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Nanoemulsion of *Coleus Scutellarioides* Leaf Extract Ameliorates Diabetes-Induced Hyperglycemia and Organ Damage in Rats

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ABSTRACT

This experimental study evaluated the effectiveness of nanoemulsion of ethanol extract of miana leaf (*Coleus scutellarioides* (L.) Benth.) in controlling type 1 diabetes mellitus in male rats. The optimal formulation (F2), obtained from a ratio of VCO: Tween 80: Propylene glycol (2:10:5), yielded a particle-sized nanoemulsion of 59.2 nm, a polydispersity index of 0.353, and a zeta potential of -32.26 mV. Male Wistar rats were induced with streptozotocin (100 mg/kg BW, intraperitoneally), and diabetes was confirmed 72 hours post-induction when blood glucose levels exceeded 250 mg/dL. The nanoemulsion was administered intraperitoneally once daily for 28 days at doses of 50, 75, and 100 mg/kg BW. The normal control group (non-diabetic) exhibited baseline urea and creatinine levels of 20.59 mg/dL and 0.26 mg/dL, respectively. By day 28, the group receiving 100 mg/kg BW showed urea and creatinine levels of 22.88 mg/dL and 0.28 mg/dL, respectively, which were not significantly different from the normal controls ($p > 0.05$). Different doses showed optimal effects on different parameters: 50 mg/kg BW was most effective for blood glucose reduction (84.3% reduction by day 28), 75 mg/kg BW provided optimal pancreatic protection (mean damage score of 2.0), while 100 mg/kg BW showed superior renal protective effects. Phytochemical screening of the ethanol extract confirmed the presence of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, and steroids. Statistical analyses were performed using one-way ANOVA followed by post hoc tests for parametric data and the Kruskal–Wallis test for non-parametric data, revealing significant differences among treatment groups ($p < 0.05$).

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to dysfunction in insulin production and function. Global prevalence is increasing threefold, with predictions showing an increase in DM cases in Indonesia from 10.7 million in 2019 to 13.7 million in 2030 [1]. The hyperglycemia in DM generates excessive reactive oxygen species (ROS), leading to oxidative stress that damages multiple organs, including the pancreas and kidneys [2-4].

Coleus scutellarioides (L.) Benth., commonly known as miana, is a medicinal plant containing bioactive compounds including alkaloids, flavonoids, essential oils, tannins, saponins, and terpenoids [5, 6]. Previous studies have demonstrated its traditional use as a cough suppressant, poison neutralizer, and diabetes treatment [6, 7]. However, conventional plant extracts face limitations including poor water solubility, low bioavailability, and chemical instability, which restrict their therapeutic efficacy.

Nanoemulsion technology offers a promising solution to overcome these limitations. Nanoemulsions are kinetically stable, transparent colloidal dispersions with droplet sizes typically ranging from 20-200 nm and a low polydispersity index (PDI < 1) [8]. The extremely small particle size of nanoemulsions significantly increases the surface area-to-volume ratio, enhancing absorption through biological membranes, particularly the small intestine wall, thereby improving bioavailability of active compounds [8, 9]. Recent advances in nanoemulsion applications for herbal medicine delivery have shown enhanced therapeutic efficacy compared to conventional formulations, particularly for lipophilic phytochemicals.

Despite the known antidiabetic properties of *C. scutellarioides* extracts [10-12], no studies have systematically investigated the effects of its nanoemulsion formulation on STZ-induced diabetes, particularly regarding multi-organ protection. Furthermore, the dose-dependent effects on different diabetic complications (hyperglycemia, nephropathy, and pancreatic damage) remain unclear.

We hypothesize that formulating *C. scutellarioides* leaf ethanol extract into nanoemulsions will more effectively reduce blood glucose levels and alleviate STZ-induced pancreatic and renal damage compared to conventional

formulations, by improving bioavailability and cellular uptake of bioactive compounds. We further hypothesize that different doses may exhibit optimal effects on different organ systems due to the multi-target nature of the phytochemical constituents.

2. MATERIALS AND METHODS

2.1 Materials

Equipment: Vacuum Rotatory Evaporator, Magnetic Stirrer (Heidolph®), Particle Size Analyzer (Horiba-SZ 100z®), Ultrasonicator (Zeta Sonic ZS-640 DB®), Transmission Electron Microscopy (TEM), UV-Vis Spectrophotometer (Single Beam®), Analytical Balance (Ohaus PA214 SKZO®), Viscometer (NDJ-8S®), pH meter, and standard laboratory glassware.

Chemicals: Virgin Coconut Oil (VCO), Tween 80, Propylene glycol, 96% ethanol, Streptozotocin (Bioworld USA), Glibenclamide powder, and analytical grade reagents.

2.2 Plant material and extract preparation

Fresh leaves of *C. scutellarioides* were collected, authenticated, dried, and powdered. Maceration was performed using 96% ethanol (1:10 w/v) for 3 × 24 hours with occasional stirring. The extract was filtered and concentrated using a rotary evaporator at 50°C under reduced pressure.

2.3 Nanoemulsion formulation

Nanoemulsions were prepared using the spontaneous emulsification method. Three formulations (F1, F2, F3) were prepared with varying ratios of VCO: Tween 80: Propylene glycol. F1 (1:10:5): VCO (1 g) was mixed with Tween 80 (10 g) and propylene glycol (5 g) containing the extract. The mixture was stirred at 1000 rpm for 30 minutes at room temperature (25±2°C), then subjected to ultrasonication at 40 kHz for 10 minutes in an ice bath to prevent temperature increase. F2 (2:10:5) and F3 (3:10:5) were prepared similarly with adjusted VCO ratios.

2.4 Nanoemulsion characterization

Particle Size Analysis (PSA): Performed using Dynamic Light Scattering (Horiba-SZ 100z®). Samples were diluted 1:100 with distilled water before measurement. Z-average diameter, PDI, and zeta potential were recorded in triplicate.

pH Measurement: Determined using a calibrated pH meter at 25°C (n = 3).

Viscosity: Measured using NDJ-8S viscometer at 25°C with spindle speed of 60 rpm (n = 3).

Transmittance: Measured at 650 nm using a UV-Vis spectrophotometer with distilled water as a blank (n = 3).

Transmission Electron Microscope (TEM): Samples were diluted and placed on copper grids, negatively stained, and observed at various magnifications (15,000×, 43,000×, and 97,000×).

2.5 Phytochemical screening

Qualitative analysis was performed using standard methods to identify alkaloids (Dragendorff's reagent), flavonoids (Mg powder-HCl test), saponins (foam test), tannins ($FeCl_3$ test), and steroids (Liebermann-Burchard test).

2.6 Animal study

Thirty male Wistar rats (Rattus norvegicus), aged 2-3 months, weighing 150-200 g, were obtained from Thirty male Wistar rats (Rattus norvegicus), aged 2-3 months, weighing 150-200 g, were obtained from Gold Mice Farm Makkasar. The animals were housed in standard polypropylene cages under controlled conditions (temperature 25±2°C, 12-hour light/dark cycle) with free access to standard pellet feed and drinking water ad libitum. This study was approved by the Research Ethics Committee of the Faculty of Medicine, Tadulako University, number: 2667/UN28.10/KL/2024.

After 7 days of acclimatization, rats were randomly divided into 6 groups (n = 5 per group). Normal Control (NC): Non-diabetic rats receiving vehicle (nanoemulsion base without extract). Negative Control (STZ): Diabetic rats receiving vehicle only. Positive Control (STZ+Glib): Diabetic rats receiving glibenclamide (0.45 mg/kg BW). Treatment 1 (STZ+NE50): Diabetic rats receiving nanoemulsion at 50 mg/kg BW. Treatment 2 (STZ+NE75): Diabetic rats receiving nanoemulsion at 75 mg/kg BW. Treatment 3 (STZ+NE100): Diabetic rats receiving nanoemulsion at 100 mg/kg BW.

After overnight fasting (12 hours), diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (100 mg/kg BW) dissolved in 0.1 M citrate buffer (pH 4.5). Normal control received citrate buffer only. Blood glucose was measured 72 hours post-injection using a glucometer (tail vein blood). Rats with fasting blood glucose >250 mg/dL were considered diabetic and included in the study.

Starting from day 7 post-induction, treatments were administered once daily via intraperitoneal injection for 28 consecutive days. All administrations were performed between 09:00 and 11:00 AM to minimize circadian variations.

2.7 Blood glucose monitoring

Fasting blood glucose (FBG) was measured on days 0 (baseline), 7 (post-induction), 14, 21, and 28. The percentage reduction in blood glucose was calculated as:

$$\%\text{Reduction} = [(\text{FBG Day7} - \text{FBG Day X}) / \text{FBG Day7}] \times 100 \quad (1)$$

2.8 Biochemical analysis

On day 28, after 12-hour fasting, rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg). Blood samples (3-5 mL) were collected via cardiac puncture into plain tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Urea and creatinine levels were measured using an automated clinical chemistry analyzer following standard protocols.

2.9 Histopathological examination

Following blood collection, rats were euthanized, and pancreatic tissues were immediately harvested, fixed in 10% neutral buffered formalin for 24 hours, processed, embedded in paraffin, sectioned at 5 μm thickness, and stained with

hematoxylin and eosin (H&E).

Three sections per animal were examined by a certified pathologist blinded to the experimental groups. Five random high-power fields (400×) per section were evaluated. Pancreatic damage was scored based on the percentage of affected tissue showing degenerative changes and necrosis in both islets of Langerhans and exocrine tissue: Score 0: No damage/normal (0%), Score 1: Mild damage (1-20%), Score 2: Moderate damage (21-50%), Score 3: Severe damage (51-75%), Score 4: Very severe damage (>75%). The scoring system was adapted from established pancreatic injury grading systems [13-15].

2.10 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. Normally distributed data were analyzed using one-way ANOVA followed by LSD or Duncan's post-hoc test. Non-normally distributed data (histopathological scores) were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney U test for pairwise comparisons. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS Statistics version 25.0.

3. RESULTS

3.1 Characterization of nanoemulsions

Values in bold in Table 1 indicate that formulation 2 (F2) was selected as the optimal formulation. Formulation 2 (F2) was selected as the optimal nanoemulsion based on comprehensive characterization. F2 exhibits a particle size of 59.2 nm, within the ideal nanoemulsion range, providing optimal surface area for enhanced bioavailability. A PDI of 0.353 indicates acceptable size distribution uniformity. A zeta potential of -32.26 mV indicates good electrostatic stability, preventing particle aggregation. A pH of 6.55 is suitable for biological applications, and a transmittance of 92.6% confirms excellent optical clarity and stability.

3.2 TEM analysis

Transmission Electron Microscope (TEM) images shown in Figures 1-3 confirm the formation of discrete nanoparticles in all formulations. F2 shows a relatively uniform particle distribution, although some particles exhibit non-spherical morphology, likely due to phase separation dynamics during emulsification or the influence of propylene glycol on droplet shape. These morphological variations do not affect the stability and functional performance of the nanoemulsions.

3.3 Blood glucose levels and percentage reduction

All treatment groups showed a significant decrease in blood glucose compared to the STZ control ($p < 0.001$), as seen in Tables 2 and 3. The 50 mg/kg dose achieved the highest percentage decrease on day 28 (84.3%), although it was not significantly different from the other treatment groups.

3.4 Renal function parameters

In Table 4, the 100 mg/kg dose showed superior renal protective effects, with creatinine (0.28±0.29 mg/dL) and urea (22.88±2.3 mg/dL) levels showing no significant difference from normal controls ($p > 0.05$) on day 28. Normal reference range: creatinine 0.2-0.8 mg/dL, urea 15-25 mg/dL.

Table 1. Physicochemical characterization of miana leaf extract nanoemulsions

Parameter	Standard Range	F1	F2	F3
Particle Size (nm)	20-500	238.0	**59.2**	13.3
PDI	<1	0.303	**0.353**	0.306
Zeta Potential (mV)	>±30	-34.9	**-32.26**	-25.1
pH	5-7	6.35	**6.55**	6.70
Viscosity (mPa·s)	1-100	258.75	**233.25**	230.25
Transmittance (%)	100	57.1	**92.6**	99.08

Table 2. Blood glucose levels (mg/dL) in experimental groups

Day	Normal Control	STZ	STZ+Glib	STZ+NE50	STZ+NE75	STZ+NE100	p-value
0	91.2±6.4	92.8±5.8	95.6±7.2	89.4±6.1	93.2±5.9	96.4±6.8	0.355
7	95.4±7.1	422.4±28.6	439.0±31.2^{b}	418.2±26.4	382.4±29.1	427.6±30.8	0.000*
14	97.0±6.8	433.0±32.4	126.8±11.2	108.2±9.8	104.8±8.6	121.8±10.4	0.000*
21	98.2±7.2	407.0±28.9	95.6±8.4	83.6±7.1	85.2±7.6	84.8±7.4	0.000*
28	96.8±6.9	399.6±26.7	74.2±6.8	65.8±5.9	68.4±6.2	78.6±7.1	0.000*

Notes: Values are mean±SD (n = 5). Different superscript letters indicate significant differences ($p < 0.05$, ANOVA + LSD). *Significant at $p < 0.05$.

Table 3. Percentage reduction in blood glucose levels

Group	Days 7-14	Days 7-21	Days 7-28
STZ	-2.5±7.9	3.6±4.5	5.4±5.1
STZ+Glib	70.7±10.2	78.2±2.5	83.1±1.9
STZ+NE50	74.1±2.5	80.0±4.7	**84.3±5.8**
STZ+NE75	72.5±2.5	79.5±3.4	83.8±3.6
STZ+NE100	71.6±5.9	80.1±3.0	81.6±1.8

Notes: Values are mean±SD (n = 5). Same superscript letters indicate no significant difference between treatment groups ($p > 0.05$). The values in Bold indicate the highest reduction.

3.5 Pancreatic histopathology

Table 5 shows that the 75 mg/kg dose provided optimal pancreatic protection with a mean damage score of 2.00, significantly lower than the 50 mg/kg dose ($p < 0.05$) and comparable to the positive control. Representative histopathology images show: Normal Control (Score 1): Intact islets of Langerhans with minimal degeneration (1-20%) STZ (Score 2-3): Extensive β-cell necrosis, islet lysis (>50%), and exocrine damage (25-50%) STZ+Glib (Score 2): Reduced but present cellular damage STZ+NE75 (Score 1-2): Well-preserved pancreatic architecture with minimal necrosis.

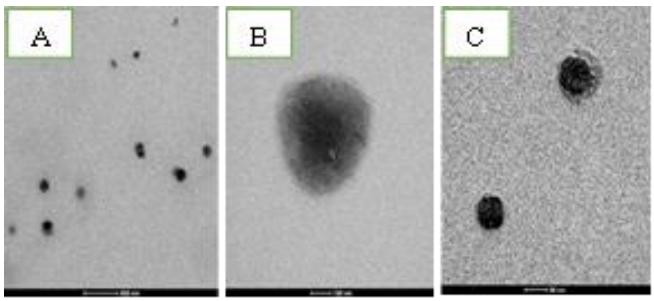

Figure 1. TEM images of formulation 1 nanoemulsion at different magnifications (A) 15,000x, (B) 43,000x, (C) 97,000x

3.6 Results of pancreatic histopathology preparation examination

Figure 4 shows pancreatic tissue preparations from white rats from various treatment groups. The normal control group (A) and the positive control group (C) showed a score of 1 with a level of degeneration and necrosis of 1-20% in one field of view. The negative control group (B) and the group given a dose of 50 mg/kgBW (D) showed a score of 2 with higher tissue damage, namely 21-50% degeneration and necrosis. Meanwhile, the group given a dose of 75 mg/kgBW (E) showed a score of 1 with a lower level of damage, namely 1-20% degeneration and necrosis in one field of view.

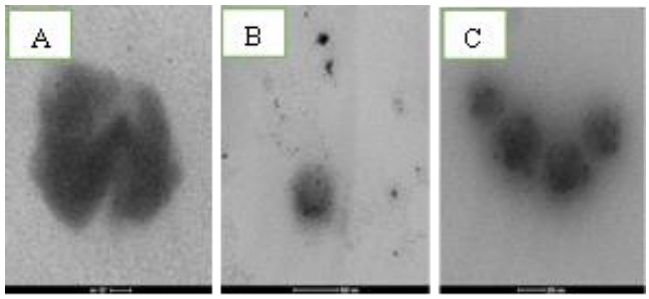

Figure 2. TEM images of formulation 2 nanoemulsion at different magnifications (A) 43,000x, (B) 19,000x, (C) 29,000x

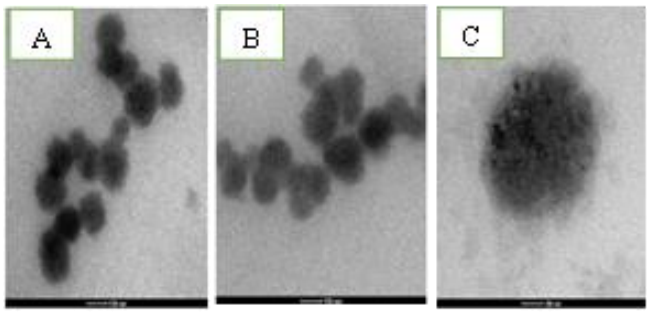

Figure 3. TEM images of formulation 3 nanoemulsion at different magnifications (A) 15,000x, (B) 43,000x, (C) 97,000x

Table 4. Serum creatinine (mg/dL) and urea (mg/dL) levels

Day	Parameter	Normal Control	STZ	STZ+Glib	STZ+NE50	STZ+NE75	STZ+NE100	p-value
0	Creatinine	0.38±0.07	0.36±0.08	0.41±0.07	0.38±0.09	0.43±0.11	0.43±1.07	0.679
7	Creatinine	0.31±0.09	1.01±0.07	0.91±0.09	0.93±0.04	0.97±0.05	0.98±0.09	0.000*
14	Creatinine	0.29±0.03	1.12±0.14	0.77±0.04	0.74±0.12	0.76±0.06	0.76±0.06	0.000*
21	Creatinine	0.26±0.02[a]	1.06±0.06	0.51±0.05	0.48±0.04	0.48±0.04	0.50±0.04	0.000*
28	Creatinine	0.26±0.02	1.07±0.08	0.28±0.07	0.43±0.03	0.39±0.03	**0.28±0.29**	0.000*
28	Urea	20.59±2.1	42.26±3.8	22.54±2.4	28.76±2.9	26.07±2.6	**22.88±2.3**	0.000*

Notes: Values are mean±SD (n = 5). Different superscript letters indicate significant differences ($p < 0.05$, ANOVA + Duncan's test). The values in Bold indicate values not significantly different from the normal control. *Significant at $p < 0.05$.

Table 5. Pancreatic damage scores

Group	Individual Scores	Mean±SD	Median	p-value
Normal Control	1, 1, 1	1.00±0.00	1.0	
STZ	3, 2, 2	2.33±0.58	2.0	0.024*
STZ+Glib	2, 1, 3	2.00±1.00	2.0	
STZ+NE50	3, 3, 2	2.67±0.58	3.0	
STZ+NE75	1, 2, 3	2.00±1.00	2.0	
STZ+NE100	2, 3, 2	2.33±0.58	2.0	

Notes: Values represent individual animal scores, mean±SD, and median (n = 3 per group). Different superscript letters indicate significant differences ($p < 0.05$, Kruskal-Wallis + Mann-Whitney U test). The values in Bold indicate optimal pancreatic protection. *Significant at $p < 0.05$.

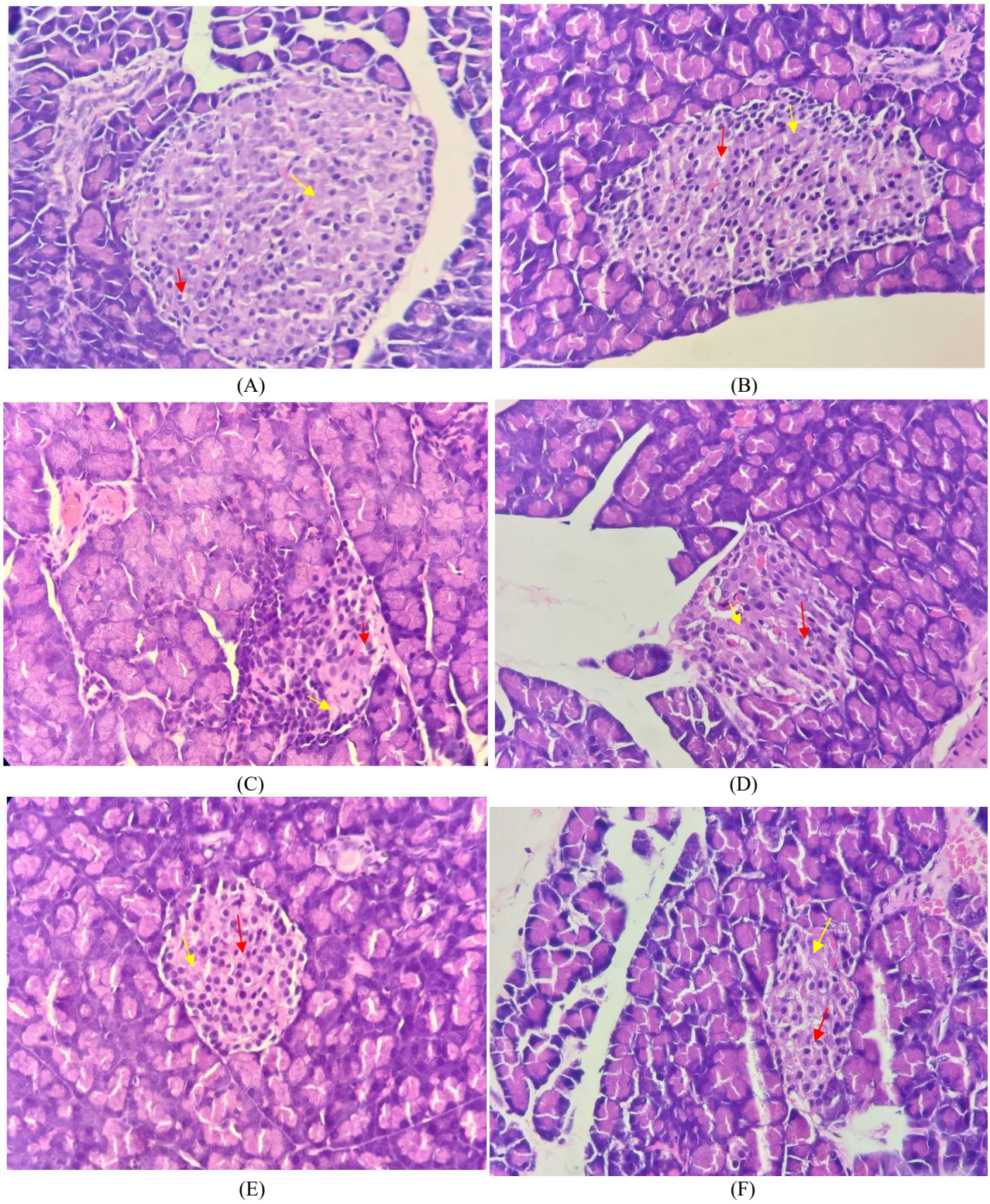

Figure 4. Preparation of pancreatic tissue from white mice with various levels of damage. (A) Normal control group showing minimal tissue damage (Score 1), (B) Negative control (STZ) group with extensive damage (Score 2-3), (C) Positive control (STZ+Glib) group showing moderate damage (Score 1), (D) Treatment group receiving 50 mg/kg BW showing significant damage (Score 2), (E) Treatment group receiving 75 mg/kg BW with reduced damage (Score 1-2), (F) Treatment group receiving 100 mg/kg BW showing moderate damage (Score 2). H&E staining, magnification 400×

Yellow arrows indicate necrosis, while red arrows indicate degeneration.

4. DISCUSSION

Formulation 2 demonstrated superior physicochemical characteristics, making it the optimal formulation. The particle size of 59.2 nm falls within the ideal nanoemulsion range (20-200 nm), providing maximum surface area for enhanced bioavailability of active compounds [8, 9]. This size is crucial because particles below 100 nm can effectively penetrate the intestinal mucosa and achieve improved oral absorption. The PDI of 0.353, although slightly higher than ideal (<0.3),

remains acceptable for pharmaceutical applications and indicates reasonably uniform size distribution [9].

The negative zeta potential (-32.26 mV) exceeds the threshold for electrostatic stability (±30 mV), preventing particle aggregation and ensuring long-term colloidal stability [16, 17]. The high transmittance (92.6%) confirms excellent optical clarity, correlating with small particle size and minimal light scattering–a hallmark of true nanoemulsions versus conventional emulsions.

TEM analysis revealed some non-spherical particle morphology in F2. This deviation from perfect sphericity may result from the specific surfactant-cosurfactant ratio affecting interfacial film flexibility, or rapid solvent evaporation during sample preparation [18-23]. Importantly, this morphological variation did not compromise nanoemulsion stability or biological performance, as evidenced by consistent therapeutic effects throughout the 28-day study period.

The nanoemulsion formulation addresses critical limitations of conventional *C. scutellarioides* extracts. The lipophilic bioactive compounds (flavonoids, terpenoids) in miana leaves have poor aqueous solubility, limiting their absorption and bioavailability [19, 20]. Nanoemulsion technology solubilizes these compounds in nano-sized oil droplets stabilized by surfactant layers, creating a thermodynamically stable delivery system that:

(1) Increases surface area: The extremely small droplet size dramatically increases the interfacial area available for absorption.

(2) Enhances permeability: Nanoparticles can penetrate through tight junctions and undergo transcellular transport across the intestinal epithelium.

(3) Protects active compounds: The oil phase shields sensitive phytochemicals from degradation in the harsh gastrointestinal environment.

(4) Improves lymphatic uptake: Lipid-based formulations promote absorption via the intestinal lymphatic system, bypassing first-pass metabolism.

This enhanced delivery explains why lower doses of nanoemulsion achieved comparable or superior effects compared to conventional extracts reported in literature [11, 13].

Our study revealed an intriguing dose-response relationship where different doses exhibited optimal efficacy for different parameters:

Blood glucose reduction (50 mg/kg optimal): The 50 mg/kg dose achieved 84.3% glucose reduction by day 28, slightly superior to higher doses, though differences were not statistically significant. This suggests a plateau effect where the 50 mg/kg dose provides sufficient active compounds to saturate insulin signaling pathways and glucose uptake mechanisms. The hypoglycemic effect likely operates through multiple mechanisms:

(1) Insulin secretagogue activity: Flavonoids in *C. scutellarioides* may stimulate residual β-cells to secrete insulin, similar to sulfonylureas [24].

(2) Enhanced insulin sensitivity: Bioactive compounds may activate the PI3K/Akt signaling pathway, promoting GLUT4 translocation in peripheral tissues [25, 26].

(3) Inhibition of hepatic gluconeogenesis: Alkaloids may suppress key gluconeogenic enzymes [27].

The highest dose (100 mg/kg) demonstrated superior nephroprotective effects, normalizing both creatinine and urea levels. This dose-dependent renal protection suggests that kidney damage in diabetes requires higher concentrations of bioactive compounds to:

(1) Scavenge ROS: Advanced glycation end-products (AGEs) generate excessive oxidative stress in diabetic kidneys. Higher doses provide more antioxidant flavonoids and phenolic compounds to neutralize free radicals [28, 29].

(2) Reduce inflammation: Tannins and saponins exert anti-inflammatory effects by suppressing NF-κB activation and pro-inflammatory cytokines (TNF-α, IL-6) [25].

(3) Preserve glomerular function: Prevention of mesangial matrix expansion and podocyte injury requires sustained high concentrations of protective phytochemicals.

The dose-response curve for renal protection appears steeper than for glucose control, explaining why 100 mg/kg was necessary to achieve normalization.

Interestingly, the intermediate dose (75 mg/kg) provided optimal pancreatic protection (score 2.00), better than both lower and higher doses. This "inverted U-shaped" dose-response may reflect:

(1) Optimal regenerative signaling: The 75 mg/kg dose may achieve ideal concentrations for activating pancreatic stellate cells and β-cell proliferation pathways without causing oxidative stress from excess compounds [30-32].

(2) Anti-apoptotic effects: Alkaloids at moderate concentrations can inhibit caspase activation and preserve β-cell viability, while excessive doses might have pro-oxidant effects [33, 34].

(3) Balanced angiogenesis: VEGF expression for pancreatic microvascular restoration may be optimally modulated at this dose [35, 36].

Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, and steroids in the nanoemulsion. These diverse secondary metabolites explain the multi-organ protective effects through complementary mechanisms:

(1) Flavonoids: Act as potent antioxidants (ROS scavenging), insulin mimetics (activating insulin receptor substrate), and anti-inflammatory agents [37].

(2) Alkaloids: Modulate glucose metabolism, inhibit α-glucosidase and α-amylase (reducing postprandial glucose), and protect against STZ-induced β-cell toxicity [38].

(3) Saponins: Enhance insulin secretion, improve lipid profiles, and exhibit nephroprotective effects through anti-inflammatory actions [39].

(4) Tannins: Provide astringent effects that reduce intestinal glucose absorption and protect against oxidative kidney damage [40].

This phytochemical synergy underlies the holistic therapeutic effects observed across multiple organ systems, characteristic of herbal medicines but challenging to achieve with single-target synthetic drugs.

Previous studies on *C. scutellarioides* have reported antidiabetic effects, but typically used conventional extracts at higher doses (200-400 mg/kg) with less comprehensive efficacy assessments [20]. Our nanoemulsion formulation achieved:

(1) Lower effective doses: 50-100 mg/kg versus 200-400 mg/kg in conventional studies.

(2) Faster onset of action: Significant glucose reduction by day 14 versus 21-28 days.

(3) Multi-organ protection: Simultaneous pancreatic and renal benefits are rarely documented together.

(4) Superior stability: Maintained therapeutic effects over 28 days without degradation.

These advantages directly result from nanoemulsion

technology, enhancing bioavailability and cellular uptake of phytochemicals. The nanoscale delivery system overcomes the bioavailability limitations that have hindered clinical translation of promising herbal medicines.

Compared to other plant-based nanoemulsions for diabetes (e.g., *Gymnanthemum amygdalinum* [26], *Moringa oleifera* [41]), our *C. scutellarioides* nanoemulsion showed comparable efficacy with the added advantage of robust renal protection–a critical consideration given that diabetic nephropathy is a leading cause of end-stage renal disease.

The differential dose-response relationships observed have important clinical implications. A combination therapy approach using 75 mg/kg for pancreatic protection supplemented with 100 mg/kg for renal protection might provide optimal multi-organ benefits. However, the 100 mg/kg dose appears most clinically relevant as it normalizes renal function (critical for long-term diabetes management), achieves substantial glucose reduction (81.6%), and provides adequate pancreatic protection (score 2.33). The nanoemulsion's stability and bioavailability make it suitable for oral formulation development, potentially as capsules or ready-to-drink preparations for diabetic patients. Future clinical trials should investigate:

(1) Pharmacokinetics: Absorption, distribution, metabolism, and elimination profiles.

(2) Safety profile: Long-term toxicity, drug interactions, and optimal therapeutic window.

(3) Efficacy in type 2 diabetes: Most human diabetes is type 2; insulin-resistant models needed

(4) Comparative studies: Head-to-head trials against standard antidiabetic medications.

Several limitations warrant consideration:

(1) STZ-induced model: Represents acute type 1 diabetes; may not fully reflect chronic type 2 diabetes pathophysiology in humans.

(2) Short duration: 28-day treatment period; longer studies needed to assess sustained efficacy and potential complications.

(3) Route of administration: Intraperitoneal injection used for standardization; oral bioavailability needs confirmation.

(4) Sample size: Small group sizes (n = 3-5) limit statistical power for histopathological analysis.

(5) Mechanism elucidation: Molecular pathways require investigation using Western blot, RT-PCR, and immunohistochemistry.

5. CONCLUSIONS

This study successfully developed and characterized a stable nanoemulsion formulation of *Coleus scutellarioides* leaf ethanol extract. The optimal formulation (F2, ratio 2:10:5 of VCO:Tween 80:Propylene glycol) exhibited ideal physicochemical properties, including particle size of 59.2 nm, PDI of 0.353, and zeta potential of -32.26 mV, meeting international nanoemulsion standards.

The nanoemulsion demonstrated significant therapeutic efficacy in STZ-induced diabetic rats with dose-dependent multi-target effects:

(1) 50 mg/kg BW: Most effective for blood glucose reduction (84.3% reduction by day 28).

(2) 75 mg/kg BW: Optimal for pancreatic tissue protection (mean damage score of 2.00).

(3) 100 mg/kg BW: Superior renal protection, normalizing creatinine (0.28 mg/dL) and urea (22.88 mg/dL) to levels statistically comparable with normal controls.

These differential dose-response relationships reveal the multi-target pharmacological nature of *C. scutellarioides* phytochemicals (alkaloids, flavonoids, saponins, tannins, steroids), operating synergistically through antioxidant, anti-inflammatory, insulin secretagogue, and cytoprotective mechanisms.

The nanoemulsion formulation represents a significant advancement over conventional extracts by:

(1) Enhancing bioavailability through nanosized drug delivery.

(2) Achieving therapeutic effects at lower doses.

(3) Providing comprehensive multi-organ protection.

(4) Maintaining long-term stability and consistent efficacy.

The 100 mg/kg dose is recommended for comprehensive diabetes management, offering balanced efficacy across hyperglycemia control, pancreatic preservation, and renal protection. This formulation holds promise for development as an adjunctive or alternative therapy for diabetes mellitus, particularly for patients experiencing multi-organ complications.

Future research should focus on oral bioavailability assessment, long-term safety profiling, mechanistic pathway elucidation, and clinical trials to translate these findings.

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