.

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DECLARATION OF INTEREST

There is no conflict of interest in this study.

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