

Research Article

Elucidating the Therapeutic and Preventive Role of Piperonal in Diabetic Nephropathy Using Albino Rat Model

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ABSTRACTS

Background: Diabetic kidney disease is associated with oxidative stress and progressive tissue injury. Piperonal is a plant-derived aromatic compound with reported biological activities, but its effects in experimental diabetic nephropathy remain insufficiently characterized. This study evaluated the effects of different piperonal doses and administration timings on histopathology and oxidative stress-related markers in diabetic rats.

Methods: Forty-two male albino rats were randomly assigned to seven groups (n=6). Diabetes was induced using nicotinamide (230 mg/kg) followed 15 minutes later by streptozotocin (65 mg/kg). Piperonal was administered orally at 30, 40, or 50 mg/kg/day either from day 1 or from day 15 through day 45. A glibenclamide-treated group and a diabetic control group were included. Kidney and liver tissues were examined histopathologically, and malondialdehyde (MDA), catalase activity, and reduced glutathione (GSH) were assessed.

Results: The diabetic control group showed marked renal and hepatic histopathological alterations and higher MDA levels than the normal control group. Early piperonal administration at 40 mg/kg/day was associated with comparatively preserved renal and hepatic architecture and lower MDA levels than the diabetic control and delayed-treatment groups. In contrast, delayed piperonal treatment showed progressively higher MDA levels with increasing dose, with the highest values observed at 50 mg/kg/day. Catalase activity and GSH levels also increased in several diabetic and delayed-treatment groups, suggesting altered or compensatory antioxidant responses rather than consistent improvement in oxidative status.

Conclusion: Piperonal administration produced timing- and dose-related differences in histopathological and oxidative-stress-related outcomes. Early administration at 40 mg/kg/day was associated with comparatively favorable histopathological and MDA findings, whereas delayed treatment showed no consistent biochemical improvement. These preliminary findings require confirmation in adequately powered studies using quantitative histopathological assessment, conventional renal-function measures, and additional mechanistic biomarkers.

Keywords: Piperonal, Diabetic Nephropathy, Streptozotocin-Nicotinamide, Oxidative Stress, Malondialdehyde, Catalase, Glutathione.

INTRODUCTION

Diabetes mellitus is a major metabolic disorder characterized by persistent hyperglycemia and progressive damage to multiple organs. Diabetic kidney disease (DKD), also referred to

as diabetic nephropathy, is one of the major complications of diabetes and is associated with progressive renal injury and chronic kidney dysfunction (1). The development and progression of diabetic nephropathy involve

complex interactions among metabolic disturbances, oxidative stress, inflammation, hemodynamic abnormalities, and renal fibrosis (2).

Oxidative stress plays an important role in the development and progression of diabetic nephropathy. Persistent hyperglycemia can increase the generation of reactive oxygen species and impair endogenous antioxidant defense mechanisms, resulting in oxidative damage to renal cells (3). Oxidative stress is also closely associated with inflammatory responses and progressive kidney injury in patients with diabetes (4). Therefore, targeting oxidative stress and strengthening endogenous antioxidant defenses have received considerable attention as potential strategies for limiting diabetic renal damage (5).

Oxidative stress-related biomarkers have been increasingly investigated to understand the severity and progression of renal injury in chronic kidney disease, particularly in diabetic nephropathy (6). Experimental studies have also demonstrated that natural polyphenol-rich extracts may exert renoprotective effects by reducing oxidative stress and kidney injury in streptozotocin-induced diabetic models (7). These findings support continued investigation of plant-derived compounds with antioxidant and protective properties.

Plant-derived antioxidants have attracted increasing interest because of their potential to counteract oxidative damage and support cellular defense mechanisms (8). Experimental animal models are particularly important for investigating the mechanisms underlying diabetic complications and for evaluating potential therapeutic compounds before clinical investigation (9).

The nuclear factor erythroid 2-related factor 2 (NRF2) is an important regulator of cellular antioxidant and cytoprotective responses in the kidney. Activation of NRF2 promotes the expression of several antioxidant and detoxifying genes and contributes to protection against oxidative injury (10). Dysregulation of oxidative stress and NRF2 signaling has been implicated in different forms of kidney injury, including diabetic renal damage (11).

Diabetic kidney disease develops through multiple molecular and cellular mechanisms, and increasing understanding of these pathways has contributed to the identification of novel therapeutic targets (12). Current and emerging treatments aim not only to control

hyperglycemia and blood pressure but also to reduce inflammation, oxidative stress, fibrosis, and other mechanisms involved in renal injury (13). Identification of specific molecular targets may therefore contribute to the development of more effective strategies for preventing or slowing the progression of diabetic kidney disease (14).

Inflammation and oxidative stress are closely interconnected processes in diabetic kidney disease. Pharmacological interventions that modify these pathways may provide additional renal protection beyond conventional metabolic control (15). In addition, natural compounds have been investigated for their ability to influence multiple molecular pathways involved in the development and progression of diabetic kidney disease (16).

Polyphenols are of particular interest because of their potential effects on oxidative stress, inflammation, fibrosis, and apoptosis. These biological activities suggest that polyphenol-based compounds may have therapeutic potential in experimental diabetic nephropathy (17). Experimental studies using streptozotocin–nicotinamide-induced diabetes have further demonstrated the usefulness of animal models for evaluating the effects of plant-derived interventions on diabetes-associated complications (18).

The NRF2/HO-1 signaling pathway has also been investigated as a potential mechanism through which therapeutic compounds may reduce oxidative stress and inflammation in diabetic nephropathy. Experimental evidence has shown that activation of this pathway can attenuate renal injury in diabetic animals (19). Although several pharmacological and natural compounds have been investigated in diabetes and its complications, their mechanisms and therapeutic effects vary considerably. For example, studies of antidiabetic agents have explored molecular pathways beyond glucose lowering, demonstrating the importance of investigating specific biological mechanisms underlying diabetes-associated tissue injury (20).

Piperonal is a naturally occurring aromatic aldehyde that has attracted scientific interest because of its diverse biological and pharmacological properties. It contains a benzodioxole structure and has been investigated for potential therapeutic applications in several experimental settings (22). Its chemical and biological characteristics make it a potentially interesting compound for

investigation in disorders associated with oxidative stress.

Despite increasing interest in antioxidant compounds and natural products for diabetic kidney disease, evidence specifically evaluating piperonal in experimental diabetic nephropathy remains limited. In particular, the effects of different piperonal doses and administration timing on renal and hepatic histopathology and oxidative stress-related biomarkers have not been adequately characterized.

Therefore, the present study was designed to investigate the effects of different doses and administration timing of piperonal on histopathological changes and oxidative stress-related biomarkers in streptozotocin–nicotinamide-induced diabetic rats. The study may provide preliminary experimental evidence regarding the potential protective effects of piperonal against diabetes-associated tissue injury and oxidative stress.

MATERIALS AND METHODS

Materials

Equipment and Chemicals

The major equipment used in the study included a blood glucose meter, blood glucose test strips, weighing balance, syringes and needles, animal cages, a microtome, glass slides, a light microscope, and a spectrophotometer. Standard laboratory equipment and protective materials, including gloves, were also used.

The chemicals and reagents used in the study included dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), sodium citrate dihydrate, citric acid monohydrate, sodium hydroxide (NaOH), normal saline (0.9% NaCl), glucose, standard laboratory feed, distilled water, piperonal (3,4-methylenedioxybenzaldehyde), streptozotocin (STZ), nicotinamide (NAD), hematoxylin and eosin (H&E) stains, ethanol, potassium phosphate buffer, ethylenediaminetetraacetic acid (EDTA), thiobarbituric acid (TBA), hydrogen peroxide (H₂O₂), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and sulfosalicylic acid.

Preparation of Sodium Citrate Buffer

Sodium citrate buffer was prepared by dissolving 0.34 g of sodium citrate dihydrate and 0.26 g of citric acid monohydrate in distilled water. The final volume was adjusted to 50 mL, and the pH was adjusted to 4.5 using NaOH or HCl. The freshly prepared buffer was used for the preparation of streptozotocin solution.

Preparation of 10% Glucose Solution

A 10% (w/v) glucose solution was prepared by dissolving 100 g of glucose in distilled water and adjusting the final volume to 1000 mL.

Study Site

The study was conducted at the Animal Research Laboratory of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan.

Experimental Animals and Study Design

Forty-two male albino rats aged 10–12 weeks were obtained from the University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, and transferred to the animal house of IMBB, The University of Lahore. The animals were housed in steel cages under controlled laboratory conditions with a 12:12-hour light/dark cycle and an ambient temperature of approximately 22°C. Standard pellet diet and drinking water were provided ad libitum. The animals were acclimatized for two weeks before the commencement of the experiment.

The animals were randomly allocated into seven experimental groups, with six animals in each group. Before diabetes induction, the animals were fasted for 16 hours with free access to water.

Inclusion and Exclusion Criteria

Male albino rats aged 10–12 weeks and weighing 150–180 g were included in the study. Only clinically healthy animals were selected.

Animals showing signs of illness or poor general health, weighing less than 150 g, or not meeting the predefined age and body-weight criteria were excluded.

Table 1. Inclusion and Exclusion Criteria for Experimental Animals

Inclusion criteria	Exclusion criteria
Male albino rats aged 10–12 weeks	Rats showing signs of illness or poor general health
Body weight of 150–180 g	Rats weighing <150 g
Clinically healthy animals	Animals not meeting the predefined age or body-weight criteria

Induction of Experimental Diabetes

Experimental diabetes was induced using a nicotinamide–streptozotocin (NAD–STZ) protocol. Nicotinamide (230 mg/kg body weight) was administered intraperitoneally in 0.9% normal saline. Fifteen minutes later, freshly prepared streptozotocin (STZ) was administered intraperitoneally at a dose of 65 mg/kg body weight after dissolution in 0.1 M sodium citrate buffer (pH 4.5).

Blood glucose levels were measured 72 hours after STZ administration and again on day 7 using a blood glucose meter. Rats with blood glucose levels >250 mg/dL were considered diabetic and were included in the diabetic groups.

Diabetes-associated renal and hepatic alterations were subsequently evaluated using

histopathological examination and oxidative-stress-related biochemical parameters.

Experimental Treatment

Following confirmation of diabetes, piperonal and glibenclamide were administered orally by intragastric gavage according to the experimental group and treatment schedule. Piperonal was dissolved in DMSO before administration.

The early-treatment group received piperonal from day 1 through day 45, whereas the delayed-treatment groups received piperonal from day 15 through day 45. Glibenclamide was administered at a dose of 5 mg/kg/day. Each experimental group consisted of six animals.

Table 2. Experimental Groups and Treatment Protocol

Group	Condition	Treatment
G1 (NC)	Non-diabetic control	No diabetes induction and no experimental treatment
G2 (ST)	Diabetic	Glibenclamide 5 mg/kg/day for 45 days
G3 (PP)	Diabetic	Piperonal 40 mg/kg/day from day 1 to day 45
G4 (DC)	Diabetic control	Control/vehicle treatment
G5 (T1)	Diabetic	Piperonal 30 mg/kg/day from day 15 to day 45
G6 (T2)	Diabetic	Piperonal 40 mg/kg/day from day 15 to day 45
G7 (T3)	Diabetic	Piperonal 50 mg/kg/day from day 15 to day 45

Tissue Collection and Processing

At the end of the experimental period, the animals were euthanized according to the approved institutional protocol. The kidneys and liver were subsequently collected for histopathological and biochemical analyses.

Each organ was divided into appropriate portions. Tissue samples intended for histopathological examination were fixed in 10% neutral buffered formalin, whereas samples intended for biochemical analysis were processed for tissue homogenization and stored at –20°C until analysis.

Histopathological Examination

Kidney and liver tissues were processed using standard paraffin-embedding techniques. Tissue samples were fixed in 10% neutral buffered formalin for approximately 24 hours, washed, dehydrated through graded concentrations of ethanol, cleared with xylene, and embedded in paraffin wax.

Paraffin blocks were sectioned using a semi-automatic microtome at approximately 5 µm thickness. The sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) according to standard histological procedures.

The stained sections were examined under a light microscope, and representative photomicrographs were captured using an Optika imaging system.

Oxidative Stress and Antioxidant Assays

Kidney and liver tissues were homogenized in cold potassium phosphate buffer (50 mM, pH 7.5) containing 1 mM EDTA. The homogenates were centrifuged at 4,000 rpm for 15 minutes at 4°C. The resulting supernatants were collected and stored at –20°C until biochemical analysis.

Malondialdehyde (MDA)

Malondialdehyde (MDA), a marker of lipid peroxidation, was determined using the thiobarbituric acid (TBA) method described. The reaction product was measured spectrophotometrically at 532 nm, and MDA levels were calculated using an appropriate standard curve.

Catalase (CAT) Activity

Catalase (CAT) activity was determined by measuring the decomposition of hydrogen peroxide (H₂O₂) spectrophotometrically at 240 nm according to the method of Chance and

Maehly. The assay mixture contained phosphate buffer and H_2O_2 as the substrate, and changes in absorbance were recorded over the defined reaction period.

Catalase activity was calculated according to the assay protocol and expressed in the appropriate unit.

Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined based on its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Tissue homogenates were deproteinized using 4% sulfosalicylic acid and centrifuged at $6,000 \times g$ for 15 minutes at $4^\circ C$.

The resulting supernatant was reacted with phosphate-buffered saline and DTNB, and absorbance was measured at 412 nm. GSH concentration was calculated using a standard GSH calibration curve according to the method of Jollow et al. (1974).

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics. When the overall ANOVA was statistically significant, Tukey's post-hoc test was used for pairwise comparisons. A p-value ≤ 0.05 was considered statistically significant.

Ethical Considerations and Reporting

The study protocol was reviewed and approved by the Departmental Bioethical, Biosafety, and Biosecurity Committee (BBBC), Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, under Ref. No. CRIMM/23/Research/33, dated 22 August 2023.

All experimental procedures were conducted in accordance with the approved institutional animal-care and experimental protocol and reported with reference to the ARRIVE 2.0 guidelines for animal research.

RESULTS

Histopathological Observations

Histopathological examination of kidney and liver tissues revealed distinct morphological differences among the experimental groups. Representative hematoxylin and eosin (H&E)-stained sections are presented in Figures 1 and 2.

Histopathology of Kidney Tissue

Kidney sections from the normal control group (G1) showed preserved renal architecture, with normal-appearing glomeruli, Bowman's spaces, renal tubules, and predominantly viable tubular cells (Figure 1A).

The standard-treatment group (G2) demonstrated relatively preserved renal architecture, with largely maintained glomerular and tubular structures. Mild lipofuscin deposition was observed (Figure 1C). The early piperonal group (G3), which received piperonal at 40 mg/kg/day from day 1, showed relatively preserved renal architecture. Glomeruli and Bowman's spaces were generally maintained, with occasional inflammatory/eosinophilic cells and minimal tubular atrophy.

In contrast, the diabetic control group (G4) exhibited marked renal morphological alterations, including basement membrane thickening, glomerular changes, widened Bowman's spaces, tubular atrophy, and cytoplasmic vacuolation. Kimmelstiel-Wilson-like lesions were also observed (Figure 1B).

The delayed piperonal groups (G5-G7), which received piperonal at doses of 30, 40, and 50 mg/kg/day, respectively, from day 15 to day 45, continued to exhibit pathological changes, including widened Bowman's spaces, inflammatory-cell infiltration, and tubular atrophy (Figure 1D). These abnormalities appeared more pronounced in G7 (50 mg/kg/day) than in G5 and G6.

Overall, the histopathological findings showed relatively preserved renal architecture in G1, G2, and G3, whereas more pronounced morphological abnormalities were observed in G4 and the delayed piperonal groups.

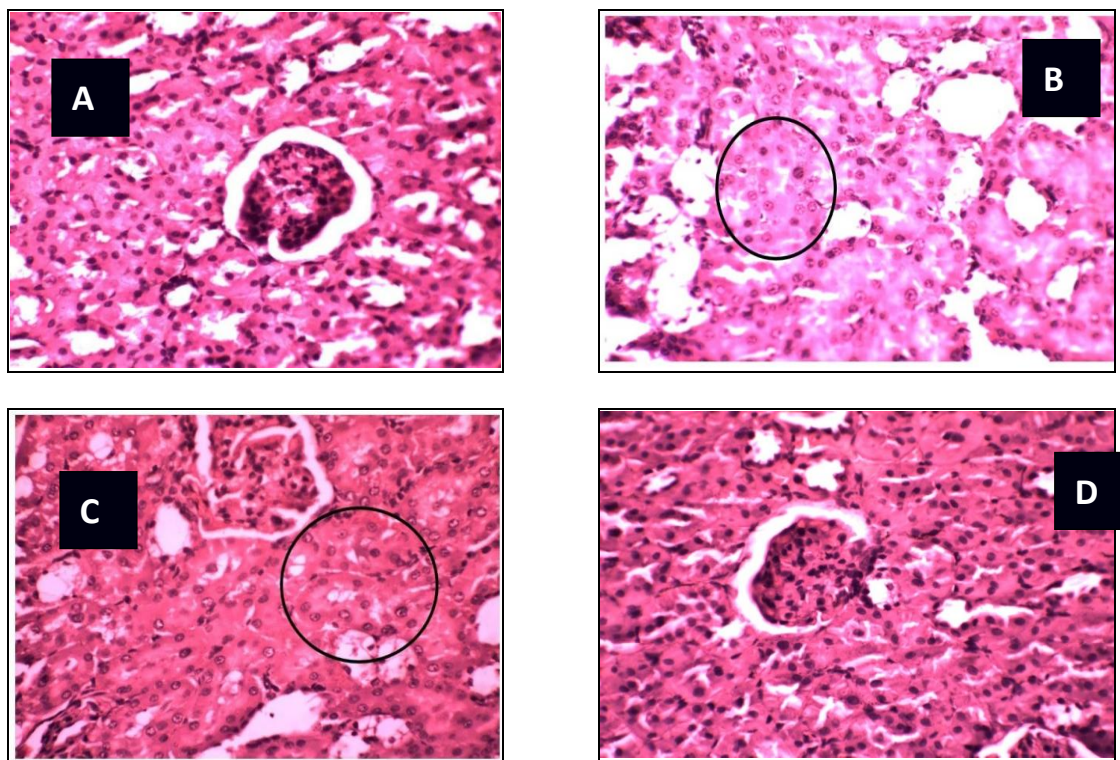


Figure 1. Representative H&E-Stained Kidney Sections from the Experimental Groups. (A) G1, Normal Control Showing Preserved Glomeruli And Renal Tubules; (B) G4, Diabetic Control Showing Glomerular Alterations, Kimmelstiel-Wilson-Like Lesions, Tubular Atrophy, and Widened Bowman's Spaces; (C) G2, Standard-Treatment Group Showing Relatively Preserved Renal Architecture; And (D) G5-G7, Delayed Piperonal Groups Showing Widened Bowman's Spaces, Inflammatory-Cell Infiltration, And Tubular Atrophy

Histopathology of Liver Tissue

Liver sections from the normal control group (G1) showed preserved hepatic architecture, with normal-appearing hepatocytes, central veins, and sinusoidal spaces (Figure 2A).

The diabetic control group (G4) demonstrated hepatic morphological abnormalities, including central-vein dilation, inflammatory-cell infiltration, and nuclear alterations such as karyolysis (Figure 2B).

The standard-treatment group (G2) showed relatively preserved hepatic architecture, with predominantly normal hepatocytes and mild lipofuscin deposition (Figure 2C).

The early piperonal group (G3), which received piperonal at 40 mg/kg/day from day 1, demonstrated comparatively preserved

hepatic architecture, although mild sinusoidal widening and a small number of degenerative or non-viable cells were observed (Figure 2E). The delayed piperonal groups (G5-G7) exhibited increased sinusoidal spaces, eosinophilic cytoplasmic changes, and nuclear abnormalities, including karyorrhexis (Figure 2D). These changes appeared more prominent in G7 (50 mg/kg/day), whereas G5 and G6 showed comparatively less pronounced alterations.

Overall, the liver findings demonstrated relatively preserved architecture in G1, G2, and G3, while more evident morphological alterations were observed in G4 and the delayed piperonal groups.

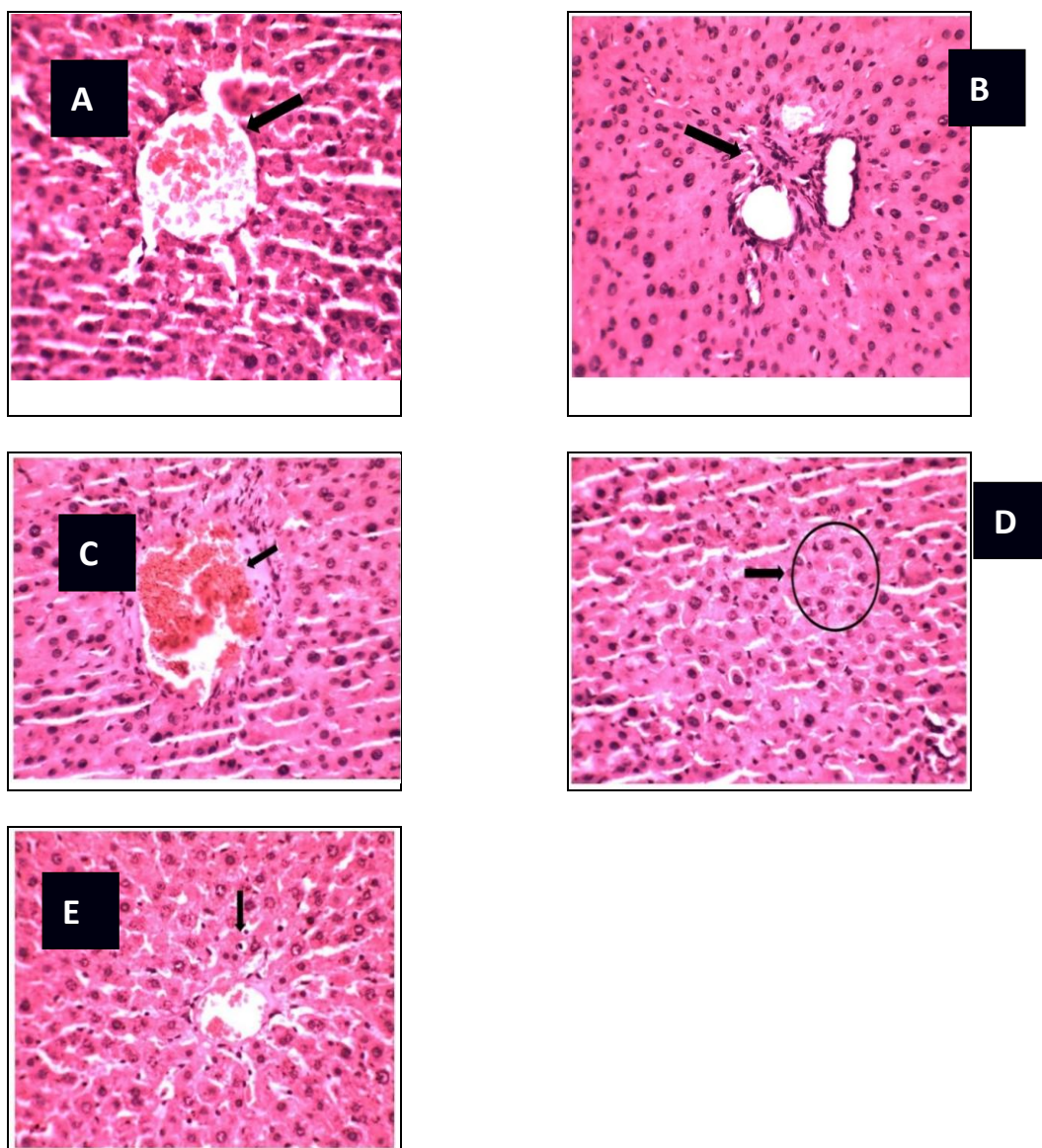


Figure 2. Representative H&E-Stained Liver Sections From the Experimental Groups. (A) G1, Normal Control Showing Preserved Hepatocytes, Central Vein, and Sinusoidal Spaces; (B) G4, Diabetic Control Showing Central-Vein Dilation, Inflammatory-Cell Infiltration, and Karyolysis; (C) G2, Standard-Treatment Group Showing Relatively Preserved Hepatic Architecture; (D) G5-G7, Delayed Piperonal Groups Showing Sinusoidal Widening, Eosinophilic Cytoplasmic Changes, And Nuclear Abnormalities; and (E) G3, Early Piperonal Group Showing Relatively Preserved Hepatic Architecture with Mild Sinusoidal Widening

Oxidative Stress and Antioxidant Markers

The concentrations of MDA, catalase, and reduced glutathione (GSH) in kidney and liver tissues are summarized in Table 5. The corresponding group-wise patterns are illustrated in Figures 3-5.

Malondialdehyde

MDA concentrations differed among the experimental groups in both kidney and liver tissues (Table 2; Figure 3). In kidney tissue, the normal control group (G1) showed the lowest MDA concentration (2.26 ± 0.39 nmol), whereas the diabetic control group (G4)

showed a substantially higher concentration (22.77 ± 0.39 nmol). The standard-treatment group (G2) and early piperonal group (G3) showed lower MDA concentrations than G4, with values of 13.03 ± 1.45 and 13.70 ± 0.27 nmol, respectively.

Among the delayed piperonal groups, kidney MDA concentrations increased from 24.06 ± 1.92 nmol in G5 to 26.11 ± 0.98 nmol in G6 and 29.80 ± 0.39 nmol in G7. The highest kidney MDA concentration was observed in G7. In liver tissue, MDA concentration was lowest in G1 (1.87 ± 0.65 nmol). G2 and G3 showed comparable concentrations of 10.20 ± 0.91

and 10.21 ± 1.09 nmol, respectively, whereas higher concentrations were observed in G4 and the delayed piperonal groups. The highest liver MDA concentration was recorded in G7 (20.26 ± 0.33 nmol).

The reported post-hoc analysis indicated significant differences among several group

comparisons. G2 and G3 did not differ significantly from each other for either kidney or liver MDA, whereas G7 differed significantly from the groups identified in the reported pairwise comparisons.

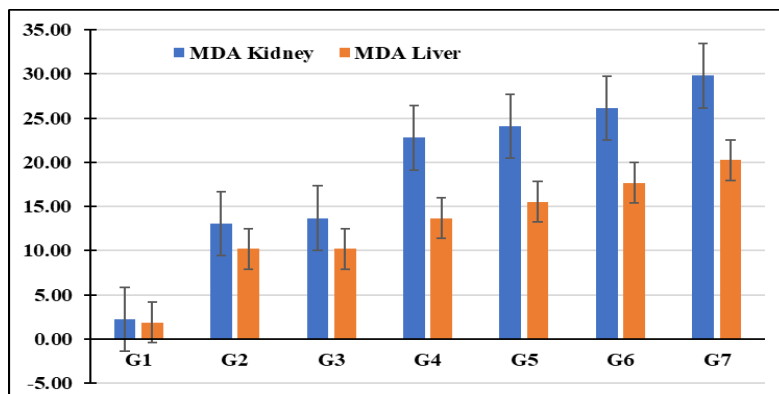


Figure 3. MDA Concentrations in Kidney and Liver Tissues across the Experimental Groups. Values Are Presented As Mean $\pm$ SD

Catalase

Catalase concentrations also differed among the experimental groups in both kidney and liver tissues (Table 2; Figure 4). Kidney catalase was 2.70 ± 0.37 U/mol in G1 and increased to 3.54 ± 0.67 and 3.76 ± 0.05 U/mol in G2 and G3, respectively. Higher values were observed in G4 and the delayed piperonal groups, with the highest kidney catalase concentration recorded in G7 (9.00 ± 0.01 U/mol).

The reported post-hoc analysis grouped G1, G2, and G3 within the same subgroup, whereas G4, G5, and G6 formed another

subgroup. G7 represented a separate subgroup and showed the highest kidney catalase concentration.

In liver tissue, catalase concentrations ranged from 2.09 ± 0.11 U/mol in G1 to 6.49 ± 0.16 U/mol in G7. G2 and G3 showed relatively lower concentrations of 2.37 ± 0.05 and 3.11 ± 0.05 U/mol, respectively, while higher values were observed in G4 and the delayed piperonal groups. The reported post-hoc analysis indicated significant differences among several groups, although G5 and G7 did not differ significantly.

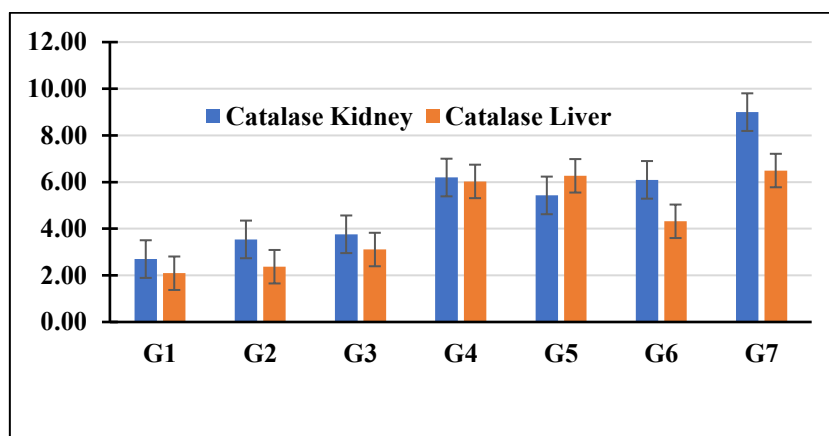


Figure 4. Catalase Concentrations in Kidney and Liver Tissues across the Experimental Groups. Values Are Presented As Mean $\pm$ SD

Reduced Glutathione

Reduced glutathione (GSH) concentrations differed among the experimental groups in

both kidney and liver tissues (Table 2; Figure 5).

In kidney tissue, G1 showed the lowest GSH concentration (0.21 ± 0.002 U/L), followed by G2 (0.65 ± 0.057 U/L) and G3 (0.68 ± 0.001 U/L). G4 showed a concentration of 1.14 ± 0.006 U/L. The delayed piperonal groups demonstrated further increases, with concentrations of 1.16 ± 0.016 , 1.31 ± 0.012 , and 1.49 ± 0.020 U/L in G5, G6, and G7, respectively.

A similar pattern was observed in liver tissue. GSH increased from 0.48 ± 0.003 U/L in G1 to 0.79 ± 0.068 U/L in G2 and 0.88 ± 0.009 U/L in G3. Higher concentrations were observed in G4 and the delayed piperonal groups, reaching the highest value in G7 (1.66 ± 0.041 U/L). The reported post-hoc analysis indicated differences among several experimental groups, with the specific subgroup patterns differing between kidney and liver tissues.

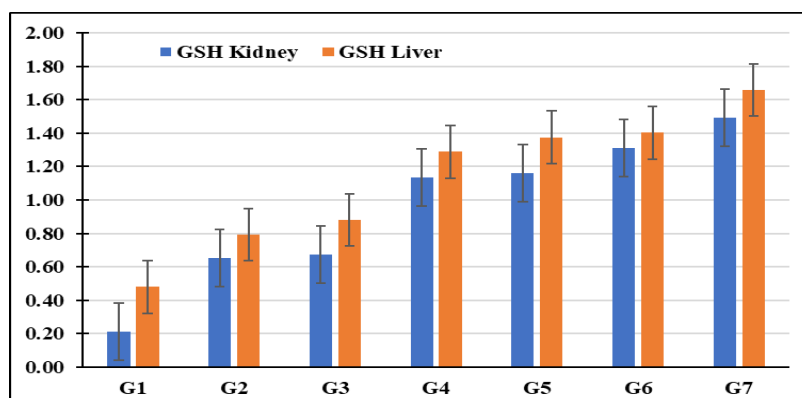


Figure 5. Reduced Glutathione (GSH) Concentrations in Kidney and Liver Tissues Across The Experimental Groups. Values Are Presented As Mean $\pm$ SD

Summary of Biochemical Findings

Overall, MDA concentrations were higher in the diabetic control and delayed piperonal groups than in the normal control group, with the highest values observed in G7. Catalase and GSH concentrations also increased across several diabetic and delayed piperonal groups (Table 3; Figures 3-5).

The early piperonal group (G3) showed lower MDA concentrations and comparatively preserved tissue architecture relative to the diabetic control and delayed piperonal groups. In contrast, the delayed piperonal groups demonstrated progressively higher MDA concentrations across increasing doses. The biochemical findings therefore showed alterations in oxidative-stress and antioxidant-related markers across the experimental groups rather than a consistent dose-dependent reduction in oxidative stress.

Correlation between MDA and Antioxidant-Related Markers

Correlation analysis demonstrated strong positive associations between MDA and antioxidant-related markers in both kidney and liver tissues (Table 2). Kidney MDA showed correlation coefficients of 0.91 with kidney catalase, 0.87 with liver catalase, 0.99 with kidney GSH, and 0.99 with liver GSH. Liver MDA showed correlation coefficients of 0.89 with kidney catalase, 0.81 with liver catalase, 0.98 with kidney GSH, and 0.97 with liver GSH. These correlation coefficients indicated strong positive relationships between MDA and the measured antioxidant-related markers in the available dataset. However, statistical significance was not inferred from the correlation coefficients alone because corresponding p-values and confidence intervals were not available.

Table 3. Correlations between MDA and Antioxidant-Related Markers in Kidney and Liver Tissues

Oxidative-stress marker	Catalase Kidney	Catalase Liver	GSH Kidney	GSH Liver
MDA Kidney	0.91	0.87	0.99	0.99
MDA Liver	0.89	0.81	0.98	0.97

Values represent correlation coefficients (r). Statistical significance should only be reported when corresponding p-values and/or confidence intervals are available.

Statistical Considerations

The biochemical analyses were based on unequal analytical sample sizes across groups (n = 2-3 per group). Therefore, the statistical

findings should be considered exploratory and interpreted cautiously. The small and unequal sample sizes limit the precision of between-group comparisons and correlation analyses.

Accordingly, the observed statistical differences should be regarded as preliminary findings and interpreted within the context of the limited analytical sample size.

Table 4. Effect of Experimental Treatments on Oxidative-Stress and Antioxidant-Related Markers in Kidney and Liver Tissues

Groups (N)	DN	MDA Kidney Nmol	MDA Liver Nmol	Cat-Kidney u/mol	Cat-Liver u/mol	GSH-Kidney U/L	GSH-Liver U/L
G1-NC (3)	–	2.26 ^a ± 0.39	1.87 ^a ± 0.65	2.70 ^a ± 0.37	2.09 ^a ± 0.11	0.21 ^a ± 0.002	0.48 ^a ± 0.003
G2-ST (2)	+	13.03 ^b ± 1.45	10.20 ^b ± 0.91	3.54 ^a ± 0.67	2.37 ^b ± 0.05	0.65 ^b ± 0.057	0.79 ^b ± 0.068
G3-PP (3)	+	13.70 ^b ± 0.27	10.21 ^b ± 1.09	3.76 ^a ± 0.05	3.11 ^c ± 0.05	0.68 ^b ± 0.001	0.88 ^b ± 0.009
G4-DC (3)	+	22.77 ^c ± 0.39	13.70 ^c ± 0.37	6.19 ^b ± 0.18	6.03 ^e ± 0.11	1.14 ^c ± 0.006	1.29 ^c ± 0.002
G5-T1 (3)	+	24.06 ^{cd} ± 1.92	15.51 ^d ± 0.74	5.43 ^b ± 1.06	6.27 ^f ± 0.49	1.16 ^c ± 0.016	1.38 ^{cd} ± 0.005
G6-T2 (3)	+	26.11 ^d ± 0.98	17.66 ^e ± 0.98	6.09 ^b ± 0.16	4.32 ^d ± 0.02	1.31 ^d ± 0.012	1.40 ^d ± 0.030
G7-T3 (3)	+	29.80 ^e ± 0.39	20.26 ^f ± 0.33	9.00 ^c ± 0.01	6.49 ^f ± 0.16	1.49 ^e ± 0.020	1.66 ^e ± 0.041

Values are presented as mean ± SD. Different superscript letters indicate significant differences according to the reported post-hoc analysis ($p < 0.05$). NC, normal control; ST, standard treatment; PP, early/preventive piperonal treatment; DC, diabetic control; T1, piperonal 30 mg/kg/day; T2, piperonal 40 mg/kg/day; T3, piperonal 50 mg/kg/day. G2 received glibenclamide, G3 received piperonal from day 1, and G5-G7 received piperonal from day 15 to day 45.

DISCUSSION

Diabetic nephropathy involves oxidative stress, inflammation, and progressive tissue injury. Hyperglycemia can increase oxidative damage and disturb antioxidant defenses (20). In the present study, streptozotocin–nicotinamide-induced diabetes produced marked renal and hepatic histopathological changes and altered MDA, catalase, and GSH levels. The response to piperonal varied according to treatment timing and dose.

Histopathological Findings

The normal control group showed preserved renal and hepatic architecture, whereas the diabetic control group demonstrated glomerular alterations, widened Bowman's spaces, tubular atrophy, inflammatory-cell infiltration, and hepatic abnormalities. These findings are consistent with diabetes-associated oxidative and tissue injury (20).

The standard-treatment group and early piperonal group (G3) showed comparatively preserved tissue architecture. In contrast, delayed piperonal groups (G5–G7) continued to show pathological changes, with more prominent abnormalities in G7. The relatively favorable morphology in G3 may suggest an association between early piperonal administration and reduced tissue injury. However, histopathology was descriptive and was not based on blinded quantitative scoring. Piperonal has been investigated for diverse biological properties, but evidence specifically addressing its effects in diabetic nephropathy remains limited (21). Therefore, the present histopathological findings should be considered preliminary.

Oxidative Stress and Antioxidant Findings

MDA was substantially higher in the diabetic control group than in the normal control group, indicating increased lipid peroxidation (20). Delayed piperonal treatment did not reduce MDA. Instead, kidney MDA increased from 24.06 ± 1.92 nmol in G5 to 29.80 ± 0.39 nmol in G7, while liver MDA increased from 15.51 ± 0.74 to 20.26 ± 0.33 nmol.

In contrast, G3 showed lower MDA than the diabetic control and delayed piperonal groups, together with comparatively preserved tissue architecture. This may indicate a potential association with early administration, although a preventive antioxidant effect cannot be confirmed from the present data.

Catalase and GSH also increased in several diabetic and delayed-treatment groups, with the highest values generally observed in G7. Because these increases occurred alongside elevated MDA, they may represent compensatory antioxidant responses rather than improved tissue protection.

The strong positive correlations between MDA and antioxidant-related markers should also be interpreted cautiously because biochemical analyses included only 2–3 observations per group and corresponding significance values were unavailable.

Possible Mechanisms and Comparison with Previous Research

Nrf2-related antioxidant signaling has been investigated as an important pathway in diabetic renal injury (20). Natural compounds and polyphenol-based interventions have also been studied for their effects on oxidative stress and inflammation in experimental diabetic nephropathy (25). Resveratrol has shown potential renoprotective effects in experimental renal disorders, including diabetic nephropathy (26), while dietary polyphenols have been investigated in type 2 diabetes (27).

Piperonal itself has documented biological properties (21); however, the present study did not evaluate Nrf2, NF- κ B, inflammation, apoptosis, or fibrosis. Therefore, these mechanisms remain hypothetical. Evidence regarding piperine (22,23) or piperonal biosynthesis (24) should not be interpreted as direct evidence of piperonal efficacy in diabetic nephropathy.

Limitations

The main limitation was the small analytical sample size, with only 2–3 observations per group for biochemical analyses. The study also

included limited oxidative-stress markers, lacked comprehensive renal-function assessment, and used descriptive rather than quantitative and blinded histopathological assessment. Molecular mechanisms were not investigated, and only male rats and one diabetes model were used.

CONCLUSION

Piperonal showed timing- and dose-related differences in histopathological and oxidative-stress-related outcomes. Early administration of 40 mg/kg/day was associated with comparatively preserved tissue architecture and lower MDA levels, whereas delayed treatment showed no consistent biochemical improvement. These preliminary findings warrant confirmation in adequately powered studies.

Implications for Future Research

Future studies should use adequately powered and balanced groups, blinded quantitative histopathological scoring, comprehensive renal-function assessment, and additional oxidative, inflammatory, and molecular biomarkers. Dose optimization, treatment timing, and safety should also be investigated.

REFERENCES

1. Sun T, Guo Y, Su Y, Shan S, Qian W, Zhang F, Li M, Zhang Z. Molecular mechanisms of diabetic nephropathy: a narrative review. *Cell Biology International*. 2024 Sep;48(9):1240-53.
2. Darenskaya M, Kolesnikov S, Semenova N, Kolesnikova L. Diabetic nephropathy: significance of determining oxidative stress and opportunities for antioxidant therapies. *International Journal of Molecular Sciences*. 2023 Aug 3;24(15):12378.
3. Jin Q, Liu T, Qiao Y, Liu D, Yang L, Mao H, Ma F, Wang Y, Peng L, Zhan Y. Oxidative stress and inflammation in diabetic nephropathy: role of polyphenols. *Frontiers in immunology*. 2023 Jul 21;14:1185317.
4. Hernandez LF, Eguchi N, Whaley D, Alexander M, Tantisattamo E, Ichii H. Anti-oxidative therapy in diabetic nephropathy. *Frontiers in Bioscience-Scholar*. 2022 May 9;14(2):14.
5. Østergaard JA, Cooper ME, Jandeleit-Dahm KA. Targeting oxidative stress and anti-oxidant defence in diabetic kidney disease. *Journal of nephrology*. 2020 Oct;33(5):917-29.

6. Vodošek Hojs N, Bevc S, Ekart R, Hojs R. Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy. *Antioxidants*. 2020 Sep 27;9(10):925.
7. Afolabi OB, Akinola AG, Raji AK, Obafemi OT, Olasehinde OR, Olorunlagba AO, Babatunde OE, Obafemi BA, Omotuyi OI, Oloyede OI, Lebelo SL. Renoprotective effect of polyphenolic-rich extract of *Phyllanthus niruri* in streptozotocin-induced diabetic nephropathy via moderation of oxidative stress and inhibition of kidney injury. *Journal of Molecular Histology*. 2026 Feb;57(1):13.
8. Yin Z, Cheng H, Liu Y, Li X, Yu X, Zhang M, Li M, Zou Y, Shi Y. Oxidative Stress in Diabetic Cardiomyopathy: Molecular Mechanisms, Current Treatment and Therapeutic Potential of Plant Antioxidants. *Antioxidants*. 2026 May 6;15(5):587.
9. Lee A. Mouse models of diabetic complications: dissecting molecular mechanisms of disease progression. *Frontiers in Bioscience-Landmark*. 2026 Mar 18;31(3).
10. Bondi CD, Hartman HL, Tan RJ. NRF2 in kidney physiology and disease. *Physiological Reports*. 2024 Mar;12(5):e15961.
11. Ng C, Kim M, Yanti, Kwak MK. Oxidative stress and NRF2 signaling in kidney injury. *Toxicological Research*. 2025 Mar;41(2):131-47.
12. Aboolian A, Urner S, Roden M, Jha JC, Jandeleit-Dahm K. Diabetic kidney disease: from pathogenesis to novel treatment possibilities. *From Obesity to Diabetes*. 2022 Mar 23:269-307.
13. Martinez Leon V, Hilburg R, Susztak K. Mechanisms of diabetic kidney disease and established and emerging treatments. *Nature Reviews Endocrinology*. 2026 Jan;22(1):21-35.
14. Maruno S, Tanaka T, Nangaku M. Exploring molecular targets in diabetic kidney disease. *Kidney Research and Clinical Practice*. 2022 Mar 15;41(Suppl 2):S33.
15. Winiarska A, Knysak M, Nabrdalik K, Gumprecht J, Stompor T. Inflammation and oxidative stress in diabetic kidney disease: the targets for SGLT2 inhibitors and GLP-1 receptor agonists. *International Journal of Molecular Sciences*. 2021 Oct 6;22(19):10822.
16. Zhou K, Zi X, Song J, Zhao Q, Liu J, Bao H, Li L. Molecular mechanistic pathways targeted by natural compounds in the prevention and treatment of diabetic kidney disease. *Molecules*. 2022 Sep 21;27(19):6221.
17. Sopian S, Budin SB, Taib IS, Mariappan V, Zainalabidin S, Chin KY. Role of polyphenol in regulating oxidative stress, inflammation, fibrosis, and apoptosis in diabetic nephropathy. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*. 2022 Apr 1;22(5):453-70.
18. Parsa H, Ghasemi F, Ranjbar K, Komaki A. The Effect of Co-Administration of *Portulaca Oleracea* and *Plantago Psyllium* Plus Submaximal Swimming Training on Memory Deficit in Streptozotocin/Nicotinamide-Induced Type 2 Diabetic Rats.
19. Antar SA, Abdo W, Taha RS, Farage AE, El-Moselhy LE, Amer ME, Monsef AS, Hamid AM, Kamel EM, Ahmeda AF, Mahmoud AM. Telmisartan attenuates diabetic nephropathy by mitigating oxidative stress and inflammation, and upregulating Nrf2/HO-1 signaling in diabetic rats. *Life sciences*. 2022 Feb 15;291:120260.
20. Kaur P, Singh T, Jena L, Gupta T, Rana MK, Singh S, Singh R, Kumar P, Munshi A. Dapagliflozin ameliorate type-2 diabetes associated neuropathy via regulation of IGF-1R signaling. *Journal of Neuroimmune Pharmacology*. 2025 Apr 3;20(1):32.
21. Ma L, Wu F, Shao Q, Chen G, Xu L, Lu F. Baicalin alleviates oxidative stress and inflammation in diabetic nephropathy via Nrf2 and MAPK signaling pathway. *Drug design, development and therapy*. 2021 Jul 21:3207-21.
22. Akash J, Dushyant C, Jasmine C. Piperonal: the journey so far. *Mini Reviews in Medicinal Chemistry*. 2020 Nov 1;20(18):1846-56.
23. Haq IU, Imran M, Nadeem M, Tufail T, Gondal TA, Mubarak MS. Piperine: A review of its biological effects. *Phytotherapy research*. 2021 Feb;35(2):680-700.
24. Li S, Lv M, Xu H. Overview of piperine: Bioactivities, total synthesis, structural modification, and structure-activity

- relationships. *Mini Reviews in Medicinal Chemistry*. 2023 May 1;23(8):917-40.
25. Jin Z, Ro DK, Kim SU, Kwon M. Piperonal synthase from black pepper (*Piper nigrum*) synthesizes a phenolic aroma compound, piperonal, as a CoA-independent catalysis. *Applied biological chemistry*. 2022 Dec;65(1):20.
 26. Ghavidel F, Amiri H, Tabrizi MH, Alidadi S, Hosseini H, Sahebkar A. The combinational effect of inulin and resveratrol on the oxidative stress and inflammation level in a rat model of diabetic nephropathy. *Current developments in nutrition*. 2024 Jan 1;8(1):102059.
 27. AlRamadneh TN, Abdulsahib WK, Ismael SH, Roopashree R, Mohanty B, Nandhini SU, Arora V, Chauhan AS, Farhood B. Renoprotective properties of resveratrol in the treatment of renal fibrosis, renal cell carcinoma, diabetic nephropathy and nephrotoxicity: a review of current evidence based on underlying cellular mechanisms. *Toxicology Mechanisms and Methods*. 2026 Mar 24;36(3):354-75.
 28. Martiniakova M, Sarocka A, Penzes N, Biro R, Kovacova V, Mondockova V, Sevcikova A, Ciernikova S, Omelka R. Protective role of dietary polyphenols in the management and treatment of type 2 diabetes mellitus. *Nutrients*. 2025 Jan 13;17(2):275.