Ind. J. Pharm. Edu. Res., 2025; 59(3):1118-1127.
https://www.ijper.org

Original Article

Nephroprotective Effects of Piperlongumine against Streptozotocin-Induced Diabetic Kidney Disease in Rats

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder defined by hyperglycemia, potentially resulting in various consequences, such as diabetic kidney disease. Diabetic kidney disease is a major health concern associated with heightened morbidity and mortality risk. **Aim/Objectives:** The current study was performed to study the beneficial effects of piperlongumine against Streptozotocin (STZ)-induced diabetic renal dysfunction and renal fibrosis in an experimental rat model. **Materials and Methods:** In the present study, rats were injected with 45 mg/kg of STZ, and subsequently administered with piperlongumine for 8 weeks. Upon conclusion of the treatments, the body weight, feed and water consumption, glucose, and insulin concentrations of the experimental rats were assessed. The biomarkers for renal impairment, such as kidney index, creatinine, urine albumin, Blood Urea Nitrogen (BUN), and oxidative markers were evaluated using commercial assay kits. The renal tissues of the experimental rats underwent histological examinations to evaluate the histological changes, renal interstitial fibrosis, and glomerulosclerosis levels in the kidneys. **Results:** The treatment of piperlongumine considerably increased body weight and subsequently reduced food and water consumption, glucose, and insulin in STZ-induced rats. In addition, the piperlongumine treatment reduced the amounts of renal function markers and considerably augmented the renal antioxidant levels in rats with diabetic nephropathy. Furthermore, the histopathological studies also evidenced that piperlongumine treatment can hinder renal fibrosis and glomerulosclerosis in diabetic nephropathy rats. **Conclusion:** The current results suggest that piperlongumine may considerably alleviate diabetic kidney disease in rats. Therefore, the present findings indicate that piperlongumine can be a useful treatment option for diabetic nephropathy.

Keywords: Glomerulosclerosis, Piperlongumine, Hyperglycemia, Streptozotocin, Insulin.

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Received: 07-11-2024;
Revised: 28-01-2025;
Accepted: 19-04-2025.

INTRODUCTION

Diabetes mellitus is a persistent metabolic condition marked by hyperglycemia, potentially resulting in many severe consequences, such as diabetic nephropathy.[1] Diabetes-associated renal dysfunction and renal fibrosis have emerged as major public health issues in recent decades. This intricate pathophysiology leads to a gradual decrease in glomerular filtration rate, proteinuria, and progressive renal function deterioration, finally resulting in End-Stage Renal Disease (ESRD).[2] Factors influencing the onset and advancement of diabetic renal impairment encompass genetic, immunological, hemorheological, biochemical, and hemodynamic pathways. Among these, non-enzymatic glycosylation of proteins and glomerular hyperfiltration seem to be the most significant pathogenic contributors.[3] Recent advancements have enhanced the understanding of the natural history of diabetic kidney disease, particularly emphasizing the stage of incipient diabetic nephropathy, which is marked by microalbuminuria. Despite existing therapeutic methods, a significant residual risk of disease initiation and progression persists, requiring extensive innovation to enhance health outcomes for patients. Overall, the burden of diabetic kidney disease is immense, contributing significantly to morbidity, mortality, and healthcare expenditures globally.[4]

The pathophysiology of diabetic nephropathy is complex and multifactorial, involving numerous signaling pathways and pathophysiological mechanisms. One of the primary contributing factors is the non-enzymatic glycosylation of proteins, which can lead to the development of advanced glycation end products that accumulate in the kidneys and induce inflammation, oxidative stress, and structural changes. Additionally, hyperglycemia-induced oxidative stress plays an essential role in the onset of diabetic nephropathy, as it can contribute to the activation of various signaling molecules and pathways.[5] The pathophysiology of diabetic nephropathy is a multifaceted process

DOI: 10.5530/ijper.20251645

that involves metabolic, hemodynamic, and signaling pathways. Targeting these various pathways and mechanisms may offer novel therapies to prevent and treat diabetic nephropathy.[6]

The current treatment options for diabetic kidney disease, like the utilization of angiotensin-converting enzyme inhibitors and angiotensin-II receptor blockers, have shown limited efficacy in hindering the advancement of renal dysfunction and fibrosis.[7] The treatment of diabetic kidney disease is further complicated by fluctuations in blood glucose homeostasis, ambiguous precision of glucose measurements, and modified kinetics of glucose-lowering drugs in patients with ESRD.[8] Recently, interest has surged in utilizing plant-derived bioactive chemicals as prospective therapeutic agents for managing diabetic kidney disease.[9] These compounds have diverse pharmacological effects, which may be advantageous in treating diabetic kidney disease.[10] Piperlongumine is an important bioactive alkaloid compound mostly present in the roots and fruits of the *Piper longum* plant, which belongs to the Piperaceae family.[11] The piperlongumine has already demonstrated several pharmacological properties, including anticancer, anti-inflammatory, antiplatelet activity, anti-atherosclerotic, antimicrobial and neuroprotective effects.[12-17] However, the beneficial effects of piperlongumine against diabetes and its associated complications have not been reported yet. Hence, the current work was performed to study the beneficial roles of piperlongumine against streptozotocin-induced diabetic renal dysfunction and renal fibrosis in experimental rats.

MATERIALS AND METHODS

Experimental rats

The eight-week-old Sprague Dawley rats were obtained from an institutional animal facility and subsequently utilized in this work. The rats were housed in sterile enclosures under laboratory conditions, with a temperature range of 21±1°C, humidity levels of 60-70%, and 12 hr cycles of alternating light and dark cycles. Animals were provided with free access to regular feed and water throughout the study.

Experimental groups

After a one-week acclimation period, the experimental rats were categorized into four experimental groups, each comprising six rats. Group I was the "normal control" or "vehicle control" group and received only regular feed without active compound. Group II rats were administered with STZ at a dosage of 45 mg/kg to induce diabetic kidney disease. Following a three-day treatment of STZ, blood glucose levels were assessed and rats exhibiting glucose levels over 250 mg/dL were classified as diabetic and selected for further experiments. Group III rats were STZ-induced and subsequently given a dosage of 50 mg/kg of piperlongumine for 8 weeks. Group IV rats were STZ-induced and received a dosage of 350 mg/kg of metformin for 8 weeks. Throughout the experiment, the body weight, feed, and water consumption of the experimental rats were accurately measured every week. On the final day of treatment, rats were housed in metabolic cages, and 24-hr urine was obtained to measure urinary albumin content using a kit (Elabscience, USA). The rats were then anesthetized using an overdose of isoflurane and sacrificed. The blood sample was obtained and centrifuged at 5000 rpm for 15 min, with serum samples preserved at -80°C for biochemical analysis. The renal tissues were obtained for histological analyses, and kidney index was computed by the formula: kidney index=kidney weight (mg)/ body weight (g).

Analysis of glucose and insulin levels

The glucose levels were assessed from the tail vein utilizing a standard glucometer (Roche, USA). Blood was extracted and centrifuged for 15 min to isolate serum; a commercial diagnostic kit was employed to measure insulin levels (Abcam, USA).

Analysis of biochemical marker levels

The serum concentrations of creatinine and Blood Urea Nitrogen (BUN) were estimated using commercial kits. The tests were performed with three replicates following the guidelines of the assay kit manufacturer (Elabscience, USA). The collected renal tissues were homogenized in iced saline, followed by centrifugation for 15 min. The resultant supernatant was utilized to estimate oxidative stress markers and antioxidant levels utilizing commercial assay kits. The antioxidants Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Malondialdehyde (MDA) was evaluated utilizing the kits. The tests were performed in triplicate and carried out according to the manufacturer's recommended protocols (Abcam, USA).

Histological analysis

The excised renal tissues were preserved in paraformaldehyde (4%) for 24 hr. Later, the tissues were dried with graded ethanol and then paraffinized. The tissues were subsequently sectioned into 5-µm sizes. The tissue slides were then stained with eosin and hematoxylin and analyzed using a light microscope. The kidney histopathology was evaluated using a renal pathological scoring method.[18]

Analysis of renal interstitial fibrosis by Masson's Trichrome staining

To analyze the renal interstitial fibrosis level, the 5 µm of renal tissue slides were stained with Masson's trichrome by using the guidelines described in the staining kit (Sigma Aldrich, USA). Following the staining, the specific fields were randomly chosen on each slide to assess the extent of interstitial fibrosis. The fibrotic region was evaluated using semiquantitative scoring as observed under a light microscope. The findings were determined using ImagePro+ software (Media Cybernetics, USA).

Analysis of glomerulosclerosis by PAS staining method

The kidney sections at 5 μm size were stained using Periodate-Schiff (PAS) following the protocol specified in the staining kit (Sigma Aldrich, USA). A semi-quantitative scoring method was employed to assess the extent of glomerulosclerosis. Each PAS-stained segment was examined, and 20 glomeruli were classified into lesions ranging from 0 to 4 as per the following measures: 0: no glomerular sclerosis; 1: <25% sclerosis; 2: 25-50% sclerosis; 3: 50-75% sclerosis; 4: 75-100% sclerosis. The Glomerulosclerosis Index (GSI) is computed using an equation: (1×N1+2×N2+3×N3+4×N4)/N×100, where N1-4 specifies the count of glomeruli assigned scores of 1, 2, 3, and 4 respectively, and N denotes the total number of glomeruli.

Statistical analysis

Each experimental result was depicted as a mean±SD from three replicates. A one-way ANOVA and Tukey's *post hoc* test were employed to assess the significance, with $p<0.05$ indicating significance.

RESULTS

Effect of piperlongumine on Body Weight, food and Water Consumption of experimental Rats

The effect of piperlongumine on body weight and feed and water intake in the rats was evaluated, and the findings are exhibited in Figure 1. The STZ rats demonstrated a considerable reduction in body weight and a subsequent increase in both food and water consumption. The treatment of piperlongumine at 50 mg/kg dosage remarkably increased body weight and considerably reduced food and water intake in the STZ rats. The activities of piperlongumine are further corroborated by the findings related to metformin treatment, which also elevates body weight and reduces feed and water consumption in STZ rats.

Effect of piperlongumine on the glucose and insulin levels in the experimental rats

The levels of glucose and insulin in the experimental rats are presented in Figure 2. The STZ rats revealed a substantial elevation in both blood glucose and serum insulin when compared with the control. The piperlongumine at 50 mg/kg concentration showed a considerable diminution in both blood glucose and serum insulin in the STZ rats. The treatment of standard drug metformin also significantly diminished the glucose and insulin in the STZ rats, so further corroborating the efficacy of piperlongumine.

Effect of piperlongumine on the renal function markers in experimental rats

The nephroprotective properties of piperlongumine in diabetic nephropathy rats were assessed by quantifying the levels of renal function markers, as indicated in Figure 3. The rats with STZ-induced diabetic nephropathy exhibited substantial increases in kidney index, urine albumin, serum creatinine, and BUN concentrations. The treatment of 50 mg/kg of piperlongumine effectively diminished the levels of kidney index, urine albumin, serum creatinine, and BUN in rats with diabetic nephropathy. The nephroprotective effects of piperlongumine are corroborated by the findings of metformin treatment, which similarly reduced these renal function markers in rats with STZ-induced diabetic nephropathy.

Effect of piperlongumine on oxidative stress in experimental rats

The rats with diabetic nephropathy exhibited diminished CAT, SOD, and GPx, alongside an elevated MDA concentration in their renal tissues (Figure 4). However, the 50 mg/kg of piperlongumine-treated rats exhibited a significant increase in their antioxidant concentrations, along with a decrease in MDA in the kidney tissues. Moreover, metformin also diminished MDA levels and elevated the antioxidants in the renal tissues of diabetic nephropathy rats, thereby supporting the antioxidant activities of piperlongumine.

Effect of piperlongumine on renal tissue histology of experimental rats

Figure 5 illustrates the pathological abnormalities in the kidneys of the experimental rats. The significant damage to the glomerular architecture, expansion of the renal interstitium, presence of vacuolar degeneration, tubular epithelial cell necrosis, and an increase in inflammatory cells within the renal interstitium was noted in the kidneys of the STZ-induced rats. In contrast to STZ rats, the renal architecture of the piperlongumine-treated rats exhibited normalized glomerular volume; smooth and intact renal tubular endothelium, normal epithelial cells, and reduced inflammatory cell infiltration in the interstitium. Similar results were also noted in the results of metformin treatment, which further supports the activity of piperlongumine.

Effect of piperlongumine on renal interstitial fibrosis in experimental rats

The Masson's trichrome-stained renal tissues of the experimental rats were examined for renal interstitial fibrosis, and findings were presented in Figure 6. The renal tissues of STZ-induced rats illustrate significant renal interstitial fibrosis, accompanied by extensive collagen fiber distribution when compared with control. Interestingly, the extent of renal fibrosis in piperlongumine-treated rats was substantially reduced than that of STZ-induced rats. Similarly, the metformin also effectively diminished the renal fibrosis in the STZ rats.

Effect of piperlongumine on glomerulosclerosis in the experimental rats

The PAS-stained kidney tissue sections of the experimental rats were examined for glomerulosclerosis (Figure 7). The kidney tissues of STZ rats exhibited substantial levels of glycoprotein within the glomerular matrix and capillary endothelium. Captivatingly, the piperlongumine treatment at 50 mg/kg effectively reduced the glycoprotein levels in the kidney tissues of STZ rats, which is also supported by the metformin treatment. Furthermore, the GSI was also considerably elevated in the STZ rats. Nonetheless, the piperlongumine and/or metformin treatment considerably diminished the GSI, which suggests that piperlongumine can prevent glomerulosclerosis in diabetic nephropathy rats.

DISCUSSION

Diabetes is a chronic metabolic condition that can lead to significant complications affecting multiple organs, including the kidneys. Diabetic nephropathy, a prevalent consequence of diabetes, is a primary contributor to ESRD. Metabolic alterations linked to diabetes, including hyperglycemia, can result in glomerular hypertrophy, glomerulosclerosis, and tubulointerstitial inflammation, which are indicative of diabetic kidney disease.[19] These pathological changes are influenced by several factors, including genetic predisposition, immunological responses, and hemodynamic alterations. Importantly, the interactions between metabolic and hemodynamic factors play a pivotal role in the onset of diabetic nephropathy.[20] Diabetes is a chronic metabolic condition defined by an improper regulation of blood sugar, and one of the common symptoms associated with this disease is unexplained weight loss. The primary reason for

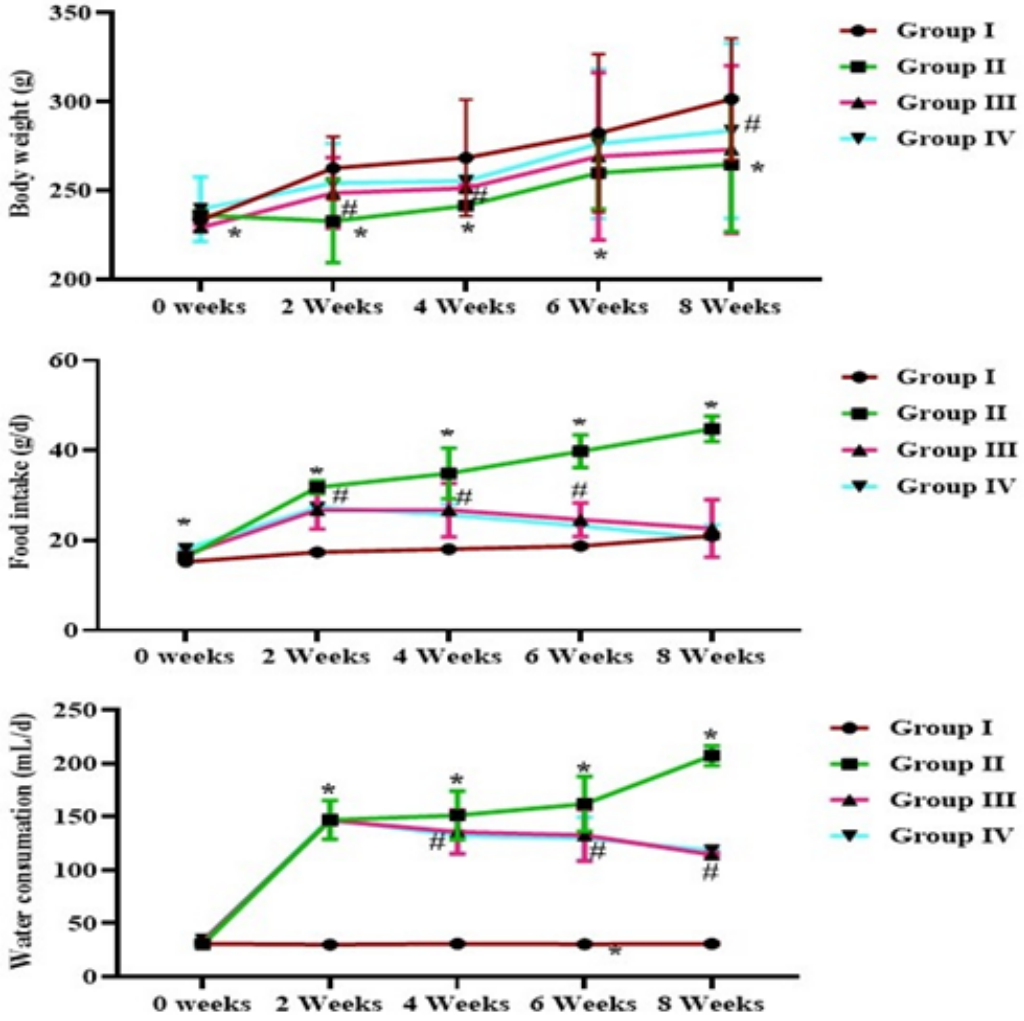

Figure 1: Effect of piperlongumine on the body weight, food, and water consumption of the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

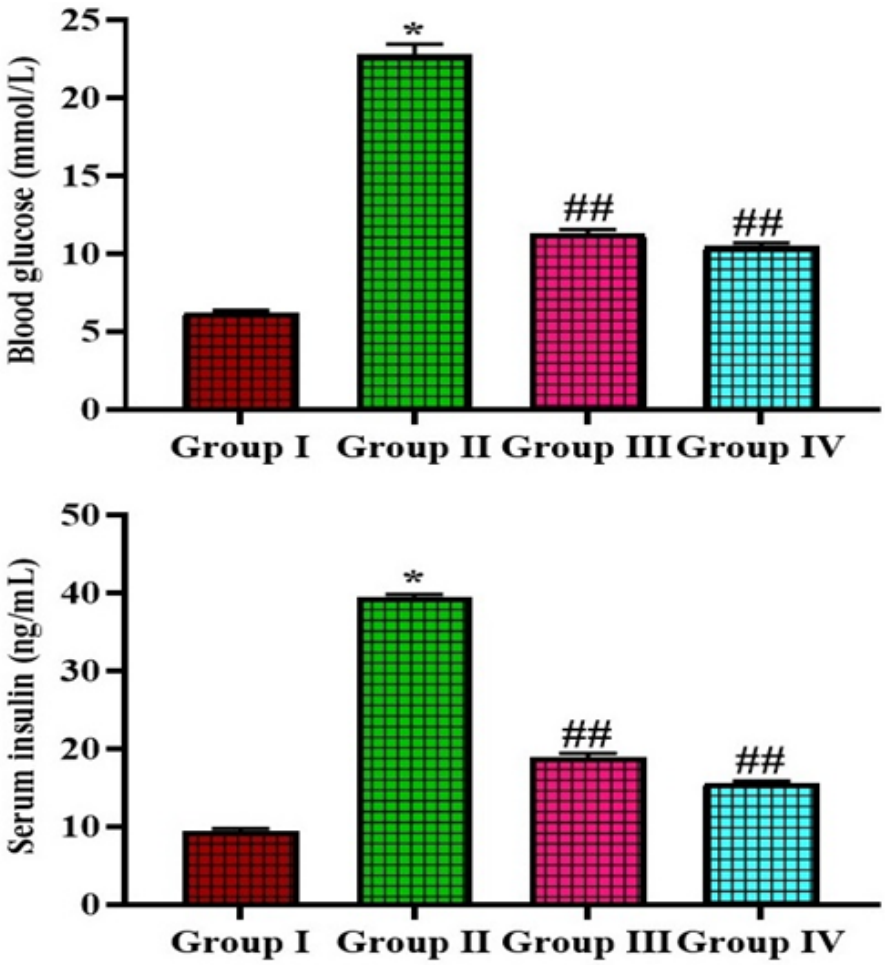

Figure 2: Effect of piperlongumine on the blood glucose and serum insulin levels in the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

this weight loss is the body's inability to effectively use glucose. In individuals with untreated or poorly controlled diabetes, the body is unable to use glucose effectively, leading to a state of starvation at the cellular level.[21] This metabolic imbalance results in the body turning to alternative energy sources, such as fat and muscle, to meet its energy needs. As a result, individuals with uncontrolled diabetes may experience significant weight loss, even in the absence of dietary restrictions or increased physical activity.[22] Food intake and dietary habits are also crucial in the management of diabetes and diabetic nephropathy. Excessive caloric intake, particularly from carbohydrates and fats, can lead to weight gain, hyperglycemia, and dyslipidemia, all of which can negatively impact kidney health. Conversely, adherence to a balanced, low-calorie diet that emphasizes whole, unprocessed foods can help improve glycemic control, reduce inflammation, and potentially slow the advancement of diabetic nephropathy.[23] Water consumption is another important factor in the diabetes management and its associated impediments. Adequate hydration can help maintain kidney function, regulate fluid balance, and flush out waste products from the body. Addressing these factors through lifestyle modifications and targeted therapies can potentially slow down the advancement of diabetic kidney disease and improve patient outcomes.[24] Here, the STZ-induced rats demonstrated a considerable diminution in body weight and a subsequent elevation in both food and water consumption. The treatment of piperlongumine significantly increased body weight and considerably reduced food and water consumption in the STZ rats.

The analysis of specific biomarkers is crucial for early diagnosis, monitoring, and management of diabetic nephropathy. The kidney index, which measures the size and function of the kidneys, can provide valuable insights into the progression of diabetic nephropathy. Urine albumin levels are a well-established marker of kidney injury, as increased albumin excretion is an early indicator of diabetic kidney disease.[25] Serum creatinine

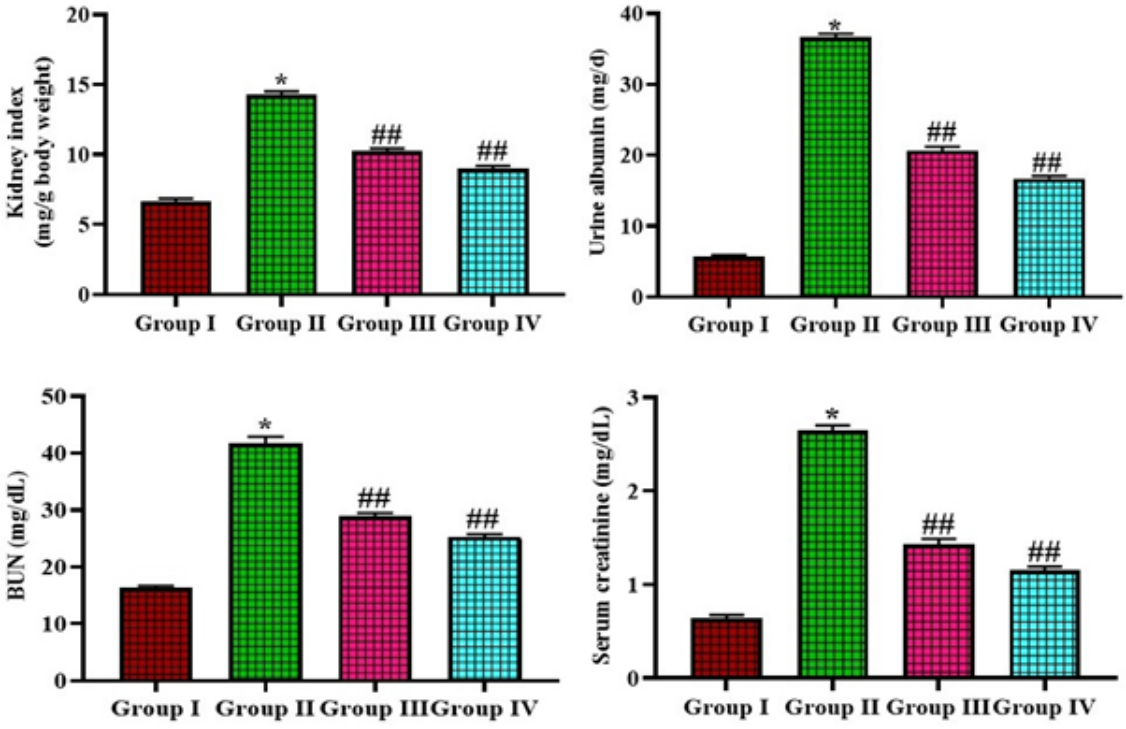

Figure 3: Effect of piperlongumine on the renal function markers in the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

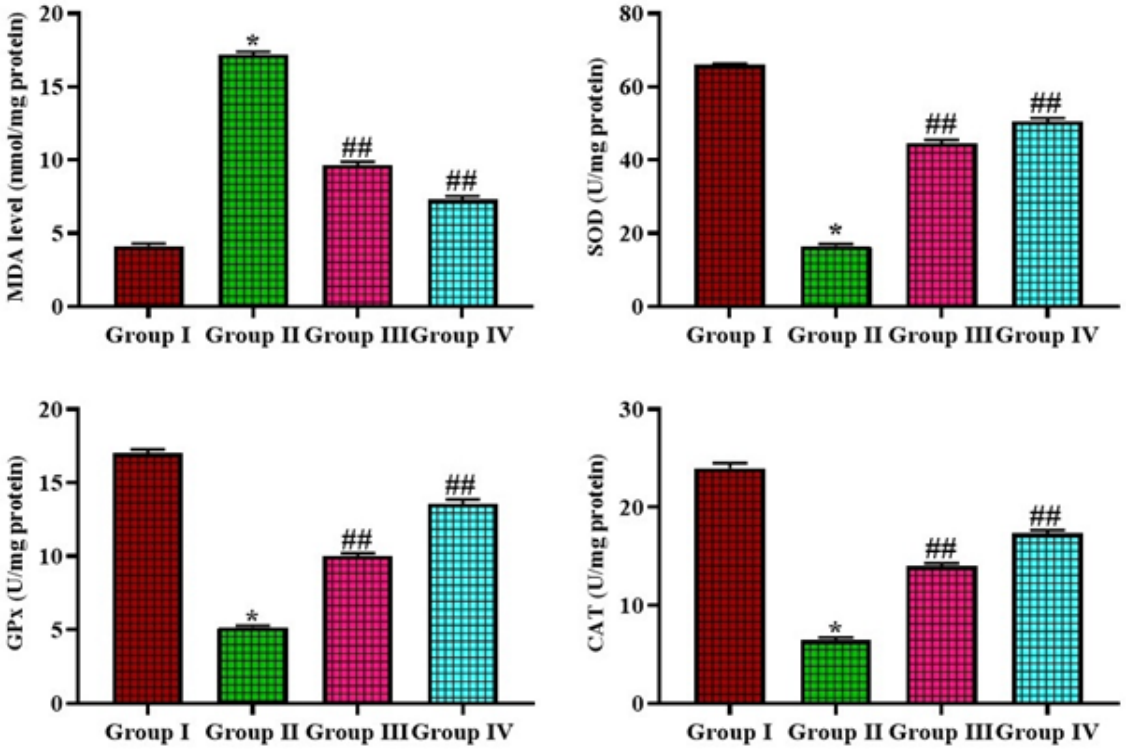

Figure 4: Effect of piperlongumine on the oxidative stress in the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

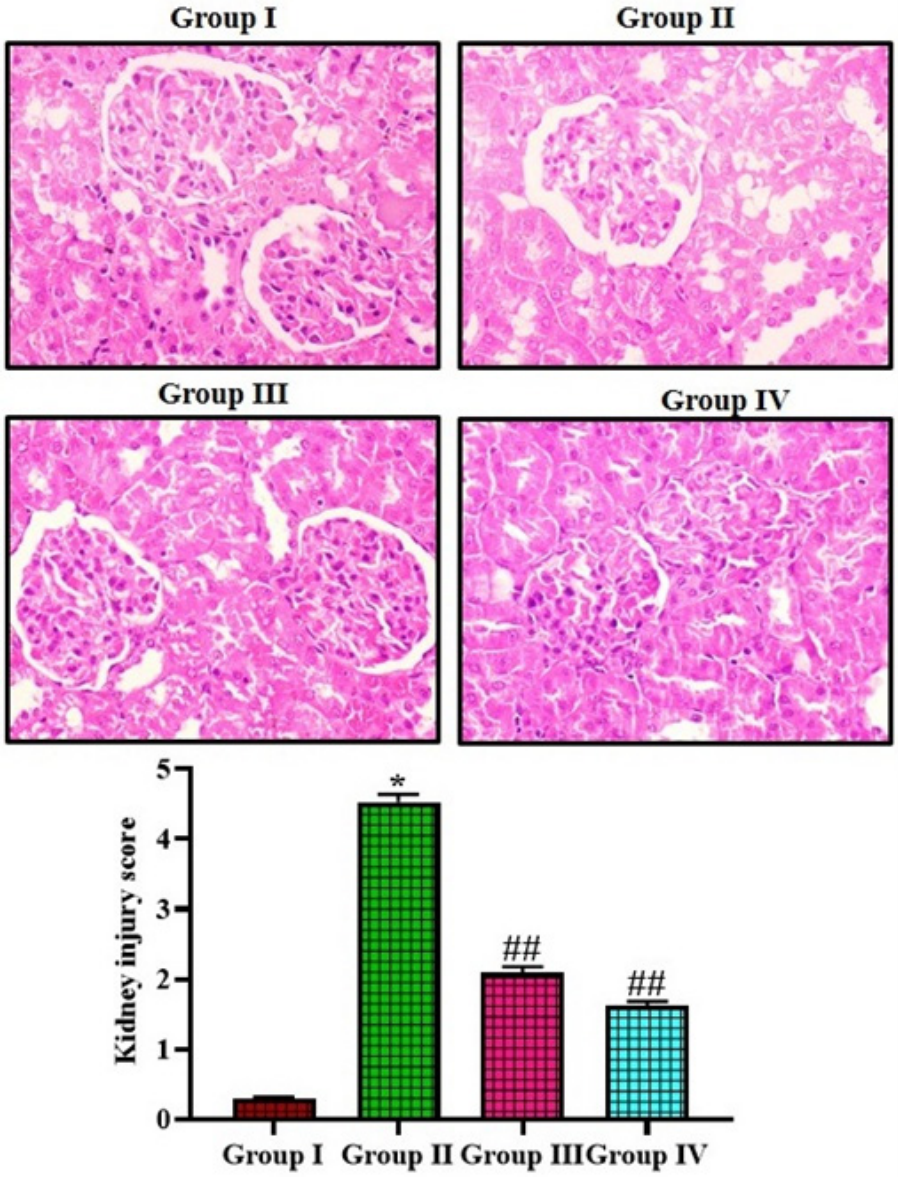

Figure 5: Effect of piperlongumine on the renal tissue histopathology of the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

and BUN, on the other hand, are indicators of overall kidney function, and their levels can help assess the severity of renal impairment.[26] Urinary tubular damage markers may elevate in diabetic individuals before the emergence of microalbuminuria, presenting a chance for early detection and intervention. Novel biomarkers may serve as more precise indicators of disease development and long-term prognosis compared to existing markers. Analyzing these biomarkers can help researchers identify individuals at risk of developing diabetic nephropathy, monitor disease progression, and guide treatment decisions.[27] In this work, the outcomes exhibited that rats with STZ-induced diabetic nephropathy exhibited considerable elevations in kidney index, urine albumin, serum creatinine, and BUN concentrations, which evidences the onset of renal dysfunction. Interestingly, the piperlongumine treatment effectively diminished the levels of kidney index, urine albumin, serum creatinine, and BUN in rats with diabetic nephropathy. These findings highlight the assumption that piperlongumine can prevent renal dysfunction in diabetic conditions.

The pathophysiology of diabetic nephropathy is intricate and multifaceted, encompassing factors like oxidative stress generated by hyperglycemia, inflammation, and endothelial dysfunction.[28] The analysis of oxidative stress-associated biomarkers provides useful insights into the pathophysiology of diabetic nephropathy. MDA is a widely used marker of lipid peroxidation, a mechanism

that occurs when ROS attacks the cellular components. CAT, GPx, and SOD are antioxidant enzymes that play a key role in protecting the cells and tissues against oxidative stress.[29] It has been demonstrated that diabetic individuals exhibit elevated MDA and decreased antioxidants, including CAT, GPx, and SOD, in the kidneys.[30] These results highlight that the assessment of these oxidative stress markers can offer important data about the onset of diabetic nephropathy and the effectiveness of therapeutic interventions targeting oxidative stress.[31] The analysis of oxidative stress-associated biomarkers in the kidneys of individuals with diabetic nephropathy is crucial for understanding the pathogenesis of this condition and developing effective treatment strategies.[32] In this work, the present results demonstrated that the rats with diabetic nephropathy exhibited diminished CAT, SOD, and GPx activities, alongside an elevated MDA concentration in their renal tissues. Fascinatingly, the piperlongumine-treated rats exhibited a significant increase in their antioxidant concentrations, along with a decrease in MDA on the renal tissues, which eventually supported the antioxidant effects of piperlongumine.

The onset of renal fibrosis is one of the key pathological features of diabetic nephropathy, which can significantly contribute to the decline in kidney function. The analysis of renal interstitial fibrosis using Masson's Trichrome staining is essential to comprehensively understand diabetic nephropathy.[33] Diabetic nephropathy is a multifaceted condition that involves various pathological changes. These metabolic alterations associated with diabetes can lead to the accumulation of extracellular matrix components, like collagen, in the renal interstitium, resulting in the onset of interstitial fibrosis. Masson's Trichrome is a widely utilized histological technique that can effectively visualize and quantify the extent of this interstitial fibrosis, providing valuable insights into the onset of diabetic nephropathy.[34] The importance of analyzing renal interstitial fibrosis using Masson's Trichrome staining in diabetic nephropathy is well reported. Interstitial fibrosis is a significant marker of renal function decline and is connected with a poor prognosis in patients with diabetic nephropathy.[35] Furthermore, the analysis of interstitial fibrosis can provide valuable insights into the underlying pathogenic mechanisms, which participate in the onset of diabetic nephropathy.[36] The current results demonstrated that the renal tissues of STZ rats reveal significant renal interstitial fibrosis, accompanied by extensive collagen fiber distribution when compared with control. Captivatingly, the extent of renal fibrosis in piperlongumine-treated rats was substantially reduced, which suggests that piperlongumine can hinder renal fibrosis.

The accurate diagnosis and monitoring of glomerulosclerosis, a hallmark of diabetic nephropathy, is crucial for effective management and treatment of diabetic nephropathy.[37] One important tool in the analysis of glomerulosclerosis is the PAS staining technique. This histological staining method allows for the visualization and quantification of the mesangial matrix expansion and glomerular basement membrane thickening. By using this staining method, researchers can acquire useful information about the progression of glomerulosclerosis and its association with other pathological changes in the kidney.[38] It has already been established that in diabetic models, glycogen deposition and vacuole formation consistently occur in the distal tubule and macula densa, indicating that in diabetic nephropathy, there is not only an augmented influx of macromolecules into the subendothelial space but also an obstruction to their efflux from the base of the glomeruli in the macula densa region. It highlights the importance of analyzing the glomerular changes using PAS staining, as it can provide information about the complex alterations that occur in the diabetic kidney.[39] This

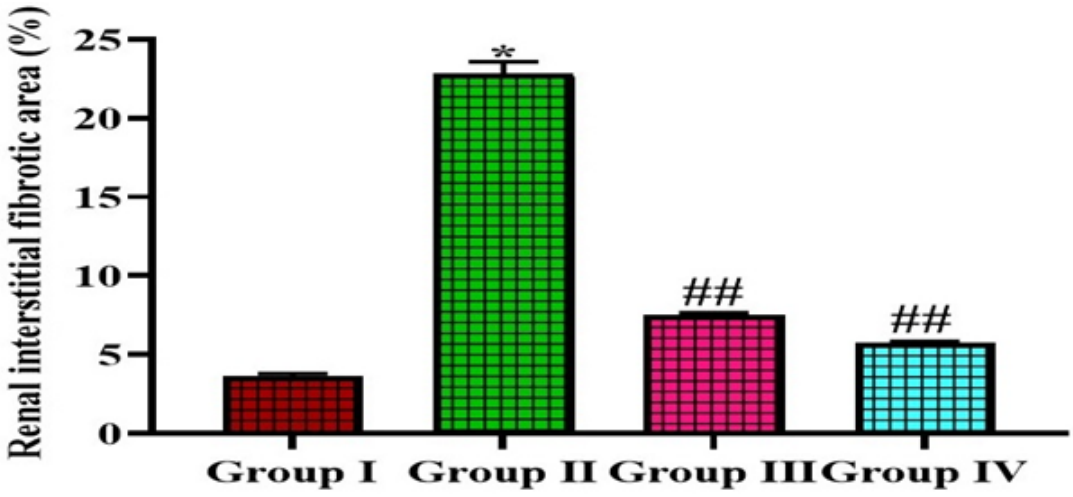

Figure 6: Effect of piperlongumine on renal interstitial fibrosis in the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

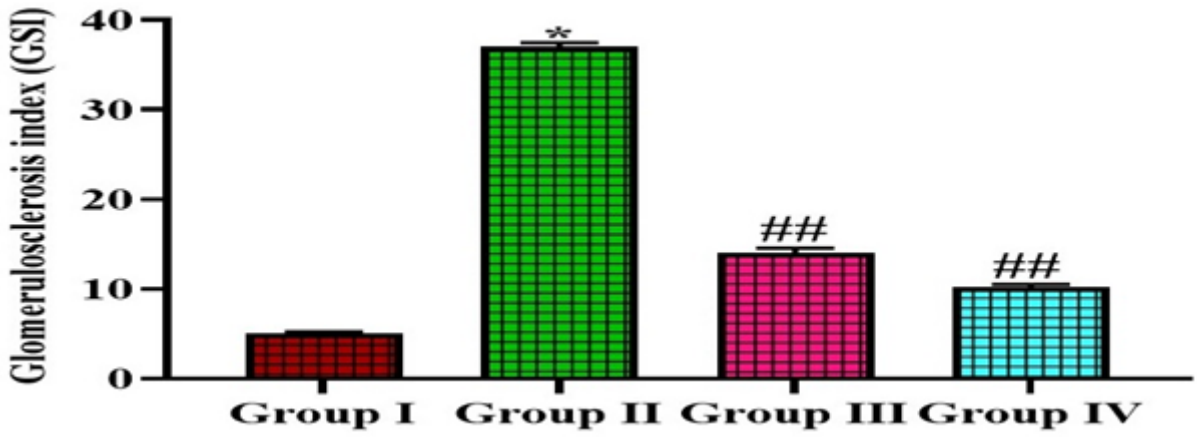

Figure 7: Effect of piperlongumine on glomerulosclerosis in the experimental rats. Group I: Control rats; Group II: STZ-induced diabetic rats; Group III: STZ-induced diabetes+piperlongumine-treated rats; Group IV: STZ-induced diabetes+metformin-treated rats. The results are shown as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* assay were utilized to evaluate statistical significance. Note: '*' designates statistical significance at $p<0.01$ when compared with control; '##' designates statistical significance at $p<0.05$ when compared with the STZ-induced group.

technique allows for the visualization and quantification of the structural changes within the glomeruli, which are central to the development and progression of this debilitating complication of diabetes.[40] The current results highlighted that the kidney tissues of STZ-induced rats exhibited substantial levels of glycoprotein within the glomerular matrix. However, the piperlongumine treatment effectively reduced the glycoprotein levels in the kidneys of STZ rats, which suggests that piperlongumine can prevent glomerulosclerosis in diabetic nephropathy rats.

CONCLUSION

The current results suggest that piperlongumine may considerably alleviate diabetic kidney disease in rats. The piperlongumine treatment remarkably decreased the glucose and insulin concentrations, and elevated body weight in the diabetic nephropathy rats. In addition, the piperlongumine treatment also effectively decreased the renal function markers level and alleviated oxidative stress by subsequently increasing antioxidant levels in rats with diabetic nephropathy. Furthermore, the histopathological studies also evidenced that piperlongumine treatment can hinder renal fibrosis and glomerulosclerosis in diabetic nephropathy rats. Therefore, the present findings indicate that piperlongumine can be a useful treatment option for diabetic nephropathy.

ACKNOWLEDGEMENT

This work was supported by the Department of Nephrology, Affiliated Hospital of Hebei University (Key Laboratory of Skeletal Metabolic Physiology for Chronic Kidney Disease in Hebei Province), Baoding, 071000, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Yang Xu and Xijie Zheng contributed equally to the work and can be considered into co-first author.

FUNDING

The project supported by: 1. Analysis of the causes of volume compliance in peri-dialysis patients in a single center, Grant No.20231495. 2. Medical Science Research Project of Hebei Health Commission (20212314).

ABBREVIATIONS

STZ: Streptozotocin; **MDA:** Malondialdehyde; **SOD:** Superoxide dismutases; **CAT:** Catalase; **GSH:** Glutathione; **GPx:** Glutathione peroxidase; **ELISA:** Enzyme Linked Immunosorbent Assay; **DN:** Diabetic nephropathy; **DM:** Diabetes Mellitus; **ROS:** Reactive oxygen species; **RAAS:** Renin-angiotensin-aldosterone system; **FBG:** Fasting blood glucose; BW: Body weight; **GR:** Glutathione reductase; **GSSG:** Oxidized glutathione; **H&E:** Hematoxylin and eosin; **Ig:** Immunoglobulin; **CML:** Carboxy methyl lysine; **HbA1c:** Glycated haemoglobin; **ATP:** Adenosine triphosphate.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work was approved by the institutional ethical committee at Hebei University, Baoding, 071000, China.

SUMMARY

The study indicates that piperlongumine shows promise in alleviating diabetic kidney disease in rats. Treatment with piperlongumine significantly reduced glucose and insulin levels, improved body weight, decreased renal function markers, and mitigated oxidative stress by enhancing antioxidant levels.

Histopathological analysis further demonstrated that it helps prevent renal fibrosis and glomerulosclerosis. These findings suggest that piperlongumine could be a potential therapeutic option for diabetic nephropathy.

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Cite this article: Xu Y, Zheng X, Hao Z. Nephroprotective Effects of Piperlongumine against Streptozotocin-Induced Diabetic Kidney Disease in Rats. Indian J of Pharmaceutical Education and Research. 2025;59(3):1118-27.