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Original Research Article

Dorema aucheri extract for reducing oxidative stress and kidney injuries in diabetic rats

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Abstract

Objective: Diabetic hyperglycemia causes oxidative stress, contributing to chronic kidney disease (CKD). This study evaluated the effects of ethanolic *Dorema aucheri* extract from aerial parts on oxidative stress, inflammation, and kidney injury in diabetic rats.

Materials and Methods: Thirty male rats were randomly assigned to five groups (n=6): non-diabetic control, diabetic control, diabetic + metformin (500 mg/kg/day), and diabetic + *D. aucheri* extract (250 or 500 mg/kg/day). Treatments were given orally for 28 days. Serum and kidney samples were analyzed for fasting blood glucose (FBS), renal function [blood urea nitrogen (BUN), creatinine (Cr)], inflammatory cytokines [interleukin-6 (IL-6), interleukin-1β (IL-1β)], oxidative stress [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)], mRNA expression [kidney injury molecule-1 (*KIM-1*), neutrophil gelatinase-associated lipocalin (*NGAL*), B-cell lymphoma 2 (*BCL-2*), Caspase-9 (*CASP-9*)], stereology, and histopathology.

Results: Treatment with *D. aucheri* hydroalcoholic extract significantly improved metabolic, inflammatory, oxidative, and renal outcomes in diabetic rats. Specifically, both 250 mg/kg and 500 mg/kg doses reduced blood glucose ($p<0.05$), BUN ($p<0.05$ and $p<0.01$) and Cr ($p<0.01$ for 500 mg/kg), IL-6 ($p<0.05$), and IL-1β ($p<0.05$ and $p<0.001$), respectively. MDA decreased ($p<0.001$ and $p<0.05$), SOD, CAT, and GPx increased ($p<0.01$). Renal *KIM-1* and *NGAL* decreased ($p<0.01$), *CASP-9* decreased ($p<0.01$ and $p<0.05$), and *BCL-2* increased ($p<0.05$), respectively. Histopathology confirmed reduced kidney damage correlating with biochemical and gene expression improvements.

Conclusion: *Dorema aucheri* extract has the potential to modulate antioxidant and apoptotic pathways and reduce kidney injury in diabetic rats. Further studies are needed to confirm its therapeutic efficacy.

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Introduction

Diabetes and hypertension are the leading causes of chronic kidney disease (CKD) (Li et al. 2021), a progressive condition characterized by structural and functional kidney abnormalities, high morbidity, and mortality. Many CKD patients advance to end-stage renal disease (ESRD), requiring dialysis or transplantation (Gheith et al. 2016). Antihypertensive drugs remain the primary treatment for diabetic nephropathy, though they only slow progression to ESRD (Mogensen et al. 2000). Key contributors to CKD and diabetic nephropathy include diabetes mellitus, hypertension, oxidative stress, and inflammation (Kishi et al. 2024; Tavafi 2016; Wang and Zhang 2024). Oxidative stress in diabetics results from increased reactive oxygen species (ROS) and reduced antioxidant capacity, exacerbating kidney damage (Bayat et al. 2020). Diabetes generates free radicals via glucose oxidation, non-enzymatic protein glycation, and glycosylated protein oxidation, disrupting the antioxidant-free radical balance. Antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase mitigate oxidative stress, while reducing glycosylated hemoglobin, malonaldehyde, and carbonyl active groups alleviates damage (Hsueh and Law 1998).

Oxidative stress stimulates the production of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), which collectively contribute to a decrease in the expression of the anti-apoptotic protein BCL2. This shift promotes apoptosis in target cells by exacerbating insulin resistance and facilitating the release of cytochrome c from the mitochondria. As a key electron carrier in the mitochondrial electron transport chain, cytochrome c plays a pivotal role in modulating ROS levels. Under conditions of cellular stress, cytochrome c translocates into the cytosol where it binds to apoptotic protease activating factor-1 (Apaf-1), initiating the formation of the apoptosome. This complex activates the initiator caspase, caspase-9, which in turn triggers the downstream activation of effector caspases, particularly caspase-3 and caspase-7, culminating in programmed cell death (apoptosis)(Al Dubayee et al. 2021; King et al. 2023; Zhou et al. 2024).

Markers such as kidney injury molecule 1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) facilitate early CKD detection (Nickolas et al. 2008). KIM-1, a type I transmembrane glycoprotein, is upregulated in the proximal tubule following kidney injury (Guo et al. 2012). NGAL, part of the lipocalin superfamily, is minimally expressed in normal tissues but overexpressed in renal injury and insulin resistance (Yan et al. 2007).

Natural antioxidants, particularly medicinal plants, may protect against oxidative damage in diabetic patients (Shrilatha and Muralidhara 2007). One such plant, *Dorema aucheri*, from the Umbelliferae family, flourishes in Yasuj, Iran. Rich in flavonoids, it exhibits potent antioxidant properties (Wollenweber et al. 1995). This study evaluates the effects of *D. aucheri* extract on CKD parameters in streptozotocin-induced diabetic rats, focusing on oxidative stress and inflammation markers and renal enzyme activity, glucose levels, and kidney gene expression.

Materials and Methods

Experimental animals

Fifty male Sprague-Dawley rats (200±20 g, 12 weeks old) from Shiraz University's Laboratory Animals Research Center were acclimated for two weeks. They were housed in controlled conditions (22–25°C, 12-hr light/dark cycles, and 55% humidity) with food and water provided *ad libitum*. The study was approved by the Institutional Animal Ethics Committee based on NIH guidelines (85-23, revised 1996).

Preparation of the hydroalcoholic extract of *D. aucheri* plant

D. aucheri was collected in March 2022 from Yasuj, Iran. Aerial parts were cleaned, air-dried, and extracted using 70% ethanol (1000 ml) via percolation for 72 hr. The filtrate was rotary-evaporated at 40°C, then vacuum-dried and stored at -20°C (yield: 16.5%). Professor F. Attar identified and authenticated the plant, which was then deposited at the Central Herbarium of Tehran University (No. 46056TUH)

Diabetes induction and body weight monitoring after treatment

Diabetes was induced via intraperitoneal injection of freshly synthesized streptozotocin (STZ, 60 mg/kg, Sigma, USA) in 0.1 mol/L citrate buffer (pH 4.5) in overnight-fasted rats (Bayat et al. 2017; Masiello et al. 1998). Fasting blood sugar (FBS) was measured on day 7 post-STZ using an Accu-Chek glucometer. Diabetes was confirmed at FBS >300 mg/dl.

Experimental design

Rats were randomly assigned to five groups (n=6) for a 28-day and all treatments were administered orally by gavage:

Control: Healthy rats given 1 ml saline orally.

STZ: Diabetic control rats receiving 1 mL saline.

STZ + Metformin: Diabetic rats given metformin (500 mg/kg).

STZ + *D. aucheri* 250/500: Diabetic rats receiving *D. aucheri* extract (250 or 500 mg/kg) (Ahmed et al. 2024; Azarnioushan et al. 2009; Nahvinejad et al. 2016).

Following treatment, rats fasted for 12 hr before blood collection via cardiac puncture under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia (Elfasan, Netherlands). Euthanasia was performed using CO_2 asphyxiation (Abed and AL-Awady 2020; Keyghobadi et al. 2024). Blood samples were centrifuged at 3000-3500 rpm for 15 min, and serum was stored at -80°C (Figure 1).

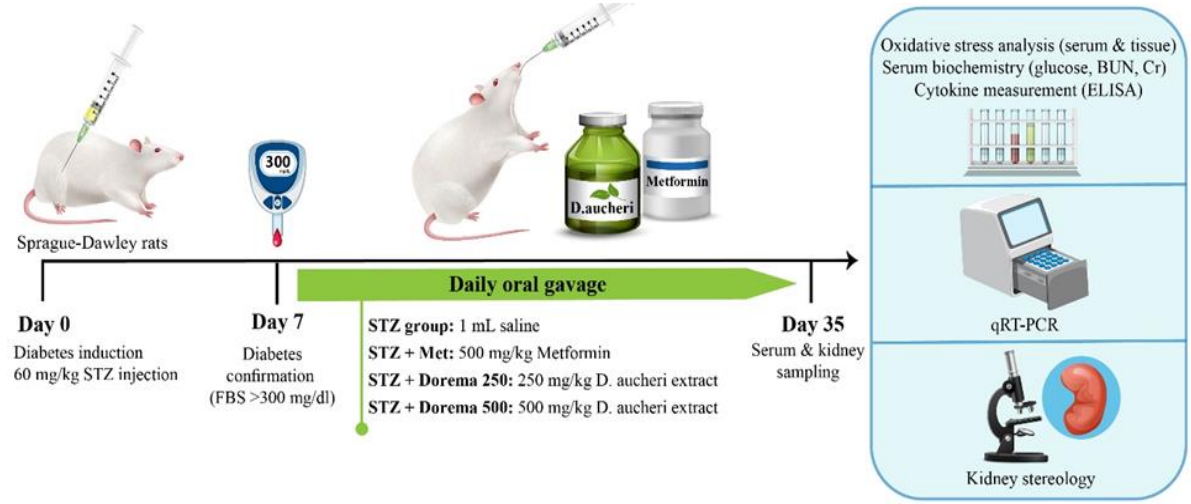

Figure 1. Experimental timeline illustrating the induction of diabetes in Sprague-Dawley rats by a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) on Day 0. Diabetes was confirmed on Day 7 by measuring fasting blood sugar (FBS > 300 mg/dl). From Day 7 to Day 35, diabetic rats received daily oral gavage treatments as follows: saline (STZ group), metformin (500 mg/kg), *Dorema aucheri* extract at 250 or 500 mg/kg. On Day 35, serum and kidney samples were collected for oxidative stress analysis, serum biochemical parameters evaluation, cytokine measurement by ELISA, qRT-PCR, and kidney stereology.

Serum glucose, BUN and Creatinine kidney analysis

Serum glucose in mg/dl (S N; 421019), creatinine in mg/dl (S N; 421341), and BUN in mg/dl (S N; 414194) levels were measured using colorimetric diagnostic kits (Biorex Fars, Shiraz, Iran) on a Prestige instrument (Hitachi, Japan). The SNs refer to the serial numbers provided by the manufacturer (Biorex Company). These numbers indicate the specific lot or batch identification used by the company for their commercial kits.

IL-6, IL-1β, and IL-10 level measurement

Serum cytokine levels were determined via ELISA kits (Karmania Pars Gene Co., Mashhad, Iran) following the manufacturer's protocol.

Evaluation of oxidative stress

Serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) were analyzed using colorimetric kits (KIAZIST Inc., Iran). Kidney tissue homogenates were processed to assess total antioxidant capacity (TAC) and MDA levels (Karmania Pars Gene Co., Kerman, Iran).

RNA isolation and cDNA synthesis

Kidney tissues were stored in RNA later solution (-80°C). An amount of 30-50 mg of kidney tissue was removed; after tissue homogenization, samples were subjected to RNA isolation using KianZol (Kiyan Danesh Co., Iran). Then, total RNA was extracted, and purity (260/280 ratio >1.8) was assessed via NanoDrop. cDNA was synthesized using a Reverse Transcription Kit (Sinaclon, Tehran, Iran) and stored for qRT-PCR.

Quantitative reverse transcription PCR (qRT-PCR)

NGAL, *KIM-1*, *BCL-2*, and *CASP-9* expression was assessed using ABI 7500 real-time PCR (Applied Biosystems, USA), with GAPDH as the housekeeping gene. Each 20 µl reaction contained 0.8 µl primers (10 pmol), 10 µl Ampliqon SYBR Green Master Mix, 7.6 µl dH_2O, and 0.8 µl cDNA. The protocol involved 10 min at 95°C, followed by 40 cycles of 95°C (15 sec), 60°C (30 sec), 72°C (30 sec), and a final elongation at 72°C (10 min). Table 1 lists the qRT-PCR primers.

Table 1. The sequence of designed primers.

Gene Description	Accession Number	Sequences (5'->3')	Product size
BCL2: Sense	NM_016993.1	GGAGGATTGTGGCCTTCTTT	100 bp
BCL2: Anti-sense		GTCATCCACAGAGCGATGTT	
Caspase-9: Sense	NM_031632.1	ACATCTTCAATGGGACCGGC	85 bp
Caspase-9: Anti-sense		TCTTTCTGCTCACCACCACAG	
NGAL: Sense	NM_ 130741	TGAACTGAAGGAGCGATTCG	86 bp
NGAL: Anti-sense		ATTGGTCGGTGGGAACAGA	
KIM-1: Sense	NM_ 173149.2	ACTCCTGCAGACTGGAATGG	214 bp
KIM-1: Anti-sense		CAAAGCTCAGAGAGCCCATC	
GAPDH :Sense	NM_ 017008.4	AAAGAGATGCTGAACGGGCA	100 bp
GAPDH :Anti-sense		ACAAGGGAAACTTGTCCACGA	

Estimating the total volume of kidney structures

Random selection was used to choose six rats for renal stereological investigation from each group. After dissection, the right kidney was removed, and its volume was calculated by immersing it in distilled water (Keyghobadi et al. 2024; Nemati et al. 2024), applying Scherle's immersion method (Koohpeyma et al. 2021), and fixing it in a formaldehyde solution that had been buffered. Nemati and et al.'s study states that the kidney volume was approximated using orientation clock to produce the isotropic uniform random (IUR) sections. The tissue was sectioned in a manner that ensured each part had an

equal opportunity to be sampled once (Nemati et al. 2024).

After that, sections that were 5 μm thick were produced, and staining procedures were used. Hematoxylin and Eosin (H&E) and Masson's trichrome staining were applied to tissue sections in order to estimate the total volume of kidney structures, which include the kidney, medulla, cortex, glomerulus, necrotic and fibrotic tissue, proximal and distal convoluted tubules (PCT and DCT), Henle's loop, collecting ducts (CD), and vessel volume.

Using the formula V (Structure) = ∑P(Structure)/∑P(Reference), where V (Structure) is the volume density of the kidney structures and "∑P(Structure)" and "∑P(Reference)" are the total points superimposed on the target structure and the kidney sections, respectively, the point counting method was used to estimate the total volume of the kidney structures at the final magnification of × 400. Next, the volume density of each structure was multiplied by the kidney's primary volume using the formula Total V (structure) = Vv (structure) × V (primary kidney), which yielded the total volume of the structures.

Histological alterations were assessed according to the scoring system described by Kocoglu et al. (2009). The degree of tubulointerstitial damage was determined by examining the presence of necrotic and apoptotic cells, loss of the tubular brush border or destruction of tubular epithelial cells, tubular dilatation, cast formation, and lymphocytic infiltration. Based on these parameters, scoring was performed as follows: score 0 was assigned when none of the mentioned changes were observed; score 1 for damage involving 0–10% of the tissue; score 2 for 11–25% damage; score 3 for 26–45% damage; score 4 for 46–75% damage; and score 5 for 76–100% damage (Kocoglu et al. 2009).

Statistical evaluation

The data is shown as the standard deviation (SD) ± the mean value. The assumptions of normality of variances were assessed using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to compare the groups, and Tukey's multiple range testing was performed post-hoc. SPSS software (version 24.0) was used for the statistical analysis, and GraphPad PRISM program (version 9.4.1) was used for the graphic design. Statistical significance was defined as p-values less than 0.05.

Results

Effects of *D. aucheri* extract on FBS, BUN, and body weight

Table 2 shows significant increases in FBS and BUN ($p<0.001$) and a decrease in body weight ($p<0.001$) in the STZ group versus controls. No significant creatinine change was observed. Compared to STZ, the metformin group had significantly lower FBS, BUN, and creatinine ($p<0.05$), with slight weight gain. *D. aucheri* extract significantly reduced FBS ($p<0.05$) and BUN ($p<0.05$ for 250 mg/kg and $p<0.01$ for 500 mg/kg) versus STZ. Although 250 mg/kg group showed no significant creatinine change, the 500 mg/kg dose of the extract significantly reduced creatinine ($p<0.01$). Weight gain was modest.

Effects of *D. aucheri* extract on serum inflammatory markers in diabetic rats

Figure 2 shows serum IL-6, IL-10, and IL-1β levels. IL-6 and IL-1β were significantly higher in diabetics than controls ($p<0.001$). *D. aucheri* and metformin significantly reduced IL-6 *D. aucheri* and metformin significantly reduced IL-6 ($p<0.05$) and IL-1β ($p<0.05$ and $p<0.001$, respectively) compared to STZ (Figures 2A and 2B). IL-10 was significantly lower in STZ versus controls ($p<0.001$) but increased with metformin and *D. aucheri* ($p<0.01$, 0.001, and 0.05, respectively) (Figure 2C).

Table 2. Biochemical parameters and body weight in the experimental groups.

Variants	Control	STZ	Metformin (500 mg/kg)	*D. aucheri* (250 mg/kg)	*D. aucheri* (500 mg/kg)
Body weight (g)	280.89±21.502	165.50±22.249***	193.80±16.533***	181.50±28.683***	172.29±15.972***
FBS (mg/dl)	134.0.45±14.058	327.911±44.389***	256.093±22.228***†	249.428±71.945***†	254.636±69.108***†
BUN (mg/dl)	23.600±2.412	52.333±9.407***	40.571±5.682***†	41.444±11.348***†	35.286±4.535*††
Creatinine (mg/dl)	0.7800±0.0788	0.8333±0.0707	0.7286±0.0488†	0.7556±0.0726	0.7000±0.0577††

All values are mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Control: non-diabetic rats received 1 ml saline; STZ: diabetic rats received 1 ml saline; Metformin (500 mg/kg): diabetic rats received metformin (500 mg/kg); *D. aucheri* (250 and 500 mg/kg): diabetic rats received respective doses of *D. aucheri* extract. p<0.05 indicates significance. * vs. Control, † vs. STZ (*, †p<0.05; **, ††p<0.01; and ***, †††p<0.001).

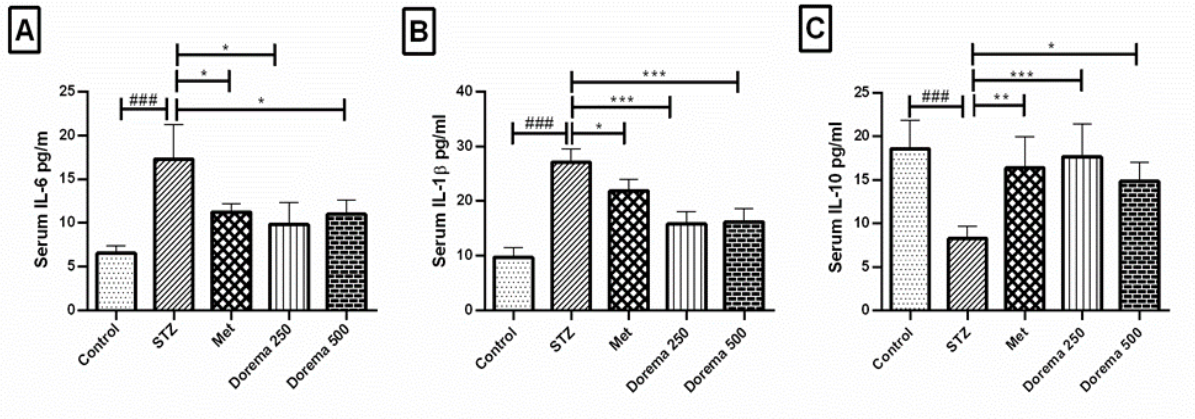

Figure 2. The impact of *D. aucheri* extract on the levels of IL-6 (A), IL-1β (B), and IL-10 (C) in the bloodstream. Control: Non-diabetic rats given saline; STZ: Diabetic rats given saline; Met: Diabetic rats given metformin (500 mg/kg); Dorema 250: Diabetic rats given *D. aucheri* (250 mg/kg); Dorema 500: Diabetic rats given *D. aucheri* (500 mg/kg). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Values are mean ± SD (n = 6). p < 0.05 indicates significance. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#p < 0.05; **,##p < 0.01; and ***,###p < 0.001)

Evaluation of the antioxidant properties of *D. aucheri* extract in diabetic rats

Diabetes significantly increased MDA(KPG-MDAK) (p<0.001) and decreased GPX(KGPX96), CAT (KCAT96), and SOD(KSOD86) levels (Figure 3). Metformin lowered MDA (p<0.001) and increased GPX, CAT and SOD (p<0.05). *D. aucheri* significantly boosted GPX, CAT and SOD (p<0.001 and p<0.01 for 250 mg/kg and 500 mg/kg, respectively) while reducing MDA (p<0.001 and p<0.05, respectively) compared to STZ.

Effects of *D. aucheri* extract on level of MDA and TAC in kidney of diabetic rats

Figure 4 shows that diabetes significantly reduced TAC (p<0.01) and increased MDA (p<0.01) in kidney tissue. *D. aucheri* and metformin significantly increased TAC and decreased MDA (p<0.01) at both doses.

Effect of *D. aucheri* extract on nephropathy biomarkers and apoptotic related genes in diabetic rats

Real-time PCR analysis (Figure 5) showed *BCL-2* downregulation (p<0.01) and increased *NGAL*, *KIM-1*, and *CASP-9* (p<0.01, p<0.01, and p<0.05 respectively) in STZ rats.

The study findings additionally demonstrated that, in comparison to the

STZ group, the renal tubular damage markers *NGAL* and *KIM-1* were downregulated following treatment with *D. aucheri* extract ($p<0.01$ and $p<0.01$, respectively) and metformin ($p<0.01$ and $p<0.05$, respectively) (Figure 5A and 5B). In terms of genes linked to apoptosis, diabetic rats treated with metformin and D. aucheri extract showed a notable down-regulation of *CASP-9* ($p<0.01$ for 250 mg/kg, $p<0.05$ for 500 mg/kg, and $p<0.01$, respectively) and a remarkable increase in *BCL-2* expression ($p<0.05$ and $p<0.01$, respectively) when compared to STZ (Figure 5C and 5D).

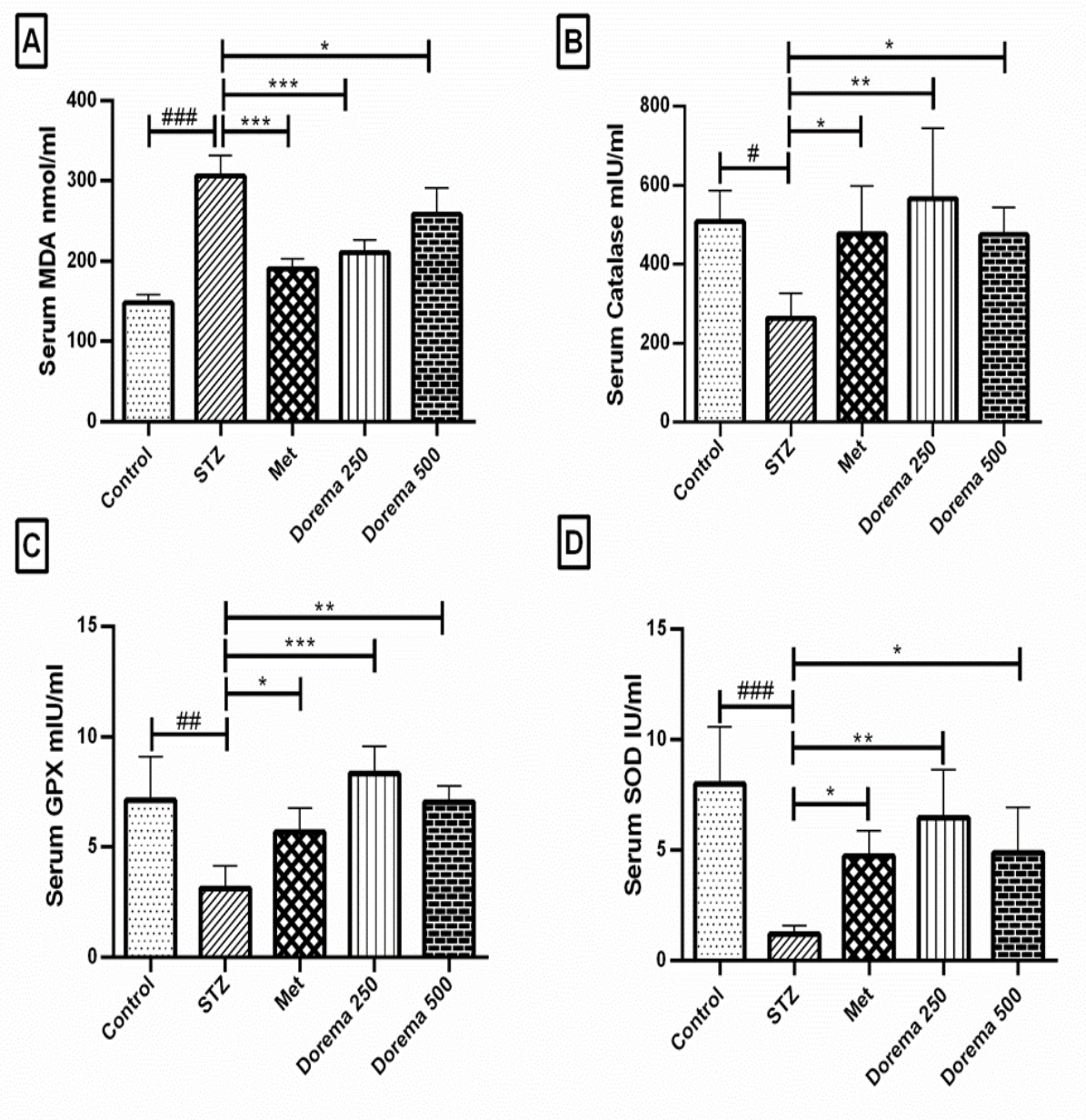

Figure 3. Serum level of MDA (A), Catalase (B), GPX (C), and SOD (D) in treated groups. Control: Rats without diabetes were given normal saline; STZ: Rats with diabetes were given normal saline; Met: Rats with diabetes were given 500 mg/kg of metformin; *Dorema 250*: Rats with diabetes were given 250 mg/kg of *D. aucheri* extract; *Dorema 500*: Rats with diabetes were given 500 mg/kg of *D. aucheri* extract. All values are presented as mean ± SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p<0.05$ denote significance level. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#$p<0.05$; **,##$p<0.01$; and ***,###$p<0.001$)

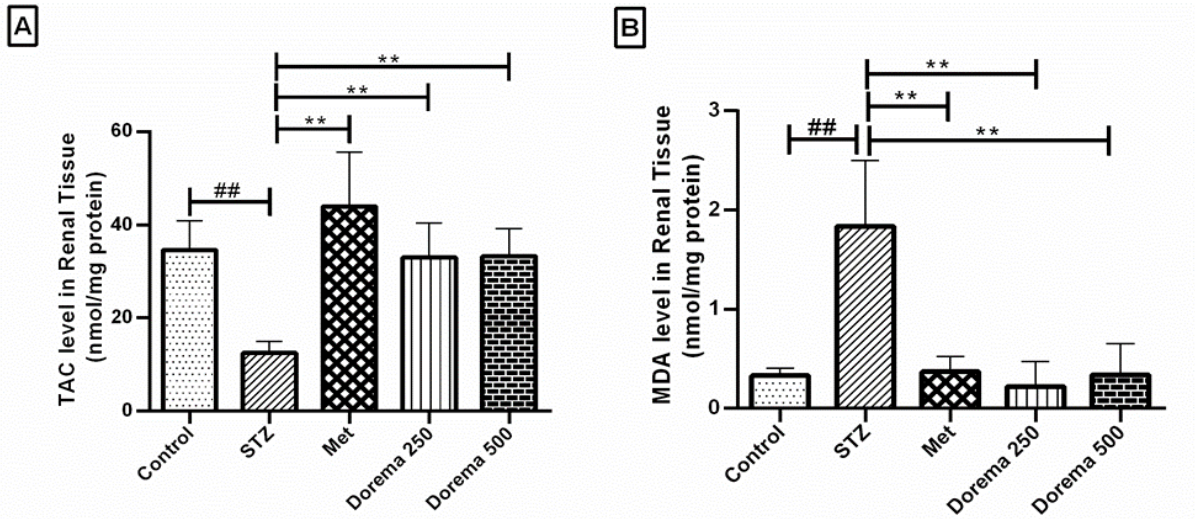

Figure 4. TAC (A) and MDA (B) level in renal tissue of treated groups. Control: Non-diabetic rats given saline; STZ: Diabetic rats given saline; Met: Diabetic rats given metformin (500 mg/kg); Dorema 250: Diabetic rats given *D. aucheri* (250 mg/kg); Dorema 500: Diabetic rats given *D. aucheri* (500 mg/kg). Values are mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p<0.05$ indicates significance. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#$p<0.05$; **,##$p<0.01$; and ***,###$p<0.001$)

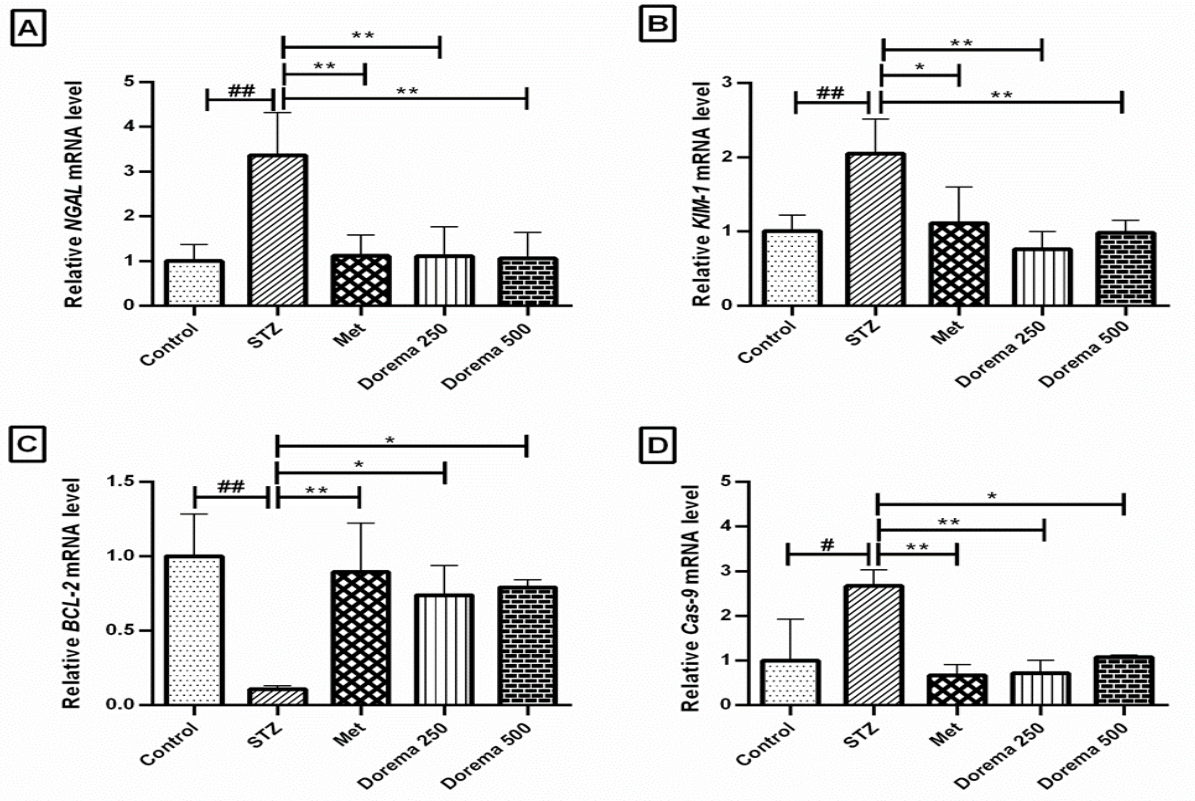

Figure 5. The mRNA expression of *NGAL* (A), *KIM-1* (B), *BCL-2* (C) and *CASP-9* (D) was examined using qRT-PCR**.** Control: Non-diabetic rats given saline; STZ: Diabetic rats given saline; Met: Diabetic rats given metformin (500 mg/kg); Dorema 250: Diabetic rats given *D. aucheri* (250 mg/kg); Dorema 500: Diabetic rats given *D. aucheri* (500 mg/kg). Values are mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p<0.05$ indicates significance. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#$p<0.05$; **,##$p<0.01$; and ***,###$p<0.001$)

Effects of *D. aucheri* extract on stereological indices of kidney in diabetic rats

Figures 6 and 7 show significant diabetes-induced reductions in kidney weight, cortex, glomerulus, PCT, DCT, Henle's loop, and vessel volume, but with no significant medulla or collecting duct changes. Diabetes significantly increased necrotic, fibrotic, and inflamed kidney tissue ($p<0.001$). *D. aucheri* and metformin significantly increased kidney weight, with 250 mg/kg showing a stronger effect ($p<0.001$) than 500 mg/kg ($p<0.01$). They also increased glomerulus, PCT, DCT, and vessel volume ($p<0.05$), with similar effects at both doses. Inflammatory, necrotic, and fibrotic tissue significantly decreased ($p<0.001$).

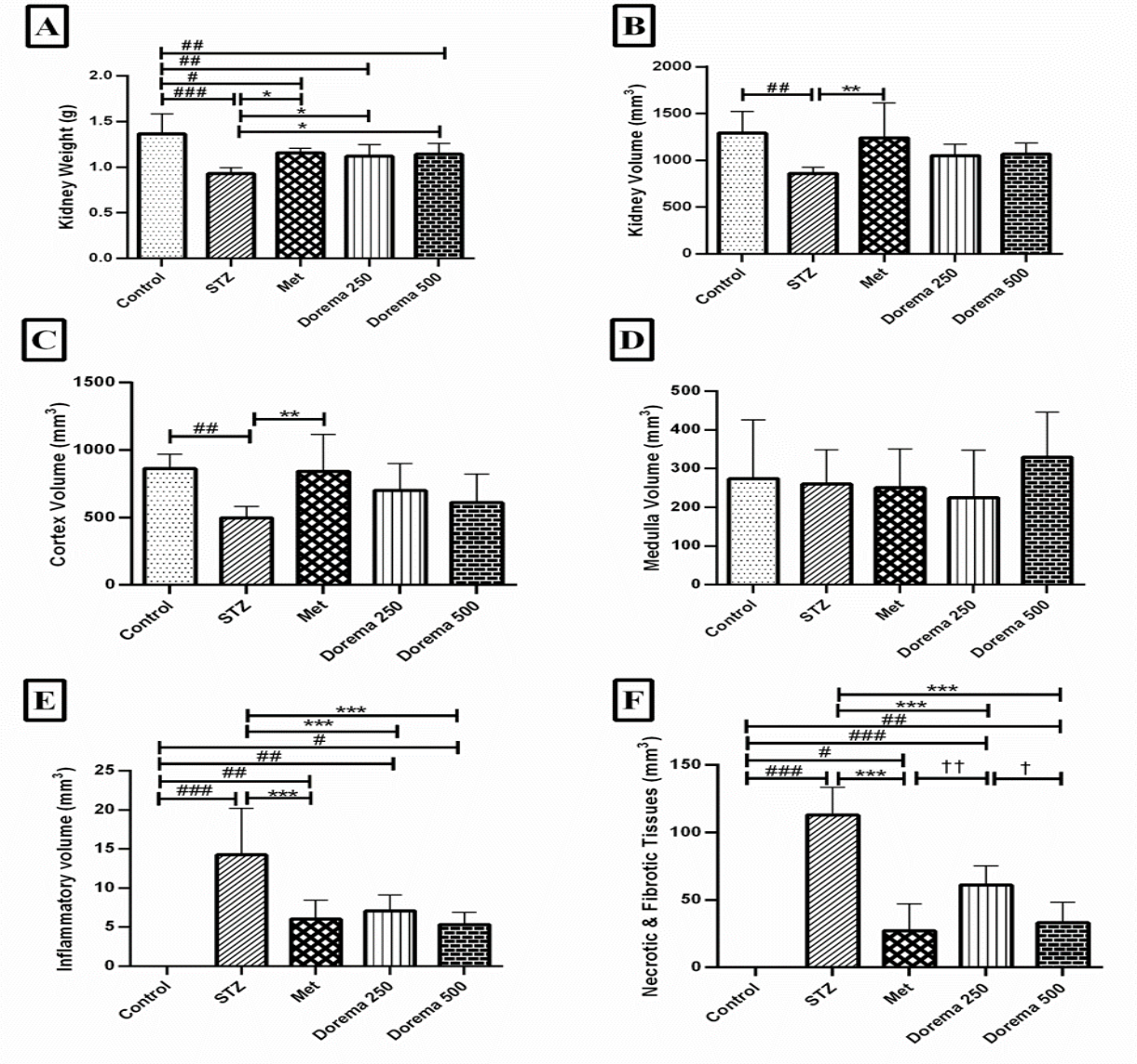

Figure 6. Comparing the average results of stereology, investigating the kidney weight, kidney volume, cortex volume, medulla volume, inflammatory volume and necrotic tissues. Control: Non-diabetic rats given saline; STZ: Diabetic rats given saline; Met: Diabetic rats given metformin (500 mg/kg); Dorema 250: Diabetic rats given *D. aucheri* (250 mg/kg); Dorema 500: Diabetic rats given *D. aucheri* (500 mg/kg). Values are mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p<0.05$ indicates significance. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#$p<0.05$; **,##$p<0.01$; ***, and ###$p<0.001$)

Effects of *D. aucheri* extract on histopathological changes in kidney tissue

Histopathological evaluation of kidney tissues stained with H&E and Masson's trichrome (Figure 8) revealed distinct alterations among the experimental groups. In the healthy control group, the renal cortex displayed normal histological architecture with intact glomeruli and well-preserved tubular structures, showing no pathological changes. In contrast, the untreated diabetic rats exhibited severe renal lesions characterized by marked interstitial inflammation, tubular epithelial degeneration, hyaline cast formation, widening of Bowman's space, extensive epithelial desquamation, and glomerulosclerosis. In the metformin-treated group, partial amelioration of renal injury was observed, although some tubules still showed epithelial degeneration and cytoplasmic vacuolization. The diabetic group treated with *Dorema aucheri* extract at 250 mg/kg showed no remarkable improvement compared with the untreated diabetic group, as degenerative changes, glomerular enlargement, congestion, and interstitial inflammation were still evident in some regions. However, treatment with *D. aucheri* extract at 500 mg/kg noticeably reduced renal tissue damage, with glomeruli and tubular structures appearing relatively preserved and closer to normal morphology.

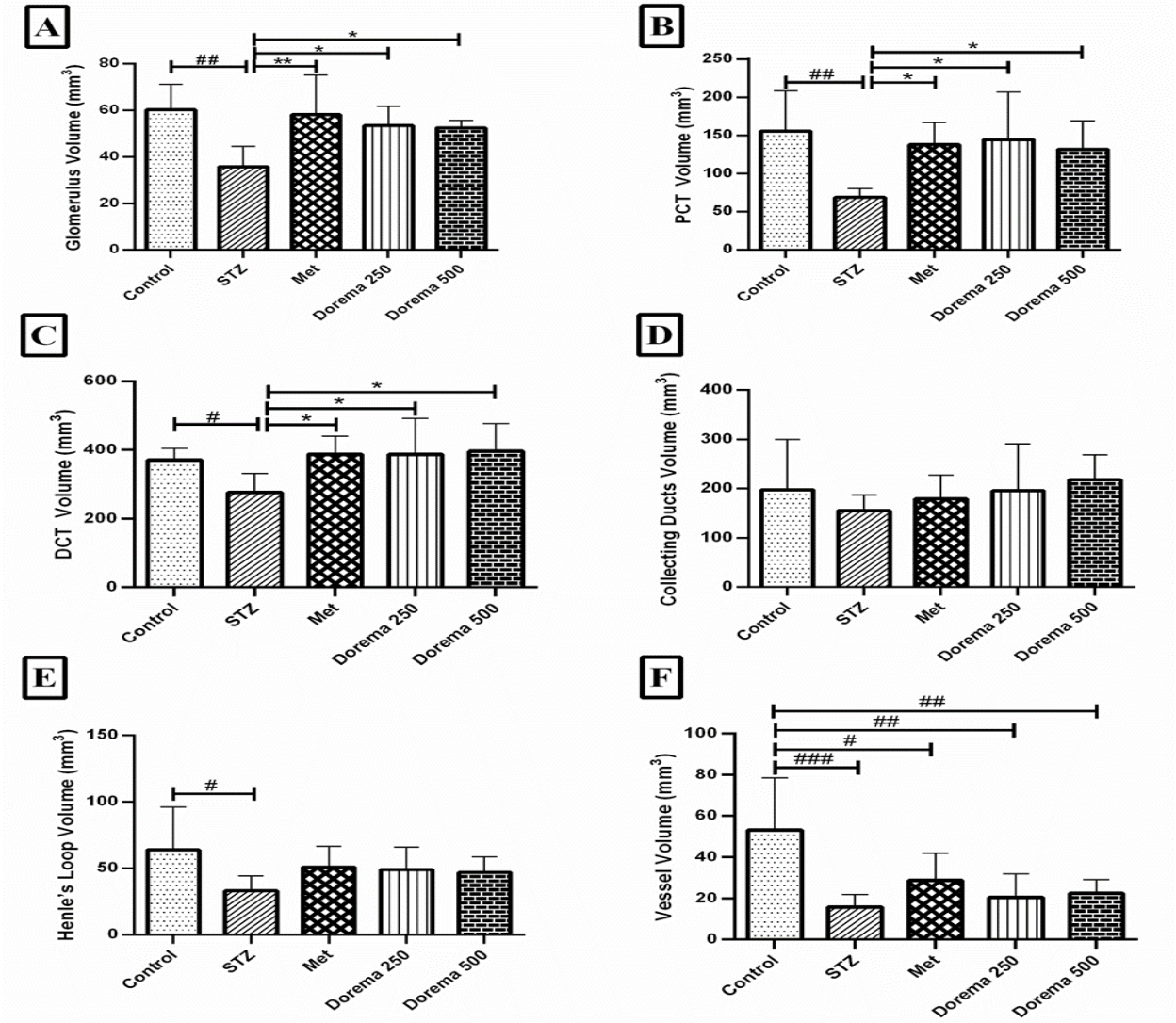

Figure 7. Comparing the average results of stereology, investigating the kidney glomerulus, PCT, DCT, collecting ducts, Henle's loop and vessel volumes. All values are presented as mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. # = significantly different between STZ and control; * = significantly different between STZ group and other treated groups. (*,#p<0.05; **,##p<0.01; ***, and ###p<0.001)

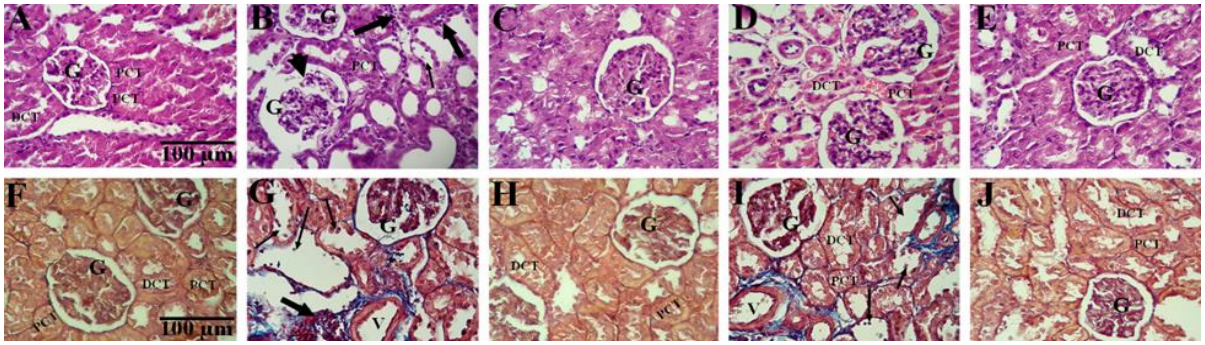

Figure 8. Histopathological features of kidney tissues in different groups. (A–E: H&E; F–J: Masson Trichrome, ×400). A, and F) Control: normal architecture with intact glomeruli (G) and tubules. B, and G) Diabetic: severe interstitial inflammation (thick arrow), tubular degeneration, hyaline casts, widened Bowman's space, epithelial desquamation (thin arrow), and glomerulosclerosis (arrowhead). C, and H) Diabetic + metformin: partial improvement; mild tubular degeneration and vacuolization remain. D, and I) Diabetic + *D. aucheri* 250 mg/kg: no marked recovery; degenerative changes, glomerular enlargement, congestion, and inflammation persist. E, and J) Diabetic + *D. aucheri* 500 mg/kg: reduced damage; glomeruli and tubules mostly preserved, approaching normal morphology.

Discussion

The main finding of our research is the potential of *D. aucheri* extract to protect renal tissue in diabetic rats by exerting antioxidant and anti-apoptotic effects. The extract mitigated oxidative stress-related damage in diabetes. Our findings confirm Ahangarpour et al.'s observation of weight loss in diabetic rats due to tissue protein degradation and muscle atrophy from insulin deficiency. However, *D. aucheri* extract-treated diabetic rats gained weight, likely due to improved insulin secretion and muscle preservation (Ahangarpour et al. 2014). Despite this, our study indicated only a trend toward increased body weight.

The findings of decreased blood glucose levels, renal enzymes, MDA and renal damage (based on histopathology), and increased antioxidant enzyme levels in diabetic rats treated with *D. aucheri* extract were in line with previous findings that *D. aucheri* extract reduces oxidative stress, inflammation, and renal dysfunction (Mousavipoor et al. 2017; Rasouli Vani et al. 2019) which are due to the presence of flavonoids in the plant. Flavonoids, a key component of *D. aucheri*, possess antioxidant and antidiabetic properties, protecting pancreatic β-cells by scavenging free radicals (Song et al. 2005). *D. aucheri* possesses antioxidant properties that can reduce oxidative stress in beta cells and enhance their functionality. This effect is likely attributed to the presence of flavonoids in this plant (Ahangarpour et al. 2014). Prior studies confirm *D. aucheri* strong antioxidant content, including flavonoids, anthocyanins, and phenolic acids (Khanahmadi et al. 2012; Mianabadi et al. 2015). Oxidative stress promotes lipid peroxidation, leading to increased MDA levels and renal cell apoptosis (Koohpeyma et al. 2021). Previous studies also showed *D. aucheri* hepatoprotective and antihyperglycemic effects in diabetic rats, improving insulin, leptin, and hepatic enzyme activity (Ahangarpour et al. 2014). Additionally, it delays SOD and CAT loss in ischemic tissue (Rasouli Vani et al. 2019) and enhances antioxidant enzyme activity in normal rats (KHOSHVAGHTI et al. 2013). These findings suggest *D. aucheri* extract mitigates chronic kidney injury by reducing oxidative stress and enhancing antioxidant defense.

Diabetic rats showed significantly elevated BUN and creatinine levels, indicating kidney damage, while *D. aucheri* treatment significantly lowered these levels. Flavonoids reduce urea and creatinine levels by preventing kidney damage through lipid membrane protection and enhanced antioxidant activity (Long et

al. 2005). Our study also showed *D. aucheri* anti-inflammatory effects, reducing IL-6 and IL-1β levels. Flavonoids are known to exert anti-inflammatory effects and alleviate insulin resistance via inflammatory signaling inhibition (Shamsudin et al. 2022). Diabetes increased renal gene expression of *KIM-1* and *NGAL*, while *D. aucheri* reduced their expression and restored BUN and creatinine levels. KIM-1 is a renal dysfunction marker, elevated in diabetics regardless of albuminuria(Nielsen et al. 2010), while NGAL is an early biomarker of renal injury (Mishra et al. 2004). Increased *KIM-1* and *NGAL* expression in diabetic nephropathy has been reported (Alter et al. 2012). Hyperglycemia induces extracellular matrix damage, increasing vascular permeability and diabetic nephropathy risk (Paolisso et al. 1993). Our findings suggest *D. aucheri* antioxidant effects play a crucial role in reducing oxidative stress-induced renal failure(Song et al. 2005). Antioxidants may exert nephroprotective effects through anti-apoptotic and antioxidant mechanisms (Huang et al. 2020). Diabetic rats exhibited increased *CASP-9* and decreased *BCL-2* expression due to hyperglycemia-induced oxidative damage. *D. aucheri* treatment lowered *CASP-9* and increased *BCL-2* expression, reducing apoptosis. Flavonoids inhibit apoptosis via death receptor and mitochondrial pathways (Guo et al. 2015). For example, galangin, a potent flavonoid from other plant sources, alleviates hyperglycemia, oxidative stress, and inflammation, while reducing Bax and caspase-3 and increasing BCL-2 expression in diabetic rat kidneys (Aladaileh et al. 2021). Flavonoids also protect pancreatic β-cells from oxidative stress-induced apoptosis (Yang et al. 2021). Histologically, *D. aucheri* improved renal morphology in diabetic rats, reducing necrosis, fibrosis, and inflammation while preserving the integrity of glomeruli, proximal, and distal tubules. Stereology analysis confirmed significant reductions in tissue damage. Metformin and *D. aucheri* treatment improved glomerular and tubular volumes.

Although *D. aucheri* modulated antioxidants, apoptosis, and kidney damage in diabetes, clinical trials are needed to confirm its therapeutic potential. Future research should explore molecular mechanisms, including protein-level validation via Western blotting.

For severe conditions like diabetes, natural antioxidant therapy can be a successful treatment. This approach may contribute to mitigating the diabetes's impact by alleviating insulin resistance, vascular injury, inflammation, and renal dysfunction. Because of its powerful antioxidant, anti-inflammatory, and anti-apoptotic properties, *D. aucheri* extract has been demonstrated to be a useful means of halting the advancement of diabetic kidney.

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Conflicts of interest

No conflict of interests

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Code of Ethics

The study's procedures were authorized by Shiraz University of Medical Sciences' Institutional Animal Ethics Committee (IR.SUMS.AEC.1401.084), in accordance with NIH publication No. 85-23, which was amended in 1996 and contains standards for the care and use of animals**.**

Authors' Contributions

Authors' contributions: All experiments, statistical analysis, and Fig.

preparation were conducted by Alireza Raeisi, Farhad Koohpeyma, Hediye Fahandezh saadi, Morvarid Siri, Pardis Negaresh, Golrokh Bahmani, and Forough Saki. The initial draft of manuscript was written by Alireza Raeisi, Sanaz Dastghaib and Mesbah Shams. All tests were set up by Alireza Raeisi, farhad koohpeyma and Sanaz dastghaib and second draft of manuscript was written by Hediye Fahandezh saadi, Morvarid Siri, Pardis Negaresh, Golrokh Bahmani. A final manuscript proof was completed by Forough Saki, Sanaz Dastghaib and Mesbah Shams. All authors have read and agreed to the published version of the manuscript.

Availability of information and resources

This article contains all data created and examined throughout this investigation. The corresponding author will provide datasets used or analyzed during the current work upon reasonable request.

Abbreviations;

Chronic kidney disease: CKD, End-stage renal disease: ESRD, Reactive oxygen species: ROS, Interleukin: IL, Activating factor of apoptosis: Apaf-1, Caspase-9: CASP-9, Kidney injury molecule 1: KIM-1, Neutrophil gelatinase-associated lipocalin: NGAL, Streptozotocin: STZ, Total antioxidant capacity: TAC, Super oxide dismutase: SOD, Catalase: CAT, Glutathione peroxidase: GPX, Malondialdehyde: MDA, Hematoxylin and Eosin: H&E, Proximal convoluted tubule: PCT, Distal convoluted tubule: DCT, Collecting ducts: CD, Standard deviation: SD, Fasting blood sugar: FBS, Carbon tetrachloride: CCl4

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