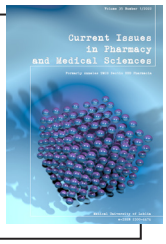
DOI: 10.12923/cipms-2026-0006 Curr. Issues Pharm. Med. Sci., Vol. 39, No. 1, Pages 34-40

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: https://czasopisma.umlub.pl/curipms



Modulation of KIM-1/TGF-β1 signaling pathway by *Ocimum gratissimum* leaf flavonoid-rich extracts in the kidneys of streptozotocin-induced diabetic rats

Basiru Olaitan Ajiboye[1], Olawale Razaq Ajuwon[2], Temitope Olawale Jeje[3], Olutayo Daniel Ijyemi[1], Abigail Faith Ogunmilade[1], Marry Abiola Okesola[4], Hossein Hosseinzadeh[5], Babatunji Emmanuel Oyinloye [6,7,8], Oluwatosin O. Ogedengbe[9], Radosław Kowalski[10*]

[1] Phytomedicine and Molecular Toxicology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Nigeria
[2] Redox Biology Research Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Nigeria
[3] Biochemical Immunology and Phytomedicine Laboratory, Department of Biochemistry, Federal University Oye-Ekiti, Nigeria
[4] Department of Chemistry and Biochemistry, Caleb University, Imota, Lagos, Nigeria
[5] Pharmaceutical Research Center, Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[6] Institute of Drug Research and Development, SE Bogoro Center, Afe Babalola University, Ado-Ekiti, Nigeria
[7] Phytomedicine, Biochemical Toxicology and Biotechnology Research Laboratories, Department of Biochemistry, College of Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[8] Biotechnology and Structural Biology Group, Department of Biochemistry and Microbiology, University of Zululand, South Africa
[9] Department of Anatomy, Federal University Oye-Ekiti, Nigeria
[10] Department of Analysis and Food Quality Assessment, University of Life Sciences in Lublin, Poland

ARTICLE INFO

Received 05 December 2024
Accepted 05 February 2026

Keywords:
diabetic nephropathy;
oxidative stress; pro-inflammatory cytokines;
KIM-1;
TGF-β1;
renal function biomarkers;
Ocimum gratissimum.

ABSTRACT

Diabetic nephropathy, one of the most prevalent complications of diabetes mellitus, is driven by hyperglycemia-induced overproduction of reactive oxygen species (ROS) and pro-inflammatory cytokines, ultimately resulting in structural and functional renal impairment. This study evaluated the renoprotective effects of a flavonoid-rich extract derived from *Ocimum gratissimum* leaves in a streptozotocin (STZ)-induced diabetic rat model. Type 2 diabetes mellitus was induced by intraperitoneal administration of STZ (45 mg/kg body weight) following one week of 20% (w/v) fructose supplementation. Rats were randomly assigned to five groups (n = 8): negative control (NC), diabetic control (DC), diabetic rats treated with low-dose (150 mg/kg) and high dose (300 mg/kg) *O. gratissimum* flavonoid-rich extract (LDOGFL and HDOGFL, respectively), and metformintreated diabetic rats (200 mg/kg; MET). On day 22, blood and kidney tissue was collected for assessment of redox status, inflammatory biomarkers, kidney function indices (creatinine, urea, and uric acid), electrolyte concentrations, kidney-specific acid phosphatase (ACP) and alkaline phosphatase (ALP) activities, mRNA expression of KIM-1 and TGF-β1, and histopathological changes. Treatment with LDOGFL, HDOGFL, or MET significantly ($p < 0.05$) improved redox balance, reduced inflammatory cytokines, and lowered creatinine, urea, and uric acid levels compared with untreated diabetic controls. Electrolyte profiles and ACP/ALP activities increased significantly ($p < 0.05$), whereas mRNA expression of KIM-1 and TGF-β1 was markedly downregulated. Histopathological examination revealed enhanced epithelial cell integrity within renal convoluted tubules and glomeruli in treated groups. Collectively, these findings indicate that the flavonoid-rich extract of *O. gratissimum* leaves confers renoprotective benefits in diabetic nephropathy by attenuating oxidative stress, suppressing inflammation, and improving renal function.

*** Corresponding author**
e-mail: radoslaw.kowalski@up.lublin.pl

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin action, or both. Insulin, a peptide hormone, plays a central role in regulating blood glucose homeostasis [1]. Chronic hyperglycemia is fundamental to the pathophysiology of diabetes and its complications, including diabetic nephropathy (DN), as it triggers the activation of multiple biochemical pathways that promote the generation of reactive oxygen species (ROS), pro-inflammatory cytokines, and progressive renal injury [2-4].

The kidney is among the organs most affected by chronic hyperglycemia-associated complications. DN develops as sustained elevations in blood glucose alter renal structure and impair glomerular and tubular function, ultimately leading to abnormalities in kidney function indices [3,6-8]. The kidneys are essential for maintaining internal homeostasis by filtering metabolic waste products, excess electrolytes, and fluids from the bloodstream, thereby regulating volume status and acid-base balance. Structural and functional compromise of these nephrons results in measurable biochemical derangements in individuals with diabetes.

Amid the global burden of metabolic diseases, medicinal plants continue to serve as an accessible, affordable, and culturally accepted source of therapeutic agents. Numerous plant-derived compounds have demonstrated glucoselowering and organ-protective properties [9]. *Ocimum gratissimum* (commonly known as "Scent Leaf") is rich in phytochemicals, particularly flavonoids, which contribute to its documented anti-inflammatory, antimicrobial, and antioxidant activities and support its traditional use in metabolic and inflammatory disorders [10-11].

Flavonoids are phenolic compounds known to exhibit diverse biological activities, including antidiabetic effects [12]. Their hypoglycemic mechanisms involve modulation of carbohydrate-digesting enzymes, such as inhibition of αglucosidase and reduced intestinal glucose transport [13], as well as enhancement of insulin signaling pathways [14]. Given the destructive impact of diabetes on renal structure and function, there is a compelling need to investigate the potential renoprotective effects of flavonoid-rich extracts of *O. gratissimum*. Therefore, this study employed a streptozotocin (STZ)-induced diabetic rat model to evaluate the ameliorative effects of a flavonoid-rich leaf extract of *O. gratissimum* on kidney function. Specifically, we assessed redox stress biomarkers, inflammatory indicators, serum creatinine, urea, uric acid, electrolyte levels, activities of alkaline phosphatase (ALP) and acid phosphatase (ACP), mRNA expression of KIM-1 and TGF-β1, and histopathological alterations in renal tissue.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Ocimum gratissimum* were purchased from Oja-Oba Market, Ado-Ekiti, Ekiti State, Nigeria, and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Chemicals, reagents, and enzyme kits

Methanol, H_2SO_4, streptozotocin (STZ), NaH_2PO_4, Na_2HPO_4, HCl, NaOH, and halothane were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Eschenstr., 82024 Taufkirchen, Germany). All biochemical assay kits were products of Randox Laboratories (Crumlin, United Kingdom).

Preparation of the flavonoid rich extract

Leaves of *O. gratissimum* were air-dried at room temperature for two weeks and ground into fine powder using an electric blender. The powder was macerated in 80% methanol for 72 hours, followed by filtration through muslin cloth. The filtrate was concentrated using a rotary evaporator. A portion of the residue (20 g) was dissolved in 200 mL of 10% H_2SO_4 and heated at 100°C for 30 minutes in a water bath. The mixture was subsequently cooled on ice for 15 minutes to precipitate the flavonoid aglycones [15].

Animals and induction of diabetes mellitus

Male Wistar rats (150-180 g) were obtained from ShowGold Animal House, Idofin, Oye-Ekiti, Ekiti State, Nigeria. Animals were housed under standard laboratory conditions (22 ± 2°C; 12-hour light/dark cycle) with *ad libitum* access to commercial chow and water. After a 7 day acclimation period, 40 rats designated for diabetes induction received a 20% (w/v) fructose solution ad libitum for 7 days [16]. Although exact fluid intake per rat was not quantified, water bottle levels were inspected twice daily to ensure adequate access.

Following an overnight fast, diabetes was induced via a single intraperitoneal injection of freshly prepared streptozotocin (STZ; 40 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5). Fasting blood glucose (FBG) was measured 3 days post-STZ injection using a glucometer (Accu-Chek Active, Roche Diagnostics). Rats with FBG ≥ 250 mg/dL were considered diabetic. All 40 STZ-treated rats met this criterion (mean FBG: 312 ± 23 mg/dL) and were included in the study.

All animal procedures adhered to the ARRIVE 2.0 guidelines and were approved by the FUOYE Faculty of Science Research Ethics Committee (FUOYEFSC 201122 – REC 2023/008).

Experimental grouping and treatment

Rats were randomly assigned into five groups:

- **Group I:** Negative Control (NC)
- **Group II:** Diabetic Control (DC)
- **Group III:** LDOGFL – Diabetic rats treated with low-dose *O. gratissimum* flavonoid-rich leaf extract (150 mg/kg body weight)
- **Group IV:** HDOGFL – Diabetic rats treated with high dose extract (300 mg/kg body weight)
- **Group V:** MET – Diabetic rats treated with metformin (200 mg/kg body weight)

Treatments were administered once daily for 21 days. On day 22, rats were euthanized under halothane anesthesia. Blood samples were collected via cardiac puncture and centrifuged at 1500 rpm for 5 minutes; serum was stored for

biochemical analyses. Kidneys were excised, homogenized in 1.0 M phosphate buffer (pH 7.4) at a 1:5 (w/v) ratio, and centrifuged at 4000 rpm for 15 minutes to obtain a clear supernatant.

Biomarker analyses

Oxidative stress biomarkers, malondialdehyde (MDA), catalase, superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx), and reduced glutathione (GSH) were quantified using commercial assay kits.

Serum electrolytes (HCO_3^-, K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}) were analyzed using corresponding Randox kits. Renal inflammatory biomarkers, including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), were measured via ELISA.

Kidney histology was assessed after hematoxylin and eosin (H&E) staining. Serum levels of creatinine, urea, and uric acid were determined using standard kits. Activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were quantified with Randox assay kits.

Relative gene expression of KIM-1 and TGF-β1

Total RNA was extracted from renal tissue using the QuickRNA MiniPrep™ Kit (Zymo Research). Contaminant DNA was removed using DNase I. The purified RNA was reverse-transcribed to cDNA, which was used for PCR amplification of the target genes. The primer sequences are presented in Table 1.

Table 1. Primer sequences

Gene	Forward Primer	Reverse Primer
KIM-1	5′-TGGCAGATTCTGTAGCTGGTT-3′	5′-AGAGAACATGAGCCTCTATTCCA-3′
TGF-β1	5′-CTTCTCCACCAACTACTGCTTC-3′	5′-GGGTCCCAGGCAGAAGTT-3′
GAPDH	5′-CATCTTCTTTTGCGTCGCCA-3′	5′-TTAAAAGCAGCCCTGGTGACC-3′

Statistical Analysis

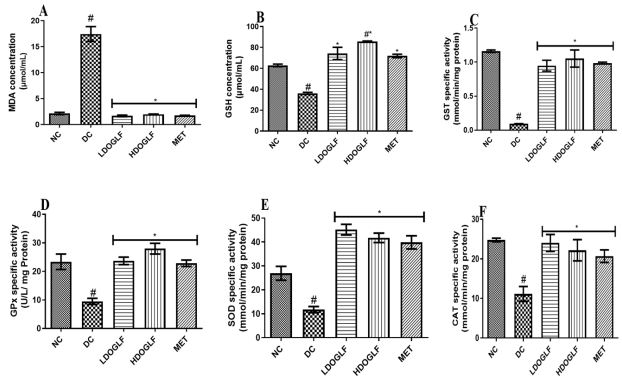
All data were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's multiple comparison test was performed using GraphPad Prism. Statistical significance was set at $p < 0.05$.

RESULTS

Kidney redox stress biomarkers in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

As shown in Figure 1, the diabetic control (DC) group exhibited a significant ($p < 0.05$) reduction in reduced glutathione (GSH) concentration and in the activities of glutathione S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) compared with the negative control (NC) group. Treatment of diabetic rats with either the low-dose (LDOGFL; 150 mg/kg) or high dose (HDOGFL; 300 mg/kg) *O. gratissimum* flavonoid-rich extract, as well as metformin (MET; 200 mg/kg), resulted in significant ($p < 0.05$) increases in GSH levels and antioxidant enzyme activities relative to the DC group.

Although GST, GPx, SOD, and CAT activities in the treated groups did not differ significantly ($p > 0.05$) from the NC group, GSH levels were significantly elevated at the high extract dose (300 mg/kg). Conversely, malondialdehyde (MDA) concentration was significantly higher ($p < 0.05$) in the DC group compared with NC. All treatment regimens significantly reduced MDA levels relative to DC; however, MDA concentrations in treated groups were not significantly different ($p > 0.05$) from NC.

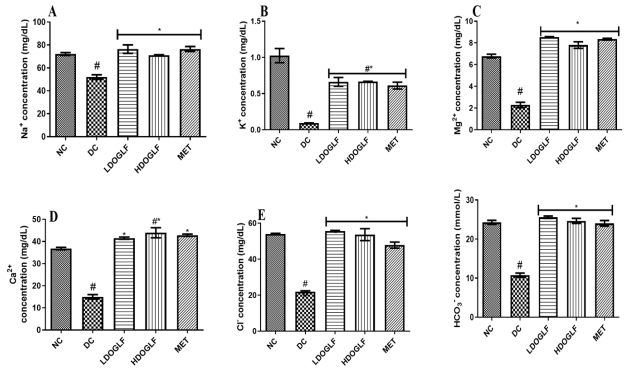


Values represent mean ± SD (n = 8). # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC. Legend: NC = Negative Control; DC = Diabetic Control; LDOGFL = 150 mg/kg extract; HDOGFL = 300 mg/kg extract; MET = 200 mg/kg metformin; MDA = Malondialdehyde; GSH = Reduced glutathione; GST = Glutathione S-Transferase; CAT = Catalase; GPx = Glutathione Peroxidase; SOD = Superoxide Dismutase

Figure 1. Kidney redox stress biomarkers in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

Electrolyte levels in STZ-induced diabetic rats treated with *Ocimum gratissimum* leaf extracts

Diabetic rats in the DC group exhibited significantly decreased ($p < 0.05$) serum electrolyte concentrations (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, and HCO_3^-) relative to the NC group (Figure 2). Treatment with LDOGFL, HDOGFL, or MET restored electrolyte balance, resulting in significant ($p < 0.05$) increases across all measured ions compared with DC. No significant differences ($p > 0.05$) were observed between treated groups and NC, except for Ca^{2+}, which was significantly higher ($p < 0.05$) in the HDOGFL group (300 mg/kg).

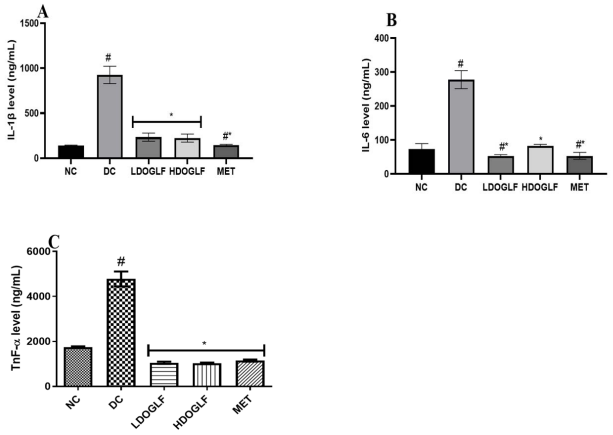


Values represent mean ± SD (n = 8). # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC. Legend: NC = Negative Control; DC = Diabetic Control; LDOGFL = 150 mg/kg extract; HDOGFL = 300 mg/kg extract; MET = 200 mg/kg metformin

Figure 2. Electrolyte levels in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

Kidney inflammatory biomarkers in STZ-induced diabetic rats treated with *Ocimum gratissimum* leaf extracts

As presented in Figure 3, pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were significantly elevated ($p < 0.05$) in the DC group compared with NC. Treatment with LDOGFL, HDOGFL, or MET significantly ($p < 0.05$) reduced the levels of all three cytokines relative to the DC group. While TNF-α levels in treated groups did not differ significantly ($p > 0.05$) from NC, a significant reduction in IL-1β was observed in the MET group, and IL-6 was significantly reduced in both the LDOGFL and MET groups.



Values represent mean ± SD (n = 8). # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC
Legend: NC = Negative Control; DC = Diabetic Control; LDOGFL = 150 mg/kg extract; HDOGFL = 300 mg/kg extract; MET = 200 mg/kg metformin

Figure 3. Kidney inflammatory biomarkers in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

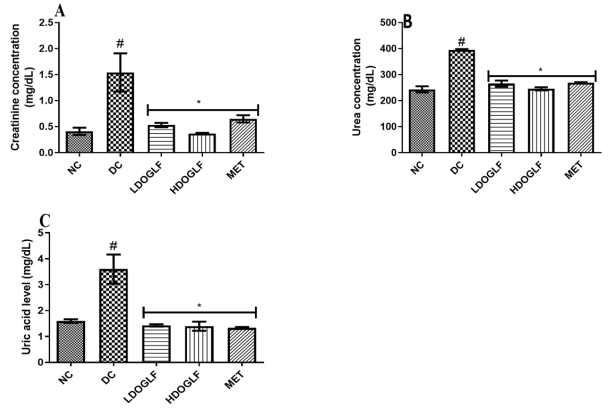
Kidney histopathology following treatment with *Ocimum gratissimum* leaf extracts

Histological evaluation (Figure 4) revealed intact renal morphology in the NC group, with normal glomeruli and well-preserved epithelial cells lining the proximal (PCT) and distal convoluted tubules (DCT). In contrast, the DC group displayed pronounced renal lesions, including widened glomerular urinary space and marked degeneration of PCT epithelial cells.

Rats treated with LDOGFL or HDOGFL showed restored glomerular architecture and improved epithelial cell integrity, indicating reversal of the pathological changes observed in diabetic controls. The MET-treated group exhibited mild degeneration within glomerular, PCT, and DCT epithelial cells, showing partial but less pronounced improvement compared with extract-treated groups.

Serum creatinine, urea, and uric acid concentrations in STZ-induced diabetic rats treated with *Ocimum gratissimum* extracts

As shown in Figure 5, serum creatinine, urea, and uric acid levels were significantly elevated ($p < 0.05$) in the DC group relative to NC. Treatment with LDOGFL, HDOGFL, or MET significantly ($p < 0.05$) reduced these renal function markers compared with the DC group. No significant differences ($p > 0.05$) were observed between treated groups and NC, indicating near-complete normalization of these indices following treatment.



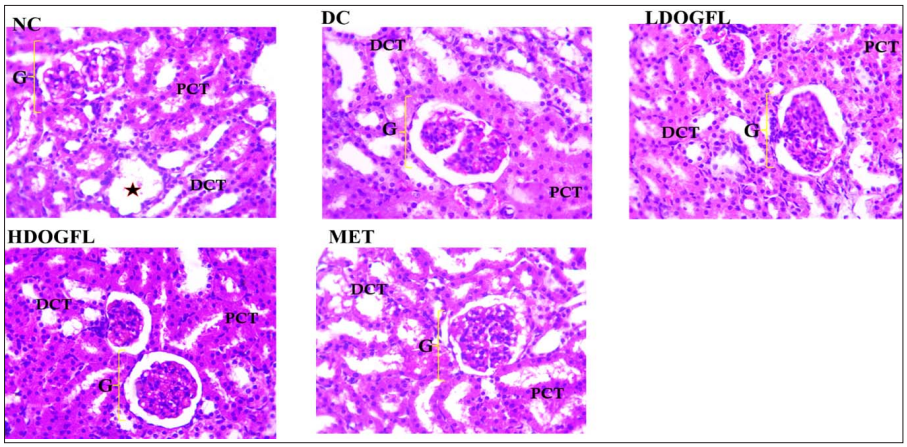
Values represent mean ± SD (n = 8), # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC
Legend: NC, DC, LDOGFL (150 mg/kg), HDOGFL (300 mg/kg), MET (200 mg/kg)

Figure 5. Serum creatinine, urea, and uric acid concentrations in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

RESULTS

Kidney phosphatase activities in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

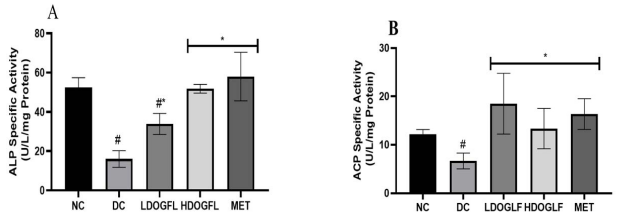
The specific activities of kidney alkaline phosphatase (ALP) and acid phosphatase (ACP) are presented in Figure 6. STZ-induced diabetic rats (DC) showed a marked and significant reduction ($p < 0.05$) in ALP and ACP activities compared with the NC group. Treatment with *O. gratissimum* flavonoid-rich extract at both low (LDOGFL; 150 mg/kg) and high doses (HDOGFL; 300 mg/kg), as well as with metformin (MET; 200 mg/kg), restored the activities of both enzymes toward normal values.



Legend: NC, DC, LDOGFL (150 mg/kg), HDOGFL (300 mg/kg), MET (200 mg/kg); H&E staining; magnification ×800

Figure 4. Photomicrographs of kidney sections from STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

However, ALP activity in the LDOGFL group remained significantly lower ($p < 0.05$) than that of NC, although it was still significantly higher ($p < 0.05$) than in DC, indicating partial but meaningful recovery. ACP activity in all treated groups returned to levels comparable with NC.



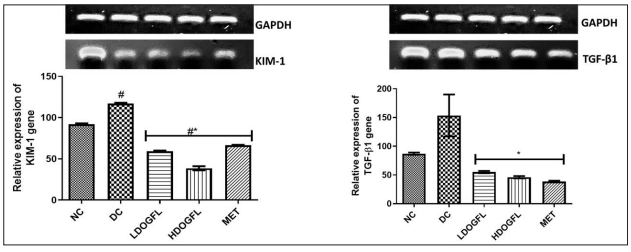
Values represent mean ± SD (n = 8). # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC; #* $p < 0.05$ *vs*. both NC and DC
Legend: NC, DC, LDOGFL (150 mg/kg), HDOGFL (300 mg/kg), MET (200 mg/kg)

Figure 6. Kidney ALP and ACP activities in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

Relative gene expression of KIM-1 and TGF-β1 in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

Figure 7 presents the mRNA expression patterns of KIM-1 and TGF-β1 across the experimental groups. KIM-1 expression was significantly elevated ($p < 0.05$) in the DC group compared with NC, confirming STZ-induced renal injury. Treatment with LDOGFL, HDOGFL, or MET resulted in a pronounced reduction ($p < 0.05$) in KIM-1 expression relative to DC. Although KIM-1 expression in treated groups remained significantly different from NC ($p < 0.05$), all treatments effectively suppressed the pathological overexpression observed in diabetic rats.

Similarly, TGF-β1 expression was significantly higher in DC compared with NC. Administration of either extract dose or metformin led to significant ($p < 0.05$) downregulation of TGF-β1 expression relative to DC, demonstrating the ability of *O. gratissimum* flavonoid-rich extract to reduce pro-fibrotic signaling in diabetic kidneys.



Values represent mean ± SD (n = 8). # $p < 0.05$ *vs*. NC; * $p < 0.05$ *vs*. DC
Legend: NC, DC, LDOGFL (150 mg/kg), HDOGFL (300 mg/kg), MET (200 mg/kg)

Figure 7. Relative mRNA expression of KIM-1 and TGF-β1 in STZ-induced diabetic rats treated with *Ocimum gratissimum* flavonoid-rich extracts

DISCUSSION

Diabetic nephropathy (DN) is strongly driven by oxidative imbalance, and structural damage in diabetic kidneys has been directly linked to redox stress [17]. Given this mechanistic basis, evaluating whether plant-derived therapeutics can restore redox homeostasis by enhancing antioxidant defenses is essential for determining their potential renoprotective effects [18]. Hyperglycemia promotes the overproduction of reactive oxygen species (ROS) through multiple biochemical pathways [2]. Under physiologic conditions, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST), along with nonenzymatic antioxidants like reduced glutathione (GSH), help neutralize ROS to prevent oxidative injury [19].

In this study, diabetic rats exhibited significant reductions in antioxidant enzyme activities and GSH concentration, consistent with earlier findings showing diminished antioxidant reserves in DN [20]. Treatment with *Ocimum gratissimum* flavonoid-rich leaf extracts markedly improved antioxidant profiles, indicating enhanced redox buffering capacity. When oxidants outweigh antioxidants, redox signaling becomes dysregulated, leading to extensive molecular injury [21]. One major target of ROS is membrane lipids, and the resulting lipid peroxidation is typically measured through malondialdehyde (MDA) levels [22]. The extract significantly lowered renal MDA levels, demonstrating its ability to mitigate oxidative membrane damage.

Electrolyte imbalance, or dyselectrolytemia, is another hallmark of diabetic renal dysfunction. Electrolytes, including sodium, potassium, magnesium, calcium, bicarbonate, and chloride are essential for maintaining cellular and systemic metabolic functions [23]. Their homeostasis is controlled by renal reabsorption and secretion along nephron tubules [24-25]. Hyperglycemia induces osmotic diuresis, volume depletion, and electrolyte wasting, contributing to dyselectrolytemia in individuals with type 2 diabetes mellitus (T2DM) [28-30]. In the present study, the diabetic group showed marked reductions in serum electrolyte levels, whereas treatment with *O. gratissimum* extracts normalized most electrolytes. Exceptions included potassium, which remained reduced, and calcium, which increased at higher extract doses, suggesting dose-dependent mineral effects.

Inflammation plays a central role in the pathogenesis and progression of DN [31-32]. Multiple reports support anti-inflammatory therapy as a promising adjunct for diabetic kidney disease [33-34]. Here, the diabetic rats demonstrated elevated serum concentrations of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, which aligns with established inflammatory patterns in experimental and clinical diabetes. Treatment with the flavonoid-rich extract significantly reduced these cytokines at both doses, reflecting the extract's potent immunomodulatory effects. TNF-α is particularly important because it can directly damage renal cells and promote indirect toxicity by increasing endothelial permeability, stimulating ROS generation, and promoting leukocyte recruitment [36-37]. TNF-α is also known to enhance sodium retention and stimulate TGFβ-mediated pathways contributing to renal hypertrophy [33]. Similarly, IL-1β and IL-6 disrupt glomerular hemodynamics and contribute to thickening of the glomerular basement membrane [4,33]. Although NFκB signaling, the primary driver of renal inflammatory responses was not examined here, the reductions in IL-1β, IL-6, and TNF-α strongly imply its involvement [3,34].

Histological evaluation supported these biochemical findings. Diabetic rats showed disrupted glomerular structure, widened urinary spaces, and severe epithelial loss in

proximal (PCT) and distal convoluted tubules (DCT), consistent with hallmarks of diabetic kidney disease (DKD) such as mesangial expansion and tubulointerstitial fibrosis [38]. Treatment with *O. gratissimum* extracts restored glomerular architecture and tubular epithelial integrity, indicating reversal of structural injury. These improvements correlate with the observed enhancement of antioxidant status and attenuation of inflammation.

Renal function indices, including creatinine, urea, and uric acid, were markedly elevated in diabetic animals, reflecting impaired glomerular filtration and possibly extrarenal metabolic derangements [8,39-40]. Treatment normalized these parameters, demonstrating that the extract restored filtration efficiency and renal metabolic handling. Consistent with this, phosphatase enzymes (ACP and ALP), which decline in renal tissue during injury [42-43], were significantly restored following treatment. Reduced renal hydrolase activity indicates damage to renal tubular cells, whereas extract-mediated normalization suggests preservation of cellular integrity [44-47]. Antioxidant compounds are known to maintain phosphatase activity in oxidative disease states [49], supporting the likelihood that *O. gratissimum* exerts protective effects at the cellular level.

Finally, the study assessed mRNA expression of two critical renal biomarkers: KIM-1 and TGF-β1. KIM-1 is a highly sensitive marker of proximal tubular injury [3,51], while TGF-β1 is a key driver of renal fibrosis due to its role in stimulating extracellular matrix accumulation [52-53]. Hyperglycemia induces overexpression of both markers in diabetic kidneys [54]. Treatment significantly downregulated their expression, suggesting that the extract reduces tubular injury and fibrotic signaling. Other antidiabetic phytochemicals have also demonstrated renoprotective effects through modulation of TGFβ pathways [55-56]. Because inflammation and oxidative stress are core drivers of fibrosis [4,57], the extract's ability to suppress these processes likely underlies the observed improvements.

These findings suggest that the *O. gratissimum* flavonoid-rich extract acts through multiple complementary mechanisms, antioxidant, anti-inflammatory, and anti-fibrotic to counteract the pathophysiological processes underlying diabetic nephropathy.

CONCLUSION

This study demonstrates that flavonoid-rich extracts from *Ocimum gratissimum* leaves exert significant renoprotective effects in streptozotocin-induced diabetic rats. The extract enhanced antioxidant defenses by elevating GSH levels and increasing the activities of GST, GPx, CAT, and SOD, while reducing lipid peroxidation. Treatment also normalized renal function parameters, including creatinine, urea, uric acid, and electrolyte levels, and improved histological architecture of the kidney. Furthermore, downregulation of TGF-β1 and KIM-1 mRNA expression indicates inhibition of fibrotic and injury-related pathways. Collectively, these findings suggest that *O. gratissimum* may serve as a promising adjunctive therapy for preventing or mitigating diabetes-related kidney complications.

ORCID iDs

Basiru Olaitan Ajiboye https://orcid.org/0000-0001-5982-2322
Olawale Razaq Ajuwon https://orcid.org/0000-0003-3958-1685
Temitope Olawale Jeje https://orcid.org/0009-0001-5469-9537
Marry Abiola Okesola https://orcid.org/0000-0001-6391-8554
Hossein Hosseinzadeh https://orcid.org/0000-0002-3483-851X
Babatunji Emmanuel Oyinloye https://orcid.org/0000-0003-2165-7936
Oluwatosin O. Ogedengbe https://orcid.org/0000-0002-1215-8521
Radosław Kowalski https://orcid.org/0000-0003-3063-8710

REFERENCES

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. *Int J Mol Sci.* 2020;21(17):62-75. https://doi.org/10.3390/ijms21176275
2. Pelle MC, Provenzano M, Busutti M, Porcu CV, Zaffina I, Stanga L, et al. Up-date on diabetic nephropathy. *Life* (Basel). 2022;12(8):1202. https://doi.org/10.3390/life12081202
3. Duan S, Lu F, Song D, Zhang C, Zhang B, Xing C, et al. Current challenges and future perspectives of renal tubular dysfunction in diabetic kidney disease. *Front Endocrinol* (Lausanne). 2021;12: 661185. https://doi.org/10.3389/fendo.2021.661185
4. Sindhughosa DA, Pranamartha AGMK. The involvement of pro-inflammatory cytokines in diabetic nephropathy: Focus on IL-1, IL-6, and TNF-α signaling mechanism. *Bali Med J.* 2017;6(1):44-51. https://doi.org/10.15562/bmj.v6i1.299
5. Ricciardi CA, Gnudi L. Kidney disease in diabetes: From mechanisms to clinical presentation and treatment strategies. *Metabolism.* 2021;124:154890. https://doi.org/10.1016/j.metabol.2021.154890
6. Vistisen D, Andersen GS, Hulman A, Persson F, Rossing P, Jørgensen ME. Progressive decline in estimated glomerular filtration rate in patients with diabetes after moderate loss in kidney function even without albuminuria. *Diabetes Care.* 2019;42(10):1886-94. https://doi.org/10.2337/dc19-0349
7. Alshawi EAAA, Mahdi AA, Obaid NH, Inam JR. Evaluate CRP, urea, uric acid, and creatinine in dialysis patients. *Tex J Med Sci.* 2023;26:37-42.
8. Tola FS, Zawdie B, Belay M. Evaluation of uric acid and its correlation with decline in estimated glomerular filtration rate among chronic kidney disease patients. *Eur J Clin Biomed Sci.* 2022;8(6):84-91.
9. Malode LL, Manwar JV, Panchale WA, Bartere SA, Bakal RL. Potential of medicinal plants in management of diabetes: An update. GSC *Adv Res Rev.* 2021;8(1):149-59. https://doi.org/10.30574/gscarr.2021.8.1.0151
10. Ijinu TP, Jacob J, Sreejith SL, Parvathy S, Pushpangadan P. Phytochemical constituents and pharmacology of *Ocimum gratissimum* L. In: Pullaiah T, editor. *Bioactives and Pharmacology of Lamiaceae.* New York: Apple Academic Press; 2023. p. 367-88. https://doi.org/10.1201/9781003346142
11. Ugbogu OC, Emmanuel O, Agi GO, Ibe C, Ekweogu CN, Ude VC, et al. A review on traditional uses, phytochemistry and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon.* 2021;7(11):e08404. https://doi.org/10.1016/j.heliyon.2021.e08404
12. Hussain T, Tan B, Murtaza G, Liu G, Rahu N, Kalhoro MS, et al. Flavonoids and type 2 diabetes: Evidence from clinical and animal studies and delivery strategies to improve therapeutic efficacy. *Pharmacol Res.* 2020;152:104629. https://doi.org/10.1016/j.phrs.2020.104629
13. Sok Yen F, Shu Qin C, Tan Shi Xuan S, Jia Ying P, Yi Le H, Darmarajan T, et al. Hypoglycemic effects of plant flavonoids: A review. *Evid Based Complement Alternat Med.* 2021;2021:2057333. https://doi.org/10.1155/2021/2057333
14. Manavi SP, Amiri T, Mozafaryan MJ. Role of flavonoids in diabetes. *J Rev Med Sci.* 2021;1(3):149-61.

15. Obafemi TO, Akinmoladun AC, Olaleye MT, Stephen OA, Amos AO. Potentials of flavonoid-rich extract. *J Ayurveda Integr Med.* 2017;8: 238-46. https://doi.org/10.1016/j.jaim.2017.01.008
16. Salau VF, Erukainure OL, Olofinsan KO, Bharuth V, Ijomone OM, Islam MS. Ferulic acid improves glucose homeostasis via modulation of diabetogenic pathways and restoration of pancreatic architecture in diabetic rats. *Fundam Clin Pharmacol.* 2023;37(2):324-39. https://doi.org/10.1111/fcp.12860
17. Miranda-Díaz AG, Pazarín-Villaseñor L, Yanowsky-Escatell FG, Andrade-Sierra J. Oxidative stress in diabetic nephropathy with early chronic kidney disease. *J Diabetes Res.* 2016;2016:7047238. https://doi.org/10.1155/2016/7047238
18. Matoba K, Takeda Y, Nagai Y, Yokota T, Utsunomiya K, Nishimura R. Targeting redox imbalance as an approach for diabetic kidney disease. *Biomedicines*. 2020;8(2):40. https://doi.org/10.3390/biomedicines8020040
19. Pervaiz S, Bellot GL, Lemoine A, Brenner C. Redox signaling in human disease and the regulatory role of autophagy. *Int Rev Cell Mol Biol.* 2020;352:189-214. https://doi.org/10.1016/bs.ircmb.2020.03.002
20. Mansoor G, Tahir M, Maqbool T, Abbasi SQ, Hadi F, Shakoori TA, et al. Increased expression of circulating stress markers and inflammatory cytokines with decreased antioxidant levels in diabetic nephropathy. *Medicina* (Kaunas). 2022;58(11):1604. https://doi.org/10.3390/medicina58111604
21. Gutteridge JM, Halliwell B. Oxidative stress, redox stress or redox success? *Biochem Biophys Res Commun.* 2018;502(2):183-6. https://doi.org/10.1016/j.bbrc.2018.05.045
22. Avery SV. Molecular targets of oxidative stress. *Biochem J.* 2011;434(2):201-10. https://doi.org/10.1042/BJ20101695
23. Uehara A, Shibagaki Y. Complications of homeostasis (electrolytes and acid-base). In: *Acute Kidney Injury and Regenerative Medicine*. Singapore: Springer; 2020. p. 14762.
24. Verschuren EH, Castenmiller C, Peters DJ, Arjona FJ, Bindels RJ, Hoenderop JG. Sensing tubular flow and renal electrolyte transport. *Nat Rev Nephrol.* 2020;16(6):337-51. https://doi.org/10.1038/s41581-020-0259-8
25. Shrimanker I, Bhattarai S. Electrolytes. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2023.
26. McGregor T, Jones S. Fluid and electrolyte problems in renal dysfunction. *Anaesth Intensive Care Med.* 2021;22(7):406-9.
27. Pattanaik L, Acharya M, Yadav M, Mishra P, Nayak M. Alterations in serum electrolyte homeostasis in type 2 diabetes mellitus patients. *J Evid Based Med Healthc.* 2020;7:3148-52. https://doi.org/10.18410/jebmh/2020/64
28. Waled A, Ahmed A. Disturbance of electrolyte (Na, K, Cl) homeostasis in type II diabetes mellitus. *Libyan J Med Res.* 2022;16(2): 153-60. https://doi.org/10.54361/ljmr.16215
29. Rana AK, Ray S. Dyselectrolytemia in hyperglycemic crisis in noninsulindependent diabetes mellitus. *Int J Med Res Health Sci.* 2017;5(2):478. Https://doi.org/10.18203/2320-6012.ijrms20170136
30. Liamis G, Liberopoulos E, Barkas F, Elisaf M. Diabetes mellitus and electrolyte disorders. *World J Clin Cases.* 2014;2(10):488-96. https://doi.org/10.12998/wjcc.v2.i10.488
31. Pérez-Morales RE, Del Pino MD, Valdivielso JM, Ortiz A, Mora-Fernández C, Navarro-González JF. Inflammation in diabetic kidney disease. *Nephron.* 2019;143(1):12-6. https://doi.org/10.1159/000493278
32. Hojs R, Ekart R, Bevc S, Hojs N. Markers of inflammation and oxidative stress in diabetic renal disease. *Nephron.* 2016;133(3):159-62. https://doi.org/10.1159/000447434
33. Jung SW, Moon JY. The role of inflammation in diabetic kidney disease. *Korean J Intern Med.* 2021;36(4):753-66.
34. Han J, Pang X, Zhang Y, Peng Z, Shi X, Xing Y. Hirudin protects against kidney damage in STZ-induced diabetic nephropathy by inhibiting the p38 MAPK/NFκB pathway. *Drug Des Devel Ther.* 2020;14:3223-34.
35. Sevak V, Chinniah R, Pandi S, Krishnaswamy SK, Karuppiah B. D*ifferential expression of pro-inflammatory cytokines in CKD patients.* Preprint. 2021.
36. Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, et al. TNF-α in autoimmune disease and TNF-α inhibitors in therapy. *Int J Mol Sci.* 2021;22(5):2719. https://doi.org/10.3390/ijms22052719
37. Chou CC, Wang CP, Chen JH, Lin HH. Antiatherosclerotic effect of *Hibiscus* leaf polyphenols against TNF-α-induced vascular smooth muscle dysfunction. *Antioxidants.* 2019;8(12):620. https://doi.org/10.3390/ijms22052719
38. Adeva-Andany MM, Adeva-Contreras L, Fernández-Fernández C, Carneiro-Freire N, Domínguez-Montero A. Histological manifestations of diabetic kidney disease and insulin resistance. *Curr Diabetes Rev.* 2023;19(1):5070.
39. Tola FS, Zawdie B, Belay M. Evaluation of uric acid and its correlation with eGFR decline in CKD. *Eur J Clin Biomed Sci.* 2022;8(6):8491.
40. Balaky HM, Kakey IS. Indications of liver and kidney function in non-insulindependent diabetes. *Iraqi J Sci.* 2021;76978.
41. Mizokami-Stout KR, Li Z, Foster NC, Shah V, Aleppo G, McGill JB, et al. Contemporary prevalence of diabetic neuropathy in type 1 diabetes. *Diabetes Care.* 2020;43(4):806-12. https://doi.org/10.2337/dc19-1327
42. Faria J, Ahmed S, Gerritsen KG, Mihaila SM, Masereeuw R. Kidney-based *in vitro* models for druginduced toxicity testing. *Arch Toxicol.* 2019;93:3397-418. https://doi.org/10.1007/s00204-019-02573-0
43. Bull H, Murray PG, Thomas D, Fraser AM, Nelson PN. Acid phosphatases. *Mol Pathol.* 2002;55(2):65-72. https://doi.org/10.1136/mp.55.2.65
44. Brzóska MM, Kamiński M, Supernak-Bobko D, Zwierz K, Moniuszko-Jakoniuk J. Chronic cadmium exposure and kidney damage in rats. *Arch Toxicol.* 2003;77:344-52. https://doi.org/10.1007/s00204-003-0450-6
45. Moss DW, Rosalki SB. *Enzyme tests in diagnosis.* London: Edward Arnold; 1986. p. 8893.
46. Guo J, Pu Y, Zhong L, Wang K, Duan X, Chen D. Lead-induced immune and tissue impairment via oxidative stress and apoptosis. *Ecotoxicol Environ Saf.* 2021;226:112857. https://doi.org/10.1016/j.ecoenv.2021.112857
47. Ertik O, Magaji UF, Sacan O, Yanardag R. *Moringa oleifera* extract protects against valproateinduced oxidative damage in muscle. *Drug Chem Toxicol.* 2023;46(6):121222. https://doi.org/10.1080/0148054.2022.2144876
48. Khedr NF, Talkan OF. Neurotoxicity of arsenic, lead and iron: MAPK activation and oxidative stress. *J Biochem Mol Toxicol.* 2022;36(6):e23040. https://doi.org/10.1002/jbt.23040
49. Balakrishnan BB, Krishnasamy K, Mayakrishnan V, Selvaraj A. *Moringa concanensis* extract ameliorates hyperglycemia-mediated oxidative stress. *Biomed Pharmacother.* 2019;112:108688.
50. Song J, Yu J, Prayogo GW, Cao W, Wu Y, Jia Z, et al. Understanding kidney injury molecule-1: An immune factor in kidney pathology. *Am J Transl Res.* 2019;11(3):121931.
51. Wu T, Ding L, Andoh V, Zhang J, Chen L. Mechanism of hyperglycemia-induced renal cell injury in diabetic nephropathy. *Life.* 2023;13(2):539. https://doi.org/10.3390/life13020539
52. Zhang Y, Dai Y, Raman A, Daniel E, Metcalf J, Reif G, et al. TGF-β1 overexpression induces renal fibrosis in PKD. *Am J Physiol Renal Physiol*. 2020;319(6):F113548. https://doi.org/10.1152/ajprenal.00366.2020
53. Zhao L, Zou Y, Liu F. TGF-β1 in diabetic kidney disease. *Front Cell Dev Biol.* 2020;8:187. https://doi.org/10.3389/fcell.2020.00187
54. D'Alessandro VF, Takeshita A, Yasuma T, Toda M, D'Alessandro Gabazza CN, Okano Y, et al. TGF-β1 overexpression and rapid fibrosis in diabetic conditions. *Int J Mol Sci.* 2022;23(22):142-65. https://doi.org/10.3390/ijms232214265
55. Qiu D, Song S, Chen N, Bian Y, Yuan C, Zhang W, et al. NQO1 alleviates fibrosis by inhibiting TLR4/NFκB and TGF-β/Smad pathways in DN. *Cell Signal.* 2023;108:1107-12. https://doi.org/10.1016/j.cellsig.2023.110712
56. Keelo RMAH, Elbe H, Bicer Y, Yigitturk G, Koca O, Karayakali M, et al. Crocin suppresses diabetic nephropathy progression via modulation of TGF-β1 and oxidative stress. *Chem Biol Interact.* 2022;351:109733. https://doi.org/10.1016/j.cbi.2021.109733
57. Charlton A, Garzarella J, Jandeleit-Dahm KA, Jha JC. Oxidative stress and inflammation in renal and cardiovascular complications of diabetes. *Biology* (Basel). 2021;10(1):18. https://doi.org/10.3390/biology10010018