RESEARCH ARTICLE

Gynura procumbens Leaf Extract Ameliorates Hyperglycemia, Oxidative Stress, and Pancreatic Islet Damage in Diabetic Rats

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Abstract

BACKGROUND: Conventional antidiabetic drugs, while effective, are often associated with adverse effects, secondary failure, and high costs, particularly in developing countries. This has driven interest in complementary plant-based therapies. *Gynura procumbens* is a medicinal plant traditionally used in Southeast Asia for diabetes and metabolic disorders, however scientific evidences supporting this use have not been elucidated well. In this study, *G. procumbens* leaf extract (GPLE) was assessed whether it could lower glucose, restore antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx), reduce the oxidative marker malondialdehyde (MDA), suppress pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, and improve pancreatic structure in diabetic rats.
METHODS: Twenty eight male Wistar rats were divided into four groups: normal rats, untreated diabetic rats, and diabetic rats given 500 or 1000 mg/kg of GPLE orally for 14 days. Blood glucose, SOD, GPx, and MDA were measured by spectrophotometry, while IL-6, IL-8, and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA). Pancreas sections were stained with H&E.
RESULTS: Administration of both extract doses significantly lowered fasting blood glucose by approximately 66% relative to the untreated diabetic control group (p<0.05). The administration of 1000 mg/kg GPLE increased SOD activity by 107% and GPx activity by 488%, while reducing MDA by 95% (p<0.05). Proinflammatory cytokines were markedly suppressed IL-6 by 76%, IL-8 by 76%, and TNF-α by 79% at the high dose. Histological examination showed that treated rats had nearly normal islet morphology, reduced vascular congestion, and almost no lymphocytic infiltration.
CONCLUSION: GPLE demonstrates promising antihyperglycemic, antioxidant, and anti-inflammatory activities alongside structural pancreatic preservation in a preclinical model. These findings suggest its potential as a candidate for further exploration in complementary metabolic therapies.
KEYWORDS: antioxidants, diabetes mellitus, *Gynura procumbens*, histopathology, inflammation, oxidative stress

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It represents a profound global health burden associated with long-term microvascular and macrovascular complications, requiring robust, continuous improvements in therapeutic and management strategies.(1,2) At the cellular and tissue levels, the clinical progression and severity of diabetes are heavily driven by chronic low-grade inflammation,

sustained systemic oxidative stress, and accelerated apoptosis of pancreatic tissues. These complex destructive pathways underscore the urgent need to explore effective cytoprotective, antioxidant, and anti-inflammatory interventions, including those derived frommedicinal plants.(3,4)

Chronic hyperglycemia leads to disturbances in carbohydrate, lipid, and protein metabolism and triggers a cascade of pathological events, including increased production of reactive oxygen species (ROS) through glucose autooxidation, protein glycation, and activation of the polyol pathway.(5) When ROS production overwhelms the body's antioxidant defense mechanisms, oxidative stress ensues, characterized by cellular damage to lipids, proteins, and DNA.(6) A key consequence is the peroxidation of membrane polyunsaturated fats, generating malondialdehyde (MDA) as a well-established marker of oxidative injury.(7) The body counteracts this damage with endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), whose activities are often depleted in diabetes.(8–10)

In addition to oxidative stress, chronic hyperglycemia activates inflammatory pathways (*e.g.*, nuclear factor-kappa (NF-κB)), leading to elevated production of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL)-6, and IL-8.(11,12) TNF-α disrupts insulin signaling by promoting serine phosphorylation of insulin receptor substrate (IRS)-1, inducing insulin resistance.(13) IL 6 is associated with β cell dysfunction and predicts the development of type 2 diabetes.(14,15) IL-8 recruits neutrophils and amplifies inflammatory cascades, exacerbating tissue damage.(16) Notably, IL-8 serves as a crucial chemokine that specifically recruits neutrophils to the injured pancreatic microenvironment, accelerating localized tissue necrosis and microvascular endothelial dysfunction. Evaluating IL-8 provides vital insights into the acute inflammatory cascades directly triggered by islet injury, which are often overlooked in standard therapeutic evaluations. The pancreatic islets, particularly insulin-producing β-cells, are highly vulnerable to this combined oxidative and inflammatory damage due to their inherently low expression of antioxidant enzymes.(11,17)

Current pharmacological approaches for diabetes management heavily rely on conventional oral hypoglycemic agents and subcutaneous insulin therapy. Despite the clinical availability of multiple drug classes including metformin, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, and sodium-glucose cotransporter 2 (SGLT2) inhibitors. These standard therapies have not fully resolved the progressive nature of diabetic complications and are frequently associated with significant medical limitations.(18) Specifically, long-term conventional treatment often triggers severe patient compliance issues due to well-documented adverse effects, including persistent gastrointestinal disturbances and diarrhea associated with metformin; substantial weight gain and high risks of acute hypoglycemia with sulfonylureas; peripheral fluid retention and potential cardiovascular risks with thiazolidinediones; and site-specific injection complications with clinical insulin dependency. This has driven growing interest in complementary and alternative medicine, especially medicinal plants with traditional use for diabetes management. Despite the availability of multiple antidiabetic drugs (metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and insulin), current therapies have not fully resolved the problem of diabetic complications. Many patients experience adverse effects such as gastrointestinal disturbances (metformin), weight gain and hypoglycemia (sulfonylureas), fluid retention (thiazolidinediones), or injection-related issues (insulin). Moreover, secondary failure loss of glycemic control despite continued treatment occurs in a substantial proportion of patients over time. Consequently, there is growing interest in complementary and alternative medicine, especially medicinal plants with traditional use for diabetes management, as they may offer better tolerability, lower cost, and multi-targeted mechanisms (e.g., combined antioxidant, anti-inflammatory, and hypoglycemic actions).(19,20)

Indonesia possesses rich biodiversity with thousands of medicinal plant species traditionally used for various ailments. *Gynura procumbens* is a medicinal plant widely distributed across Southeast Asia, particularly in Indonesia, Malaysia, and Thailand. Traditionally, the leaves have been used for treating diabetes, hypertension, hyperlipidemia, and inflammatory conditions.(21,22) Phytochemical studies have identified numerous bioactive compounds in *G. procumbens* leaves, including flavonoids (kaempferol, quercetin, rutin, astragalin), alkaloids, tannins, saponins, and phenolic acids.(22,23) Flavonoids, particularly flavonol glycosides, have demonstrated antihyperglycemic activity through multiple mechanisms including enhancement of insulin secretion, protection of pancreatic β cells, inhibition of α glucosidase, and improvement of peripheral insulin sensitivity. Saponins may contribute by inhibiting intestinal glucose absorption.(24) Previous studies have reported various biological activities of *G. procumbens*, including antioxidant, anti-inflammatory, antihypertensive, and

antihyperlipidemic effects.(22,25) However, comprehensive studies simultaneously evaluating its effects on multiple parameters blood glucose, antioxidant enzyme activities, lipid peroxidation, proinflammatory cytokines, and pancreatic histopathology remain limited. Furthermore, dose-response relationships and comparative efficacy of different extract concentrations have not been thoroughly investigated.

Accordingly, the present study was designed to evaluate the impact of low and high dosages of *G. procumbens* leaf extract (GPLE) on fasting blood glucose, the activities of endogenous antioxidant enzymes (SOD and GPx), the lipid peroxidation biomarker MDA, and key pro-inflammatory cytokines (TNF-α, IL-6, and IL-8) in Wistar rats with streptozotocin-induced diabetes. The specific inclusion of IL-8 as an evaluative parameter serves as a critical novelty in this study, as this chemokine is directly responsible for acute neutrophil recruitment, localized microvascular endothelial dysfunction, and accelerated tissue necrosis within the damaged pancreatic microenvironment following chemical insult. Concurrently, these biochemical modulations were correlated directly with structural and histopathological alterations in pancreatic islet architecture. By establishing this multi-targeted profiling, the findings of this study provide rigorous scientific evidence supporting the traditional, empirical use of *G. procumbens* for diabetes management and contribute substantially to the development of evidence-based complementary therapeutic strategies.

Methods

Preparation of GPLE

G. procumbens leaves were collected and taxonomically authenticated as described previously by the Department of Biology, University of Indonesia (No. 96/UN2.F3.11/PDP.02.00/2023).(26) One kilogram of fresh leaves was washed, cut, dried at 35°C, and extracted with 70% ethanol (Cat #100983; Merck, Darmstadt, Germany) using a Soxhlet apparatus for 10 h. The extract was concentrated by solvent evaporation using a rotary evaporator at 50°C, yielding a thick, concentrated extract of G. procumbens. The final yield was 8.6% (w/w) relative to the dried leaf material. For oral administration, the extract was freshly dissolved in distilled water to a stock concentration of 100 mg/mL such that the desired doses (500 mg/kg and 1000 mg/kg body weight) could be delivered in a volume of 5 mL/kg body weight.

Phytochemical Screening of GPLE

Qualitative phytochemical screening of GPLE was performed using standard procedures to detect the presence of major secondary metabolites. For flavonoids, 1 g of extract was dissolved in 10 mL distilled water, followed by addition of 2 mL of 10% sodium hydroxide (Cat #106498; Merck); a deep yellow color that became colorless after adding dilute hydrochloric acid (Cat #100317; Merck) indicated a positive result. Alkaloids were tested using Dragendorff's reagent (Cat #44507; Sigma-Aldrich, St. Louis, MO, USA): 1 g of extract was mixed with 5 mL of 1% HCl (Cat #100317; Merck), heated gently, filtered, and then two drops of the reagent were added; formation of an orange-red precipitate confirmed alkaloids. Tannins were identified by adding 2–3 drops of 5% ferric chloride (Cat #103943; Merck) to 1 g of extract dissolved in 10 mL distilled water; a blue-black or dark green coloration was considered positive. Saponins were detected by the froth test: 1 g of extract was shaken vigorously with 5 mL distilled water in a stoppered test tube for 30 seconds; persistent foam of at least 1 cm height for 15 minutes indicated saponins. All tests were performed in triplicate with appropriate positive and negative controls (quercetin for flavonoids, atropine for alkaloids, tannic acid for tannins, and quillaja saponin for saponins).(27) It is acknowledged that this phytochemical analysis serves as a preliminary, qualitative and semi-quantitative profiling strategy to establish the presence of broad secondary metabolite classes. While highly useful for initial biological correlations, absolute quantification of individual compound profiles was outside the scope of this baseline screening.

Experimental Animals

Twenty-eight male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 180–220 g at the start of the experiment, were used. The animals were housed in standard polypropylene cages (3–4 rats per cage) under controlled environmental conditions: temperature 22±2°C, relative humidity 50±10%, and a 12-h light/dark cycle. Rats had free access to standard rodent chow and tap water ad libitum. All animals were acclimatized for 7 days before the start of the experiment. Following acclimatization, the twenty-eight rats were allocated into four experimental groups (n=7 per group) using a stratified randomization procedure based on baseline body weight. An online random number generator was utilized by an independent technician not involved in the subsequent biochemical assays to ensure unbiased allocation and true experimental randomization. All procedures involving animals were ethically approved

and were conducted in accordance with the ARRIVE guidelines.

Sample Size and Grouping

A minimum of 6 rats per group was required; to account for potential dropouts, 7 rats per group were included, giving a total of 28 rats. The animals were randomly assigned to four groups: normal rats that received distilled water (Normal Group), diabetes-induced rats that received distilled water (Diabetic Group), diabetes-induced rats that received 500 mg/kg body weight per day GPLE (GPLE500 Group), and diabetes-induced rats that received 1000 mg/kg body weight per day GPLE (GPLE1000 Group). Treatments were administered orally by gastric gavage once daily for 14 consecutive days (Figure 1).

Induction of Diabetes Mellitus

After a 16-hour fast, diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (Cat #S0130; Sigma-Aldrich) at a dose of 65 mg/kg body weight, freshly dissolved in 0.1 M citrate buffer (Cat #C8532; Sigma-Aldrich; pH 4.5). STZ was administered within 5 minutes of preparation to maintain stability. This specific single high-dose STZ protocol was utilized to induce targeted pancreatic β-cell destruction, thereby establishing an experimental model primarily characterized by severe insulin deficiency. To prevent hypoglycemic shock, rats were provided with 5% glucose solution (Cat #G7528; Sigma-Aldrich) for 24 hours post-injection. Diabetes was confirmed 72 hours after STZ administration by measuring fasting blood glucose from the tail vein using a glucometer (Accu-Chek Active, Cat #06955248; Roche Diagnostics, Mannheim, Germany). Rats with fasting blood glucose >200 mg/dL were considered diabetic and included in the study. The primary parameter measured for confirmation was blood glucose level; no other parameters were used for inclusion.

Blood Sample Collection

Before any blood sample collection, the rats were fasted for 12 hours. Three mL blood samples were collected from the retro-orbital plexus under light anesthesia using microhematocrit capillary tubes (Cat #9000104; Hirschmann Laborgeräte, Eberstadt, Germany). Blood was transferred into microtubes and centrifuged at 3000 rpm for 10 minutes at 4°C using a refrigerated centrifuge (Eppendorf 5810R,

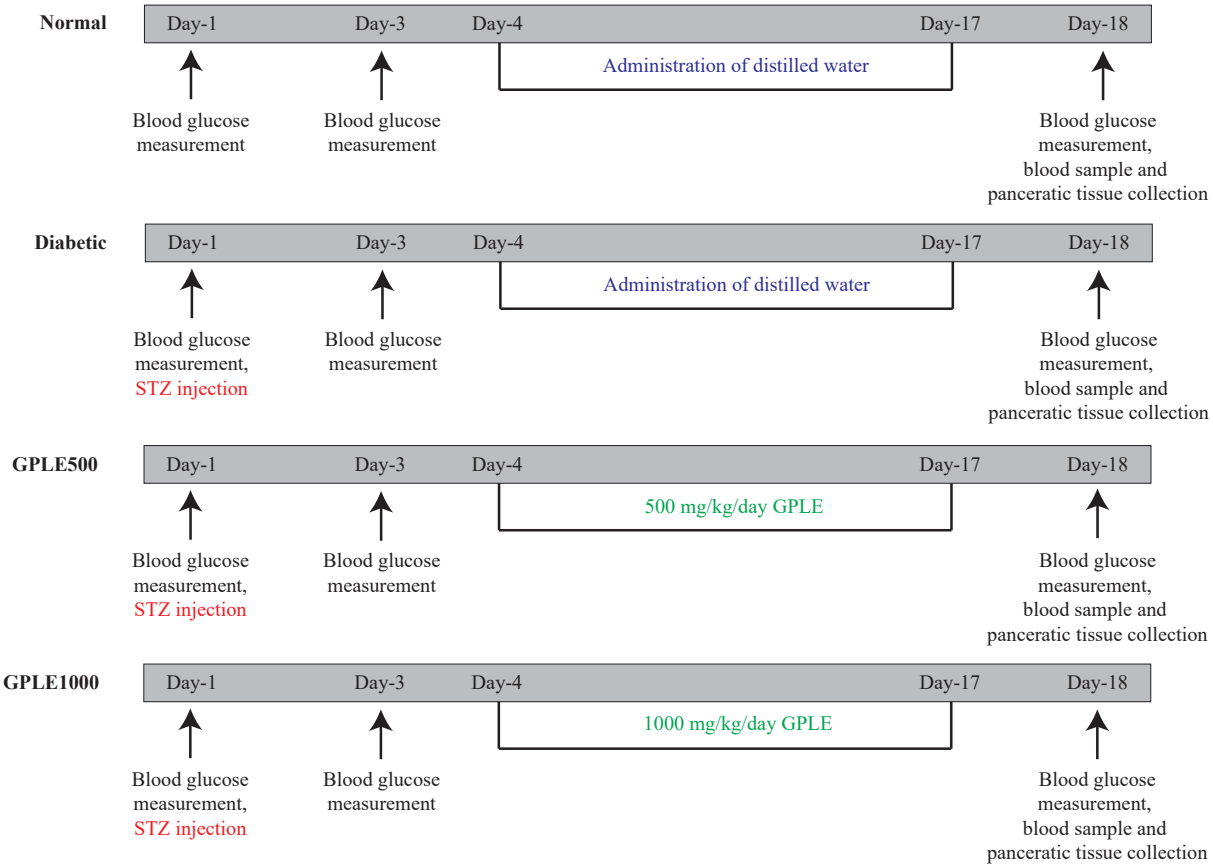

Figure 1. Schematic diagram of the treatment for each experimental group.

Cat #5810000015; Eppendorf, Hamburg, Germany). Serum was separated and stored at −20°C until analysis.

Blood Glucose Measurement

Fasting blood glucose was measured at three time points: before STZ induction (baseline), 72 hours post-induction (confirmation of diabetes), and 14 days post-treatment. Blood glucose was determined using the enzymatic Glucose Oxidase-Peroxidase Aminoantipyrine (GOD-PAP) method with a commercial kit (Cat #1 3000 99 10 123; DiaSys Diagnostic Systems, Holzheim, Germany) following the manufacturer's instructions.

Measurement of SOD Activity

The nitroblue tetrazolium (NBT) reduction method was employed to quantify SOD activity. To perform the assay, 0.06 mL of serum was mixed into 2.7 mL of 50 mM sodium carbonate buffer (Cat #S7795; Sigma-Aldrich; pH 10) that contained 0.1 mM EDTA (Cat #E9884; Sigma-Aldrich), followed by sequential addition of 0.06 mL of 10 mM xanthine (Cat #X7375; Sigma-Aldrich), 0.03 mL of 0.05% bovine serum albumin (Cat #A7030; Sigma-Aldrich), and 0.03 mL of 2.5 mM NBT (Cat #N5514; Sigma-Aldrich). The reaction commenced upon adding 0.04 units of xanthine oxidase (Cat #X1875; Sigma-Aldrich). Following a 30-minute incubation at ambient temperature, the mixture's absorbance was recorded at 560 nm using a Shimadzu UV-1800 spectrophotometer (Cat #206-24300-91; Shimadzu Corporation, Kyoto, Japan). SOD values were then calculated and expressed in U/mL.(28) The spectrophotometric assays for SOD activity were carried out in triplicate.

Measurement of GPx Activity

GPx activity was measured with a sample volume of 200 µL combined with 200 µL of 0.1 M phosphate buffer (Cat #P5244; Sigma-Aldrich; pH 7.0) that contained 0.1 mM EDTA, along with 200 µL of 10 mM reduced glutathione (GSH) (Cat #G4251; Sigma-Aldrich) and 200 µL of 2.4 units glutathione reductase enzyme (Cat #G3664; Sigma-Aldrich). This mixture was then kept at 37°C for 10 minutes. After this pre-incubation, 200 µL of 1.5 mM NADPH (Cat #N1630; Sigma-Aldrich) was introduced, and the mixture was left for another 3 minutes at the same temperature. The enzymatic reaction was triggered by adding 200 µL of 1.5 mM hydrogen peroxide (Cat #H1009; Sigma-Aldrich), and the reduction in absorbance at 340 nm was recorded over a 3-minute period. GPx activity levels were ultimately reported in units per liter (U/L).(29) All enzymatic GPx reactions were monitored and analyzed in triplicate.

Measurement of MDA Levels

The thiobarbituric acid (TBA) assay was used to quantify MDA levels. In this procedure, 0.5 mL of serum was combined with 2 mL of a chilled reagent mixture consisting of 0.25 N HCl (Cat #100317; Merck), 15% trichloroacetic acid (TCA) (Cat #T6399; Sigma-Aldrich), 0.38% TBA (Cat #T5500; Sigma-Aldrich), and 0.5% butylated hydroxytoluene (BHT) Cat #W218405; Sigma-Aldrich). The resulting solution was then heated in a water bath at 80°C for 60 minutes to allow formation of the MDA-TBA complex. After the samples had cooled to room temperature, they were centrifuged at 3500 rpm for 10 minutes. The absorbance of the clear supernatant was read at 532 nm using the Shimadzu UV-1800 spectrophotometer. Tetraethoxypropane (TEP) (Cat #86368; Sigma-Aldrich) served as the reference standard, and MDA concentrations were reported as nmol/mL.(30) The lipid peroxidation assays were executed in triplicate for each serum sample.

Measurement of Proinflammatory Cytokines (IL-6, IL-8, TNF-α)

Cytokine levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits following the instructions provided by the manufacturer (Elabscience Biotechnology Inc., Houston, TX, USA). The specific kits used were Rat IL-6 ELISA Kit (Cat #E-EL-R0015) with a detection range of 7.81–500 pg/mL and a sensitivity of 4.69 pg/mL, Rat IL-8/CXCL15 ELISA Kit (Cat #E-EL-R0814) with a detection range of 15.63–1000 pg/mL and a sensitivity of 9.38 pg/mL, and Rat TNF-α ELISA Kit (Cat #E-EL-R2856) with a detection range of 15.63–1000 pg/mL and a sensitivity of 9.38 pg/mL. In the assay procedure, 100 µL of each standard and sample were pipetted into antibody-precoated wells and left to incubate for 90 minutes at 37°C. After removing unbound material by washing, 100 µL of biotinylated detection antibody was added to each well, followed by a 60-minute incubation. The wells were washed again, then 100 µL of HRP conjugate was introduced and allowed to incubate for 30 minutes. Following a final wash, 90 µL of substrate solution was added, and the plate was kept in the dark for 15 minutes. The enzymatic reaction was terminated by adding 50 µL of stop solution, and the absorbance was read at 450 nm using a Bio-Rad iMark microplate reader (Cat #1681130; Bio-Rad Laboratories, Hercules, CA, USA). Concentrations of the cytokines were derived from standard curves and reported in pg/mL. The

manufacturer's protocol was followed without modification. All ELISA standards and experimental serum samples were rigorously loaded and evaluated in triplicate.

Histopathological Examination of Pancreas

Pancreatic tissues were fixed in 10% neutral buffered formalin (Cat #HT501128; Sigma-Aldrich) for 24 h, processed through graded alcohol, cleared in xylene (Cat #108297; Merck), and embedded in paraffin (Cat #39601006; Leica Biosystems, Nussloch, Germany). Sections of 5 µm thickness were cut using a microtome (Leica RM2255, Cat #1491000EP; Leica Biosystems) and stained with Hematoxylin and Eosin (H&E) (Cat #H3136 and Cat #HT110232; Sigma-Aldrich).(31) Histopathological examination was performed by a pathologist blinded to the treatment groups using a light microscope (Olympus CX23, Cat #CX23LEDRFS1; Olympus, Tokyo, Japan) at 100× and 400× magnifications. The following parameters were assessed using a semi-quantitative scoring system: islets of Langerhans morphology (0 = normal round shape; 1 = mild alteration; 2 = moderate alteration; 3 = severe alteration), blood vessel congestion (0 = none; 1 = mild; 2 = moderate; 3 = severe), and lymphocytic infiltration (0 = none; 1 = mild/few scattered cells; 2 = moderate/focal aggregates; 3 = severe/diffuse infiltration).(32)

Statistical Analysis

Data were presented as mean±standard deviation (SD) for continuous variables. Prior to inferential statistical analysis, the distribution of all biochemical and physiological datasets was evaluated for normality using the Shapiro-Wilk test, and homogeneity of variances was assessed via Levene's test. Datasets were statistically analyzed with One-Way Analysis of Variance (ANOVA), followed by Tukey's HSD post-hoc or Kruskal Wallis test based on the data distribution and homogeneous variances. Statistical analyses were performed using SPSS software version 26.0 (IBM Corporation, Armonk, NY, USA). A $p<0.05$ was considered statistically significant across all tests.

Results

Phytochemical Profile of GPLE

Qualitative phytochemical screening of GPLE revealed the presence of flavonoids, alkaloids, tannins, and saponins, while steroids were absent. The results were presented in semi quantitative form in Table 1 based on colour intensity, precipitate formation, and foam stability.

GPLE Lowered Blood Glucose in Diabetic Rats

All rats was considered to have normal blood glucose before the diabetic-induction, which could be proven by the blood glucose below 200 mg/dL in all groups during the baseline measurement. After the STZ injection, rats in Diabetic, GPLE500, and GPLE1000 had blood glucose levels exceeding 200 mg/dL at 72 hours post diabetic-induction, indicating that the diabetic-induction was sucessfully performed. Meanwhile, the normal rats remains to have blood glucose at 90.21±9.27 mg/dL (Table 2).

After 14 days with and without GPLE treatment, both GPLE500 and GPLE1000 groups showed significant reductions in blood glucose levels compared to the Diabetic group. The reduction in GPLE500 was 97.49±9.82 mg/dL, while in GPLE1000 was 97.93±24.89 mg/dL. Unlike the groups treated with GPLE, the Normal and Diabetic groups had elevated blood glucose instead (Table 2). Blood glucose level in GPLE500 and GPLE1000 also hasd significant different compared to the Diabetic control after the GPLE administration ($p<0.05$)

GPLE Restored SOD, GPx and Reduces MDA

At the end of study, lipid peroxidation, measured by MDA, was dramatically increased in Diabetic (62.95±29.11 nmol/

Table 1. Semi quantitative phytochemical profile of GPLE.

Phytochemical Class	Test Performed	Result	Intensity Scale
Flavonoids	Sodium hydroxide test	Positive	+++
Alkaloids	Dragendorff's test	Positive	++
Tannins	Ferric chloride test	Positive	++
Saponins	Froth test	Positive	+++
Steroids	Salkowski test	Negative	–

Intensity scale: − = absent; + = weak; ++ = moderate; +++ = strong; ++++ = very strong. Scoring was based on color intensity (flavonoids: deep yellow; tannins: dark green), amount of precipitate (alkaloids), and foam height (saponins: >1 cm stable foam).

Table 2. Blood glucose levels in experimental groups.

Group	Mean±SD (mg/dL)		
	Baseline	72 Hours Post STZ-Induction	Post Treatment
Normal	86.57±11.38[b,c,d]	90.21±9.27[b,c,d]	116.14±15.99[b]
Diabetic	128.57±15.80[a,c,d]	268.40±31.50[a]	287.43±68.49[a,c,d]
GPLE500	149.14±32.52[a,d]	271.20±28.70[a]	97.29±18.88[b]
GPLE1000	145.86±40.94[a,c]	263.50±35.20[a]	97.43±10.31[b]
p-value	0.003*	0.042*	0.032*

Different lowercase superscript letters within a row indicate statistically significant differences based on Kruskall Wallis test followed by post-hoc test (Mann-Whitney). *$p<0.05$ is considered statistically significant. [a]$p<0.05$ *vs*. Normal; [b]$p<0.05$ *vs.* Diabetic; [c]$p<0.05$ *vs.* GPLE500; [d]$p<0.05$ *vs.* GPLE1000.

mL) compared to Normal (5.58±1.05 nmol/mL), confirming oxidative damage. Both GPLE500 and GPLE1000 markedly reduced MDA levels, with GPLE1000 (2.98±1.97 nmol/mL) lowering MDA to values even below those of the Normal control (Table 3). There were statistically significant differences of MDA level across groups ($p<0.007$), underscoring the protective effect of the treatments against membrane lipid peroxidation. This suggest that the two treatment regimens, especially GPLE1000, exerted potent antioxidant effects.

GPLE1000 also significantly enhanced SOD (4.33±0.07 U/mL) and GPx activities (2.82±1.01 U/L) while nearly normalizing MDA levels (2.98±1.97 nmol/mL), after the diabetic-induction. This higher dose demonstrated superior efficacy compared to GPLE500 (Table 3). These findings suggested that GPLE1000 effectively counteracts oxidative stress by boosting enzymatic antioxidant defences and reducing cellular damage, offering stronger protective potential than GPLE500 in this experimental model.

GPLE Suppressed IL-6, IL-8, and TNF-α

There was significant difference of IL-6, IL-8, and TNF-α levels between all groups at the end of the study. The Diabetic group showed markedly elevated levels of all three cytokines, confirming that inflammatory state was observed after the diabetic-induction. Treatment with both doses of GPLE500 and GPLE100 showed significant differences of all pro-inflammatory cytokine compared to Diabetic group, with GPLE1000 group (13.81±7.29, 74.04±41.61, and 278.94±85.88 pg/mL for IL-6, IL-8, and TNF-α, respectively) showing levels comparable to or even lower than the Normal group (Table 4).

GPLE Preserves Pancreatic Islet Morphology

Histopathological observations of the pancreas of rats were conducted using the Hematoxylin-Eosin (H&E) staining method. Rats with diabetes mellitus showed differences in histopathological appearance when compared to normal rats. These differences were seen in changes in the shape of the islets of Langerhans, the number of congested blood vessels, and the large number of inflammatory lymphocytes.

The Normal group showed normal pancreatic histoarchitecture with well defined round islets of Langerhans, no blood vessel congestion, and no lymphocytic infiltration. In contrast, the Diabetic group exhibited severe histopathological changes including irregular shaped

Table 3. SOD and GPx activities in experimental groups.

Parameter	Mean±SD				*p*-value
	Normal	Diabetic	GPLE500	GPLE1000	
SOD (U/mL)	2.17±1.05[d]	2.09±0.61[d]	2.24±0.47[d]	4.33±0.07[a,b,c]	0.002*
GPx (U/L)	4.39±0.08[b,c,d]	0.48±0.19[a]	1.40±0.96[a,b,d]	2.82±1.01[a,b,c]	0.003*
MDA(nmol/mL)	5.58±1.05[b,c]	62.95±29.11[a,c,d]	13.01±5.07[a,b,d]	2.98±1.97[b,c]	0.007*

Different lowercase superscript letters within a row indicate statistically significant differences based on Kruskall Wallis test followed by post-hoc test (Mann-Whitney). *$p<0.05$ is considered statistically significant. [a]$p<0.05$ *vs*. Normal; [b]$p<0.05$ *vs.* Diabetic; [c]$p<0.05$ *vs.* GPLE500; [d]$p<0.05$ *vs.* GPLE1000.

Table 4. Proinflammatory cytokine levels in experimental groups.

Cytokines	Mean±SD (pg/mL)				*p*-value
	Normal	Diabetic	GPLE500	GPLE1000	
IL-6	13.48±4.88[b,c]	58.70±3.17[a,c,d]	26.33±6.21[a,b]	13.81±7.29[b,c]	0.001*
IL-8	48.92±17.18[b,c]	307.74±21.51[a,c,d]	89.42±37.98[b,d]	74.04±41.61[b,c]	0.007*
TNF-α	377.44±86.29[b,c]	1348.92±100.32[a,c,d]	794.99±173.06[a,b]	278.94±85.88[b,c]	0.003*

Different lowercase superscript letters within a row indicate statistically significant differences based on Kruskall Wallis test followed by post-hoc test (Mann-Whitney). *$p<0.05$ is considered statistically significant. [a]$p<0.05$ *vs.* Normal; [b]$p<0.05$ *vs.* Diabetic; [c]$p<0.05$ *vs.* GPLE500; [d]$p<0.05$ *vs.* GPLE1000.

islets of Langerhans, marked blood vessel congestion, and moderate lymphocytic infiltration, indicating diabetes induced pancreatic damage. Treatment with 500 mg/kg of GPLE resulted in partial improvement, with mild islet alterations, mild congestion, and mild lymphocytic infiltration. The 1000 mg/kg treatment of GPLE showed remarkable protection, with normal islet morphology, mild congestion, and no lymphocytic infiltration, closely resembling the Normal group (Figure 1).

Histopathological analysis of the pancreas revealed that the Normal group had normal islet morphology, clear boundaries, minimal vascular congestion, and no lymphocytic infiltration (score 0). In contrast, the Diabetic group showed marked abnormalities: irregular islet shape, moderate blood vessel congestion, and moderate lymphocytic infiltration, confirming diabetes-induced pancreatic injury (score 2). Treatment with GPLE500 partially improved these parameters (mild islet alteration, mild congestion, mild infiltration) to score 1, while GPLE1000 achieved near-complete restoration, with normal islet roundness, only mild congestion, and no lymphocytic infiltration (score 0 and 1) (Table 5). These findings demonstrate dose-dependent pancreatic protection by the extract, attributable to its antioxidant and anti-inflammatory properties.

Discussion

In this study, *G. procumbens* leaf extract showed the presence of flavonoids, which indicated potential antioxidant and anti-inflammatory properties; alkaloids, which suggested possible analgesic or antimicrobial activities; tannins, which supported wound healing and astringent effects; and saponins, that pointed to immunomodulatory or hypocholesterolemic actions.(33) Meanwhile, the absence of steroids implies that the extract may not exert significant steroid related hormonal effects.

The current study results indicate that GPLE possesses notable antihyperglycemic, antioxidant, and anti-inflammatory properties, with the high dose (1000 mg/kg) showing superior effects on most parameters except fasting blood glucose, where both doses were equally effective. STZ induces diabetes mellitus by selectively destroying pancreatic β-cells through DNA alkylation and free radical generation, leading to impaired insulin production.(17) STZ enters β-cells via GLUT2 transporters, causing reduced insulin biosynthesis and elevated blood glucose levels.(34) In this study, both GPLE doses significantly reduced blood glucose in diabetic rats, with no significant difference between 500 and 1000 mg/kg. This absence of a dose-dependent glucose-lowering effect suggests a possible ceiling effect under the present experimental conditions. Potential explanations though not directly tested here include saturable intestinal absorption of bioactive flavonoids, maximal inhibition of GLUT2 or α-glucosidase at 500 mg/kg, or near-complete rescue of residual β-cell function within the 14-day treatment period. Importantly, the higher dose (1000 mg/kg) provided superior antioxidant and anti-inflammatory effects (SOD, GPx, MDA, IL-6, TNF-α) and better pancreatic protection, justifying its use for preventing diabetic complications despite the lack of additional glucose reduction. This dissociation between glycemic control and cytoprotection has not been highlighted previously and suggests that different bioactive compounds or mechanisms may underlie each effect.

Flavonoids, including kaempferol, quercetin, and rutin identified in *G. procumbens*, are known to enhance insulin secretion from remaining β-cells, increase insulin sensitivity in peripheral tissues, and inhibit hepatic glucose production.(22) Additionally, saponins in the extract may reduce glucose absorption by inhibiting α glucosidase and GLUT2 transporters in the intestinal epithelium.(34) These multiple mechanisms could collectively contribute to the observed blood glucose reduction, although the present

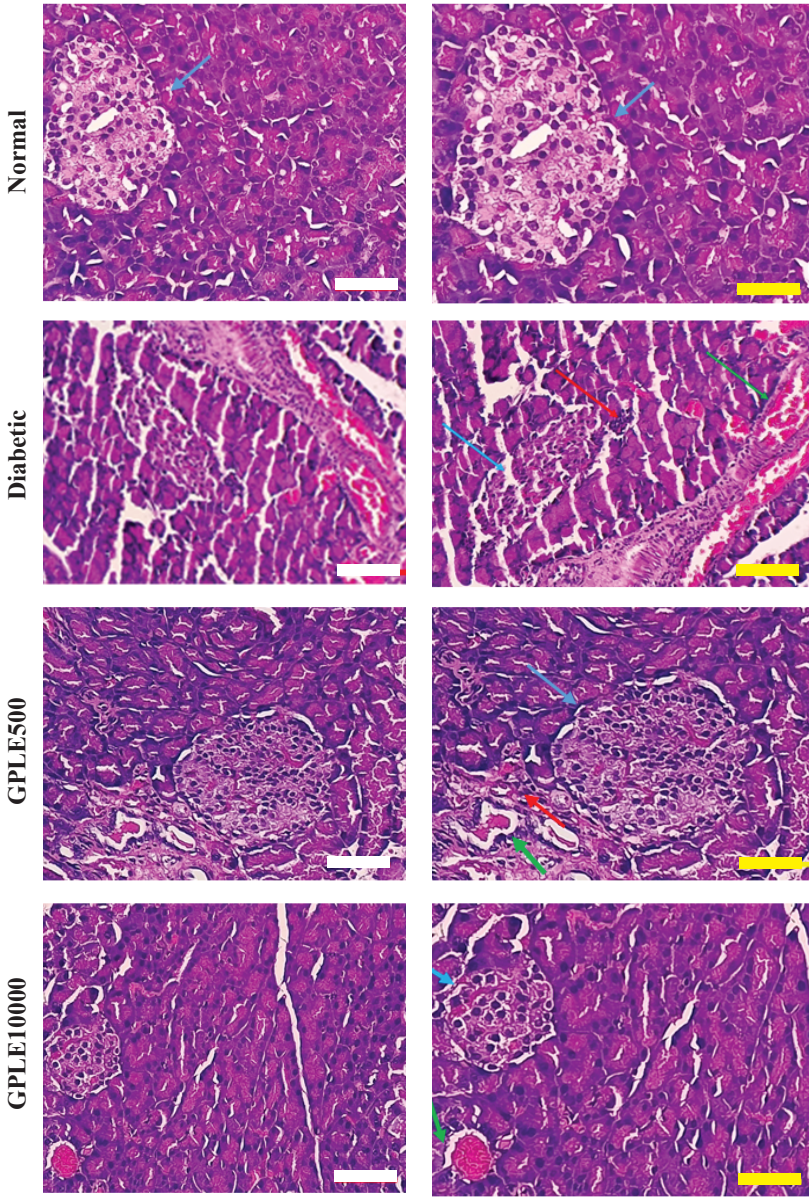

Figure 2. Representative pancreatic histopathology (H&E staining). Normal: normal islet morphology with round shape, no congestion, no infiltration. Diabetic: irregular islet shape, moderate vascular congestion, moderate lymphocytic infiltration. GPLE500: mild improvement with mild islet alteration. GPLE1000: near-normal islet morphology with mild congestion and no infiltration. Blue arrow: Islets of Langerhans; Red arrow: Inflammatory cells; Green arrow: Congestion. White bar: 50 µm; Yellow bar: 100 µm.

study did not directly measure insulin levels or glucose transporter activity; therefore, conclusions regarding the relative contribution of each mechanism remain speculative. Oxidative stress plays a central role in the pathogenesis of diabetes and its complications. Hyperglycemia promotes ROS production through glucose autooxidation, protein glycation, and activation of the polyol pathway.(10,17) The body's defense against oxidative stress includes antioxidant enzymes such as SOD and GPx, which are often depleted in diabetes.(5,10) Theresults showed that STZ induced diabetes significantly decreased SOD and GPx activities while increasing MDA levels, confirming the presence of oxidative stress. Treatment with *G. procumbens* extract, particularly at 1000 mg/kg (GPLE1000), significantly

Table 5. Histopathological scoring of pancreatic tissue.

Group	Islets of Langerhans Morphology	Blood Vessel Congestion	Lymphocytic Infiltration
Normal	0 (normal round shape)	0 (none)	0 (none)
Diabetic	2 (moderate alteration)	2 (moderate)	2 (moderate)
GPLE500	1 (mild alteration)	1 (mild)	1 (mild)
GPLE1000	0 (normal round shape)	1 (mild)	0 (none)

restored antioxidant enzyme activities and reduced lipid peroxidation. The GPLE1000 group showed SOD and GPx levels comparable to or higher than the Normal group, indicating substantial antioxidant activity.

These findings are consistent with previous studies reporting that flavonoids in *G. procumbens* enhance endogenous antioxidant defenses by upregulating gene expression of antioxidant enzymes and directly scavenging free radicals.(35) The phenolic hydroxyl groups in flavonoids donate electrons to stabilize free radicals, interrupting chain reactions of lipid peroxidation.(36) The marked reduction in MDA levels with the high-dose group showing levels even lower than normal controls demonstrates the extract's effectiveness in preventing oxidative damage to cellular membranes. This protection is crucial for preserving β-cell function and preventing diabetic complications.(37) However, the magnitude of GPx recovery (488% increase from diabetic levels) and the reduction of MDA to below normal values (2.98 *vs.* 5.58 nmol/mL) are greater than those observed in earlier studies. This discrepancy may be due to differences in extraction method, animal model, or treatment duration.(36) The marked reduction in MDA levels with the high-dose group showing levels even lower than normal controls demonstrates the extract's effectiveness in preventing oxidative damage to cellular membranes. This protection is crucial for preserving β-cell function and preventing diabetic complications.(37) A critical interpretation of the below-normal MDA is that it may reflect either genuine superior protection or, alternatively, a methodological artifact such as interference from residual polyphenols in the TBA assay. Future studies using high performance liquid chromatography (HPLC)-based measurements are needed to confirm this finding.

Chronic inflammation is increasingly recognized as a key component in the pathogenesis of type 2 diabetes. Hyperglycemia activates inflammatory pathways, leading to increased production of proinflammatory cytokines such as TNF-α, IL-6, and IL-8.(14,29) These cytokines contribute to insulin resistance, β-cell dysfunction, and the development of diabetic complications.TNF-α impairs insulin signaling by promoting serine phosphorylation of IRS-1, inhibiting insulin action.(38) In our study, diabetic rats (Diabetic group) exhibited markedly elevated levels of all three cytokines. Treatment with *G. procumbens* extract significantly reduced these inflammatory markers, with the high dose (GPLE1000) showing effects comparable to or better than the Normal group. The dose-dependent reduction in IL-6 and TNF-α suggests that the anti-inflammatory effect is related to the concentration of bioactive compounds. The anti-inflammatory mechanism of GPLE potentially involves inhibition of the NF-κB pathway, which regulates proinflammatory cytokine expression; flavonoids such as quercetin and kaempferol have been shown by others to suppress NF-κB activation.(25,35) Additionally, by reducing oxidative stress, the extract may indirectly decrease inflammation, as ROS are known to activate inflammatory pathways.(36) Nevertheless, because NF-κB activation was not directly measured in this study, these mechanistic interpretations remain hypothetical.

Histopathological examination provided direct evidence of pancreatic protection by GPLE. Diabetic rats showed characteristic changes including irregular islet morphology, blood vessel congestion, and lymphocytic infiltration, indicating ongoing inflammation and tissue damage.(10) Treatment with GPLE, particularly at 1000 mg/kg, preserved normal islet architecture, reduced vascular congestion, and prevented inflammatory cell infiltration. The near normal appearance of pancreatic tissue in the GPLE1000 group suggests that the extract not only lowered blood glucose and oxidative stress but also protected pancreatic structure from diabetes induced damage. This protective effect may result from the combined antioxidant and anti inflammatory activities of the extract. While both GPLE doses showed beneficial effects, the 1000 mg/kg dose generally demonstrated superior outcomes, particularly in antioxidant enzyme restoration (SOD), reduction of IL 6 and TNF α, and pancreatic histopathology. This pattern suggests a dose dependent relationship for some parameters. However, for blood glucose and IL 8, both doses were equally effective, indicating that maximum glycemic control may be achievable at lower doses in this model. The absence of observable toxicity at the higher dose suggests a favorable safety profile, though further studies are needed to establish the therapeutic window and optimal dosing regimen.

Several factors should be considered when interpreting these findings. The relatively small number of subjects restricts both the statistical power and the applicability of the results to broader populations. The 14 day treatment period may not adequately capture long term effects or potential adverse effects of chronic administration. Additionally, the absence of insulin level measurements prevents confirmation of whether the observed blood glucose reduction stems from enhanced insulin secretion or improved insulin sensitivity. Furthermore, this study did not directly investigate the underlying molecular pathways responsible for the observed effects (*e.g.*, NF κB activation, GLUT2 expression, α glucosidase activity). It is also important to note that the phytochemical analysis was qualitative and semi quantitative only; while this approach is acceptable for a preliminary screening study, it does not provide absolute

quantification of individual bioactive compounds. Therefore, correlations between specific compound concentrations and the observed biological effects cannot be established from the present data. Subsequent investigations should focus on clarifying the specific signaling cascades through which GPLE exerts its actions, employing quantitative analytical methods (*e.g.*, HPLC MS), and assessing its prolonged safety and efficacy. And as these results are derived from a preclinical model, additional studies, including toxicity assessments and clinical trials, are necessary before any therapeutic application can be considered.

Conclusion

This study indicates that GPLE exerts notable antihyperglycemic, antioxidant, and anti-inflammatory effects in streptozotocin-induced diabetic rats. The extract lowers blood glucose levels, enhances SOD and GPx activities, reduces MDA levels, and suppresses the proinflammatory cytokines IL-6, IL-8, and TNF-α in a dose-dependent manner. Histopathologically, treatment preserves normal islet morphology and reduces pancreatic inflammatory infiltration, with the GPLE1000 dose generally showing superior effects to GPLE500. These findings may support the traditional use of *G. procumbens* for diabetes management and suggest that it may be potential as a complementary approach to diabetes management.

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Authors Contribution

EA contributed to conceptualization, methodology, investigation, formal analysis, writing original draft, visualization, and project administration. EG was responsible for methodology, validation, resources, writing review and editing, and supervision. RI performed formal analysis, data curation, writing review and editing, and validation. All authors have read and approved the final manuscript.

Ethical Statement

All procedures involving animals were ethically approved by the Animal Research Ethics Committee of Universitas Sumatera Utara (Approval No. 070/KEPH-FMIPA/2023) and were conducted in accordance with the institutional guidelines for the care and use of laboratory animals, as well as the ARRIVE guidelines.

Conflict of Interest

The authors declare no conflicts of interest.

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