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Antidiabetic Effects of Miana (*Coleus atropurpureus*) Leaf Ethanol Extract on Blood Glucose and Pancreatic-Renal Histopathology in Diabetic Rats

Efek Antidiabetik Ekstrak Etanol Daun Miana (*Coleus atropurpureus*) terhadap Glukosa Darah dan Histopatologi Pankreas-Ginjal pada Tikus Diabetes

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ABSTRACT

This study evaluated the antidiabetic potential of ethanol extract of miana leaves (*Coleus atropurpureus* Benth.) and its protective effects on pancreatic and renal tissues in streptozotocin-induced diabetic rats. Thirty male Wistar rats were allocated to six groups: normal control, negative control (diabetes + Na-CMC), positive control (diabetes + glibenclamide), and three groups receiving miana extract at 150, 200, or 250 mg/kg body weight. Diabetes was induced by intraperitoneal streptozotocin injection (40 mg/kg body weight). Phytochemical screening detected flavonoids, alkaloids, tannins, and saponins. On day 28, 250 mg/kg produced the greatest blood glucose reduction (102 ± 9 mg/dL), numerically lower than glibenclamide (123 ± 25 mg/dL), although not statistically significant ($p = 0.248$). Histopathological analysis showed pancreatic and renal protection, with damage scores of 0.8 ± 0.447 and 0.4 ± 0.548, respectively. These findings indicate that 250 mg/kg was the most effective dose in this model.

ABSTRAK

Penelitian ini mengevaluasi potensi antidiabetes ekstrak etanol daun miana (*Coleus atropurpureus* Benth.) serta efek protektifnya terhadap jaringan pankreas dan ginjal pada tikus diabetes yang diinduksi streptozotocin. Tiga puluh tikus jantan Wistar dibagi ke dalam enam kelompok: kontrol normal, kontrol negatif (diabetes + Na-CMC), kontrol positif (diabetes + glibenklamid), dan tiga kelompok perlakuan ekstrak miana dosis 150, 200, atau 250 mg/kg BB. Diabetes diinduksi melalui injeksi streptozotocin intraperitoneal (40 mg/kg BB). Skrining fitokimia menunjukkan adanya flavonoid, alkaloid, tanin, dan saponin. Pada hari ke-28, dosis 250 mg/kg menghasilkan penurunan glukosa darah terbesar (102 ± 9 mg/dL), secara numerik lebih rendah daripada glibenklamid (123 ± 25 mg/dL), tetapi tidak berbeda signifikan ($p = 0,248$). Analisis histopatologi menunjukkan perlindungan pankreas dan ginjal, dengan skor kerusakan masing-masing 0,8 ± 0,447 dan 0,4 ± 0,548. Temuan ini menunjukkan bahwa 250 mg/kg merupakan dosis paling efektif dalam model ini.

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1. INTRODUCTION

Diabetes mellitus (DM) remains a major global health challenge. According to International Diabetes Federation data reported by the Perkumpulan Endokrinologi Indonesia (PERKENI, 2021), Indonesia ranked fifth worldwide in 2021, with approximately 10.3 million people living with diabetes. This prevalence is projected to increase substantially, reaching 21.3 million cases by 2030 (PERKENI, 2021). DM is a metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Chronic hyperglycemia can lead to serious complications in multiple organs, particularly the pancreas and kidneys. Its pathophysiology involves complex interactions between pancreatic β-cells and metabolic pathways, in which hyperglycemia-induced oxidative stress disrupts the balance between reactive oxygen species (ROS) and antioxidant defense systems. This imbalance contributes to cellular injury, impaired renal filtration, and progressive renal dysfunction (Aji et al., 2019; Ayu et al., 2020; Tandi, Danthy, et al., 2019; Tandi, Palinggi, et al., 2019).

Current diabetes management relies largely on conventional pharmacological therapies. Although these therapies are clinically useful, long-term use may be associated with cost limitations and adverse effects, particularly in resource-limited settings. These limitations have encouraged increasing interest in plant-based therapeutic candidates. Miana (*Coleus atropurpureus* Benth.), a member of the Lamiaceae family, has been traditionally used for various health purposes and has potential antidiabetic activity because of its phytochemical constituents, including flavonoids, alkaloids, tannins, and saponins (Wakhidah & Silalahi, 2018; Tandi et al., 2024). These compounds are associated with antioxidant activity and may contribute to the protection of pancreatic β-cells against oxidative injury while supporting residual insulin-related metabolic pathways.

The selection of *C. atropurpureus* over other Lamiaceae species with reported antidiabetic potential, such as *Plectranthus amboinicus* and *Ocimum basilicum*, is supported by several considerations relevant to the present study. First, *C. atropurpureus* has documented ethnomedicinal relevance in Indonesian communities. For example, miana has been reported in traditional medicinal use among communities in West Halmahera, North Maluku, supporting its ethnopharmacological importance as a medicinal plant (Wakhidah & Silalahi, 2018). Second, the characteristic deep-purple pigmentation of *C. atropurpureus* leaves suggests the presence of antioxidant-associated phytoconstituents that may be relevant to oxidative stress-mediated pancreatic β-cell injury in streptozotocin-induced diabetes. Third, a related study by the same research group reported that miana leaf ethanol extract contained total alkaloids of 12.66% w/w, flavonoids of 0.2163% w/w, and strong antioxidant activity, with an IC_{50} value of 56.96 ppm (Tandi et al., 2024). These findings provide a phytochemical basis for selecting *C. atropurpureus* as the plant material investigated in the present study.

A previous study reported that ethanol extract of miana leaves reduced urea and creatinine levels, with 250 mg/kg body weight identified as the optimal nephroprotective dose, producing average reductions of 18.8 mg/dL and 0.64 mg/dL, respectively (Tandi et al., 2022). However, studies evaluating the combined effects of miana leaf extract on blood glucose regulation, pancreatic β-cell preservation, and renal tissue protection remain limited. This gap supports the need for a systematic investigation of the antidiabetic and organ-protective effects of miana leaf ethanol extract, including dose optimization and histopathological assessment of pancreatic and renal tissues under diabetic conditions. Therefore, this study aimed to evaluate the antidiabetic efficacy of miana leaf ethanol extract at different doses and to assess its protective effects on pancreatic and renal histopathology in streptozotocin-induced diabetic rats.

2. METHODS

2.1. Study Design and Animals

This study employed a randomized controlled animal design with serial blood glucose measurements and histopathological assessment at the endpoint. Thirty male Wistar rats, which weighed 150–200 g and were aged 2–3 months, were obtained from the animal provider of Tadulako University, Palu, Central Sulawesi, Indonesia. The animals were acclimatized for 7 days under controlled laboratory conditions at 25 ± 2 °C, 55–60% relative humidity, and a 12-hour light/dark cycle.

Before streptozotocin induction, the animals were randomly allocated into six groups, with five rats in each group. Randomization was performed using simple random allocation with body weight stratification to obtain comparable baseline characteristics across groups. Group I served as the normal control; Group II served as the negative control and received diabetes induction plus 0.5% Na-CMC; Group III served as the positive control and received diabetes induction plus glibenclamide at 0.45 mg/kg body weight; and Groups IV, V, and VI received diabetes induction plus miana leaf ethanol extract at doses of 150, 200, and 250 mg/kg body weight, respectively.

The sample size of five animals per group was based on previous streptozotocin-induced antidiabetic studies conducted by the same research group, in which this sample size was sufficient to detect between-group differences (Tandi, Danthy, et al., 2019; Tandi, Palinggi, et al., 2019; Tandi et al., 2020). This sample size was also considered in relation to the 3Rs principle for ethical animal research. However, no formal a priori power calculation was performed; therefore, this should be considered a methodological limitation and addressed in future confirmatory studies.

All animal procedures were approved by the Ethics Committee of the Faculty of Medicine, Tadulako University, under approval number 6019/UN28.10/KL/2025 and were conducted in accordance with accepted principles for the care and use of laboratory animals.

2.2. Tools

The instruments used in this study included glassware (Pyrex), stirring rods, maceration vessels, porcelain dishes, animal cages, a rotary evaporator (Heidolph), a tabletop centrifuge (PLC 03 Series), an Accu-Chek glucometer, an oral gavage probe (OneMed Health Care), injection syringes (OneMed Health Care), rat drinking bottles, plain blood collection tubes (Vacutainer), Eppendorf tubes, an analytical balance (Ohaus), and a water bath (Denville).

2.3. Materials

The materials used in this study included distilled water, 70% alcohol, aluminum foil, 2 N hydrochloric acid, citrate-buffered saline, Dragendorff reagent, ethylenediaminetetraacetic acid, 96% ethanol, miana leaf ethanol extract, ferric chloride, glibenclamide, filter paper, magnesium powder, 0.5% Na-CMC, sodium hydroxide, sodium chloride, sodium salicylate, sodium nitroprusside, sodium hypochlorite, sodium citrate, phosphate buffer, streptozotocin, creatinine reagent kit, urea reagent kit, and standard animal feed.

2.4. Plant Material and Extract

Fresh miana leaves were collected in Alitupu Village, Poso Regency. Identification was performed at the Central Sulawesi Biological Resources Technical Implementation Unit (UPT), Tadulako University, under authentication number 78/UN.28.UPT-SDHS/LK/2021. The dried leaves were ground into powder and passed through a 40-mesh sieve, then extracted by maceration with 96% ethanol at a ratio of 1:3 w/v for 3 × 24 h with periodic stirring. The filtrate was concentrated using a rotary evaporator at 40–60 °C and further evaporated in a water bath at 60 °C until a thick extract was obtained.

A total of 1,000 g of dried simplicia powder was extracted, yielding 67 g of thick ethanol extract, corresponding to an extraction yield of 6.7% w/w. The simplicia yield from fresh leaves was 20% w/w. These values characterized the specific extract batch used in the present study.

2.5. Phytochemical Screening

Qualitative phytochemical screening was performed to identify the major classes of secondary metabolites present in the miana leaf ethanol extract (Shaikh & Patil, 2020). Flavonoids were detected using the magnesium–hydrochloric acid reaction, indicated by the formation of a red to orange color. Alkaloids were detected using Dragendorff reagent, indicated by the formation of an orange or reddish precipitate. Tannins were detected using ferric chloride, indicated by the formation of a blue-black or green-black color. Saponins were detected using the foam formation test, indicated by stable foam formation after shaking.

2.6. Diabetes Induction

Group I served as the normal control and received no treatment. Groups II–VI were induced with diabetes using streptozotocin at a dose of 40 mg/kg body weight, administered intraperitoneally. Seven days after streptozotocin injection, fasting blood glucose levels were measured from tail vein blood samples using an Accu-Chek glucometer to confirm hyperglycemia.

A fasting blood glucose threshold of ≥200 mg/dL was applied as the group-level confirmation criterion. All five streptozotocin-induced groups showed mean fasting blood glucose levels ≥200 mg/dL on day 7, with group means ranging from 203 to 254 mg/dL, indicating successful induction of hyperglycemia at the group level. Individual glycemic responses varied, with day-7 values ranging from 124 to 391 mg/dL across streptozotocin-induced groups. This variability is consistent with the known variability of streptozotocin-induced diabetes models. All 25 streptozotocin-induced animals were retained for analysis because the group-level glycemic profiles confirmed hyperglycemia. No animals were excluded based on individual day-7 glucose values. No animals died or were removed during the 28-day treatment period, and no replacements were required. All 30 animals completed the study and contributed to the final analysis.

A single dose of streptozotocin at 40 mg/kg body weight primarily induces insulin-deficient hyperglycemia through pancreatic β-cell destruction; therefore, this model more closely resembles insulin-deficient diabetes than classical insulin-resistant type 2 diabetes (Lenzen, 2008; Szkudelski, 2001). This model was selected because of its reproducibility and relevance to progressive β-cell failure in advanced diabetes.

2.7. Treatment Administration and Histopathological Analysis

Na-CMC 0.5%, glibenclamide, and ethanolic extract of miana leaves were administered orally using a 3-mL oral tube once daily. Treatment began after the rats were confirmed to have hyperglycemia, defined as fasting blood glucose levels >200 mg/dL on day 7 after streptozotocin induction. Blood glucose levels were measured from fasting tail vein blood samples using a standard glucometer protocol on days 0, 7, 14, 21, and 28. After the treatment period ended on day 28, the animals were euthanized, and pancreatic and kidney tissues were collected for histopathological examination.

Euthanasia was performed by first anesthetizing the animals with ether inhalation anesthesia. The animals were placed in a sealed jar containing cotton soaked in ether. The depth of anesthesia was confirmed by applying a pain stimulus to the paws. If the animals did not respond, adequate anesthesia was considered to have been achieved. After loss of consciousness, euthanasia was performed by cervical dislocation, which involved simultaneous pressure on the neck and traction of the tail until death. The pancreas and kidneys were immediately fixed in 10% formalin for 24 h. The tissues were then processed through graded dehydration, cleared with xylene, embedded in paraffin, sectioned at 4–5 µm thickness using a microtome, and stained with hematoxylin and eosin.

Histopathological evaluation was performed under a light microscope at 400× magnification by a pathologist blinded to treatment group allocation. Group allocation was conducted

before streptozotocin induction using simple randomization with body weight stratification. The allocation sequence was generated by the supervising researcher independently of the personnel administering treatment. For histopathological assessment, tissue sections were coded and provided to the evaluator without group labels, ensuring blinding of the primary histological outcome. Blood glucose measurements were performed by a single operator using a standardized protocol. Formal blinding was not applied to glucose measurement because the outcome was obtained using an objective glucometric readout. Tissue damage was assessed using a 0–4 scoring system based on the severity of morphological changes.

2.8. Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Normally distributed data with homogeneous variance were analyzed using one-way analysis of variance followed by the least significant difference post hoc test. Non-normally distributed data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test for post hoc pairwise comparisons. Statistical significance was set at $p < 0.05$.

For blood glucose data, one-way analysis of variance was applied independently at each measurement time point on days 0, 7, 14, 21, and 28 to evaluate between-group differences in glycemic status. This approach was used to compare groups at each specific time point rather than to model longitudinal trajectories. Because repeated measurements were obtained from the same animals over time, these observations were not fully independent. Therefore, repeated-measures analysis of variance or a linear mixed-effects model would be more appropriate for future longitudinal analysis. The time-point-based approach was used in the present study to identify the most effective dose at the study endpoint; however, future studies should apply longitudinal statistical models to more rigorously evaluate within-subject changes in glycemic response.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Composition

Phytochemical screening confirmed the presence of four major secondary metabolite groups in the miana leaf ethanol extract, namely flavonoids, alkaloids, tannins, and saponins (**Table 1**). Flavonoids showed a positive reaction with magnesium–hydrochloric acid, indicated by brick-red color formation. Alkaloids produced a red-orange precipitate with Dragendorff reagent, tannins produced a blue-black color with ferric chloride, and saponins showed persistent foam formation for 5 min. These findings indicate that the extract contains several phytochemical groups that may contribute to its antidiabetic and tissue-protective activities.

The phytochemical profile observed in this study is consistent with previous reports indicating that miana and related *Coleus* species contain bioactive secondary metabolites, particularly polyphenolic compounds and alkaloids (Wakhidah & Silalahi, 2018; Tandi et al., 2024). A related study by Tandi et al. (2024), using the same plant material source and extraction protocol, reported that miana leaf ethanol extract contained total alkaloids of 12.66% w/w, tannins of 0.9152% w/w, saponins of 0.5510% w/w, and flavonoids of 0.2163% w/w. These data provide supporting phytochemical context for the present qualitative findings, although they should not be interpreted as direct quantitative characterization of the extract batch used in this study.

Table 1. Phytochemical Screening Results of Miana Leaf Ethanol Extract

No	Secondary Metabolite	Reagent	Result	Status
1	Flavonoid	Magnesium-HCl	Brick-red color formation	+
2	Alkaloid	Dragendorff	Red-orange precipitate	+
3	Tannin	$FeCl_3$	Blue-black color	+
4	Saponin	Distilled water	Persistent foam (5 min)	+

Description: (+) indicates presence of the tested compound group; (-) indicates absence

The detected compound groups may contribute to the biological effects observed in this study through complementary mechanisms. Flavonoids are associated with antioxidant activity and may reduce reactive oxygen species-mediated injury in pancreatic β-cells and peripheral tissues (Wen & Li, 2025). Alkaloids may contribute to β-cell functional preservation; however, the present histopathological findings should be interpreted as structural preservation rather than confirmed β-cell regeneration because hematoxylin–eosin staining cannot directly demonstrate cellular proliferation or regeneration (Bourgeois et al., 2024). Tannins have been associated with modulation of intestinal glucose transport and glucose absorption (Brus et al., 2021), whereas saponins have been reported to support antidiabetic activity through effects on glucose handling and insulin-related pathways (Elekofehinti, 2015). Therefore, the combined presence of flavonoids, alkaloids, tannins, and saponins may partly explain the glycemic and histological improvements observed in the treated groups.

3.2. Blood Glucose Regulation: Dose-Response Relationship and Comparative Efficacy

Blood glucose analysis showed a dose-related pattern of response across the treatment period (**Figure 1**). Baseline blood glucose levels did not differ significantly among groups on day 0 ($p = 0.118$), indicating comparable initial glycemic conditions before streptozotocin induction. After induction, significant differences were observed on day 7 ($p = 0.024$), confirming the development of hyperglycemia in the diabetic groups. Significant between-

group differences were also observed on days 14 (p = 0.012), 21 (p = 0.016), and 28 (p = 0.003).

On day 28, the 250 mg/kg group showed the lowest mean blood glucose level, at 102 ± 9 mg/dL. This value was numerically lower than that of the positive control group treated with glibenclamide, which showed a mean blood glucose level of 123 ± 25 mg/dL. However, direct pairwise comparison between these two groups showed no statistically significant difference (p = 0.248, Mann–Whitney U test). Therefore, the glycemic effect of the 250 mg/kg dose should be interpreted as comparable to, rather than superior to, glibenclamide under the conditions of this study.

Descriptively, all extract-treated groups showed reductions in blood glucose from day 7 to day 28. The 150 mg/kg group decreased from 234 ± 51 to 146 ± 55 mg/dL, the 200 mg/kg group decreased from 236 ± 94 to 154 ± 47 mg/dL, and the 250 mg/kg group decreased from 203 ± 31 to 102 ± 9 mg/dL. In contrast, the negative control group remained hyperglycemic throughout the observation period. Because the statistical analysis was performed independently at each time point, these changes should be interpreted descriptively rather than as formal longitudinal effects.

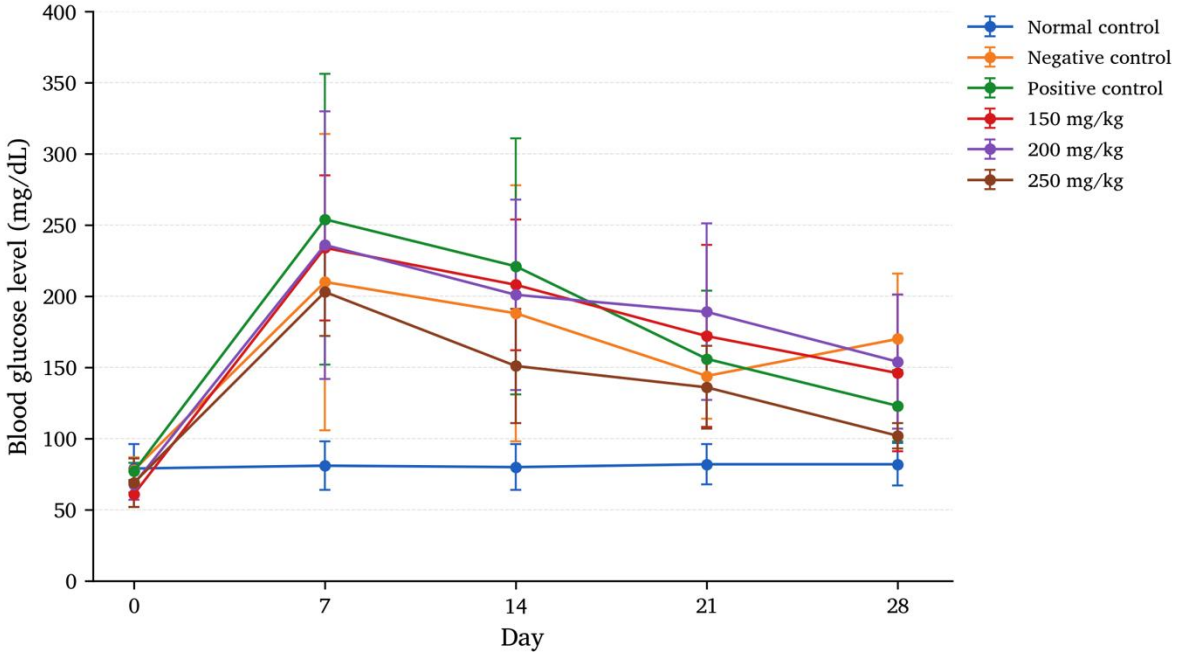

Figure 1. Changes in blood glucose levels during the treatment period. Mean blood glucose levels with standard deviation error bars are shown for each experimental group on days 0, 7, 14, 21, and 28. The 250 mg/kg group showed the most consistent reduction over the treatment period and reached the lowest mean blood glucose level on day 28. Values are expressed as mean ± SD; n = 5 rats per group.

The glycemic response observed in the 250 mg/kg group may be related to the combined activity of multiple phytochemical classes in the extract. Unlike glibenclamide, which primarily stimulates insulin secretion through closure of ATP-sensitive potassium channels in pancreatic β-cells, miana extract may act through several complementary mechanisms, including antioxidant protection, modulation of glucose absorption, support of insulin-related pathways, and preservation of pancreatic tissue structure. Similar multi-component effects have been reported for other plant extracts rich in antioxidant phytochemicals, including *Hibiscus sabdariffa* leaf flavonoid extract and a combination of *Andrographis paniculata* and *Moringa oleifera* extracts in streptozotocin-induced diabetic rats (Aji et al., 2019; Ajiboye et al., 2024).

The dose-related pattern observed in this study suggests that the 250 mg/kg dose provided the most favorable glycemic response among the tested doses. This finding is consistent with previous work reporting that the same dose of miana leaf ethanol extract reduced serum urea and creatinine levels in diabetic rats (Tandi et al., 2022). Nevertheless, because toxicity testing, pharmacokinetic assessment, and formal longitudinal modeling were not performed, the designation of 250 mg/kg as the most effective dose should be considered preliminary and specific to this experimental model.

3.3. Pancreatic Protection: Structural Preservation of Islet Architecture

Pancreatic histopathological scoring showed significant differences among groups (p = 0.001, Kruskal–Wallis test; **Figure 2**). The negative control group showed the highest pancreatic damage score, 2.0 ± 0.000, indicating moderate tissue injury characterized by islet necrotic changes and exocrine cell lysis. In contrast, the positive control and 250 mg/kg groups both showed lower damage scores of 0.8 ± 0.447, suggesting better preservation of pancreatic tissue architecture.

Representative pancreatic photomicrographs supported the scoring results (**Figure 3**). The normal control group showed intact islets of Langerhans with well-defined cellular boundaries and normal exocrine acinar cells. The negative control group showed islet vacuolation, nuclear pyknosis, and exocrine cell lysis. The

positive control group showed partial preservation of islet structure. Among the extract-treated groups, the 150 mg/kg group showed mild-to-moderate islet disruption, the 200 mg/kg group showed moderate improvement, and the 250 mg/kg group showed near-normal islet architecture with minimal vacuolation and preserved exocrine integrity.

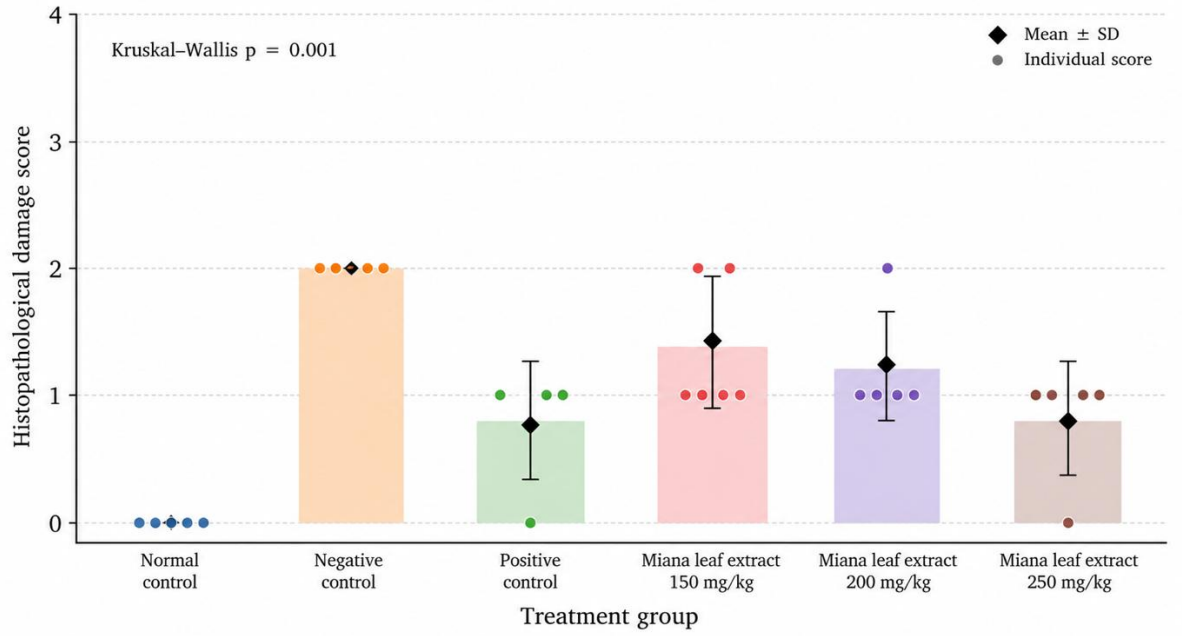

Figure 2. Pancreatic histopathological damage scores across experimental groups. Individual scores and mean ± SD are shown for semi-quantitative pancreatic injury scoring. The negative control group showed the highest damage score, whereas the positive control and miana 250 mg/kg groups showed lower scores. Overall group differences were significant by the Kruskal–Wallis test (p = 0.001; n = 5 rats per group).

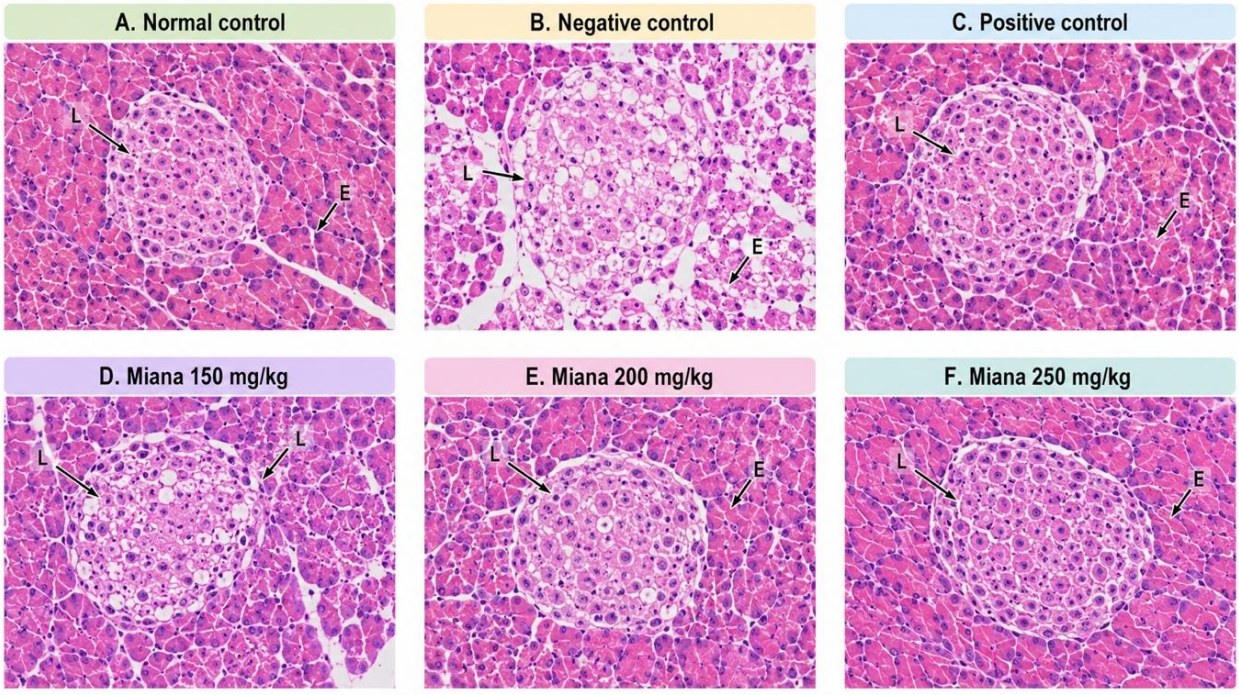

Figure 3. Representative pancreatic histopathology across experimental groups. Hematoxylin–eosin-stained pancreatic sections at 400× magnification showing normal control (A), negative control (B), positive control (C), miana 150 mg/kg (D), miana 200 mg/kg (E), and miana 250 mg/kg (F). The negative control group shows pronounced islet vacuolation and exocrine disruption, whereas the positive control and miana-treated groups demonstrate improved pancreatic tissue preservation, most notably at 250 mg/kg. L = islets of Langerhans; E = exocrine acinar cells.

These findings suggest that miana leaf ethanol extract may contribute to preservation of pancreatic tissue architecture in streptozotocin-induced diabetic rats. Similar findings have been reported for other antidiabetic plant extracts. Ethanol extract of

purple eggplant (*Solanum melongena*) peel improved pancreatic histological architecture in diabetic rats, with islet preservation associated with improved glucose control (Tandi, Danthy, et al., 2019). Similarly, *Moringa oleifera* leaf extract showed pancreatic protection in streptozotocin-induced diabetic rats, which was attributed to its phytochemical and antioxidant properties (Tandi, Palinggi, et al., 2019). These findings support the interpretation that antioxidant-containing plant extracts may help protect pancreatic tissue under diabetic conditions. However, the present study cannot confirm β-cell regeneration because immunohistochemical markers such as insulin, Ki-67, or PDX-1 were not evaluated.

3.4. Renal Protection: Histological Preservation and Nephroprotective Implications

Renal histopathological scoring also showed significant differences among groups ($p < 0.001$, Kruskal–Wallis test; **Figure 4**). The negative control group showed the highest renal damage score, 2.0 ± 0.000, indicating tubular and glomerular injury associated with untreated diabetic conditions. The positive control group showed the lowest score, 0.2 ± 0.447, whereas the 250 mg/kg group showed a low renal damage score of 0.4 ± 0.548. The 250 mg/kg group differed significantly from the negative control group ($p = 0.004$) but did not differ significantly from the positive control group ($p = 1.000$).

Representative renal photomicrographs further supported the scoring results (**Figure 5**). The normal control group showed intact glomerular tufts with patent capillary loops and normal tubular epithelium. The negative control group showed glomerular congestion, tubular epithelial swelling, cytoplasmic vacuolation, and loss of brush border integrity. The positive control group showed largely preserved renal morphology. Among the extract-treated groups, the 150 mg/kg group showed mild-to-moderate tubular and glomerular changes, the 200 mg/kg group showed mild tubular changes with preserved glomerular architecture, and the 250 mg/kg group showed near-normal glomerular and tubular morphology with minimal residual changes.

The renal histopathological findings indicate that miana leaf ethanol extract may have nephroprotective potential in streptozotocin-induced diabetic rats. These findings are consistent with a previous study reporting that miana leaf ethanol extract at 250 mg/kg reduced serum urea and creatinine levels in diabetic rats (Tandi et al., 2022). In the present study, the histological findings provide structural support for renal tissue protection. However, because renal biochemical markers were not the main endpoint presented in this section, the results should be interpreted as histological evidence of renal preservation rather than definitive evidence of restored renal function.

Comparable nephroprotective effects have been reported for other plant extracts containing antioxidant phytochemicals. Ajiboye et al. (2024) reported that *Hibiscus sabdariffa* leaf flavonoid extract exerted nephroprotective effects in streptozotocin-induced rats through mechanisms involving KIM-1 and TGF-β1 pathways. Aji et al. (2019) also reported that a combination of *Andrographis paniculata* and *Moringa oleifera* extracts reduced urea and creatinine levels in streptozotocin-induced diabetic rats. These findings support the possibility that antioxidant-rich plant extracts may reduce diabetes-related renal injury. Nevertheless, direct comparison of protective magnitude across studies should be made cautiously because experimental models, extract composition, outcome measures, and histopathological scoring systems may differ.

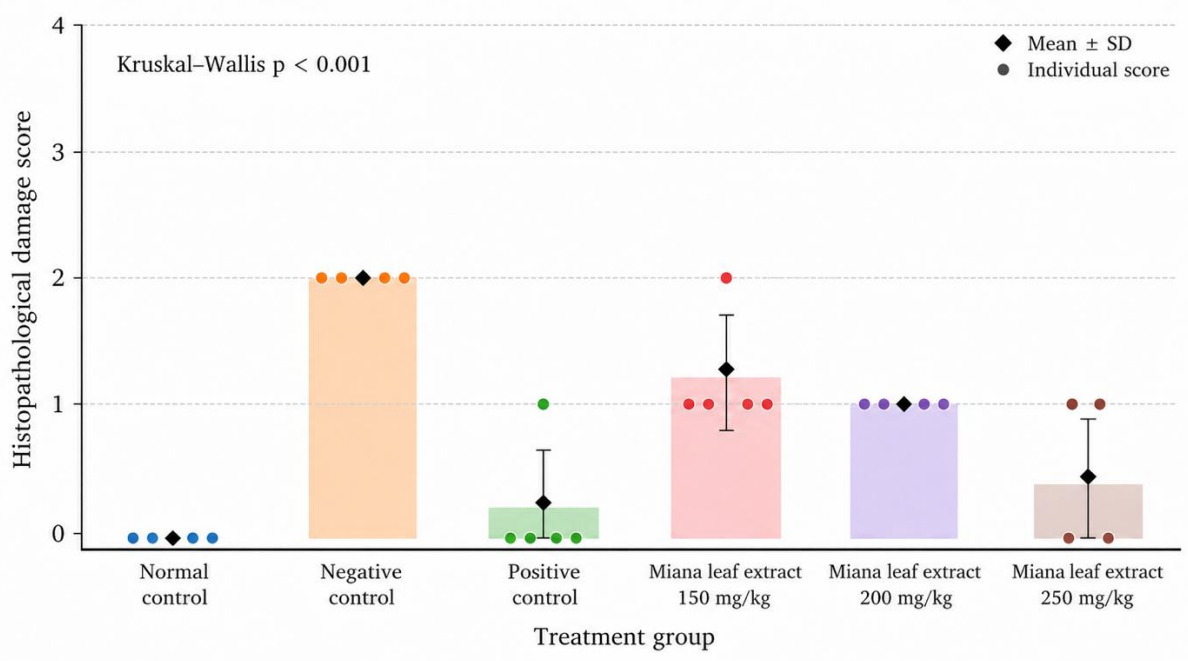

Figure 4. Renal histopathological damage scores across experimental groups. Individual scores and mean ± SD are shown for semi-quantitative renal injury scoring. The negative control group showed the highest damage score, whereas the positive control and miana 250 mg/kg groups showed lower scores. Overall group differences were significant by the Kruskal–Wallis test ($p < 0.001$; $n = 5$ rats per group).

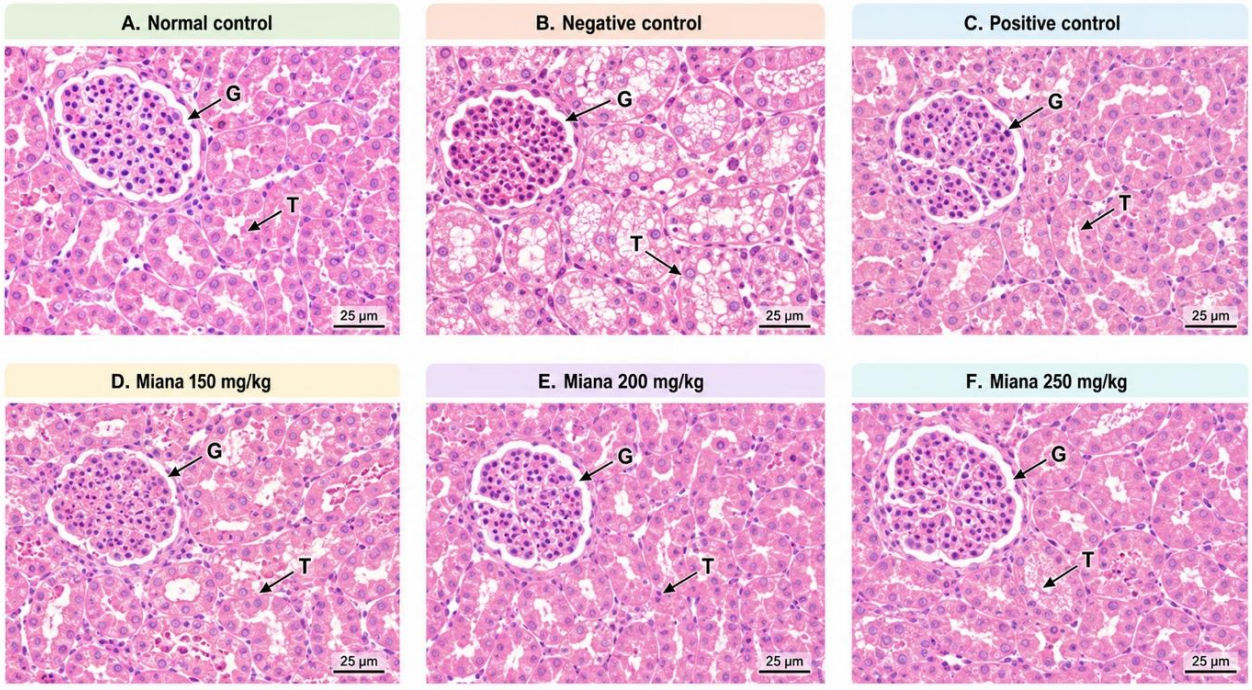

Figure 5. Representative renal histopathology across experimental groups. Hematoxylin–eosin-stained renal sections at 400× magnification showing normal control (A), negative control (B), positive control (C), miana leaf extract 150 mg/kg (D), miana leaf extract 200 mg/kg (E), and miana leaf extract 250 mg/kg (F). Renal tissue alterations were most evident in the negative control group, whereas milder changes were observed in the positive control and miana 250 mg/kg groups. G = glomerulus; T = renal tubule. Scale bars = 25 µm.

3.5. Integrated Therapeutic Mechanisms and Study Limitations

The overall findings suggest that miana leaf ethanol extract at 250 mg/kg produced the most favorable outcomes among the tested doses. This dose was associated with the lowest mean blood glucose level on day 28, pancreatic damage scores comparable to the positive control, and low renal histopathological damage scores. These outcomes indicate a consistent dose-related pattern across glycemic and histological endpoints. The combined presence of flavonoids, alkaloids, tannins, and saponins may contribute to these effects through complementary pathways, including antioxidant protection, modulation of glucose absorption, insulin-related activity, and preservation of pancreatic and renal tissue architecture (Alam et al., 2022; Brus et al., 2021; Elekofehinti, 2015; Wen & Li, 2025).

These mechanisms remain inferential because the present study did not directly measure oxidative stress markers, insulin levels, glucose transporter expression, inflammatory markers, or pharmacokinetic parameters. Therefore, the mechanistic interpretation should be regarded as biologically plausible rather than conclusive. Further pharmacodynamic and pharmacokinetic studies are required to confirm whether these proposed mechanisms operate directly in the present model.

Several limitations should be considered. First, the sample size was small, with five animals per group, which limits statistical power and may contribute to variability in several outcomes. Second, no formal a priori power calculation was performed. Third, the 28-day observation period does not allow conclusions regarding long-term efficacy or safety. Fourth, the absence of immunohistochemical markers such as insulin, Ki-67, and PDX-1 limits interpretation of pancreatic findings to structural preservation rather than β-cell regeneration. Fifth, blood glucose data were analyzed at separate time points, which does not account for within-subject correlations across repeated measurements. Future studies should use repeated-measures ANOVA or linear mixed-effects models to better evaluate longitudinal glycemic changes.

Finally, the streptozotocin-induced diabetes model primarily reflects β-cell injury and insulin-deficient hyperglycemia rather than the insulin resistance that characterizes many cases of type 2 diabetes. Therefore, translation of these findings to human type 2 diabetes should be made cautiously. Future research should include standardized extract characterization, toxicity and pharmacokinetic testing, insulin-resistant animal models, and molecular endpoints. If supported by further evidence, miana leaf ethanol extract may be considered for future development as a complementary antidiabetic candidate with potential glycemic and organ-protective effects.

4. CONCLUSION

This study demonstrates that ethanol extract of miana leaves (*Coleus atropurpureus* Benth.) has promising antidiabetic and organ-protective effects in streptozotocin-induced diabetic rats. Among the tested doses, 250 mg/kg produced the most favorable outcomes, as indicated by the lowest mean blood glucose level on day 28 (102 ± 9 mg/dL), which was numerically lower than that

of glibenclamide (123 ± 25 mg/dL), although the difference was not statistically significant (p = 0.248). This dose was also associated with lower pancreatic and renal histopathological damage scores, at 0.8 ± 0.447 and 0.4 ± 0.548, respectively, compared with the untreated diabetic control group. These findings suggest that the antidiabetic and tissue-protective effects of miana leaf ethanol extract may be related to the combined activity of flavonoids, alkaloids, tannins, and saponins. However, because this study was limited to a short-term insulin-deficient animal model, further research involving standardized extract characterization, toxicity evaluation, pharmacokinetic analysis, insulin-resistant diabetic models, and clinical studies is required before miana leaf extract can be recommended for therapeutic use in diabetes management.

AUTHOR CONTRIBUTIONS

Conceptualization, T.W.H., Mry., and C.C.; methodology, T.W.H., Mry., and C.C.; validation, J.T. and Mgf.; formal analysis, T.W.H., Mry., and C.C.; investigation, T.W.H. and Mry.; resources, T.W.H. and J.T.; data curation, C.C. and Mgf.; writing—original draft preparation, C.C.; writing—review and editing, T.W.H., Mry., J.T., C.C., and Mgf.; visualization, C.C. and Mgf.; supervision, J.T.; project administration, T.W.H. and Mry.; funding acquisition, T.W.H. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Ethics Committee of the Faculty of Medicine, Tadulako University, Indonesia, under approval number 6019/UN28.10/KL/2025.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the entire content to ensure that it accurately represents their own ideas, data, analyses, and interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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