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RESEARCH ARTICLE

# Renoprotective Potential of Robustaflavone Against Diabetes Induced Kidney Damage Via Regulating Oxidative Stress, Inflammation and Apoptosis

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**ABSTRACT**

Diabetes mellitus (DM) is considered a persistent metabolic disorder in humans and animals that impacts more than 170 million people around the world. DM is associated with nephrotoxicity through ROS overproduction. Robustaflavone (RF) is a bioactive biflavonoid sourced from *Nandina domestica*. This work was carried out to investigate the therapeutic potential of RF against DM-provoked renal injury in rats. Albino rats were portioned into 4 treatment-groups each having 12 rats as follows: Control, DM (65mg/kg), DM + RF (65mg/kg+150mg/kg), and RF (150mg/kg). Current study outcomes displayed that DM-induction increased the levels of glucose, HbA1c, inflammatory, pro-apoptotic, renal injury and oxidative stress markers, while lowering the activities of antioxidants as well as insulin and anti-apoptotic marker levels. Moreover, DM induction instigated numerous severe histological anomalies in renal tissues. However, RF lowered the glucose and HbA1c levels while upregulating the insulin concentration. RF administration enhanced the enzymatic activities of antioxidants, including glutathione peroxidase (GPx), superoxidase dismutase (SOD), glutathione S-transferase (GST), glutathione (GSH), catalase (CAT), and glutathione reductase (GSR) but malonaldehyde (MDA) level and reactive oxygen species (ROS) were lowered. RF administration also lowered the concentration of creatinine, urea, NGAL, urinary proteins, kidney injury molecule-1 (KIM-1), and urobilinogen. Simultaneously, there was an increase in creatinine clearance concentration. Moreover, RF supplementation lowered inflammatory cytokines levels [nuclear factor-kappa B (NF-κB), Interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), cyclooxygenase-2 (COX-2), and interleukin one beta (IL-1β)]. RF administration also lowered pro-apoptotic markers (Bax & Caspase-3) levels, while increasing anti-apoptotic marker (Bcl-2) levels and enhancing histological architecture of renal tissues. These outcomes demonstrate that RF confers significant protection against DM-induced renal injury via targeting oxidative stress, inflammatory and apoptotic pathway.

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## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease affecting around 347 million people all over the globe in 2008 and it is estimated to rise to 694 million by 2050 (Subramanian *et al.,* 2024). Epidemiological studies reveal that DM not only affects humans but also causes significant damage in animals, especially pet animals (Waite *et al.,* 2025). Hyperglycemia is the clinical manifestation of DM, caused by impaired insulin production, release, or activity in humans as well as animals (Mohandes *et al.,* 2023). Chronic hyperglycemia in DM is directly linked to elevated OS (Almousawi, 2025) and inflammation (Kumar *et al.,* 2023). DM, particularly when poorly managed or exacerbated by daily habits such as imbalanced diets, smoking, low physical activity, and liquor consumption, can lead to multisystem organ damage (De Geest and Mishra, 2022). Nonetheless, pancreas is a central player in the pathogenesis of diabetes, diabetic nephropathy is a major reason for end-stage kidney failure and remains as an important therapeutic target (Byrne *et al.,* 2018). Among various organs affected by diabetes, kidney is particularly vulnerable to chronic hyperglycemia-induced oxidative stress, making diabetic nephropathy one of the most severe

and prevalent long-term complications of diabetes (Wang and Zhang, 2024). The kidney is continuously exposed to high glucose flux and metabolic stress, which promotes oxidative damage, inflammation, and apoptosis in renal cells (Turkmen, 2017). Chronic hyperglycemia disrupts renal hemodynamics by inducing glomerular hyperfiltration, mesangial expansion, and thickening of the basement membrane, ultimately leading to progressive renal dysfunction (Wu *et al.,* 2023).

Kidney is notably vulnerable to severe damage due to rise in circulating blood glucose concentrations (Elliott and Oyama, 2025). DM-induced oxidative stress plays a vital function in renal injury by enhancing ROS generation, impairing antioxidant defenses, and leading to mitochondrial dysfunction (Roy *et al.,* 2025). Excessive ROS activates fibrotic and inflammation cascades with NF-κB as a key regulator, thereby promoting deposition of extracellular proteins, and glomerulosclerosis (Jung *et al.,* 2022; Aremu, 2023). Furthermore, chronic inflammation in DM elevates inflammation mediators, which exacerbate renal tubular injury and podocyte apoptosis (Sourris *et al.,* 2024). Morpho-functional alterations in the diabetic kidney are reflected in elevated circulating creatinine as well as urea, along with increased urinary albumin, which serve as clinical biomarkers of renal impairment (Xue *et al.,* 2023). Moreover, diabetic kidneys show increased infiltration of leukocytes, leading to persistent oxidative and nitrosative damage (Mansoor *et al.,* 2022). Collectively, OS, inflammation, and perturbation of mitochondrial function synergistically drive diabetic kidney damage, ultimately progressing to kidney failure (Mitra *et al.,* 2024). Therefore, OS, apoptosis and inflammation are the key parameters that a drug or compound may target to mitigate diabetes associated nephropathy.

Medicinal plants are regarded as an effective source of medication to treat several diseases (Chen *et al.,* 2022; Peng *et al.,* 2023). Biflavonoids are natural compounds with marvelous pharmacological potential and are considered a major part of drug development in pharmacology (Regginato *et al.,* 2021; Haque *et al.,* 2024; Karmakar *et al.,* 2025). Robustaflavone (RF) is a naturally occurring biflavonoid, derived from *Nandina domestica,* with potential pharmacological characteristics. It has been reported that RF possesses anti-viral, anti-allergic, anti-inflammatory, and anti-tumor activities (Sim *et al.,* 2020; Ambarwati *et al.,* 2022; Wu *et al.,* 2023; Singh *et al.,* 2024). These pharmacological attributes suggest that RF may exert protective effects against diseases characterized by oxidative stress and inflammation, including diabetic complications. Hence, the current work was intended to elucidate the therapeutic potential of RF to counteract DM-induced kidney dysfunction.

## MATERIALS AND METHODS

**Chemicals and drugs:** Streptozotocin (STZ) and RF were procured from Merck (USA).

**Animals:** Male adult Sprague-Dawley albino rats (6-7 weeks old, 180-200g weight) were obtained from the animal breeding unit of the University of Agriculture, Faisalabad (UAF), Pakistan, and kept under standardized experimental conditions (23-25℃ temperature, 55-60% humidity and 12h dark/light cycle) and well-ventilated enclosures at experimental facility of the UAF. Animals were given a commercial diet and free access to tap water. All procedures complied with EU-approved guidelines for laboratory animal welfare (Directive 2010/63/EU) and this research was duly approved by the graduate studies and research board (GSRB) of the university (No. DGS/207085-88).

**Experimental induction of diabetes:** Type 1 DM was induced using one-time dose of freshly prepared STZ (65 mg/kg in 0.1M citrate buffer) via i.p. injections. Before STZ injections the animals were fasting for overnight while being given only water (Alsabaani *et al.,* 2024). After administering STZ, rodent chow, water as well as a 5% dextrose solution were supplied to animals overnight to prevent hypoglycemic shock. After 48 hours from the STZ injection, diabetes development was confirmed by determining fasting blood glucose level. Animals with a fasting blood sugar level > 200mg/dL were chosen for the experiment. In the present study, STZ successfully induced diabetes in all treated animals, and no mortality was observed during the experimental period.

**Assessment of Serum Glucose, Insulin and HbA1c:** The blood glucose concentration was determined with a spectrophotometer using the Triner (1969) procedure by using a commercial kit Randox Laboratories, UK. Sandwich ELISA kits obtained at Linco Research, USA were employed to gauge insulin levels. The glycated hemoglobin (HBA1c) concentration was measured through the Helena GLYCO-Tek affinity column protocol supplied by Helena laboratories, USA. HbA1c was included as a parameter of long-term glycemic control since it reflects the average blood glucose level over several weeks and provides an integrated index of chronic hyperglycemia (Sherwani *et al.,* 2016).

**Experimental protocol:** Experimental rats were categorized into 04 groups, with each group containing twelve rats, i.e., Control, DM (65mg/kg), DM+RF (65mg/kg+150mg/kg), and RF (150mg/kg). Diabetes was first induced in the DM and DM+RF groups and after the confirmation of hyperglycemia the RF was initiated in DM+RF group and RF alone group. The rats were anesthetized after 8 weeks using i.p. injection of ketamine (60mg/kg) and xylazine (6mg/kg) and thereafter dissected. Heparinized syringes were utilized to withdraw blood. The kidneys were taken out, with the one kidney fixed in 10% neutral buffered formalin to be subjected to histological analysis and the other one in a Zip-lock bag to be stored at -20℃ to check oxidative stress, inflammatory, antioxidant as well as apoptotic parameters.

**Assessment of oxidants/antioxidants markers:** The enzymatic activity of CAT, GSH, and GSR, were estimated from renal tissues using the methods Chance and Maehly (1955), Jollow *et al.* (1974), and Carlberg and Mannervik (1975), respectively. Moreover, the activities of GPx, SOD, and GST, were quantified using established methods of Lawrence and Burk (1976), Kakkar *et al.* (1984), and Couri and Abdel-Rahman (1979), respectively. MDA and ROS

levels were gauged by methods of Placer *et al.* (1966), and Iqbal *et al.* (1996).

**Assessment of levels of kidney function parameters:** The concentrations of kidney parameters were determined from blood serum samples using ELISA kits. Albumin (ab108789), Urea (ab83362), creatinine (ab65340) and creatinine clearance ELISA kits were from Abcam (UK), and Urinary protein (MBS008020), KIM-1 (MBS564137), urobilinogen (MBS2548454), and NGAL (MBS260195) ELISA kits were from MyBioSource (US). The guidelines provided by manufacturer were strictly followed during the analysis.

**Assessment of levels of inflammatory parameters:** ELISA kits were used to determine inflammatory indices [COX-2 (CSB- E13399r), IL-6 (CSB-E04640r), TNF-α (CSB-E11987r), NF-κB (CSB-E13148r), and IL-1β (CSB-E08055r)] from the renal tissues, and the procedure was performed according to the guideline manual provided with the kits brought from Cusabio, USA.

**Assessment of levels of apoptotic parameters*:*** The levels of Bax (ELK5698), Caspase-3 (ELK1528), caspase-9 (ELK1531), and Bcl-2 (ELK9198) were measured from the renal tissues by using commercially available ELISA kits (ELK Biotechnology, USA) as per the directions provided by the manufacturer.

**Histopathological examination:** Samples of renal tissues were immersed in 10% formaldehyde for 10 days after which they were dehydrated using increasing grades of alcohol (70, 80, 90, and 100% ethanol). The tissues were then cleared in xylene and fixed in paraffin-wax. Thin paraffin sections (4µm thickness) were prepared via microtome. The staining of slides was done with Hmetoxylin & Eosin method and visualized with the help of optical microscope to assess histopathological abnormalities.

**Statistical analysis:** The entire set of data was shown as mean ± SE. Using the Graph Pad Prism J software, ANOVA (one-way) and Tukey's post hoc test were carried out for pair-wise group comparisons with statistical significance kept at $P<0.05$.

## RESULTS

**Influence of DM and RF on glucose, HbA1c, and insulin:** DM induction substantially ($P<0.05$) increased the serum glucose and HbA1c levels while downregulating the insulin production. Furthermore, DM group co-administered with RF notably ($P<0.05$) reversed all the above-mentioned parameters, as compared to DM-induced rats. Moreover, non-significant variations were spotted between RF alone and the control group (Table 1).

**Table 1:** Protective role of RF on glucose, HbA1c and insulin profile

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | DM | DM+ RF | RF |
| Glucose (mg/dl) | 94.1±1.82$^{c}$ | 384.32±5.20$^{a}$ | 146.18±5.41$^{b}$ | 92.12±1.62$^{c}$ |
| HbA1c (%) | 5.01±0.05$^{b}$ | 12.96±0.30$^{a}$ | 5.94±0.49$^{b}$ | 4.99±0.04$^{b}$ |
| Insulin (Mu/l) | 42.20±1.30$^{a}$ | 8.34±0.68$^{b}$ | 41.23±1.01$^{a}$ | 42.72±1.17$^{a}$ |

Note: Distinct superscripts (a/b/c) on various values (Mean±SE) demonstrate discrepancies among groups.

**Influence of DM and RF on biochemical parameters:** DM induction significantly ($P<0.05$) downregulated antioxidant enzymes activities (GST, SOD, GPx, CAT, GSH along with GSR) and elevated MDA and ROS levels. Furthermore, administration of RF to DM group notably ($P<0.05$) restored above-mentioned enzymes' activities, whereas lowered OS parameters as compared to DM-induced rats. Moreover, non-significant variations were spotted between RF alone and the control group (Table 2).

**Table 2:** Protective role of RF on antioxidant and oxidative stress profile

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | DM | DM+ RF | RF |
| CAT (U/mg protein) | 17.73±0.83$^{a}$ | 8.61±0.49$^{b}$ | 16.57±0.71$^{a}$ | 18.47±0.91$^{a}$ |
| SOD (U/mg protein) | 13.54±0.55$^{a}$ | 7.46±0.44$^{b}$ | 14.21±0.42$^{a}$ | 14.70±0.69$^{a}$ |
| GSR (nM NADPH oxidized/min/mg tissue) | 7.59±0.29$^{a}$ | 1.57±0.34$^{b}$ | 7.16±0.41$^{a}$ | 8.43±0.63$^{a}$ |
| GPx (U/mg protein) | 34.96±0.88$^{a}$ | 15.89±1.02$^{b}$ | 33.59±0.53$^{a}$ | 37.19±1.02$^{a}$ |
| GSH (U/mg protein) | 28.39±0.64$^{ab}$ | 11.53±0.52$^{c}$ | 26.21±1.02$^{b}$ | 29.58±0.55$^{a}$ |
| GST (U/mg protein) | 43.01±0.76$^{a}$ | 17.39±0.87$^{b}$ | 40.47±0.67$^{a}$ | 44.07±1.32$^{a}$ |
| MDA (nmol/g) | 0.96±0.47$^{b}$ | 4.16±0.34$^{a}$ | 1.45±0.29$^{b}$ | 0.70±0.39$^{b}$ |
| ROS (nmol/g) | 2.09±0.41$^{b}$ | 6.76±0.28$^{a}$ | 1.93±0.21$^{b}$ | 1.78±0.40$^{b}$ |

Note: Distinct superscripts (a/b/c) on various values (Mean±SE) demonstrate discrepancies among groups.

**Influence of DM and RF on renal injury markers:** DM induction substantially ($P<0.05$) compromised kidney efficiency by increasing level of serum urea, concentration of serum creatinine, urine protein, urobilinogen, KIM-1, and NGAL, simultaneously reducing the concentration of serum albumin, along with creatinine clearance versus non-treated animals. Nonetheless, supplementation of RF in DM induced group notably ($P<0.05$) restored their levels towards the values observed in the control group, as compared to the DM group. Furthermore, the above-mentioned indices showed no alterations in RF and control rats (Table 3).

**Table 3:** Protective role of RF on kidney injury markers

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | DM | DM+ RF | RF |
| Urea (mg/dL) | 25.05±1.04$^{bc}$ | 67.79±1.65$^{a}$ | 29.89±1.41$^{b}$ | 19.50±0.59$^{c}$ |
| Creatinine (mg/dL) | 1.12±0.33$^{b}$ | 7.23±0.12$^{a}$ | 2.04±0.10$^{b}$ | 0.83±0.38$^{b}$ |
| Creatinine Clearance (mL/min) | 2.35±0.17$^{a}$ | 0.23±0.11$^{b}$ | 2.15±0.23$^{a}$ | 2.65±0.24$^{a}$ |
| Albumin (mg/dl) | 8.28±0.35$^{a}$ | 1.03±0.42$^{b}$ | 7.32±0.41$^{a}$ | 8.50±0.40$^{a}$ |
| Urinary proteins (mg/dl) | 12.05±0.86$^{c}$ | 66.59±0.89$^{a}$ | 16.92±0.82$^{b}$ | 11.45±0.69$^{c}$ |
| Urobilinogen (mg/dl) | 3.47±0.35$^{c}$ | 17.65±0.28$^{a}$ | 6.89±0.46$^{b}$ | 3.2±0.39$^{c}$ |
| KIM-1 (ng/day) | 0.37±0.25$^{b}$ | 6.00±0.42$^{a}$ | 1.63±0.27$^{b}$ | 0.28±0.21$^{b}$ |
| NGAL (mg/ml) | 1.03±0.27$^{c}$ | 7.87±0.23$^{a}$ | 2.58±0.34$^{b}$ | 0.83±0.31$^{c}$ |

Note: Distinct superscripts (a/b/c) on various values (Mean±SE) demonstrate discrepancies among groups.

**Influence of DM and RF on inflammatory parameters:** DM induction remarkably ($P<0.05$) elevated inflammatory cytokine levels with respect to control group. However, provision of RF in DM induced group markedly ($P<0.05$) lowered aforementioned indices level relative to DM-exposed animals. Moreover, the control and RF only supplemented groups did not present noticeable alterations (Table 4).

**Table 4:** Protective role of RF on inflammatory markers

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | DM | DM+ RF | RF |
| NF-kB (ng/g tissue) | 33.44±0.97$^{bc}$ | 74.06±0.86$^{a}$ | 36.87±0.84$^{b}$ | 31.75±1.19$^{c}$ |
| TNF-α (ng/g tissue) | 27.33±0.64$^{bc}$ | 63.89±1.01$^{a}$ | 30.56±0.70$^{b}$ | 26.53±0.71$^{c}$ |
| IL-1ß (ng/g tissue) | 23.40±0.61$^{c}$ | 57.71±0.84$^{a}$ | 26.67±0.66$^{b}$ | 22.57±0.69$^{c}$ |
| IL-6 (ng/g tissue) | 13.94±0.73$^{c}$ | 51.19±1.05$^{a}$ | 19.21±0.82$^{b}$ | 12.97±0.93$^{c}$ |
| COX-2 (ng/g tissue) | 38.65±0.80$^{c}$ | 96.39±1.25$^{a}$ | 47.88±0.85$^{b}$ | 38.02±0.88$^{c}$ |

Note: Distinct superscripts (a/b/c) on various values (Mean±SE) demonstrate discrepancies among groups.

**Influence of DM and RF on apoptotic indices:** DM induction remarkably (P<0.05) elevated the levels of Bax, caspase-9, and caspase-3 and lowered Bcl-2 levels with respect to control-group. Conversely, DM+RF combined group the levels of caspase-3, caspase-9 and Bax were significantly (P<0.05) lowered, and Bcl-2 levels were elevated. Additionally, no appreciable variations were noted in above-said indices levels across control and RF only treated group (Table 5).

**Table 5:** Protective role of RF on apoptotic markers

| Parameters | Groups | | | |
|---|---|---|---|---|
| | Control | DM | DM+ RF | RF |
| Bax (pg/mL) | 5.58±0.45$^{c}$ | 15.40±0.50$^{a}$ | 7.43±0.39$^{b}$ | 5.22±0.51$^{c}$ |
| Bcl-2 (ng/ mL) | 13.99±0.70$^{a}$ | 3.52±0.43$^{b}$ | 12.53±0.84$^{a}$ | 15.85±1.40$^{a}$ |
| Caspase-3 (pg/ mL) | 4.92±0.43$^{b}$ | 18.96±0.97$^{a}$ | 6.69±0.26$^{b}$ | 4.59±0.48$^{b}$ |
| Caspase 9 (pg/mL) | 6.58±0.47$^{c}$ | 25.54±1.06$^{a}$ | 10.35±0.55$^{b}$ | 6.14±0.63$^{c}$ |

Distinct superscripts (a/b/c) on various values (Mean±SE) demonstrate discrepancies among groups.

**Influence of DM and RF on histopathological parameters:** In comparison to control group, DM intoxication was linked to dilation, vacuolation, degeneration, widened Bowman's capsule and necrosis. Nonetheless, RF provision counteracted the severity of the histopathological anomalies relative to DM-induced animals. Moreover, no meaningful distinctions were identified between the RF-only provided and no-treatment rats (Fig. 1).

## DISCUSSION

DM has been considered a global health concern and a major risk factor, which usually leads to severe morbidity and mortality (Gao *et al.,* 2022). Renal tissues are highly vulnerable to diabetes-induced glycaemic disruptions, which are reflected by disruption in mitochondrial processes in kidney (Gal *et al.,* 2023). The plant-based compounds have elicited interest among the scientific community owing to their exceptional curative efficacy to cure numerous ailments (Ahmed *et al.,* 2025). RF, a compound extracted from plants, possesses the potential to counter oxidative stress, inflammation and improve the antioxidant system of the body. As a result, this work was sought to quantify the renoprotective potency of RF to ameliorate diabetes prompted renal toxicity via regulating oxidative stress, inflammation, and apoptosis.

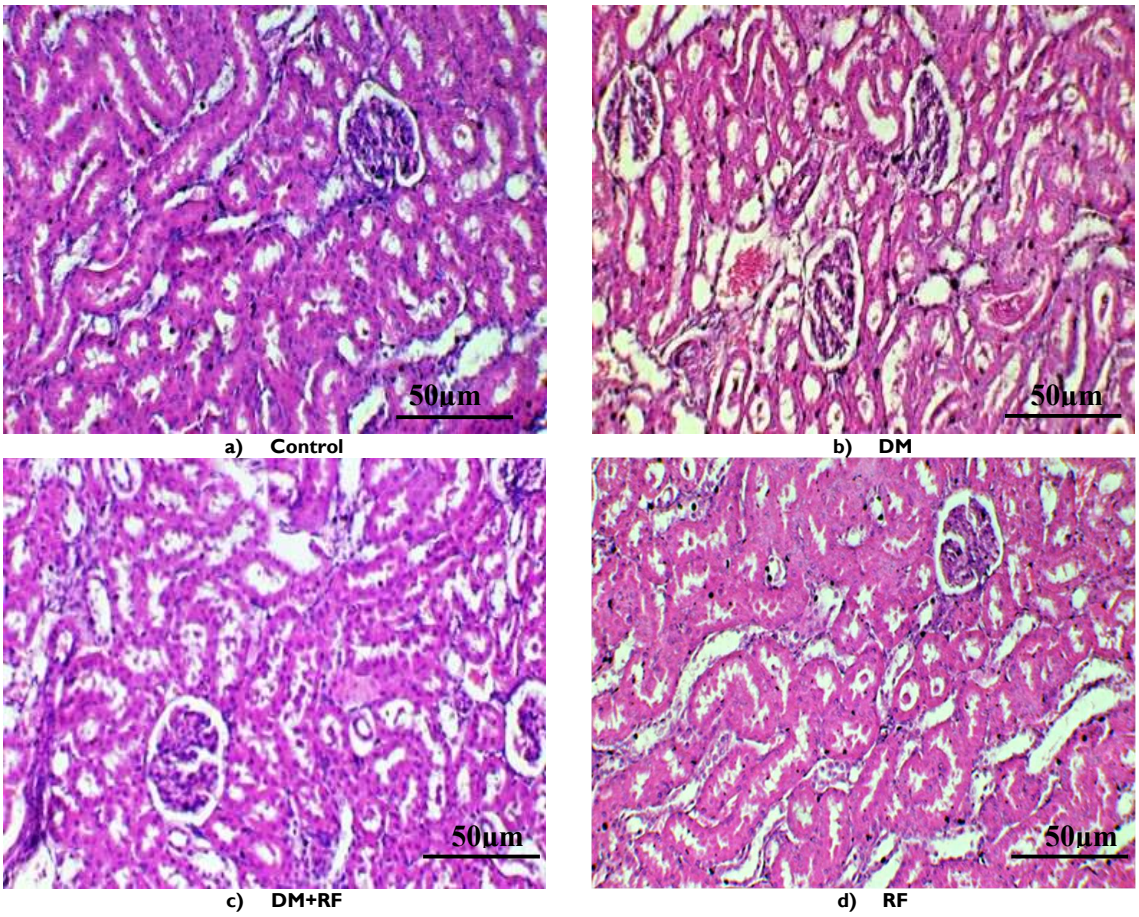


**Fig. 1:** Photomicrographs shows the influence of DM and RF on renal tissues (H&E, 400X); **a)** control group, displaying normal histological structure. b) DM treated group, showing dilation, vacuolation, degeneration, and widened Bowman's capsule necrosis in the kidney tissues. c) DM+RF-treated group showing improved histoarchitecture with reduced degenerative architecture in renal tissues. d) RF-treated group showing normal renal histoarchitecture.

Our findings demonstrated that DM induction increased the levels of serum glucose and HbA1c while decreasing the insulin concentration. Also, it reduced the antioxidant enzymes (CAT, GPx, SOD, GSR & GSH) while increasing the levels of ROS & MDA. Antioxidant enzymes are primary barriers against oxidative damage (Öner *et al.,* 2023). A study on veterinary animals showed a strong relationship between ROS and cellular injury provoked by DM (Esteves-Monteiro *et al.,* 2025). Excessive ROS impairs the functionality of important enzymes and damages cell structure, hence responsible for the development of different diseases (Şahin *et al.,* 2024). Besides, elevated free radicals lower the functioning of antioxidants, compromising the internal defense system of the cell. It has been shown that induction of DM lowers antioxidants activity due to reduced NADH availability coupled with higher $O_2^-$ radical (Ezz *et al.,* 2023). Current study findings are supported by Andonova *et al.* (2023) who stated that DM-treatment decreases the enzymatic activity of antioxidants along with elevating lipid peroxidation. Nevertheless, concurrent treatment of DM+RF notably improved the activities of antioxidant enzyme and decreased MDA and ROS levels due to the antioxidant capacity of RF. The effect of RF may be attributed to the free radical scavenging and redox-modulating properties of biflavonoid, like RF.

This investigation showed that DM induction compromised kidney function by increasing the levels of urea, creatinine, urinary protein, urobilinogen, KIM-1, and NGAL, and reducing the concentration of serum albumin, and creatinine clearance. Biomarkers of kidney injury reflect renal damage; a slight rise in their concentration within the bloodstream signifies serious kidney disorder (Kim *et al.,* 2024). These results are in line with a recent study, in which it was demonstrated that DM induction leads to renal dysfunction, as evidenced by escalated concentrations of renal injury parameters (de Andrade *et al.,* 2023). More importantly, administration of RF with DM lowered renal injury marker levels, thus displaying renoprotective efficacy of RF.

Current study confirmed that diabetes induction escalated the concentrations of inflammatory parameters in kidney. In diabetic nephropathy, inflammatory pathways (i.e., NF-κB) are stimulated in the response to excessive OS, which results in the production of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α). This activation of inflammatory pathways further increases the severity of renal damage (Li *et al.,* 2018). NF-κB is an important stimulator that enhances the synthesis of these inflammatory mediators, ultimately promoting chronic inflammation and subsequent oxidative impairments (Aremu, 2023). COX-2 is also one of the key inducers of inflammation in the kidney (Thai *et al.,* 2023). Our results support the fact that DM increases the inflammation indices, thereby reflecting inflammation within the kidneys. However, co-administration of RF with DM effectively reduced the levels of the above-mentioned markers of inflammation. This remarkable reduction in the levels of inflammatory markers suggest that RF exerts potential anti-inflammatory effects via the suppression of ROS production as well as inhibition of inflammatory signaling cascades, importantly NF-κB pathway. These outcomes suggest that RF exhibits similar anti-inflammatory properties as shown by other reported flavonoids i.e., didymin (Hamza *et al.,* 2025), sudachitin (Hayat *et al.,* 2024), and isorhoifolin (Ijaz *et al.,* 2025).

Our research anticipated that DM induction caused an elevation in the levels of Bax, caspase-9, and caspase-3. In contrast, it decreased Bcl-2 levels in renal tissues. Oxidative stress and inflammation are closely associated with mitochondrial damage and induction of apoptotic pathways (Sinha *et al.,* 2013). The disruption in mitochondrial outer membrane due to OS alters proapoptotic (Bax) and anti-apoptotic (Bcl-2) ratio. The production of Bax increases and Bax as well as related proteins translocate to external membrane of mitochondria, rising its permeability as well as facilitating the Cyt c discharge, caspase proenzymes activation, and stimulation of other apoptotic mediators. Reduced Bcl-2 expression further enhances Cyt c release into the cytoplasm, where it binds Apaf-1 to form the apoptosome. This complex activates caspase-9, which triggers downstream effector caspases, such as caspase-3, culminating in apoptosis (Araj-Khodaei *et al.,* 2024). In our study, DM significantly upregulated the levels of caspase-3, caspase-9, and Bax accompanied by reduced Bcl-2 in kidney tissues, thereby promoting apoptosis. These findings are consistent with previous reports demonstrating diabetes-induced pro-apoptotic effects in the kidney, characterized by altered Bax/Bcl-2 ratios and caspase activation (Belhan *et al*., 2023). However, RF treatment effectively restored these parameters almost near to the control group, thereby demonstrating its anti-apoptotic potential. The regulation of apoptotic markers by RF shows that RF may safeguard renal tissues from apoptosis via sustaining the integrity of mitochondria. These anti-apoptotic properties of RF are comparable to other natural flavonoids i.e., vitexin (Noor *et al.,* 2022; Ghafoor *et al*., 2024).

Microscopic examination of renal sections from the control group revealed well-preserved kidney architecture, characterized by intact glomeruli, normal Bowman's space, and regularly arranged epithelial cells. In contrast, kidneys from DM-induced rats exhibited pronounced histopathological abnormalities, including cellular edema, glomerular contraction, disruption of the Bowman's capsule, and widening of the capsular space. These structural derangements are indicative of DM-induced oxidative stress and inflammatory-mediated renal injury. Comparable renal alterations, such as glomerular shrinkage, epithelial degeneration, and interstitial inflammatory infiltration following DM exposure, have also been documented by Elbadawy *et al.* (2024). Notably, administration of RF produced significant restoration of renal architecture and morphological improvement. The observed renoprotective effects of RF are likely attributable to its potent free radical scavenging capacity, along with its antioxidant and anti-inflammatory properties.

An important observation in the current research was the presence of non-significant differences in the results of the control and RF-only groups. These outcomes suggest that RF did not produce any deleterious impact (i.e., inflammation, apoptosis, cytotoxicity and oxidative stress) on the renal tissues of rats. The maintenance of the levels of these parameters near to control suggests that RF is biologically safe to use at the administered dose and does not interfere with cellular homeostasis. Therefore, the

current outcomes show the favorable safety profile of RF and supports its potential as a medicinal agent against diabetic nephropathy.

The nephroprotective effects of RF observed in the current research may involve direct regulation of antioxidant, apoptotic and inflammatory signaling and indirect enhancement of renal physiological homeostasis. The remarkable modulation of inflammatory cascades (NF-κB pathway), oxidative stress and mitochondria associated apoptosis by RF demonstrates that RF protects the renal tissues from hyperglycemia-stimulated diabetic nephropathy. Additionally, RF may produce indirect physiological effects via protective hemodynamic stability of kidney. OS and inflammation are reported to disrupt the glomerular filtration via causing endothelial dysfunction and lowering the availability of nitric oxide (Papi *et al.,* 2019). So, by restoring the redox balance, RF may enhance the renal microcirculation and endothelial function, which may further improve the filtration efficiency as well as glomerular blood flow. This mechanism was partially explained by the observed normalization of serum creatinine and urea levels, following RF treatment, suggesting the indirect renoprotective mechanism of RF. Collectively, it suggests that RF mediates renal toxicity via a multi-targeted approach involving the attenuation of oxidative stress, inflammation and apoptosis, that are the major pathological events in diabetes-induced renal injury.

Although this study discusses an important aspect to counter diabetes induced damages in renal tissues, there are some limitations in this study. The present study followed a cross-sectional experimental design, in which parameters were evaluated at the end of the treatment period rather than longitudinally in the same animals before and after RF administration. Although this approach is commonly employed in experimental pharmacological studies, it does not account for individual baseline variations among animals, which may influence the observed outcomes. A longitudinal design assessing parameters in the same animals before and after treatment would provide stronger evidence for causal therapeutic effects and should be considered in future investigations.

**Conclusions:** In conclusion, this work revealed that RF attenuated DM induced renal toxicity. RF improved the antioxidant defense, and histological structure of renal tissues. Moreover, RF lowered the levels of inflammatory, apoptotic and oxidative stress parameters. These results suggest that RF may modulate antioxidant, inflammatory and apoptotic pathways and protect the renal tissues from diabetes induced damage i.e., diabetic nephropathy. However, this research was a cross-sectional study, further longitudinal and clinical studies are required to validate its therapeutic in human as well as animals.

**Authors contribution:** NG and NE conceived and designed the study. NE supervised the experimental procedures. NG drafted the initial manuscript. NG and NE performed the statistical analysis, critically revised the manuscript for important intellectual content, and approved the final version for submission.

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