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Research Article

Polyphenol-Standardized *Aphanamixis polystachya* Leaf Extract Ameliorates Diabetes, Oxidative Stress, Inflammation, and Fibrosis in Streptozotocin-Induced Diabetic Rats

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Background. Diabetes is a rising disorder that affects millions of people annually. It also creates more complications, such as neuropathy, oxidative stress, and hepatic and kidney impairment. *Aphanamixis polystachya* plant, which possesses multiple medicinal values, is used in this study to explore its potential in treating diabetes. *Methods.* A single dose (65 mg/kg) of intraperitoneal streptozotocin injection was utilized to mediate diabetes in Sprague-Dawley rats. Diabetic animals were treated orally with 250 or 500 mg/kg of standardized leaves' extract of *A. polystachya* (AP) for 28 days to evaluate the antidiabetic and organ-protective effects of the plant. Different biochemical and histological markers are measured according to the established protocol. *Results.* Our results demonstrated a significant decrease in blood glucose ($p < 0.001$) and HbA1c ($p < 0.05$) levels in the diabetic animal after administering AP (250 and 500 mg/kg doses) compared to the control groups. AP can also regularize lipids, glycogen, alanine aminotransferase, and aspartate aminotransferase. Furthermore, serum urea nitrogen and creatinine decreased after treatment with AP in diabetic rats. AP also reduced oxidative stress markers and showed a substantial elevation in antioxidant enzymes in diabetic animals. Overall, AP at 500 mg/kg revealed comparable results against the standard antidiabetic drug glyburide. Besides, the histological investigation showed the AP's efficacy in attenuating kidney tissue inflammation and damage. HPLC data elucidated that the AP leaf extract contains polyphenols with potential antioxidant, antidiabetic, and organ protective agents: gallic acid, rutin hydrate, and quercetin hydrate. *Conclusion.* Taken together, AP may be one of the potential sources of antidiabetic agents.

1. Introduction

Diabetes is a widespread disease that affects millions of individuals. This medical condition affects about 9% of the population (roughly, 463 million people). Moreover, the number is rapidly increasing each year. By 2030, it is anticipated to reach 578 million people, and by 2045, it will have nearly doubled (700 million) [1]. Diabetes mellitus is caused by inadequate insulin production or an inability to effectively use insulin, resulting in impaired lipid, carbohydrate, and protein metabolism. As a result, hyperglycemia (high blood glucose levels), HbA1c (glycated hemoglobin), dyslipidemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activity, and diabetic nephropathy (DN) develop [2–5].

Diabetes is characterized by the presence of high blood glucose levels and the manifestation of different problems and dysfunctions in several organs. Type 1 diabetes is initiated by the destruction of cells via apoptosis, while type 2 diabetes is primarily mediated by increasing insulin resistance (IR). The primary cause of diabetes is hyperglycemia or an increase in glucose load [6]. The absorption of glucose by beta (β) cells is facilitated by the glucose transporter 2 (GLUT2) transporter, which arises in response to hyperglycemia within the cells. Free radicals such as reactive oxygen species (ROS) are produced when glucose is metabolically processed excessively through glycolysis and glucose auto-oxidation. The increased vulnerability of β cells to oxidative stress promotes the occurrence of necrosis and apoptosis. Inflammation-activated pathways, such as nuclear factor kappa beta (NF-κB) and mitogen-activated protein kinases (MAPKs), are additional factors contributing to the development of IR [7]. Eventually, each of these pathways aids in the onset of diabetes.

Streptozotocin (STZ) is extensively used to construct animal models by inducing diabetes in animals. STZ can cause diabetes in animals by causing oxidative stress in pancreatic β cells by producing nitric oxide and, subsequently, ROS [8]. The heightened sensitivity of β cells to glucose facilitates the diffusion of STZ into these cells with the aid of the GLUT2 transporter, hence leading to the eventual manifestation of STZ toxicity [9]. Nevertheless, glucose stimulates the entry of STZ into β cells, perhaps leading to detrimental effects due to the presence of the nitrosourea moiety [10].

Glucotoxicity can be associated with excessive nitric oxide (NO) production and oxidative stress. It may also exacerbate diabetes pathogenicity [11]. The DNA-protecting enzyme, aconitase, is inhibited by the hazardous levels of NO released by STZ, which causes DNA alkylation and damage [3, 11]. The activity of aconitase is reduced, and the modulation of mitochondrial respiratory complexes is observed in response to STZ. In murine models of diabetes, there is an observed augmentation in calcium ion (Ca^{2+}) absorption and a modification in mitochondrial transmembrane potential. It might be a major cause of the irregularities in mitochondrial bioenergetics. Studies have attributed the overproduction of advanced oxidation protein contents and oxidative stress to vascular and hepatic abnormalities [12–14]. Researchers discovered a link between inflammation and diabetes during these experiments [15, 16]. As a result, lowering hyperglycemia, oxidative stress, and inflammation is an excellent therapeutic approach for treating the pathogenesis of diabetes and its complications.

Medicinal plants have been used for therapeutic purposes worldwide since ancient times. They are a good source of modern medicine, traditional remedies, food additives, and other active chemicals [17–20]. In comparison to chemicals, plant extracts are thought to be less hazardous [21]. *Aphanamixis polystachya* (Wall.) R. Parker (AP), a timber tree belonging to the family of Meliaceae and mainly growing in Asia's low-altitude tropical regions, including Bangladesh, India, China, Indonesia, and Malaysia, is documented for its antioxidant, analgesic, hepatoprotective, anticancer, and antidepressant characteristics [22–24]. In Bangladesh and India, it is referred to as "Pithraj" and "Amoora," respectively [25]. This plant is traditionally utilized for the cure of a number of ailments such as tumors, ulcers, rheumatism, and liver disorders [26]. A study uncovered that limonoid isolated from this plant has antifeedant properties against a variety of agricultural pests [27]. An additional investigation discovered 25 limonoids having herbicidal, fungicidal, and insecticidal actions [28]. The experiment on the cerebral ischemia mouse model, which demonstrated the plant extract's ability to ameliorate stroke outcomes in a dose-dependent way, led to the discovery of the therapeutic usefulness of this medicinal plant. A breast adenocarcinoma cell line was highly sensitive to extracted triterpenoids and steroids isolated from the roots of *A. polystachya* in an in vitro investigation [29]. A recent study demonstrated that the treatment of the plant has a protective effect against liver injury in CCl$_4$-administered rats, possibly by alleviating inflammation, fibrosis, and oxidative stress and also enhancing the activity of the antioxidant enzymes [26]. Furthermore, Tiwari et al. [30] explored the antidiabetic effect of the methanolic extract of A. polystachya on STZ-induced diabetic rats exerted by significantly decreasing the level of blood glucose, body weight, TC, TP, and TG and increasing the level of HDL. Additionally, the study suggested that the antidiabetic effect of the phenolic and flavonoid contents in the methanolic extract of A. polystachya leaves may be due to their antioxidant properties [30].

Despite the fact that several studies have shown the therapeutic potential of *A. polystachya*, no intense research has been done so far to identify its antidiabetic properties and possible mechanistic pathways using the ethanolic extract of *A. polystachya*. Numerous studies have found a link between specific drugs with anti-inflammatory properties and easing diabetes-related problems. This study aims to characterize the phytoconstituents in the ethanolic leaves extract of *A. polystachya* and investigate its therapeutic potential to provide evidence-based recommendations for *A. polystachya* as an antidiabetic agent.

2. Materials and Methods

2.1. Chemicals and Reagents.
Gallic acid (GA), myricetin (MC), (+)-catechin hydrate (CH), pyrogallol (PG), syringic acid (SA), vanillic acid (VA), caffeic acid (CA), (−)-epicatechin (EC), trans-ferulic acid (FA), vanillin (VL), rosmarinic acid (RA), p-coumaric acid (PCA), ellagic acid (EA), quercetin hydrate (QU), rutin hydrate (RH), and kaempferol (KF) were bought from Sigma-Aldrich (St Louis, MO, USA). Acetonitrile (HPLC), acetic acid (HPLC), methanol (HPLC), and ethanol were obtained from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was purchased from Sigma-Aldrich (Germany). Reduced glutathione (GSH) was obtained from JI Baker (USA). Total triglyceride (TG), cholesterol (TC), high-density lipoprotein (HDL), aspartate transaminase (AST), low-density lipoprotein (LDL), alanine transaminase (ALT), serum creatinine, and BUN diagnostic kits were purchased from Human GmbH (Germany). All other solvents, chemicals, and reagents utilized in the current investigation were of analytical grade.

2.2. Collection, Identification, and Extraction of Plant Materials.
A. polystachya leaves were collected from Mymensingh, Bangladesh, and certified by the National Herbarium in Mirpur, Dhaka (DACB Accession No. 45274). The leaves were washed, shade-dried, crushed to a powder, macerated with ethanol, condensed with a rotary evaporator, and stored in the refrigerator until needed. Briefly, 400 grams of the A. polystachya leaves' powder were taken and soaked in raw ethanol (96%) in a 1 : 3 ratio (1 part A. polystachya powder in 3 parts ethanol). The maceration was carried out at room temperature for 7 days and repeated twice. Finally, the ethanolic extract was dried in a rotary evaporator at 37–40°C and yielded 35.67 grams of the crude leaves' extract (yield 8.92%). We have carried out extractions in another four batches of the leaves similarly and obtained 8.94%, 8.91%, 8.96%, and 8.90% yield, respectively.

2.3. HPLC Detection and Quantification of Polyphenolic Compounds.
HPLC-DAD analysis has been used to detect and quantify specified phenolic compounds in the A. polystachya ethanol extract, with some modifications described by Szkudelski et al. [8]. The experiment was carried out on a Dionex UltiMate 3000 system with a quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). Then, a 20 μl volume of the sample was injected and separated by using an Acclaim® C18 (5 m) Dionex column (4.6 × 250 mm) at 30°C (flow rate of 1 ml/min).

Gradient elution was conducted of three mobile phase systems consisting of solvent A (acetonitrile), solvent B (acetic acid solution pH 3.0), and solvent C (methanol). The elution pattern was 5%A/95%B (0–10 min), 10%A/90%B (11–15), 15%A/70%B/15%C (16–25), 20%A/60%B/20%C (26–30 min), 30%A/40%B/30%C (31–35 min), 40%A/50%B/10%C (36–40), and 5%A/95%B (41–45 min). The UV detector was set at 280 nm for 25.0 min, changed to 320 nm for 32.0 min, and further changed to 280 nm for 3 min and 380 nm for 35 min. The rest of the analysis with the diode array detector was set at an acquisition range of 200–700 nm.

For the preparation of the calibration curve, stock solutions of gallic acid, myricetin, rosmarinic acid, caffeic acid, vanillic acid, syringic acid, vanillin, trans-ferulic acid, kaempferol, (+)-catechin hydrate, (−)-epicatechin, pyrogallol, ellagic acid, p-coumaric acid, rutin hydrate, and quercetin hydrate were prepared in methanol. A solution of AP leaves was prepared in ethanol at a concentration of 10 mg/ml. To eliminate ghost peak or noise, all the solutions, including standards and AP samples, were properly filtered (0.20 μm syringe filter, Sartorius, Germany) and the mobile phases degassed in an ultrasonic bath (Hwashin, Korea) for 15 min. Data acquisition, peak integration, and calibrations were calculated with Dionex Chromeleon software (Version 6.80 RS 10).

We have repeatedly carried out the HPLC studies in all five batches of the leaf extracts for the quantification of polyphenolic compounds. We have identified polyphenolic compounds in the A. polystachya leaf extracts from the retention time and quantified them in mg/100 g of dry extracts from the calibration curve (peak area vs. concentration).

2.4. Experimental Animals.
Both male and female Sprague-Dawley rats (3 male and 3 female rats) weighing 180–200 grams were used for diabetic experiments. The animals were accustomed to standard conditions, including a 12 hour light-dark cycle at 22°C and 55% humidity. All the rats were allowed a normal pellet diet and clean water. The Institutional Animal Care and Use Committee (IACUC) approved the study at North South University in Bangladesh (2019/OR-NSU/IACUC-No.0902).

2.5. Induction of Diabetes.
The same approach was used to induce type-1 diabetes in the experimental animals, utilizing an earlier recognized experimental model [21]. In essence, animals were given a single intraperitoneal (i.p.) injection of freshly prepared STZ (65 mg/kg, b.w.) in a 0.5 M citrate buffer (pH 4.5) after starving overnight. The experimental rats' fasting glucose levels were tested one week later. Rats with fasting blood glucose levels of 8–12 mM/L have been selected for this experiment.

2.6. Study Design.
30 rats were selected for the experiment and categorized into five groups containing six in each. Group-I (normal control): The rats' were given citrate buffer by single intraperitoneal (i.p.) injection and 0.5% carboxymethyl cellulose (CMC) in water (vehicle) for 28 days. Group-II (diabetic control): Diabetes was produced by a single i.p. administration of STZ (65 mg/kg, b.w.) following 28 days of vehicle administration (p.o.). Group-III (standard treatment with glibenclamide suspension at 10 mg/kg in 0.5% CMC in water): Diabetic rats were treated with oral glibenclamide once daily for 28 days. Group-IV (AP 250 mg/kg): Diabetic rats were provided with AP 250 mg/kg suspended in 0.5% CMC once daily for 28 days. Group-V (AP, 500 mg/kg): The diabetic rats were treated with AP, 500 mg/kg, suspended in 0.5% CMC in pure water, once daily for 28 days. After providing the full course of the treatments, the

animals were given anesthesia (ketamine 100 mg/kg dose), and then, blood samples were collected in heparinized tubes. Finally, the experimental rats were euthanized (using ketamine 300 mg/kg dose) as previously described [21] and their organs were gathered. All of the samples were labeled and preserved properly for our next investigations.

2.7. Estimation of Blood Glucose. On days 0, 7, 14, 21, and 28, correspondingly, the fasting blood glucose levels of the tested rats were measured by using a commercially available glucometer (Accu-Chek, USA). Blood samples were gathered from the rat's tail puncture using a sterile needle.

2.8. Assessment of Glycosylated Hemoglobin (HbA1c). On the 28th day of the sacrifice of the rats, the most crucial biomarker in the evaluation of diabetes, HbA1c, was measured. Blood samples from the juvenile veins were collected, and HbA1c was estimated using an autoblood analyzer according to the manufacturer's guidelines.

2.9. Estimating Plasma Biochemical Markers. The blood samples undergo centrifugation at a speed of $8000 \times g$ for a duration of 15 minutes at a temperature of 4°C. Subsequently, the plasma component was isolated and preserved in a freezer at a temperature of −20°C until it was ready for utilization. LiquiUV diagnostic kits (HUMAN GmbH, Germany) and an Erba Chem v3 semi-auto biochemistry analyzer were used to investigate total cholesterol, HDL, TG, LDL, aspartate transaminase (AST), alanine transaminase (ALT), serum creatinine, and BUN in plasma samples according to standard manufacturer protocols.

2.10. Estimation of Liver Glycogen. As described previously [31], the liver glycogen was determined. In a nutshell, 1 g of fresh liver was taken from an experimental rat and placed in a test tube with KOH, which was then heated to boil in a water bath for 30 minutes. 4 ml of ethanol was added, mixed thoroughly, cooled, and centrifuged at $3000 \times g$, and the pellet was separated, which was then resuspended in distilled water. After that, an anthrone reagent was added, and the intensity of the green color was measured at 620 nm. The exact process was repeated for blank and standard glucose solutions. The following formula was used to estimate the amount of liver glycogen:

$$\text{Liver glycogen} = \left(U \times \frac{100}{S} \times 1.11 \right) \frac{\text{mg}}{\text{g}} \text{ of the liver}, \quad (1)$$

where U and S are the optical densities of the test sample and glucose solution, respectively, and 1.11 is the factor for converting glucose to glycogen.

2.11. Tissue Homogenate Preparation and Investigation of Oxidative Stress Markers and Antioxidant Enzyme Activities. For the preparation of samples, homogenization of 1 gram of the liver tissue in 10 mL of phosphate buffer (pH 7.4) was followed by centrifugation at $8000 \times g$ for 15 minutes at 4°C.

For further examination, the supernatant was aliquoted and stored at −20°C. NO levels and advanced oxidative protein products (AOPP) were measured to quantify oxidative stress. NO was assessed using the previously established procedures [32], in which absorbance was estimated at 540 nm and standardized to the blank solutions. A standard curve was used to calculate the NO level in nM/g of the tissue. Furthermore, AOPP levels were reached using a modified version of the method previously described [33]. Using PBS (100 μl) and 50 μl acetic acid and KI each, blank readings were measured. The chloramine-T absorbance was noted at 340 nm within the linear range of 0–100 nM/ml, and finally, AOPP levels were calculated as nM/ml chloramine-T equivalents.

2.12. Study of Antioxidant Enzyme Activities. The antioxidant activity of liver tissue homogenates was determined by measuring superoxide dismutase (SOD) and reduced glutathione (GSH). SOD was measured in tissue samples using a well-established technique [34]. In a nutshell, 90 litres of PBS were added to 10 litres of tissue supernatants, followed by 100 litres of epinephrine. At 480 nm, absorbance was measured at various time intervals and differences were estimated. Simultaneously, a control reaction containing all ingredients except the enzyme was studied. Furthermore, GSH levels were calculated using an earlier approach [35].

2.13. Histopathological Examination. Following the sacrifice of the experiment rats, liver and kidney tissues were collected and harvested in neutral buffered formalin, embedded in paraffin, sliced at a 5 m thickness, and stained with hematoxylin/eosin and Sirius red staining to visualize inflammation and fibrosis in the hepatic and kidney tissues, respectively. A light microscope was used to examine the stained samples at a magnification of 40× (Zeiss Axioscope). The density of the inflammation and fibrosis in the liver and kidney was measured using ImageJ (NIH, USA) software according to the previous process [36, 37].

2.14. Statistical Analysis. Values were expressed as the mean ± standard error of the mean (S.E.M.). Data were subjected to one-way analysis of variance (ANOVA) followed by t-Student-Newman-Keuls's as a post-hoc test using the statistical software GraphPad Prism (version 9.5). $p < 0.05$ was considered a statistically significant value(s), while $p < 0.0001$ was considered the most significant value(s).

3. Results

3.1. Polyphenolic Compounds in the AP Leaf Extract. The *A. polystachya* leaves' extract was standardized against sixteen phenolic components and quantified using reverse-phase HPLC. Supplementary Figures 1 and 2 demonstrate the chromatograms of the standard polyphenols and polyphenols identified in *A. polystachya* leaves' extracts, respectively. Supplementary Table 1 shows the average quantification of phenolic components detected in all five

batches of *A. polystachya* leaf extract in mg/100 g dry weight of the extract.

According to the findings, the ethanolic leaf extract of *A. polystachya* contains a modest amount of gallic acid and rutin hydrate (40.97 and 26.38 mg/100 g dry weight, respectively). Considering all five batches of the leaf's extracts, the average content of polyphenolic presence in the *A. polystachya* leaf was 40.97 : 26.38 : 7.11 of gallic acid (GA) : rutin hydrate (RH) : quercetin hydrate (QU) (Supplementary Table 1).

3.2. Effect of AP on Glycemic Control and Body Weight.

AP showed a significant decrease in the blood glucose level in the oral glucose challenge rats. Data are shown in the Supplementary Table 2. Table 1 provides information about the effects of the standardized ethanolic leaves extract of *A. polystachya* (AP) on glycemic control in diabetic animals at various treatment intervals. During the experiments, body weight (Figure 1) and fasting blood glucose (Table 1) were calculated, and it was shown that the body weight of STZ-mediated diabetic animals (the negative group) was significantly diminished compared to the nondiabetic animals in the control group ($p < 0.01$). In contrast, the treatment groups that were given AP showed a substantial improvement ($p < 0.05$) in body mass compared to the negative group in a dose-dependent manner.

As shown in Table 1, fasting blood glucose levels are progressively enhanced ($p < 0.0001$) in STZ-mediated diabetic animals. On the other hand, the oral administration of AP demonstrated a considerable decrease in blood glucose levels, restoring them to normal values in diabetic rats by the conclusion of the trial, namely, on the 28th day. At the same time, the most common and significant indicators to evaluate diabetes are fasting blood glucose and glycosylated hemoglobin (HbA1c) [3–5]. Figure 2 shows that AP reduced HbA1c levels dose-dependently and demonstrated a substantial decrease ($p < 0.0001$) of HbA1c at a dosage of 500 mg/kg in diabetic rats. The findings of this study reveal strong evidence for the efficacy of AP in regulating glycemic control and body weight in diabetic animal models.

3.3. Effect of AP on Liver Enzyme Functions.

STZ administered intraperitoneally causes diabetes-induced hepatic impairment. Previous studies showed increased serum ALT and AST activities due to hepatic dysfunction [4, 5, 38]. Figure 3 depicts the effect of AP on the liver enzyme activity. In STZ-mediated diabetic animals, plasma ALT levels were considerably higher ($p < 0.0001$) than in control rats. The diabetic rats' ALT levels were markedly lowered by the oral treatment of AP at doses of 250 or 500 mg/kg ($p < 0.0001$). Similarly, when diabetic rodents were treated with AP at 250 or 500 mg/kg, it alleviated AST enzyme activity (Figure 3(b); $p < 0.0001$), which was similar to the standard medication Gli (10 mg/kg). Furthermore, STZ treatment markedly reduced the level of liver glycogen in diabetic animals ($p < 0.0001$). As demonstrated in Figure 3(c), oral therapy with AP at 250 mg/kg ($p < 0.05$) or 500 mg/kg ($p < 0.0001$) considerably raised glycogen levels.

3.4. Effect of AP on Lipid Profiles.

Lipid imbalances are associated with diabetes [4, 5]. Thus, the plasma lipid levels in diabetic rats were measured to see the ability of AP to correct their lipid profile. Diabetic rats showed a significant ($p < 0.0001$) elevation in serum TGs (Figure 4(a)), total cholesterol (Figure 4(b)), and LDL cholesterol (Figure 4(c)) as compared to the control rats. In contrast, HDL cholesterol was significantly ($p < 0.0001$) reduced in diabetic animals, as displayed in Figure 4(d). Treatment of the diabetic animals with 250 mg/kg AP significantly ($p < 0.0001$) reduced serum TG and total cholesterol, while LDL cholesterol and HDL cholesterol were nonsignificantly ($p > 0.05$) affected. Conversely, a 500 mg/kg dose of AP significantly decreased serum TG ($p < 0.0001$), total cholesterol ($p < 0.0001$), and LDL cholesterol ($p < 0.0001$) and increased serum HDL cholesterol levels ($p < 0.001$).

3.5. Effect of AP Leaves' Extract on the Kidney Profile.

DN is a frequent diabetic consequence. BUN and creatinine levels are commonly used to diagnose acute and chronic renal disease [5]. The investigation of the blood creatinine and BUN levels was carried out to see whether the plant extract may help lower or normalize those kidney markers. There was a significant ($p < 0.0001$) increase in serum creatinine (Figure 5(a)) and BUN levels (Figure 5(b)) in the kidneys of diabetic animals as compared to the control group. The observed improvements in the kidneys of diabetic rats with a dosage of 500 mg/kg of AP were found to be statistically significant ($p < 0.0001$).

3.6. Effect of AP on Oxidative Stress and Antioxidant Enzymes.

The levels of NO and AOPP in the liver homogenates of experimental animals were used to assess oxidative stress. The livers of diabetic rats demonstrated a remarkable ($p < 0.0001$) enhancement in NO (Figure 6(a)) and AOPP (Figure 6(b)) as compared to the control group. Treatment with 250 mg/kg and 500 mg/kg of AP considerably alleviated NO ($p < 0.0001$) and AOPP ($p < 0.05$; $p < 0.0001$) in the liver of diabetic rodents. Rats given Gli exhibited significantly ($p < 0.0001$) reduced liver NO (Figure 6(a)) and AOPP levels (Figure 6(b)). At the same time, GSH levels (Figure 6(c)) and activity of SOD (Figure 6(d)) significantly ($p < 0.0001$ and $p < 0.001$, respectively) dropped in the liver of diabetic rats as compared to the control group. Diabetic rats treated with 500 mg/kg AP showed a rough improvement in liver GSH ($p < 0.05$) and SOD ($p < 0.05$).

A parallel therapeutic effectiveness of AP was found in altering oxidative stress and antioxidant enzyme markers in the diabetic kidneys of the investigational rats. In these cases, a dose of 500 mg/kg has given remarkable restoring results, which are analogous to the effect of Gli (Figure 7).

3.7. Effects of AP on Histological Alteration of the Liver and Kidney.

To estimate the anti-inflammatory and antifibrotic effects of AP on the liver and kidney in diabetic rats, H & E and Sirius red staining were utilized, respectively. Figure 8(a) shows normal hepatocytes without inflammation in the

TABLE 1: Fasting oral glucose levels of different treatment groups observed in different intervals on diabetic rats.

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	5.32 ± 0.18	5.38 ± 0.21	5.4 ± 0.17	5.48 ± 0.16	5.55 ± 0.18
STZ	8.28 ± 0.09	8.43 ± 0.16[a]	14.83 ± 0.67[a]	17.17 ± 0.77[a]	15.67 ± 0.88[a]
STZ + GLI	8.58 ± 0.17	8.45 ± 0.36	7.68 ± 0.49[b]	6.72 ± 0.25[b]	5.95 ± 0.18[b]
STZ + AP250	8.05 ± 0.05	8 ± 0.17	13.18 ± 0.46[d]	12.72 ± 0.31[f]	9.18 ± 0.21[f]
STZ + AP500	8.07 ± 0.11	7.45 ± 0.18[c]	12.00 ± 0.28[e]	10.68 ± 0.57[g]	6.77 ± 0.28[g]

Values are expressed as means ± S.E.M. ($n = 6$); a = STZ vs. control ($p < 0.0001$); b = STZ vs. STZ + GLI ($p < 0.0001$); c = STZ vs. STZ + AP500 ($p < 0.05$); d = STZ vs. STZ + AP250 ($p < 0.05$); e = STZ vs. STZ + AP500 ($p < 0.001$); f = STZ vs. STZ + AP250 ($p < 0.0001$); g = STZ vs. STZ + AP500 ($p < 0.0001$).
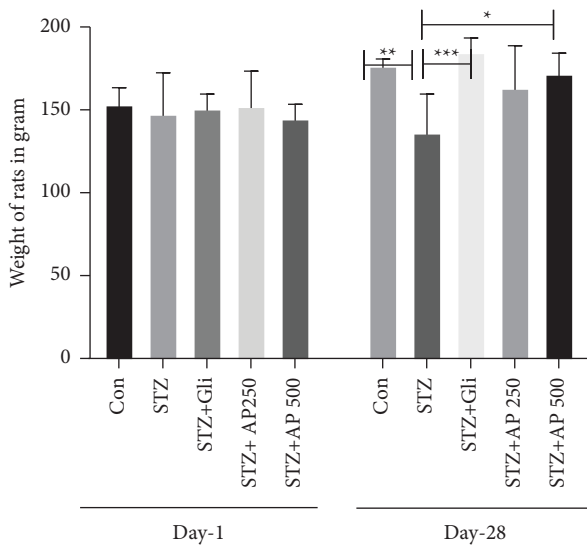

FIGURE 1: Effect of AP on body weight in diabetic rats (values are expressed as means ± S.E.M. ($n = 6$); $^{**}p \leq 0.01$ vs. control; $^{***}p \leq 0.001$ vs. STZ; and $^{*}p \leq 0.05$ STZ-induced diabetic rats).
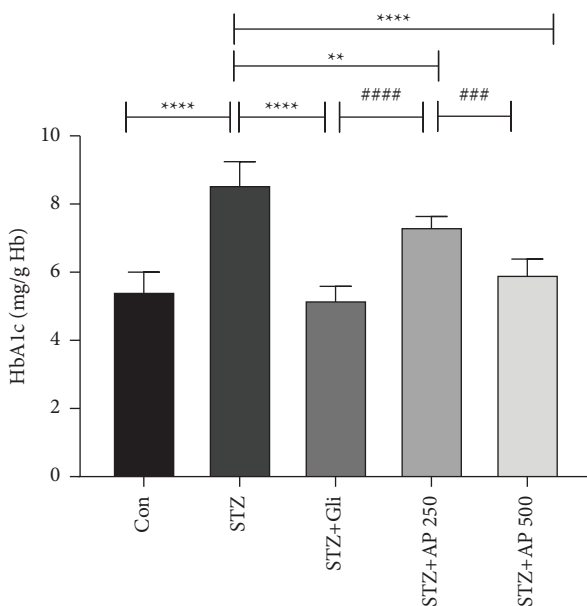

FIGURE 2: Therapeutic effect of AP on HbA1c management (values are expressed as means ± S.E.M. ($n = 6$); $^{****} = p \leq 0.0001$ vs. control or STZ; $^{**} = p \leq 0.01$ vs. STZ; $^{####}p \leq 0.0001$ vs. STZ + AP 250; and $^{###}p \leq 0.001$ vs. STZ + AP 250).

control group. Inflammatory cell infiltration (indicated by long arrows) was seen in STZ-mediated diabetic animals (Figure 8(b)). Oral treatment with AP reduced the migration of inflammatory cells (Figures 8(d) and 8(e)). STZ administration increased inflammatory cell infiltration, as shown by large arrows (Figure 8(b)), and AP oral gavage restored hepatocytes in the diabetic rats and ameliorated inflammation (Figures 8(d) and 8(e)).

STZ induction is linked to severe collagen accumulation and fibrosis (marked as red areas in Figure 9(b)) in diabetic animals compared to the control group (Figure 9(a)). The co-administration of STZ and AP extracts removed excess collagen deposition and improved liver fibrosis in the diabetic animals. The antifibrotic results of the AP extract are comparable with those of the standard drug treatment group (Figures 9(d) and 9(e)). Our findings appear to have clarified the role of AP leaf extract in reducing hepatic inflammation and damage as well.

STZ administration increased collagen deposition, as indicated by the red-marked area (large arrows in Figure 9(b)), and AP oral gavage ameliorated collagen absorption in the diabetic rats (Figures 9(d) and 9(e)). Similarly, STZ induction increased inflammation with swelling of the tubules and inflammatory cells repositioning in the kidney (Figure 10(b)). While different doses of AP extracts ameliorate the sloughing of tubules and thus inflammation (Figures 10(d) and 10(e)), Likewise, STZ induction increased collagen agglomeration in the kidney tubules, causing kidney fibrosis (Figure 11(b)), and oral administration of AP reduced collagen mass in the kidney (Figures 11(d) and 11(e)), thus improving kidney damage in the diabetic rats. Our data showed comparable results with the standard drug (Gli) group (Figures 10(c) and 11(c)).

STZ administration increased swelling of the tubules and inflammatory cell migration in the kidney, as indicated by large arrows (Figure 9(b)), and AP oral gavage restored tubules and reduced inflammation (Figures 9(d) and 9(e)).

STZ administration increased collagen deposition in the kidney, as indicated by large arrows (Figure 11(b)), and oral treatment with AP decreased collagen mass and thus reduced fibrosis (Figures 11(d) and 11(e)).

4. Discussion

Hyperglycemia can cause oxidative stress, which can start the main processes that lead to the start and progression of microvascular and macrovascular complications of diabetes,
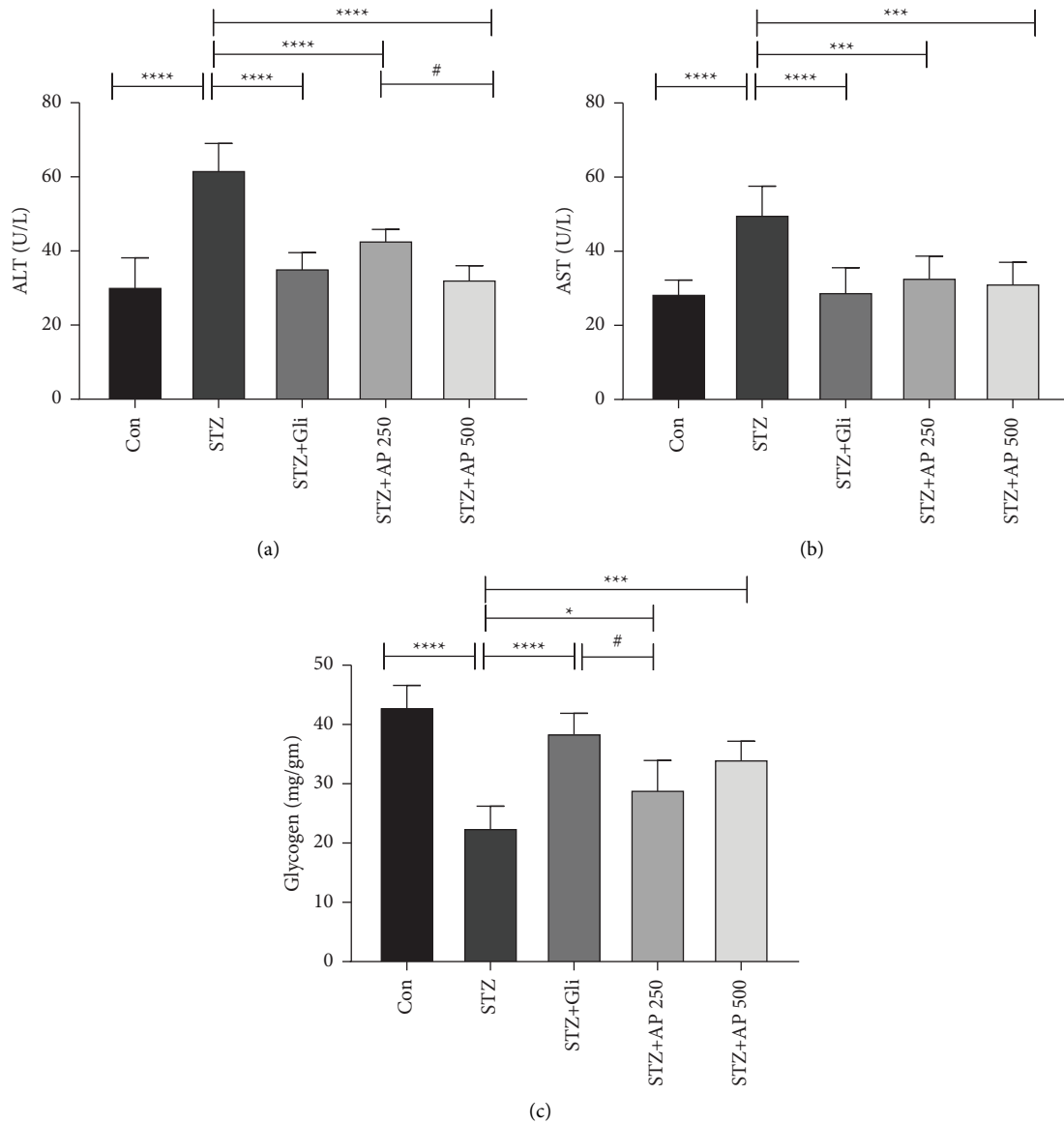
FIGURE 3: Efficacy of AP on liver functions. (a) ALT, (b) AST, and (c) glycogen in the STZ-induced diabetic rats. Values are expressed as means ± S.E.M. ($n = 6$); ****$p \leq 0.0001$ vs. control or STZ; ***$p \leq 0.001$ vs. STZ; *$p \leq 0.05$ vs. STZ-induced diabetic rats; and #$p \leq 0.05$ vs. STZ + AP 250.

such as DN [39]. Growing interest has been shown in the valuable therapeutic function of AP in the prevention, cure, and regulation of diabetes and its consequences. Therefore, the observation was conducted at how well ethanolic leaf extract of AP protected the liver and kidney of STZ-mediated diabetic rats against oxidative stress, inflammation, and apoptosis.

Phenolic compounds represent the predominant group of antioxidant chemicals found in many dietary sources and are widely consumed as part of the human diet [40]. Certain polyphenols, whether consumed as supplements or from dietary sources, have been shown to enhance insulin resistance through several mechanisms. These processes encompass the reduction of postprandial glucose levels, modulation of glucose transport, influence on insulin

signaling pathways, and protection against insulin-related damage [41].

These bioactive chemicals encompass a wide range of fruits, coffee, wine, vegetables, cocoa, tea, and grains [42]. The phenolic acids can be classified into two distinct categories: hydroxybenzoic acids, such as gallic acid, and hydroxycinnamic acids, such as ferulic, caffeic, and coumaric acid [43]. Phenolic compounds offer various advantageous effects in the management of blood glucose levels in individuals with diabetes. These effects include decreasing the absorption of dietary carbohydrates in the gut, regulating enzymes related to glucose metabolism, developing the activity of β-cells and insulin action, promoting insulin secretion, and possessing antioxidative and anti-inflammatory features [15]. The compelling evidence on
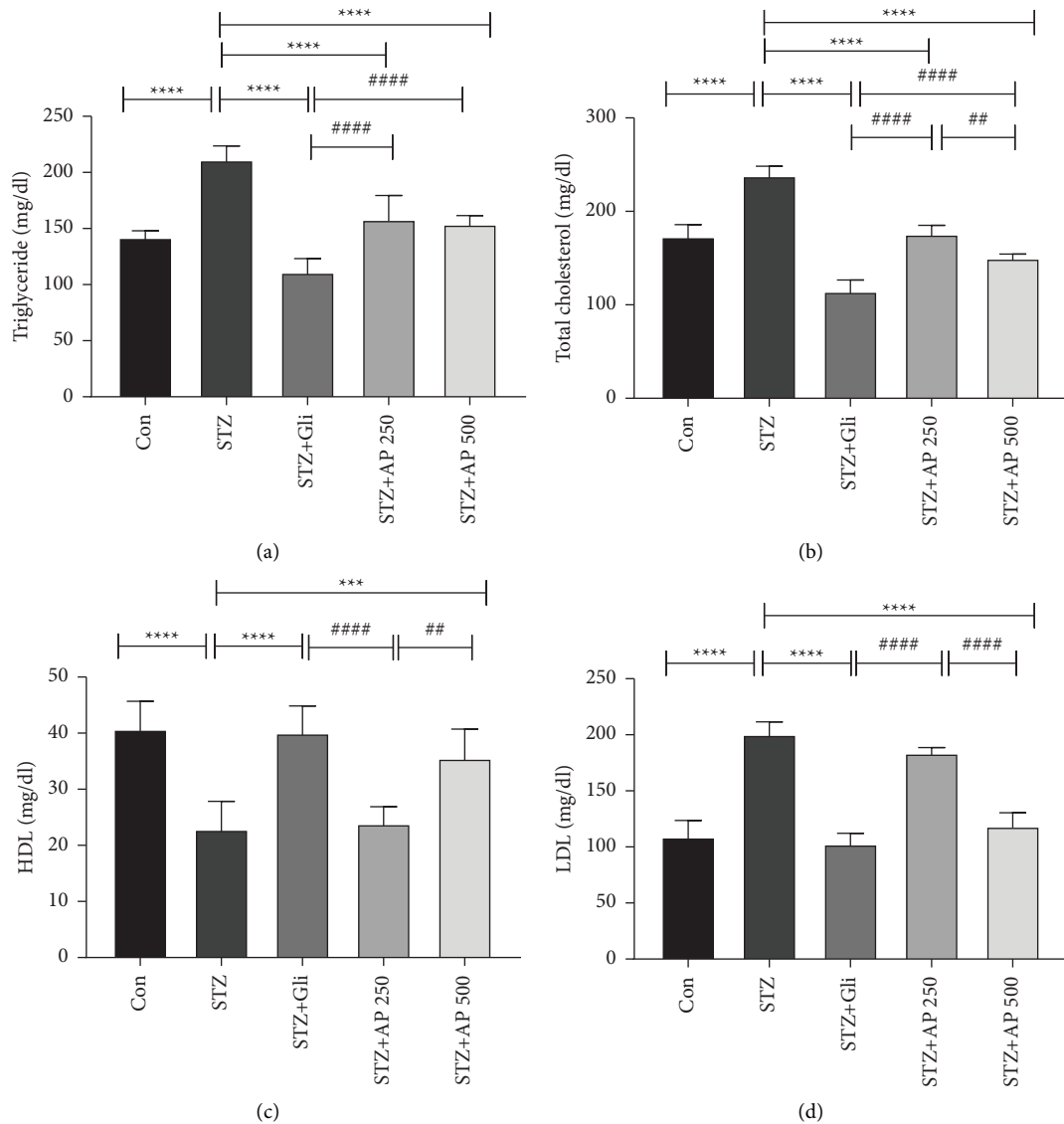
FIGURE 4: Effects of AP on the lipid profile. (a) Total cholesterol, (b) TG, (c) LDL, and (d) HDL in diabetic rodents. Values are expressed as means ± S.E.M. ($n = 6$); **** $p \leq 0.0001$ vs. control or STZ; *** $p \leq 0.001$ vs. STZ; #### $p \leq 0.0001$ vs. STZ + AP 250 or STZ + AP 500; and ## $p \leq 0.01$ vs STZ + AP 250 diabetic rats.

the preventive properties of phenolic compounds against diabetes has provided novel perspectives for the advancement of functional meals and nutritional supplements enriched with polyphenols [4].

Phenolic chemicals primarily have hypoglycemic effects by reducing the absorption of dietary carbs in the intestines. These entities participate in the control of enzymes associated with glucose metabolism, enhancement of β-cell activity and insulin action, and promotion of insulin secretion [15, 16]. Phenolic compounds exhibit robust antioxidant activity, serving as a protective mechanism against oxidative stress mediated by the overproduction of ROS [13, 14]. The implementation of strategies aimed at inhibiting the generation of intracellular free radicals presents a viable therapeutic approach for mitigating oxidative stress and associated pathogenic states [17]. Phenolic compounds

exert inhibitory effects on the synthesis of ROS through the inactivation of enzymes responsible for their generation. Several enzymes can be cited as examples, including xanthine oxidase, lipoxygenase, cyclooxygenase, mitochondrial succinoxidase, microsomal monooxygenase, NADH oxidase, and glutathione-S-transferase [18]. Furthermore, research has demonstrated that phenolic compounds have the ability to diminish the postprandial blood glucose response. This characteristic renders phenolic compounds potentially valuable agents in addressing type II diabetes [15, 16].

A nitrosourea-alkylating substance called STZ destroys DNA and is especially harmful to islet β cells. Necrosis of β cells is caused by STZ injection, which is followed by hyperglycemia and hypoinsulinemia. The current study found that STZ-treated rats had significantly higher HbA1c% and levels of blood glucose, which was in agreement with earlier
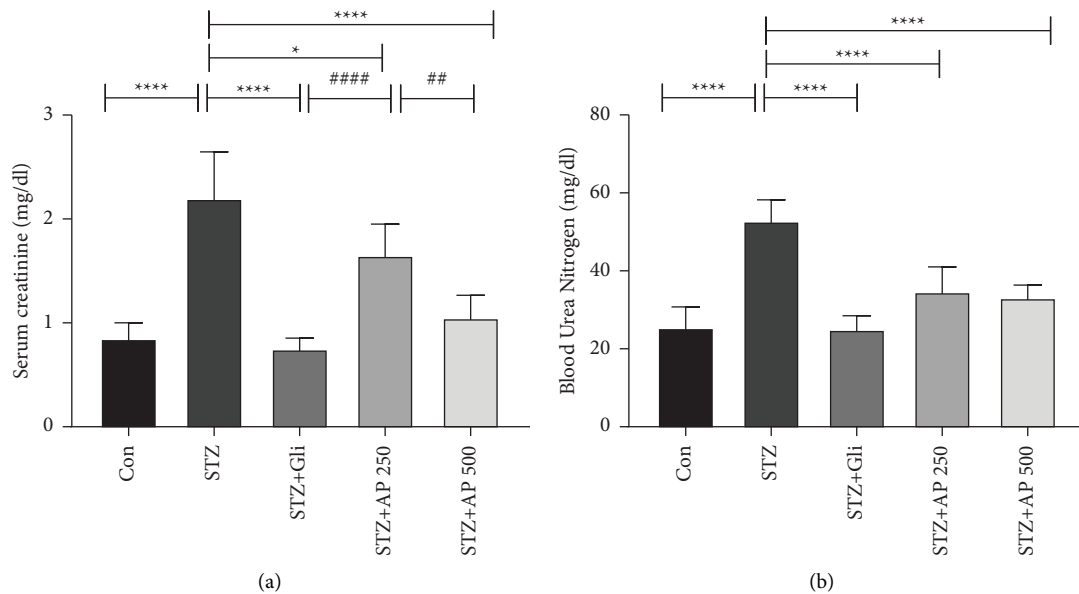
FIGURE 5: Effectiveness of AP on kidney functions. (a) Serum creatinine and (b) BUN. Values are expressed as means ± S.E.M. ($n = 6$); ****$p \leq 0.0001$ vs. control or STZ; *$p \leq 0.05$ vs. STZ; ####$p \leq 0.0001$ vs. STZ + AP 250; and ##$p \leq 0.01$ vs. STZ + AP 250 in diabetic rats.

research [44]. Different organs are chronically injured and damaged by hyperglycemia [11]. Therefore, to avoid or postpone the onset of diabetic complications, it is crucial to have appropriate glycemic control.

Both male and female Sprague-Dawley rats in our experiment were chosen to avoid gender bias. Although the metabolic and hormonal systems provide a safe guard for females against diabetes, different researchers used both sexes of animals in their studies [45, 46]. Other studies showed that over 40 mg/kg of STZ IP injection successfully produced diabetes in both male and female rats [47, 48]. NIH (National Institute of Health, USA) also suggests gender equality in animal research to avoid gender bias in animal studies [49]. Another study showed comparable results in diabetes-induced kidney problems in both male and female experimental animals [50].

In our study, the administration of STZ altered the glycemic control and body weight of the experimental rats. Oral ingestion of AP leaf extract restored those glycemic markers. The rats gained considerable weight after the therapy with AP leaf extract at 500 mg/kg (Figure 1). Compared to the control rats, STZ treatment raised their serum blood glucose and HbA1c levels. Glibenclamide (Gli) was used as a referral drug to evaluate and compare the results. Gli treatment significantly decreases the circulated glucose concentration in diabetic rats. It was discovered that Gli reduced hepatic gluconeogenesis and increased peripheral glucose consumption. The fasting blood glucose has measured because this marker is more significant for the detection of diabetes [51]. On the contrary, HbA1C itself is an important indicator for the diagnosis and prognosis of diabetes [52]. Thus, these two important biomarkers for diabetes were carried out.

Our findings support the idea that AP has antidiabetic and organ-protective potential and are in line with other research [4, 8, 12] for the management of diabetes. In this investigation, the results showed that AP leaf extract could guard against the damage to liver tissue caused by STZ-induced diabetes. The liver uses a few processes such as gluconeogenesis, glycogenolysis, glycogen synthesis, and glycolysis to stabilize blood sugar levels. However, the interruption of these metabolic pathways in diabetes results in hyperglycemia [14]. Common indicators of liver function include the hepatic enzymes AST, ALT, and ALP. In diabetes, impaired glucose consumption increases hepatic AST and ALT activities, which signify hepatic dysfunction. High levels of AST and ALT are, therefore, indicators of the etiology of diabetes. Furthermore, STZ-induced diabetes is characterized by the death of pancreatic beta cells and the reduction of liver glycogen levels, mediated by IR and impaired glycogen synthesis. Here, the comparison was done between control rats with diabetic rats to observe considerably lower levels of liver glycogen and remarkably higher levels of serum ALT and AST after the rats received an intraperitoneal injection of STZ. Oral administration of AP leaf extract at doses of 250 and 500 mg/kg considerably reduced the levels of these hepatic enzymes, indicating that AP leaf extract could guard against the damage to liver tissue by STZ-induced diabetes. Furthermore, liver glycogen significantly increased after treatment with AP at a dose of 500 mg/kg (Figure 1(c)). These outcomes are comparable to the earlier works [13, 14, 20].

Another problem associated with hyperglycemia and IR in diabetes is lipid abnormalities. The treatment of diabetic problems should thus consider medicines that potentially alter lipid profiles [5, 14, 53]. In the current investigation, oral gavage of AP at a dosage of 500 mg/kg markedly diminished TC, TG, and LDL cholesterol levels and raised the amount of HDL in diabetic rats. These findings align with earlier research on the therapeutic potential of AP for
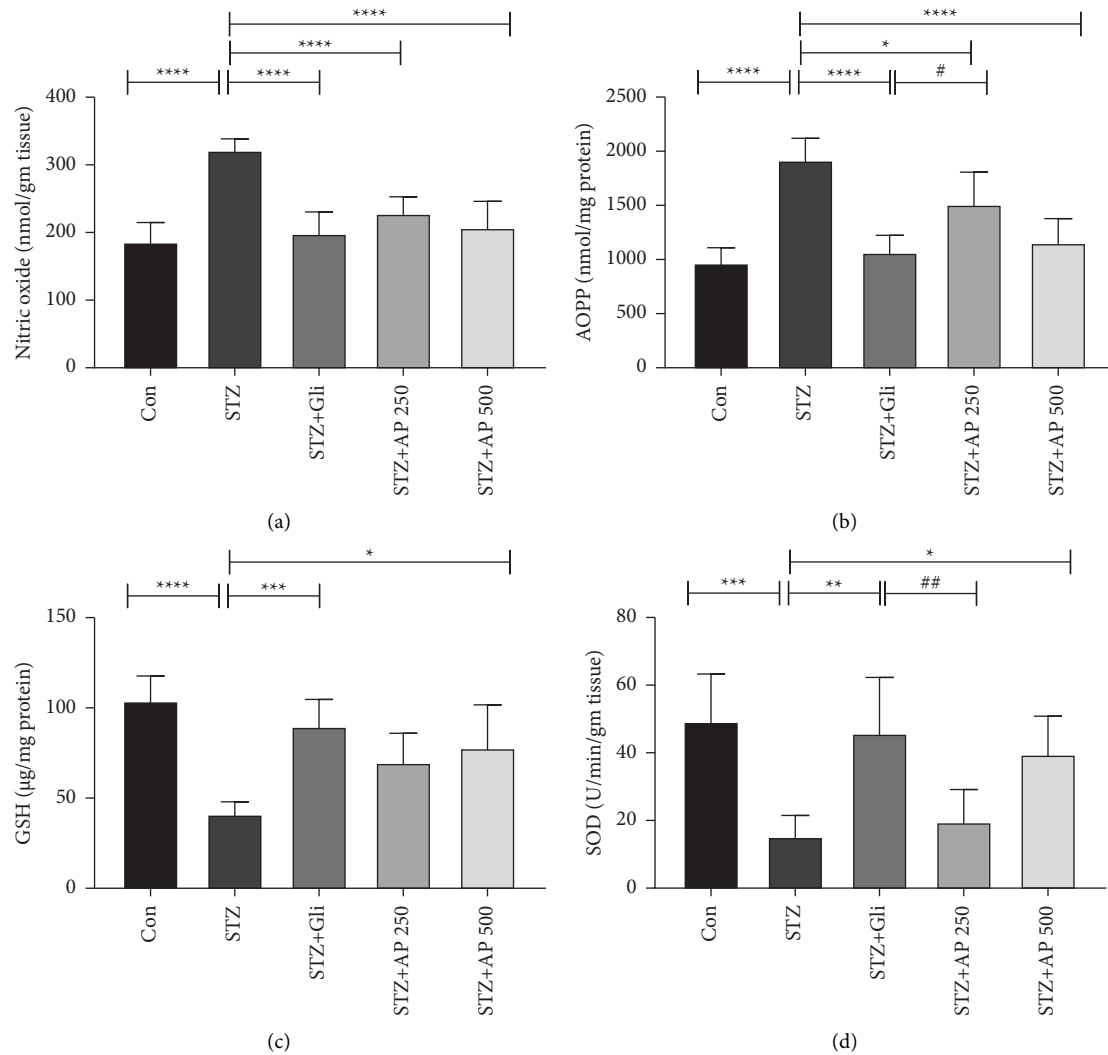
Figure 6: Effect of AP on liver oxidative stress. (a) Nitric oxide, (b) AOPP and antioxidant enzymes, (c) GSH, and (d) SOD. Values are expressed as means ± S.E.M. ($n = 6$); $****p \leq 0.0001$ vs. control or STZ; $***p \leq 0.001$ vs. control or STZ; $**p \leq 0.01$ vs. STZ; $*p \leq 0.05$ vs. STZ; $^{\#\#}p \leq 0.01$ vs. STZ + AP 250; and $^{\#\#}p \leq 0.05$ vs. STZ + AP 250 in diabetic rats.

controlling diabetes, including [5, 13, 14, 20, 53]. Diabetes also has a direct impact on the kidneys, and it causes kidney illnesses as a secondary effect. BUN and creatinine are commonly employed as primary diagnostic tools for the detection of renal abnormalities. Our investigation indicated that oral treatment of AP considerably reduced blood BUN and creatinine levels in tested animals, which was equivalent to glibenclamide, showing the plant's deep therapeutic efficiency in DN. The existing clinical and preclinical data provide evidence supporting the advantageous effects of phenolic compounds on glucose homeostasis. Specifically, it has been observed that a single oral dose of 1.562 g of GA equivalents, following an overnight fasting period, yields positive outcomes. The study observed a decrease in postprandial insulin levels and an enhancement in insulin sensitivity [54].

The existing literature confirmed that GA, an important biomarker found in *A. polystachya* leaves extract in our study, aids the protective mechanism of antioxidants, specifically protecting the renal tissue from complications related to diabetes [55–57]. GA is already proven to provide significant protection against oxidative damage [58, 59] by reducing glial activation and protein aggregates [60]. GA was previously found to scavenge free radicals and reactive species and avoid neuronal death [61]. Thus, in certain pathophysiological circumstances, GA and its derivatives have the ability to control inflammation, apoptosis, or oxidative stress. GA's antioxidant and anti-inflammatory effects have been claimed to have antihyperglycemic potential [62, 63]. Treatment with GA increased the FRAP content and reduced MDA levels in serum and renal tissues; this might be due to the antioxidant functions of GA. It was also shown to activate nuclear factor erythroid 2-related factor 2 (Nrf2), restoring the antioxidant and inflammatory conditions to normal levels [64, 65]. It also combats metabolic diseases such as dyslipidemia, IR, and obesity by exerting anti-inflammatory actions [66]. Other studies show that renal injury can be caused by inflammation, oxidative stress
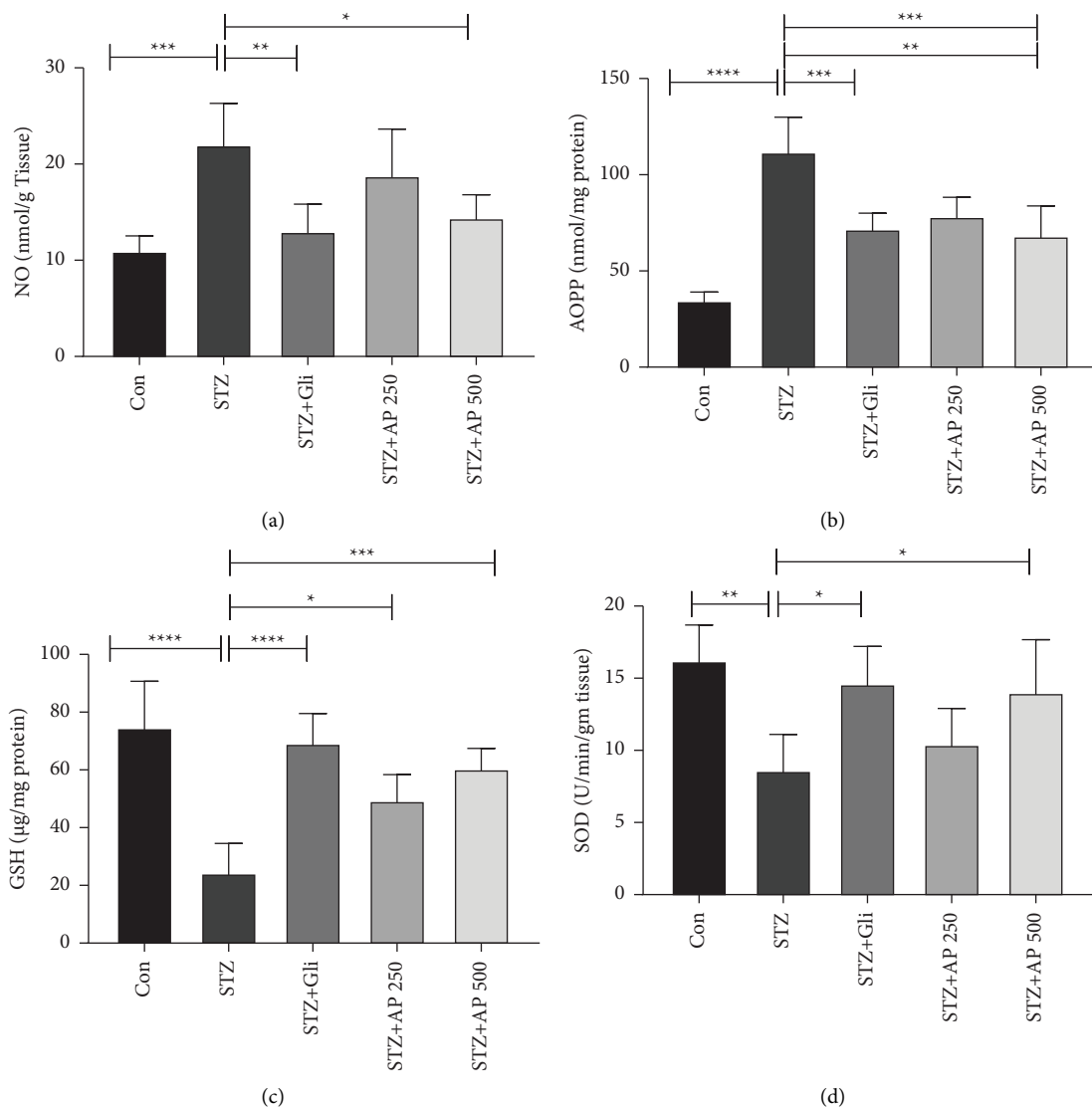
(a)

(b)

(c)

(d)

FIGURE 7: Effect of AP on kidney oxidative stress. (a) Nitric oxide, (b) AOPP and antioxidant enzymes, (c) GSH (liver), and (d) SOD. Values are expressed as means ± S.E.M. ($n = 6$); **** $p \leq 0.0001$ vs. control or STZ; *** $p \leq 0.001$ vs. control or STZ; ** $p \leq 0.01$ vs. STZ; and * $p \leq 0.05$ vs. STZ-induced diabetic rats.

(the formation of ROS), or direct tubular injury [67]. Gallic acid administration noticeably reduced uric acid, creatinine, and urea content, proving that it has ameliorative effects against kidney injury. Due to its antioxidant properties, it is associated with its capability to defend nephrons and enhance GFR levels [68]. These findings, in line with prior studies [69, 70] and our recent study findings, strongly suggest that gallic acid in *A. polystachya* leaves extract has a major role to play in treating DN.

Other studies also found a flavanol, named rutin, in *A. polystachya* leaves extract that has significant antioxidant and anti-inflammatory properties [71]. It also hinders elevated glucose-mediated renal glomerular endothelial hyperpermeability by hindering the Roha/ROS/ROCK molecular signaling pathway [72], as well as lowering the risks of diabetic neuropathy by reducing the level of plasma glucose and lowering oxidative stress by inhibiting Nrf2

signaling [73]. It was also found to relieve renal damage by lowering oxidative stress [74, 75] and protecting β cells, which increases insulin production and lowers plasma glucose concentration by scavenging free radicals and suppressing lipid peroxidation [76, 77].

In vascular and renal cells, it has been shown that elevated ROS, glucose, and the hexosamine pathway activate the RhoA/ROCK attenuating signaling pathway [78, 79]. Here, rutin inhibited HG-mediated hyperpermeability, while Nrf2 knockdown effectively eliminated junction protein abnormalities, ROS generation, and activation of the RhoA/ROCK signaling pathway. Rutin prevented the disruption of renal endothelial barrier function caused by HG by blocking the RhoA/ROCK signaling pathway, which was induced via lowering ROS [72]. In one study, rutin treatment raised the content of nonenzyme antioxidants as well as the activity of enzymatic antioxidants in the diabetic liver and kidney [80].
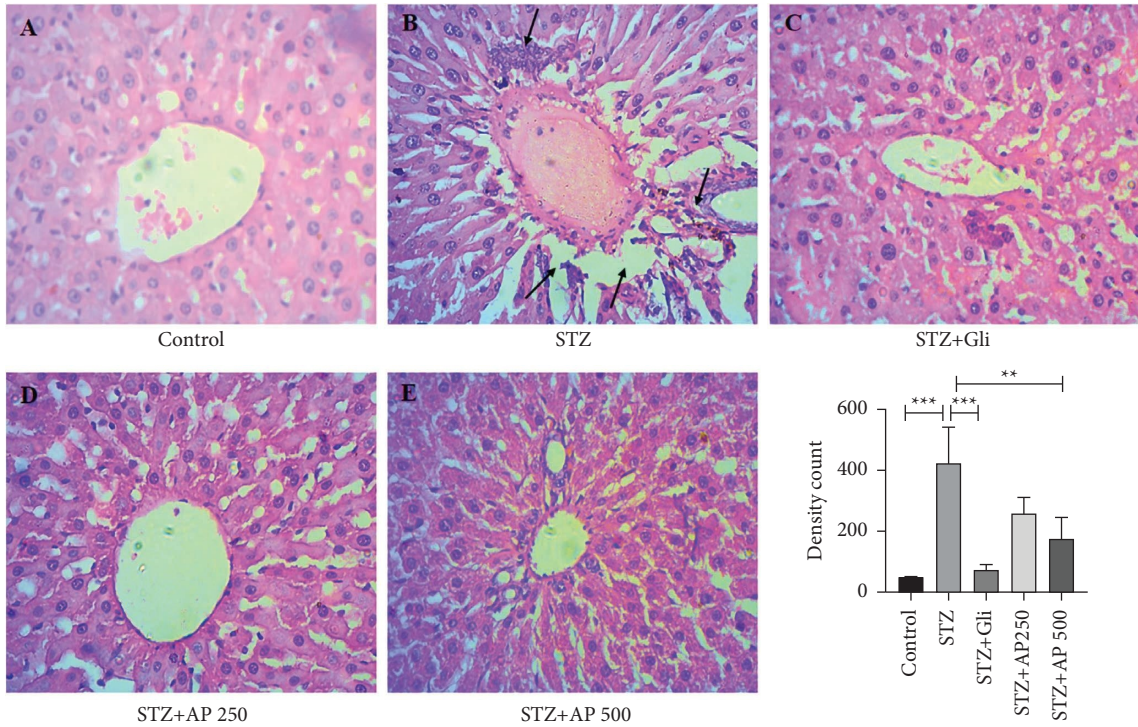
FIGURE 8: Effect of AP on liver inflammatory cell infiltration (the comparison of inflammatory cell infiltration and morphological changes was carried out in liver tissues using Image J software. $^{***}p \leq 0.001$ vs. control or STZ; $^{**}p \leq 0.001$ vs. STZ ($n = 3$)).
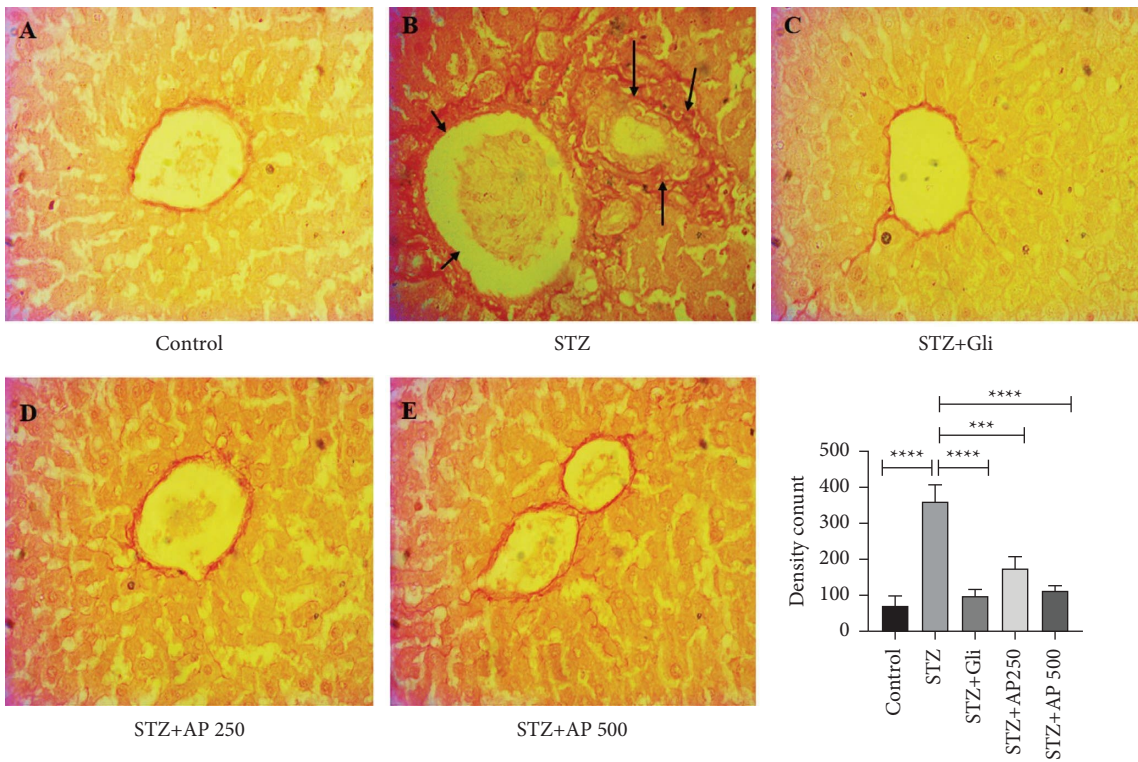

FIGURE 9: Effect of AP on liver fibrosis (the comparison between deposition of collagen and morphological changes in liver tissues was done using ImageJ software. $^{****}p \leq 0.0001$ vs. control or STZ; $^{***}p \leq 0.01$ vs. STZ ($n = 3$)).

Figure 10: Effect of AP on kidney inflammation (the comparison between inflammation and morphological changes in kidney tissues was done using ImageJ software. $^{***} p \leq 0.001$ vs. control or STZ; $^{**} p \leq 0.001$ vs. STZ ($n = 3$)).

Figure 11: Effect of AP on kidney fibrosis (the comparison between deposition of collagen and morphological changes in kidney tissues was done using ImageJ software. $^{****} p \leq 0.0001$ vs. control or STZ; $^{***} p \leq 0.01$ vs. STZ ($n = 3$)).
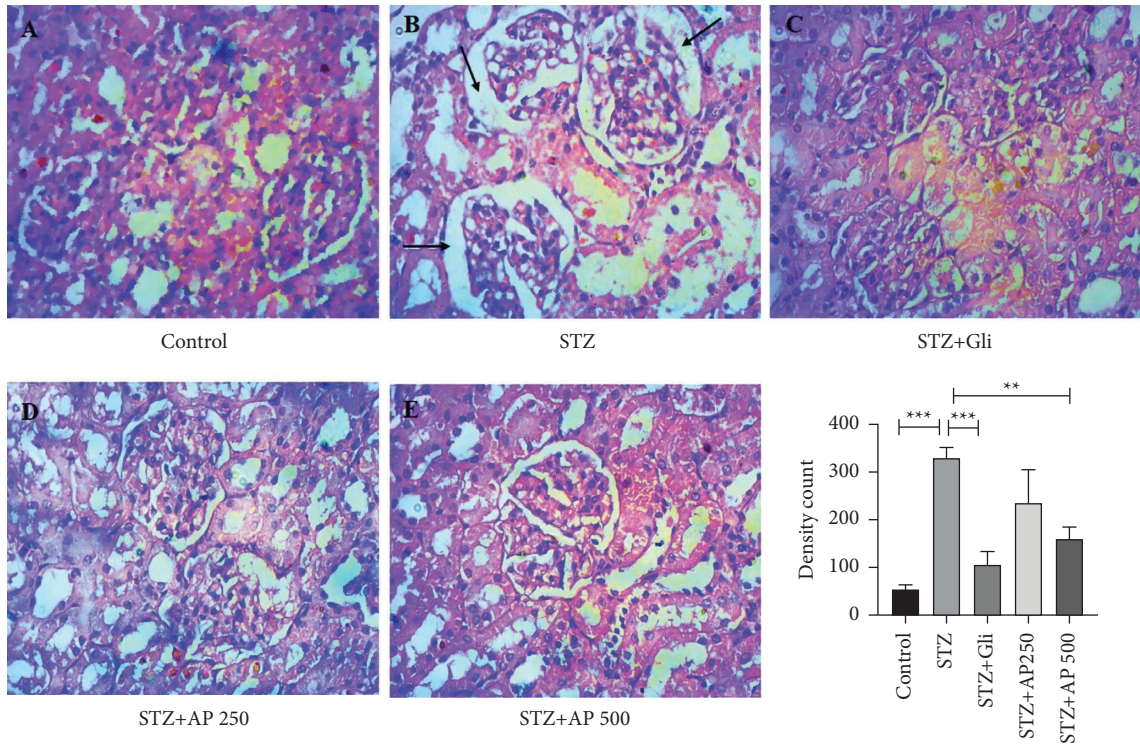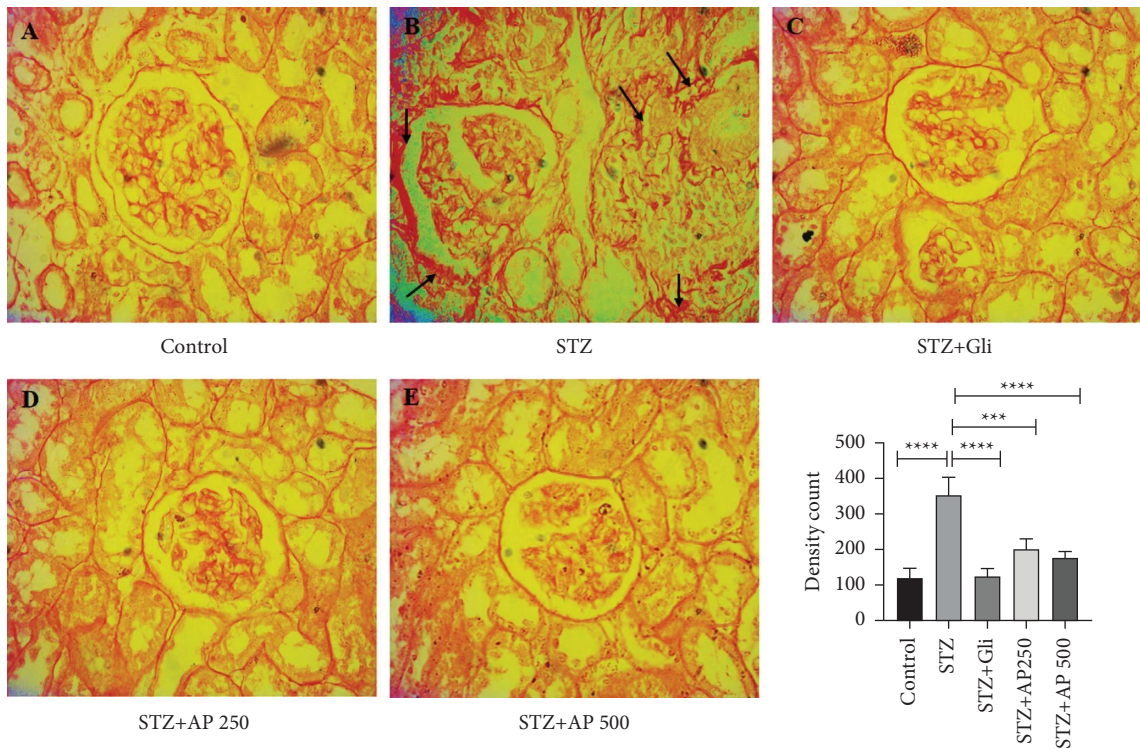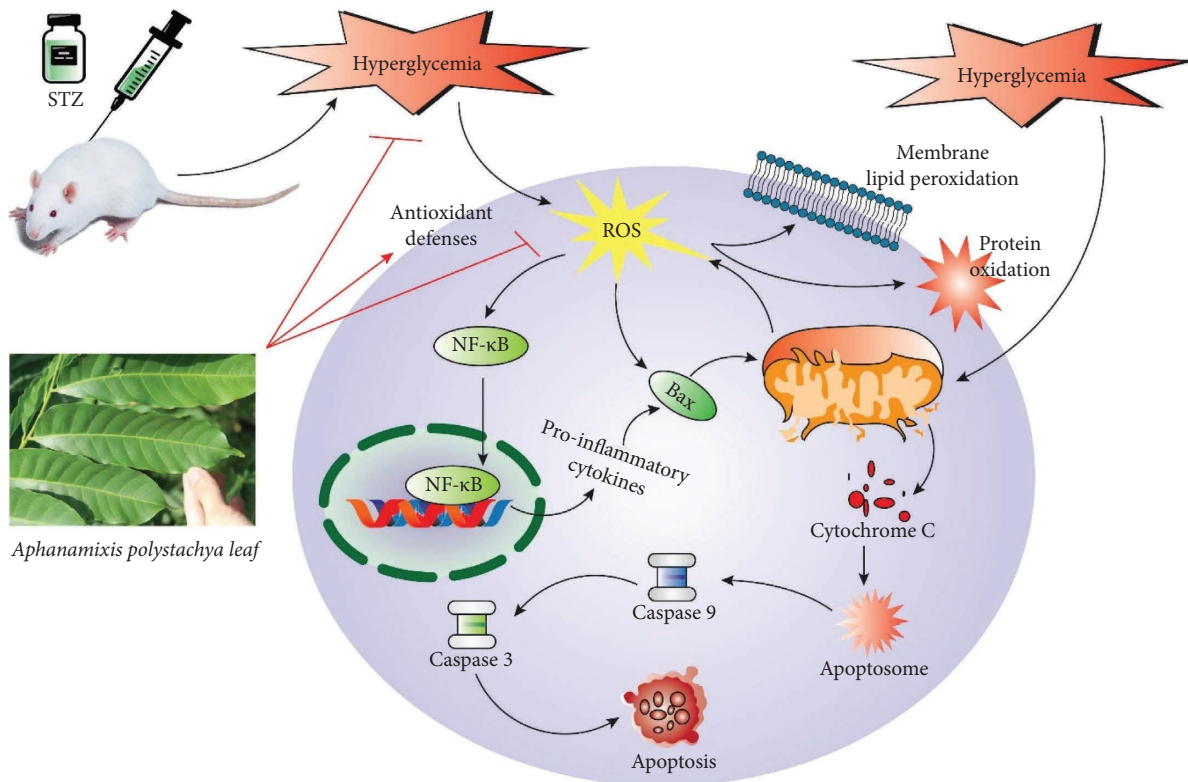
FIGURE 12: A schematic representation of the possible protective mechanism of *Aphanamixis polystachya* leaf extract against STZ-induced diabetic rats (bax: Bcl-2-associated X protein; NF-κB: nuclear factor-kappa B; ROS: reactive oxygen species; STZ: streptozotocin).

Rutin was also found to decrease plasma glucose levels along with preventing symptoms of diabetic neuropathy [81]. Rutin may have a positive impact on diabetic neuropathy because of its capacity to reduce neuroinflammation [82].

Biomarkers such as quercetin were previously found to significantly decrease FBG (fasting glucose) and increase insulin levels. Our study also found quercetin in AP leaf extract has similar properties to a well-known diabetic drug, metformin, thus proving to be a potential compound in the treatment of diabetes. It was revealed that quercetin facilitates glucose uptake by the AMPK pathway in the skeletal muscle cell line [31]. Pretreatment with quercetin showed a 2-fold enhancement in the cellular AMP : ATP ratio that could lead to the activation of AMPK and contribute to the initiation of glucose uptake [32, 33]. The mechanism of action of quercetin-stimulated glucose uptake is reported to be similar to other prior studies that showed the promotion of GLUT4 translocation through simultaneously elevating phosphorylation of AMPK and AKT [34]. However, quercetin was found to elicit activation of both insulin- and AMPK-dependent pathways. The translocation of GLUT4 is primarily regulated by pathways that are dependent on insulin and AMPK. This regulatory mechanism ultimately leads to a reduction in high glucose levels [83].

Previous studies also indicated that quercetin also aids in alleviating DN by inhibiting renal oxidative stress [35, 38, 82, 84–87]. The SCAP-SREBP2-LDLr pathway was found to be involved in the mitigation of renal lipid buildup by quercetin in diabetic mice [88]. It also elevated levels of

rCPT1, rPPAR, and rOCTN2, which are responsible for fatty acid metabolism [89], and reduced diabetic renal lipid content [90, 91]. Prior research has also demonstrated that the amount of NF-kB and the production of p65 protein in the kidney, a crucial transcription factor involved in the initiation of inflammation and the development of DN [92–94], are significantly downregulated by quercetin [35, 95].

According to previous research, giving rats STZ increased their levels of plasma nitric oxide (NO), which caused beta-cell death in the pancreas [96]. Notably, liver pathogenicity in diabetes is related to glucotoxicity and oxidative stress. Early studies have shown that oxidative stress is a significant cause of complications related to diabetes mellitus [34, 97]. The defense in the pancreas is weak, due to which glucose auto-oxidation leads to the production of other free radicals that can cause glucotoxicity. In type 2 diabetes, polyphenolic-rich compounds such as quercetin have been found to demonstrate recovery from oxidative stress, which is similar to our findings [55, 98]. Diabetes was linked to micro- and macrovascular and hepatic problems, including increased generation of AOPPs and oxidative stress. Together, our current study gives strong evidence that STZ-induced diabetic mice had significantly higher NO and AOPP levels than control rats.

Previous studies showed that STZ-induced type-2 diabetic animals suffered organ damage because their levels of GSH and SOD were low. In line with the idea that AP could be used to treat hepatic problems caused by diabetes, our

research showed that giving AP significantly increased the antioxidant enzymes GSH and SOD. This claim can be supported by a previous study that shows quercetin has protective antioxidant properties that aid in reducing FBG (fasting glucose) levels and liver and kidney marker enzymes [99]. The role of antioxidants has previously been found to improve the oxidative status by mediating liver enzyme activities and histopathological changes [97]. Additionally, a prior study demonstrated that the ingestion of quercetin has been shown to be efficacious in regulating postprandial blood glucose levels in mice induced with STZ [56].

Our findings showed that the AP had hepato- and reno-protective effects and a normalizing impact on biochemical and histopathological indicators in STZ-mediated diabetic mice. The administration of AP had a mitigating effect on hyperglycemia-mediated inflammation, oxidative stress, and apoptotic cell death in the kidneys and livers of diabetic rats. The summarized molecular pathways can be observed in Figure 12. The main phytoconstituents of *A. polystachya* leaves have been shown in in silico studies to block inflammatory and diabetic mediators implicated in the illness's etiology [21]. Overall, our results point to solid therapeutic potential for AP in treating complications of diabetes.

A limitation of this study is that data are required to gain insights into the underlying mechanisms of liver and kidney damage. Further investigation is necessary to elucidate the molecular mechanisms behind the hepatoprotective effects of either of these components. Additional research could be conducted to address these concerns.

5. Conclusion

Our results showed clear evidence of the antidiabetic effects of AP in diabetic model rats. Besides, AP also protects against liver and kidney abnormalities associated with diabetes. These data strongly indicate the ethanolic extract of *A. polystachya* as an evidence-based use of the plant in folk or traditional medicine. Phenolic compounds (GA, quercetin, and rutin) rich in *A. polystachya* are able to scavenge oxidative stress and also exhibit an antioxidant role, which ultimately gives therapeutic efficacy to ameliorate diabetes and associated complications. Further studies are required to explore the molecular pathways of the leaf extract of *A. polystachya* in diabetes.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Supplementary Materials

Supplementary Figure 1: HPLC chromatogram of a standard mixture of polyphenolic compounds (Peaks: 1, gallic acid; 2, pyrogallol; 3, (+)-catechin hydrate; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, (−)-epicatechin; 8, vanillin; 9, p-coumaric acid; 10, trans-ferulic acid; 11, ellagic acid; 12, rutin hydrate; 13, rosmarinic acid; 14, myricetin; 15, quercetin hydrate; 16, kaempferol). Supplementary Figure 2: HPLC chromatogram of ethanol extract of *Aphanamixis polystachya*. (Peaks: 1, gallic acid (GA); 2, rutin hydrate (RH); 3, quercetin hydrate (QU)). Supplementary Table 1: Contents of polyphenolic compounds in the ethanolic leaf extract of *Aphanamixis polystachya* ($n = 5$). Supplementary Table 2: Blood glucose levels observed in different treatment groups of rats after glucose challenged at different time intervals. (*Supplementary Materials*)

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