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RESEARCH ARTICLE

Quercetin Mitigates Diabetic Nephropathy in Rats via Keap1/Nrf2/HO-1 Signaling Pathway

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Abstract

A severe diabetic complication, diabetic nephropathy, progresses to terminal kidney disease. A chronic hyperglycemia-related excess of reactive oxygen species results in the advancement of diabetes complications. Through streptozotocin-induced diabetic nephropathy in rats, the present study investigated Quercetin's reno-protective effect by upregulating nuclear factor-erythroid-related factor 2 (Nrf2) to cope with oxidative stress. During eight weeks study, daily food-water and weekly body weight were evaluated while biochemical, antioxidant parameters and genetic expression (Nrf2, Hemeoxygenease-1, Nuclear factor kappa B, Interlukin-6, and Caspase-3) were assessed at the end. The outcomes were interpreted using ANOVA, and the significance was determined using Dunnett's test. Quercetin treatment for eight weeks significantly controlled hyperglycemia, dyslipidemia, and downregulated inflammatory activators NFκB, IL-6, and Caspase-3. The significant upregulation of Nrf2 gene expression reduced oxidative damage by promoting Antioxidant response elements and initiating downstream cascade (HO-1 and antioxidant enzymes). The results are supported by histopathology. Experimental evidence suggests that Quercetin can fight metabolic disorders and their related microvascular diseases by activating Nrf2.

Keywords: Caspase-3, Diabetic Nephropathy, HO-1, Nrf2, NFκB, Oxidative Stress, Quercetin

1. Introduction

Diabetic Nephropathy (DN), recently described as Diabetic Kidney Disease (DKD), typically affects individuals with diabetes mellitus type 1 (DM-1) or type 2 (DM-2) without a lengthy history of the condition[1]. DKD affects 20% of 400 million diabetics worldwide[2]. However, the current regimen that targets lowering glucose levels is ineffective in attenuating renal damage. Additionally, co-treatment using reno-protective drugs has limited efficacy owing to its toxicities[3]. Hence, current research has diverted toward the development of effective medicines that aims myriad targets to combat microvascular complications accompanying diabetes.

Multifactorial pathogenesis is involved in diabetic nephropathy. It is mainly attributed to hyperglycemia, which causes the unwarranted production of free radicals[4,5]. According to research on renal cells, hyperglycemia significantly increases oxidative stress, leading to DN[6,7]. Nuclear factor-erythroid-related factor 2 (Nrf2) is a developing therapeutic target for numerous ailments such as cancer[8], neurodegenerative diseases[9] and diabetes[10]. It is among the essential strategies for coping with oxidative damage. It regulates several antioxidant enzymes and proteins that help the body maintain homeostasis and boost cell survival by detoxifying xenobiotics and scavenging free radicals[11]. Various genes are the target of Nrf2, namely "Heme Oxygenase-1" (HO-1), "NAD(P)H Quinone Oxidoreductase" (NQO1), "γ-Glutamyl Cysteine Synthetase" (γGCS), and "Glutathione S-Transferase" (GST)[10].

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In recent years, the significance of Nrf2 in diabetes-induced complications has emerged. For example, the activation of Nrf2 by Sulforaphane restored the biochemical abnormalities of vascular endothelium induced by hyperglycemia and Reactive Oxygen Species (ROS)[12]. Furthermore, He and colleagues demonstrated that Nrf2 protects primary cardiomyocytes against hyperglycemia-induced oxidative stress isolated from Nrf2+/+ and Nrf2−/− mice[13]. Additionally, in-vivo studies have identified Nrf2 as a protective factor against diabetes induced by streptozotocin (STZ)[14,15]. Furthermore, glucose is redirected to various metabolic pathways by oxidative stress, including Protein Kinase C, hexosamine, polyol, and Advanced Glycation End product pathways, which produce inflammatory cytokines that contribute to DKD, including Tumor Necrosis Factor (TNF) and IL (interleukin)-1, IL-6, IL-18[16].

In the last decade, current research has shifted to controlling oxidative stress and regulating inflammatory cytokines to combat diabetic nephropathy[17-19]. The studies also concluded that natural bioactive substances handle oxidative stress excellently and suppress inflammatory markers[17-22].

An established dietary component, Quercetin (Q), a widely occurring secondary metabolite, was studied to determine if it could activate Nrf2 and slow diabetes-induced nephropathy progression in STZ-treated rats. The phytonutrient Quercetin belongs to a member of the flavanol group, commonly found in citrus fruits, red wine, apples, red onions, teas, and berries[23]. It is a crucial antioxidant agent that neutralizes oxygen radicals that cause necrosis, membrane peroxidation, endometriosis[24], autoimmune disease[25], cardiovascular disease[26,27], cancer[28], asthma[29], and neurological disorders[23]. According to earlier research, Quercetin suppresses lipid peroxidation, xanthine oxidases, plasma glucose levels, and serum lipid profiles via regenerating vitamin E, islet cells, and regulating lipogenic genes[30,31]. Additionally, it increases Antioxidant enzyme activity in C57BL/6J mouse livers[30] and the DM-2 rat model[32]. According to Wang and his colleagues, Quercetin at 25 - 100 mg/kg inhibited the activation of the renal NLRP3 inflammasome, alleviating the associated nephrotoxicity in streptozotocin-induced diabetic rats[33]. Quercetin's effect on the Nrf2-Keap-1 pathway has been extensively investigated in numerous diseases, such as diquat-induced oxidative stress in porcine enterocytes[34], drug-induced hepatotoxicity[35], and chemoprotection in human HaCaT cells and BJ foreskin fibroblasts[36]. In numerous animal models, the antidiabetic activities of Quercetin were investigated[33,37]. Nevertheless, no scientific data on a prototype accurately represents the progression of DM-2 in individuals, from insulin resistance to dysfunctional cells. Thus, a need arises to study the preventive role of Quercetin in DKD through multiple approaches.

2. Materials and Methods

2.1 Drug, Chemicals, Kits, and Preparation of Solutions

Analytical-grade chemicals were obtained from Sigma Aldrich Pvt. Ltd., including glucose in pure form. All biochemical analyses were conducted with kits acquired from i-chem, Jeev Diagnostics Pvt. Ltd. Eurofins Genomics, Bangalore, provided the primers. Applied Biosystems, Thermoscientific Inc., Ahmedabad, provided the qRT-PCR Kit.

Metformin as a gift sample by SUN Pharmaceutical Ind. Ltd. Streptozotocin (STZ) and Quercetin (Q) procured from Sigma Aldrich Pvt. Ltd. A fresh suspension of Quercetin and Metformin was prepared by suspending them in a 0.5% carboxymethyl cellulose (CMC) solution.

In this experiment, the amount of streptozotocin (STZ; Sigma) needed was estimated. The final dose of 20 mg/kg/rat was calculated, and the required amount was previously weighed in a microcentrifuge tube, dissolved in sufficient sodium citrate buffer, pH 4.5, and covered from light for no more than 15 to 20 minutes.

2.2 Experimental Animals and Diet

Forty-eight male Sprague Dawley (SD) rats weighing 170-200 g with good health were acquired from Zydus Research Centre, Ahmedabad. SD rats were categorized as three per hutch under strictly controlled 22 ± 2 °C temperature, 55 ± 5 % humidity, and day-night rotation of 12 hours. A standard laboratory diet was provided to the animals, as well as unrestricted water access. Institutional Animal Ethical Committee approved Protocol APC/2021-IAEC/2114 following clearance by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying Department of Animal Husbandry and Dairying, Government of India.

All animals had free access to either a conventional food or the High Fat Diet (HFD), comprising 22.7% vegetable shortening, 45.5% standard chow, 9% sucrose, 22.7% lard, and water. The regular chow diet provided 3.97 calories per gram, while the HFD delivered 6.25 calories per gram[38]. The produced HFD was refrigerated at 7 ± 2 °C for up to four days.

2.3 Induction of Experimental Diabetes[38]

Chronic hyperglycemia was established by exposing the islets of Langerhans to persistent stress by the Double Sequence of Repetitive Injections (DSRI) of STZ at varying time intervals with a set dose. A dose of STZ (20 mg/kg body weight) was injected intraperitoneally on days 1, 3 and 5 of the first set and after 15 days (on days 21, 23 and 25) of the second set. A portable glucose meter was used to measure fasting glucose levels within 48 hours of STZ injection, and animals with serum glucose levels above 250 mg/dl were included in the experiment.

2.4 Treatment Protocol for Diabetic Rats

All animals were arbitrarily assigned into six groups depending on their serum glucose levels, with n = 8 animals per group. Normal Control (NC) + Vehicle (1ml p.o) had unrestricted access to regular chow and RO water - Group I (NC), Diabetic Control (DC) (HFD + STZ: 20 mg/kg) + Vehicle (1ml p.o) - Group II (DC), HFD + STZ + Std metformin (120 mg/kg, p.o) - Group III (DM + Met 120), HFD + STZ + Quercetin dose 1 (25mg/kg, p.o) - Group IV (DM + Q25), HFD + STZ + Quercetin dose 2 (50 mg/kg, p.o) - Group V (DM + Q50), HFD + STZ + Quercetin dose 3 (100mg/kg, p.o) - Group VI (DM + Q100). The drug treatment continued for eight weeks.

Each group's initial and final body weights were recorded throughout the trial. In addition, the weekly consumption of food and water was tracked. After the therapy (after eight weeks), animals were kept under fasting conditions for the next 24 hours. A clean, dry centrifuge tube was used for obtaining blood samples under anesthesia from the retro-orbital plexuses. After centrifuging at 3000 rpm for 10 minutes, the serum was kept at -20 °C for investigation. Serum samples using commercially available kits were analyzed for biochemical parameters such as glucose, creatinine, uric acid, BUN, Urea, albumin, triglyceride, cholesterol, and HDL in the eighth week by autoanalyzer (Turbochem 100, USA) as per manufacturer directions. LDL (Eq-1) and VLDL (Eq-2) were calculated using the following formula[39].

$$\mathbf{LDL} = \frac{Total\ Cholesterol - (Triglycerides + HDL\ Cholesterol)}{5} \quad \text{Eq-1}$$

$$\mathbf{VLDL} = \frac{Triglycerides}{5} \quad \text{Eq-2}$$

2.5 Evaluation of Insulin Sensitivity[40,41]

2.5.1 Evaluation of Serum Insulin and HOMA-IR

Using a commercial kit, the Enzyme-Linked Immunosorbent Assay (ELISA) technique was employed to quantify plasma insulin levels as per the manufacturer's instruction, and the absorbance was measured. The luminescence method was used to determine the results of the rat insulin, and the glucose oxidase method was used to measure the blood glucose. In order to calculate the insulin sensitivity index, the following formula was used.

$$\mathbf{HOMA\ IR} = \frac{serum\ insulin\ (mmol/L) \times (blood\ glucose (mmol/L)}{22.5} \quad \text{Eq-3}$$

2.5.2 Evaluation of OGTT

Prior to OGTT, 12 hour fasting was ensured in all animals. Blood glucose was measured before glucose administration using glucometer. Then, 2g/kg body weight of glucose solution (40 per cent) was administered via oral gavage and glucose levels were measured at 30, 60, 90, 120 and 180 minutes.

2.6 Evaluation of Lipid Peroxidation and Antioxidant Enzymes

Animals were humanely euthanized before the kidneys were rapidly removed, cleaned in an ice-cold saline solution and blotted. A homogenizer was used to homogenize kidney tissues at 10% w/v using Tris-HCl (0.1 M, pH 7.4). The suspension was centrifuged at 4°C for 10 minutes at 1000 g. The concentration of antioxidant enzymes, including GSH, Catalase and SOD, was estimated. Additionally, lipid peroxidation was evaluated by measuring MDA levels.[17].

Table 1. RT PCR amplification gene sequence

Genes	Sequence
β-Actin_ F	CCCGCGAGTACAACCTTCTTG
β-Actin_ R	GTCATCCATGGCGAACTGGTG
Nrf2_F	TTGTAGATGACCATGAGTCGC
Nrf2_R	CAGGGGTGGTGAAGACTGAG
HO-1_F	GTAAATGCAGTGTTGGCCCC
HO-1_R	ATGTGCCAGGCATCTCCTTC
IL-6_F	GCTCTGGTCTTCTGGAGTTCC
Il-6_R	GGAGAGCATTGGAAGTTGGG
Caspase-3_F	GAGCTTGGAACGCGAAGAAA
Caspase-3_R	TAACCGGGTGCGGTAGAGTA
NFκB_F	GAGGCCATTGAAGTGATCCAG
NFκB_R	TGAGTTTGCGGAAGGATGTCT

2.7 Real-Time PCR

The Quantitect SYBR green qRT-PCR Kit was used to accomplish both cDNA synthesis and PCR at the same time. The QuantStudioTM 5 Real-Time PCR system was used for quantitative RT-PCR (Applied Biosystems, Foster, California, USA). TRIzol reagent helped in retrieving total RNA from kidney tissue. The β-Actin coding gene from the housekeeping gene was utilized as an internal control. A relative cycle threshold (Ct) method (2-ΔΔCt) helped measure the relative expression. Table 1 shows the forward and reverse gene amplification sequences.

2.8 Statistical Analysis

The outcomes are depicted in the form of a mean ± SEM. In graph pad prism 6.0, the statistically significant heterogeneity amongst the groups was examined using ANOVA and Dunnett's posthoc test.

3. Results and Discussion

3.1 Effect of Quercetin on Body Weight in Diabetic Nephropathy

At the beginning of the investigation, the weights of all the animals were 200 ± 50 g. However, in the fifth week, HFD therapy increased body weight in all groups. After DSRI of STZ, the model group showed a substantial reduction in weight owing to severe catabolism that may be caused by compensatory hyperinsulinemia contrary to hyperglycemia and a delayed insulin synthesis, subsequently the partial annihilation and depletion of β-cells, which mimics the natural genesis of DM-2. However, the most prominent reduction in body weight was seen in the fifth week (226.3 ± 6.614, $p < 0.0001$) compared to the normal rats (302 ± 10.34). Even though HFD was maintained throughout therapy, Quercetin (25, 50 and 100 mg/kg, respectively) and Metformin (120 mg/kg) medication prevented a rise in body weight compared with model control groups (Figure 1).

With n = 8 animals in each group, the findings were represented as mean ± SEM. $p < 0.05$ and < 0.01, <0.001, and <0.0001 were chosen as significance levels. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

3.2 Effect of Quercetin on Blood Glucose in Diabetic Nephropathy

Hyperglycaemia leads to ROS generation culminating in long-term complications; therefore, the optimal glycaemic level is essential for preventing diabetic complications[13]. Thus, when serum glucose levels were assessed in the eighth week, it was observed that negative control rats had a substantial rise in glucose levels $p < 0.001$ relative to normal control. Our study's results agree with previous studies of similar designs[17,42]. Ironically, treatment with Metformin (120 mg/kg) reduced the glucose levels, while Quercetin (25, 50, and 100 mg/kg p.o respectively) substantially prevented this rise in glucose level compared to model control animals. This action of Quercetin can be attributed to its α-glucosidase inhibitory potential, which reduces the postprandial glucose rise following a carbohydrate-rich diet[43] (Figure 2).

With n = 8 animals in each group, the findings were represented as mean ± SEM. $p < 0.05$ and < 0.01, <0.001, and <0.0001 were chosen as significance levels. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

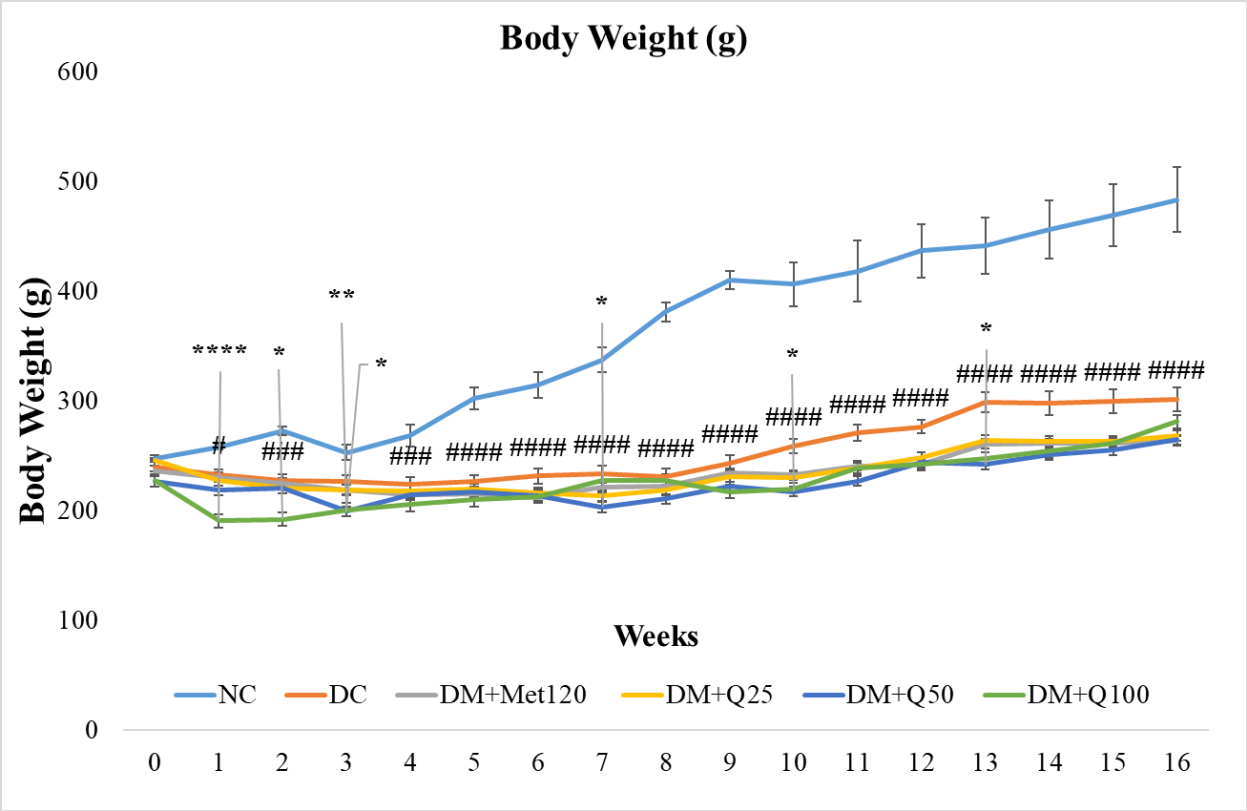

Figure 1. Effect of quercetin on body weight in diabetic nephropathy in rats.

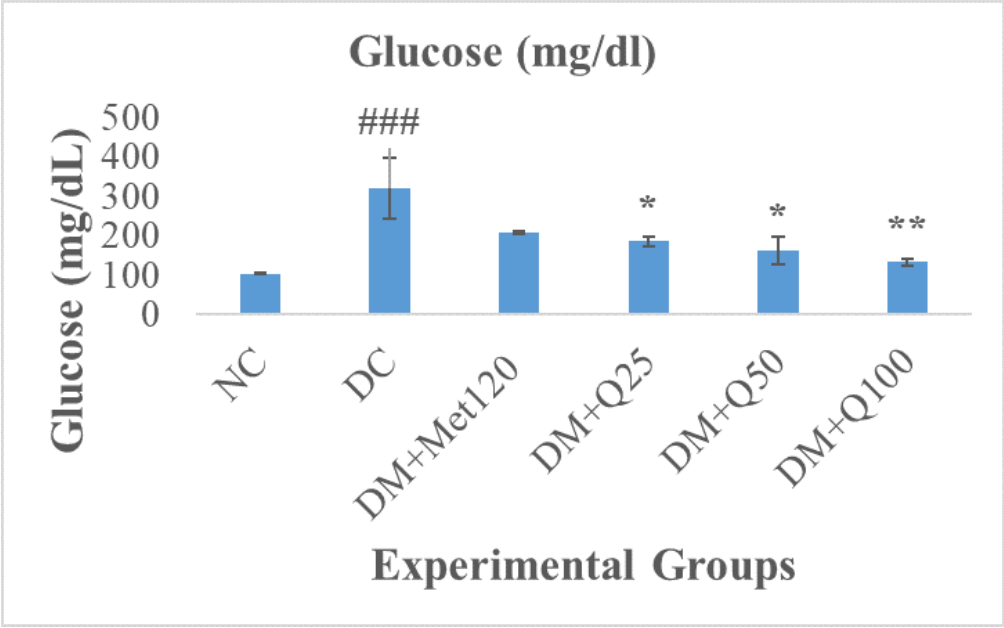

Figure 2. Effect of quercetin on serum glucose in diabetic nephropathy in rats.

3.3 Effect of Quercetin on Lipid Profile in Diabetic Nephropathy

DM-2 patients frequently have dyslipidemia, as indicated by high TG, LDL, and low HDL levels, which raises the risk of atherosclerosis and mortality[27]. Thus, in the eighth week, the serum lipid profile was measured. Model rats exhibited a substantial rise in serum cholesterol (106.1 ± 9.664, $p < 0.01$), serum triglycerides (84.21 ± 5.908, $p < 0.01$), an insignificant decline in serum HDL, a substantial rise in LDL levels (52.77 ± 14.3, $p < 0.05$ and VLDL (17.64 ± 1.071, $p < 0.01$) relative to normal control (60.46 ± 5.018), (51.41 ± 8.645), (19.75 ± 5.818) and (10.28 ± 1.729) respectively. Therefore, it can be concluded that our results mimic the previously reported studies[37]. However, Metformin treatment (120 mg/kg) insignificantly prevented this rise in serum cholesterol level. In contrast, a highly significant decline in triglycerides (25.95 ± 2.137, $p < 0.0001$), LDL (19.1 + 4.336, $p < 0.05$), VLDL (6.456 + 1.022, $p < 0.0001$) and rise in HDL levels (80.52 ± 9.597, $p < 0.01$) was observed. While Quercetin (25, 50, and 100 mg/kg) treatment substantially prevent this rise in serum cholesterol level (69.5 ± 10.99, $p < 0.05$, 64.52 ± 8.743, $p < 0.01$, 74.3 ± 5.405, $p < 0.05$, respectively), a nonsignificant decline in triglycerides was observed in Quercetin (25 and 50 mg/kg). In comparison, Quercetin (100 mg/kg) exhibited a substantial decline in triglycerides (55.12 ± 7.834, $p < 0.05$), and Quercetin (25 mg/kg and 50 mg/kg) exhibited a nonsignificant rise in HDL. In contrast, treatment with Quercetin (100 mg/kg) exhibited a highly relevant rise in the HDL levels (92.16 ± 4.032, $p < 0.001$), and Quercetin (25, 50, and 100 mg/kg) treated showed an insignificant decrease in LDL, Quercetin (100 mg/kg) treated demonstrated a significant decline in VLDL (10.03 ± 0.9633, $p < 0.01$). In contrast, Quercetin (25 and 50 mg/kg) showed an insignificant decline in VLDL compared to model control animals. Therefore, Quercetin's anti-dyslipidemia action can be attributed to its ability to downregulate the expression of "sterol regulatory element-binding protein-1c" (SREBP-1c) and "peroxisome proliferator-activated receptor-α" (PPAR-α) in the liver, resulting in decreased triglycerides synthesis[30]. Our findings are consistent with earlier research[42] (Figure 3).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.0001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

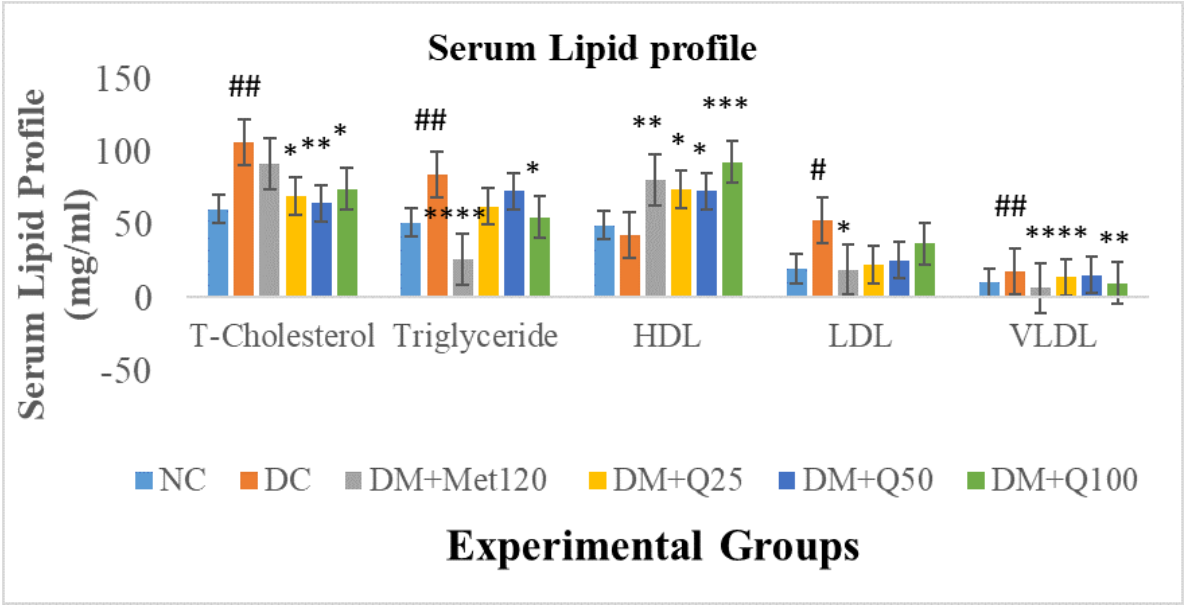

Figure 3. Effect of quercetin on serum lipid profile in diabetic nephropathy in rats.

3.4 Effect of Quercetin on Kidney Function in Diabetic Animals

The diabetic rats with no treatment developed advanced pathological changes like glomerulosclerosis, tubular degeneration, and mononuclear cell infiltration in their kidneys, which were linked to changes in kidney function parameters like elevated levels of creatinine and Urea, which is a crucial indicator of DN progression and is primarily caused by glomerular filtration barrier damage that reduces GFR[44].

3.4.1 Serum Creatinine and Uric Acid

In the eighth week, serum Creatinine and Uric acid were measured. Model rats exhibited a substantial rise in creatinine levels (0.935 ± 0.01384, P < 0.001) and Uric acid levels (2.795 ± 0.06602, P < 0.01) relative to normal control (0.7108 ± 0.05082) and (1.425 ± 0.1931) respectively. Therefore, it is our opinion that our results are consistent with those reported earlier[45]. However, treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) substantially ($p < 0.05$, < 0.01, <0.001, < 0.0001) prevented this rise in Serum Creatinine (0.702 ± 0.03787, $p < 0.0001$) and (0.714 ± 0.01327, $p < 0.001$, 798 ± 0.02634, $p < 0.05$ and 0.658 ± 0.03865, $p < 0.0001$ respectively) and Uric acid level significantly reduced with Metformin treatment (1.2 ± 0.08165, $p < 0.01$) and Quercetin treatment (25 mg/kg) (0.975 ± 0.2394, $p < 0.001$) compared to negative control animals (Figure 4).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

3.4.2 Nitrogenous Waste Products

Estimating nitrogenous waste products are essential to assess kidney function[18]. Thus, serum Urea and BUN levels were measured. Model rats exhibit a substantial rise in Urea (50.47 ± 3.257, $p < 0.001$) and BUN (23.58 ± 1.522, $p < 0.001$) relative to normal control (26.56 ± 5.475) and (12.41 ± 2.559), respectively. The results of our study are consistent with those of the previous study[45]. Conversely, treatment with Metformin (120 mg/kg) significantly

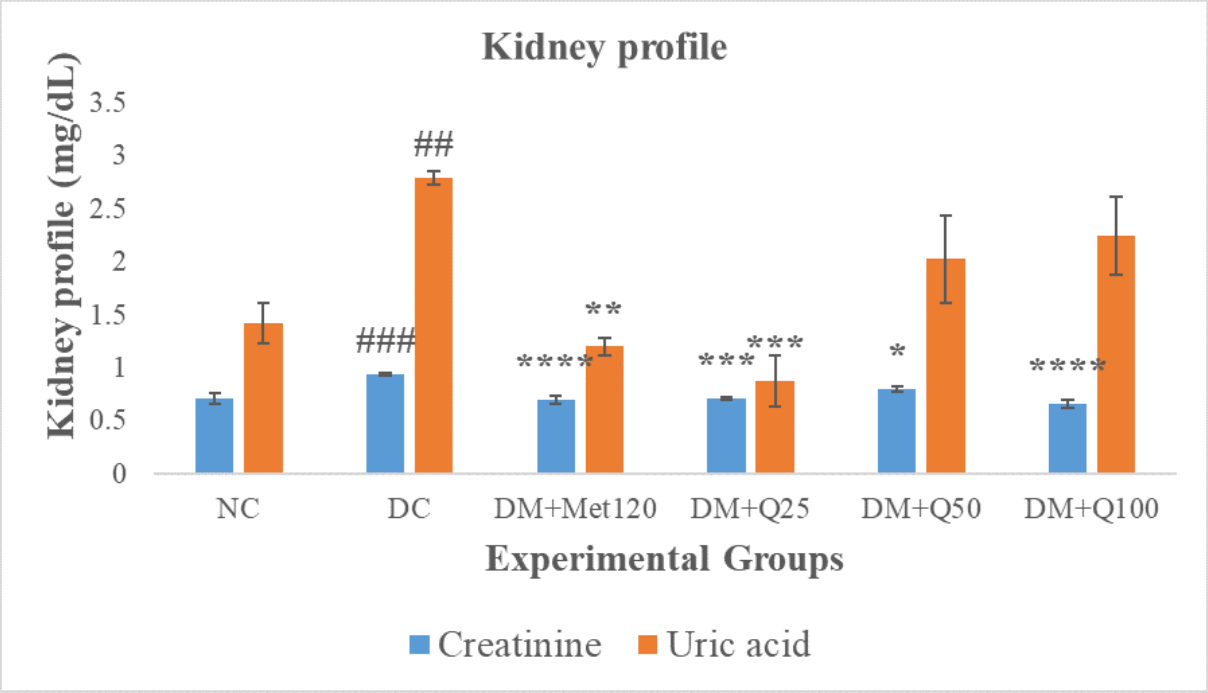

Figure 4. Effect of quercetin on serum creatinine and uric acid levels in diabetic nephropathy in rats.

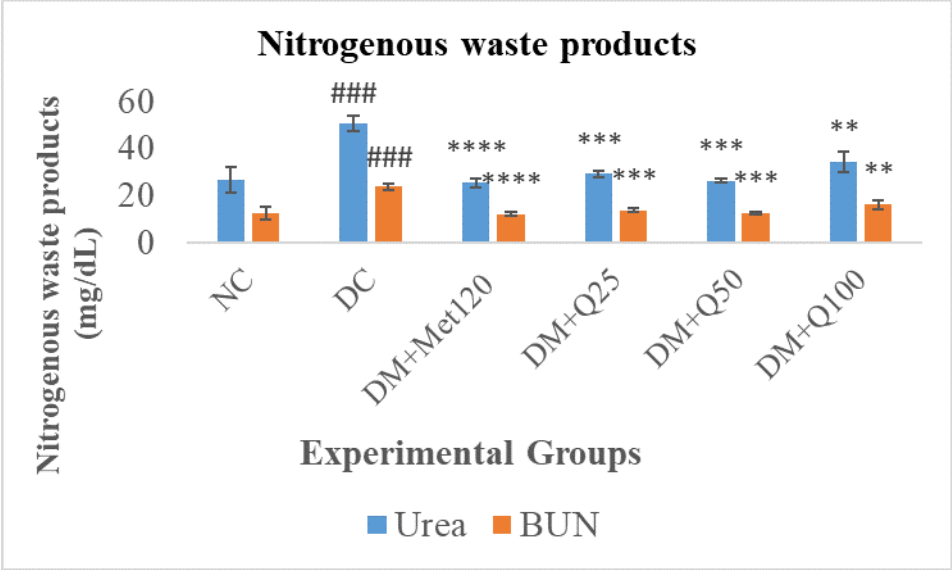

Figure 5. Effect of quercetin on serum urea and BUN levels in diabetic nephropathy in rats.

prevented this rise in Urea (25.26 ± 1.757, $p < 0.0001$) and BUN levels (11.8 ± 0.8208, $p < 0.0001$), respectively, Whereas Quercetin (25, 50, and 100 mg/kg) substantially reduced the Urea (29.06 ± 1.516, $p < 0.001$, 26.2 ± 0.8099, $p < 0.001$ and 34.16 ± 4.231, $p < 0.01$ respectively) and BUN (13.58 ± 0.7083, $p < 0.001$, 12.24 ± 0.3785, $p < 0.001$ and 15.96 ± 1.977, $p < 0.01$ respectively) relative to model control animals. Quercetin treatment reduces creatinine, BUN, and uric acid, protecting against ROS-induced focal changes in renal histology[45] and enhancing kidney function (Figure 5).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

3.5 Effect of Quercetin on Insulin Sensitivity in Diabetic Nephropathy

Beta cell feedback stimulation results in hyperinsulinemia in response to hyperglycemia and insulin resistance. In addition, it leads to insulin receptor dysfunction at the beta cell membrane and other tissues, thereby impairing the glucose uptake by the liver, ultimately leading to glucose intolerance[41].

3.5.1 Insulin ELISA

According to many studies, HFD caused insulin levels to dramatically increase in diseased rats indicating hyperinsulinemia and treatment with multiple low doses of STZ caused β-cell dysfunction[41]. Similar reported studies support our findings[30]. Therefore, after the eighth week, Insulin levels were determined for each group. Model rats exhibited a substantial rise in insulin levels (25.56 ± 2.541, $p < 0.001$) relative to normal control (14.83 ± 1.347), respectively. Treatment with Metformin (120 mg/kg) (12.78 ± 0.5638, $p < 0.001$) and Quercetin (25, 50, and 100 mg/kg) substantially prevent this rise in the insulin levels (12.83 ± 1.323, $p < 0.001$, 10.78 ± 0.6961, $p < 0.0001$ and 11.11 ± 0.6827, $p < 0.0001$ respectively) compared to model control animals (Figure 6).

With n = 8 animals in each group, the findings were represented as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.0001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the model control group.

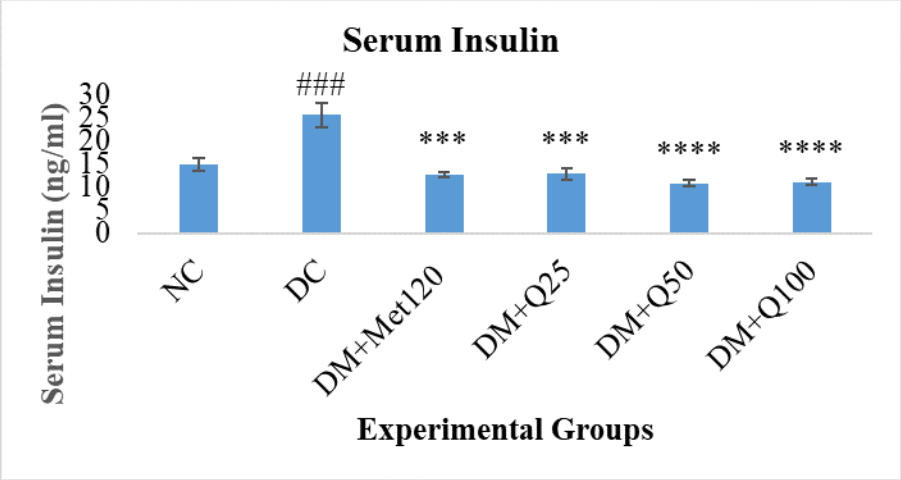

Figure 6. Effect of quercetin on insulin levels in diabetic nephropathy in rats.

3.5.2 OGTT

Due to insulin resistance, glucose absorption by hepatocytes is hindered in DM-2 individuals, resulting in glucose intolerance[41]. The OGTT was therefore estimated in each group at the end of the protocol. Model control rats showed a rise in glucose level from 30 min onwards, with a substantial rise at 60 min (257.3 ± 30.8, $p < 0.05$) relative to normal control rats (134.2 ± 8.092). In contrast, Metformin (120 mg/kg) and Quercetin (25 and 100 mg /kg) exhibited an insignificant decline in glucose from 60 min onwards compared to model control rats. These results indicated that Quercetin-treated animals had greater glycemic control owing to augmented insulin release from preserved β-cells and improved glucose uptake and utilization. Our results are consistent with similar previous studies[32] (Figure 7).

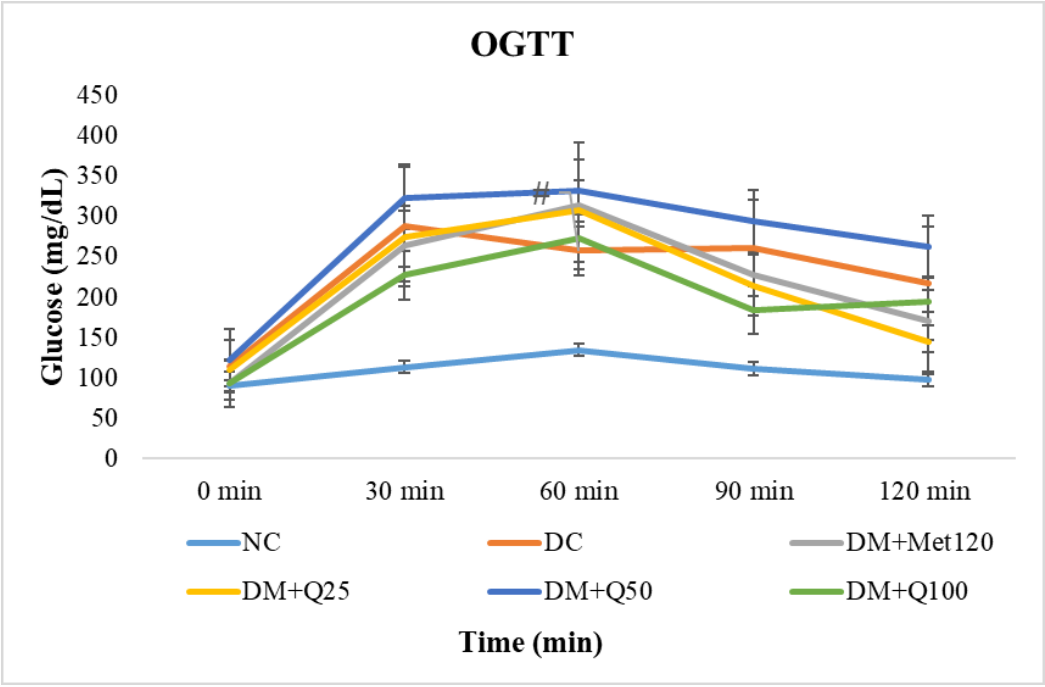

Figure 7. Effect of quercetin on OGTT in diabetic nephropathy in rats.

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.001 were chosen as levels of significance, respectively. #, ##, ###, #### Denote that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the model control group.

3.5.3 HOMA-IR

HOMA-IR is a predictive indicator for insulin sensitivity and β-cells function, routinely employed in clinical practice to measure human glucose homeostasis[41]. After eight weeks, insulin resistance was measured using HOMA-IR, a mathematical model largely dependent on fasting Insulin and glucose levels. Model control rats were significantly found to be more Insulin resistant (272.4 ± 81.04, $p < 0.05$) relative to normal control (72.67 ± 5.22). Our study demonstrates similarity with the previous studies[41]. In contrast, treatment with Metformin (120 mg/kg) and Quercetin (25 mg/kg) insignificantly exhibited improvement in insulin sensitivity, while Quercetin (50 and 100 mg/kg) significantly exhibited improvement in insulin sensitivity (105.2 ± 31.31, $p < 0.05$ and 91.42 ± 8.327, $p < 0.05$ respectively) comparative to model animals. Thus, the present study showed an elevated HOMA-IR index in diabetic animals. However, treatment with Quercetin reduced the HOMA-IR index, indicating improved glucose metabolism and reduced insulin resistance at tissue levels[41] (Figure 8).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001, and < 0.0001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

3.6 Effect of Quercetin on Antioxidant Levels in Diabetic Nephropathy

Obesity and oxidative stress are interrelated. Fluctuations in oxidative stress indicators such as MDA and the Antioxidant defense barriers suggest the oxidative stress-induced reduction in the nephron's innate defense system[46]. In the current study, negative control rats demonstrated an insignificant decline in SOD activity, GSH activity, and a substantial decline in catalase activity (0.388 ± 0.1262, $p < 0.0001$) relative to normal control (4.044 ± 0.6417). Our results align with the previously reported studies[47]. However, treatment with Metformin (120 mg/kg) exhibited an insignificant increase in SOD activity, GSH activity, and catalase activity. Quercetin treatment (25, 50, and 100 mg/kg) substantially increased SOD activity (69.4 ± 7.23, $p < 0.001$, 59.74 ± 12.08, $p < 0.01$ and 68.05 ± 12.82, $p < 0.001$ respectively). While treatment Metformin (120 mg/kg) and Quercetin (50 and

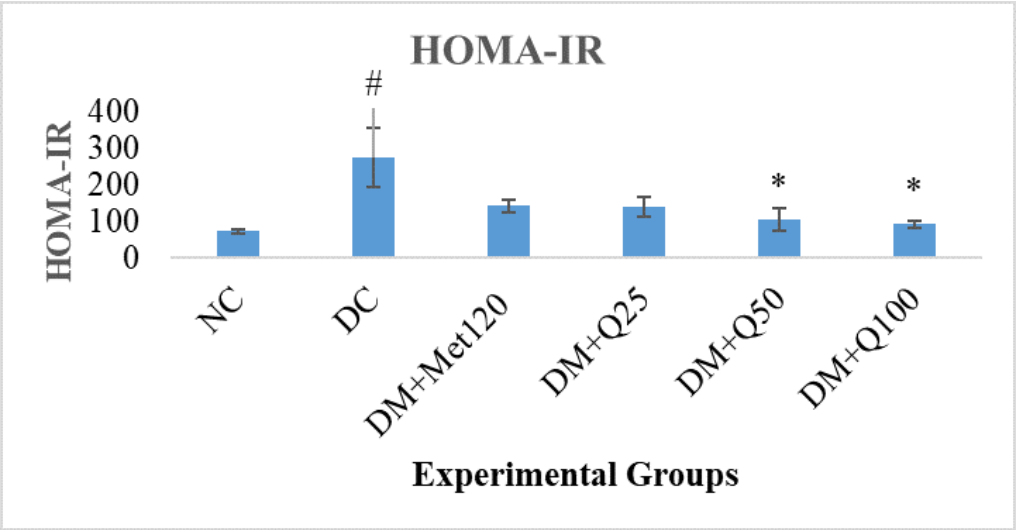

Figure 8. Effect of quercetin on HOMA-IR in diabetic nephropathy in rats.

100 mg/kg) insignificantly increased the GSH activity. While, Quercetin (25 mg/kg) exhibited a substantial rise in GSH activity (8.887 ± 0.6569, $p < 0.01$) as compared to model control animals. Metformin (120 mg/kg) and Quercetin treatment (25 and 50 mg/kg) exhibit an insignificant increased catalase activity. While Quercetin treatment (100 mg/kg) significantly raised the catalase activity (1.718 ± 0.12, $p < 0.05$) relative to model control animals. A substantial rise in MDA levels was detected in Model control rats (0.148 ± 0.03089, $p < 0.0001$) relative to normal control (0.026 ± 0.007483). Treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) significantly prevented this rise in MDA level (0.076 ± 0.004, $p < 0.01$) and (0.04 ± 0.003162, $p < 0.0001$, 0.026 ± 0.004, $p < 0.0001$ and 0.036 ± 0.007483, $p < 0.0001$ respectively) relative to negative control animals (Figures 9 (a), (b), (c), (d)).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001 and < 0.0001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the negative control group.

3.7 Effect of Quercetin on Genetic Expression Nrf2 and HO-1 in Diabetic Nephropathy

Chronic inflammation and oxidative stress lead to cellular damage and subsequent organ failure. When protecting against damage caused by stress, the Nrf2 system is one of the first lines of protection. Furthermore, there is a feedback loop whereby Nrf2 causes the production of downstream signaling molecules like HO-1, which alleviate cellular stress[19]. Thus, Nrf2 system activators can safeguard physiological processes[10]. The downregulated Nrf2 and HO-1 expression indicates inflammation and oxidative stress in the kidney tissues. Our results align with the previous study[10]. The Nrf2 expression (the fold of expression) in the model control group (0.6644 ± 0.08906, $p < 0.01$ was substantially downregulated compared to the normal control (1.436 ± 0.08135). Conversely, treatment with Metformin (120 mg/kg) and Quercetin (50 mg/kg) insignificantly upregulated the Nrf2 expression (the fold of expression). In contrast, Quercetin (25 and 100 mg/kg) substantially upregulated the Nrf2 expression (1.429 ± 0.2262, $p < 0.01$ and 2.335 ± 0.214, $p < 0.0001$ respectively) relative to model control animals (Figure 10 (a)).

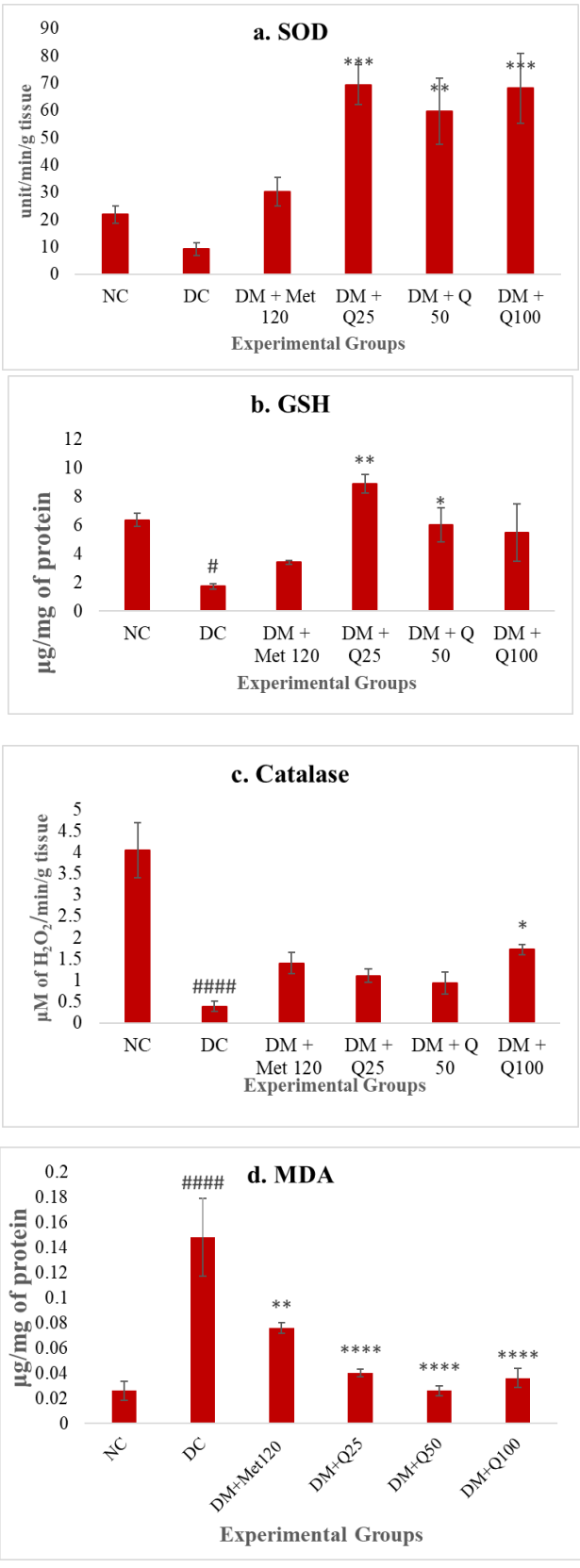

Figure 9. Effect of quercetin on (**a**). SOD, (**b**). GSH, (**c**). Catalase, and (**d**). MDA level in the kidney of diabetic rats.

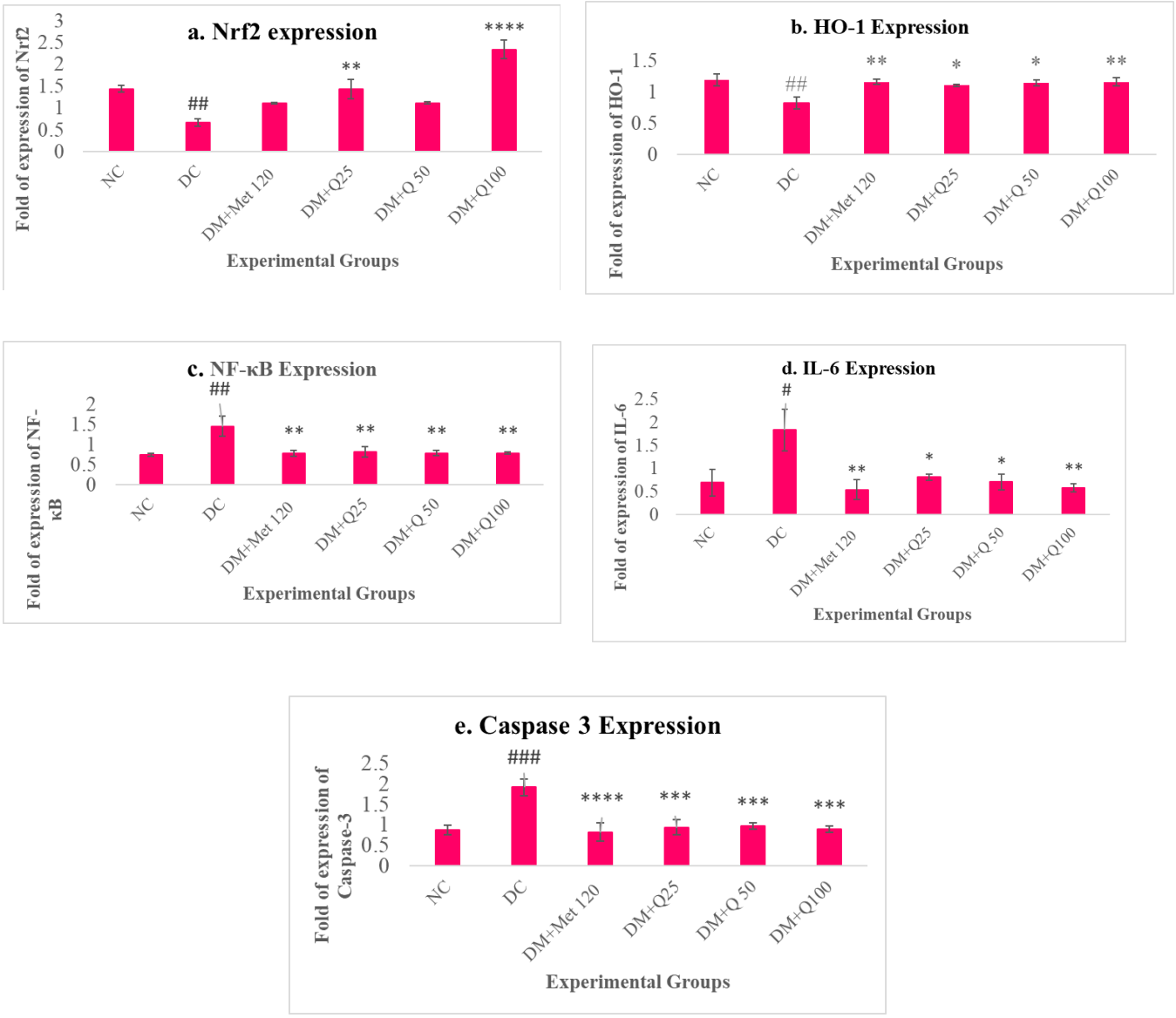

Figure 10. Effect of quercetin on (**a**). NrF2, (**b**). HO-1, (**c**). NF-κB, (**d**). IL-6, and (**e**). Caspase-3 expression in the kidney of diabetic rats.

The HO-1 expression (the fold of expression) in the model control group (0.8215 ± 0.09864, $p < 0.01$) was substantially downregulated compared to the normal control (1.19 ± 0.09776). However, treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) significantly and) upregulated the HO-1 expression (the fold of expression) (1.162 ± 0.0441, $p < 0.01$) and (1.104 ± 0.01748, $p < 0.05$, 1.144 ± 0.04789, $p < 0.05$ and 1.162 ± 0.06227, $p < 0.01$ respectively) respectively relative to model control animals (Figure 10 (b)).

3.8 Effect of Quercetin on Genetic Expression Inflammatory and Apoptotic Markers in Diabetic Nephropathy

Inflammatory reactions are significantly activated by various inflammatory mediators, including "Nuclear Factor Kappa B" (NF-κB) and "IL-6"[27]. The upregulation of NF-kB and IL-6 expression in diabetic rats indicates inflammation and oxidative stress in the kidney[27,35].

According to reported studies, the Nrf2/Keap-1 pathway can restrict NF-κB activation by degrading IKKb (inhibitory of nuclear factor κ B (IκB) Kinase complex) via ubiquitin[48]. The NFκB Expression (the fold of expression) in the model control group (1.446 ± 0.2504, $p < 0.01$) was substantially upregulated compared to the normal control (0.7304 ± 0.0364). Conversely, treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) significantly downregulated in NFκB Expression (the fold of expression) (0.7728 ± 0.06898, $p < 0.01$) and (0.8015 ± 0.1295, $p < 0.01$, 0.777 ± 0.06743, $p < 0.01$ and 0.7744 ± 0.02659, $p < 0.01$ respectively) respectively relative to model control animals (Figure 10 (c)).

The IL-6 expression (the fold of expression) in the model control group (1.83 ± 0.4517, $p < 0.05$) significantly upregulated relative to the normal control (0.6883 ± 0.2887). However, treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) significantly downregulated the IL-6 expression (the fold of expression) (0.5385 ± 0.2161, $p < 0.01$) and (0.8061 ± 0.06588, $p < 0.05$, 0.7028 ± 0.1675, $p < 0.05$ and 0.5751 ± 0.09099, $p < 0.01$ respectively) respectively relative to model control animals (Figure 10(d)).

The Caspase-3 expression (the fold of expression) in the model control group (1.907 ± 0.1941, $p < 0.001$) was substantially upregulated compared to the normal control (0.8623 ± 0.1144). The upregulated Expression of Caspase-3 in renal tissues indicated higher levels of apoptosis, possibly causing renal damage. In contrast, treatment with Metformin (120 mg/kg) and Quercetin (25, 50, and 100 mg/kg) significantly downregulated the Caspase-3 expression (the fold of expression) (0.8208 ± 0.2292, $p < 0.0001$) and (0.9359 ± 0.1792, $p < 0.001$, 0.9703 ± 0.07221, $p < 0.001$ and 0.8896 ± 0.08027, $p < 0.001$ respectively) respectively relative to model control animals (Figure 10 (e)).

With n = 8 animals in each group, the findings were denoted as mean ± SEM. $p < 0.05$, <0.01, <0.001 and < 0.0001 were chosen as levels of significance, respectively. #, ##, ###, #### denotes that the group differs considerably from the normal control group. *, **, ***, **** denotes that the group differs considerably from the model control group. Quercetin's anti-inflammatory activity was confirmed by its ability to downregulate NF-kB and IL-6 Expression in the kidneys. Similarly, it upregulated Nrf2 Expression, which activated the antioxidant response elements, ultimately leading to the overexpression of HO-1 and the subsequent decline in oxidative stress. To that end, Quercetin may mediate in mitigating renal alterations. However, Caspase-3 expression in diabetic rats was also higher in the kidney, indicating higher levels of apoptosis, possibly leading to renal damage. Conversely, Quercetin treatment downregulated the Expression of Caspase -3 in renal tissue, thereby protecting against the damage. These findings shed light on Quercetin's function in preventing the progression of diabetic nephropathy.

4. Conclusion

Quercetin modulates oxidative stress and advancement of experimentally instigated DN by governing the transcriptional activity of Antioxidant defense barriers such as SOD, GSH, and catalase via upregulation of Nrf2-HO-1 Expression. In conclusion, our study demonstrates that induction by HFD and DSRI of low-dose STZ led to disturbance in all physical, biochemical, and oxidative stress statuses by downregulating Nrf2-HO-1 Expression and upregulation of inflammatory markers, notably NFκB and IL-6. These outcomes propose that the mechanism responsible for the nephroprotective effect of Quercetin is a consequence of the restoration of Antioxidant defense barriers along with biochemical and physical parameters. It opens a new avenue for treating DN; however, clinical trials of this relatively safe natural compound should be undertaken.

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