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Research Article

Cerium Oxide Nanoparticles Attenuate Diabetic Nephropathy in Rats by Reducing Oxidative Stress, Improving Dyslipidemia, and Modulating PKM_2 and KIM-1

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ABSTRACT

Objectives: Oxidative stress and inflammation play important roles in the pathophysiology of diabetic nephropathy (DN). Nanoparticles, including cerium oxide nanoparticles (CeO_2NPs), which reduce oxidative stress and inflammation, are increasingly utilized in disease treatment. This study aims to assess the preventive potential of CeO_2NPs in a DN animal model by examining their effects on glucose levels, lipid profiles, oxidative stress, and kidney damage markers.

Methods: Diabetes was induced in rats using streptozotocin (STZ). Rats were divided into three groups: normal control (N-Cnt), diabetic control (D-Cnt), and CeO_2NP-treated (D-CeO_2, 60 mg/kg) groups. Fasting blood glucose (FBG) levels were measured at baseline and on day 35. Additionally, serum lipid profiles (total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)) were assessed. In kidney tissue, activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)), levels of malondialdehyde (MDA) and total antioxidant capacity (TAC), as well as mRNA expression of pyruvate kinase M2 (PKM_2) and kidney injury molecule-1 (KIM-1) were analyzed.

Results: CeO_2NP treatment in D-CeO_2 rats significantly reduced FBG and improved lipid profiles (decreased TC, TG, LDL-C; increased HDL-C, $P < 0.05$). CeO_2NPs also attenuated oxidative stress (increased SOD, CAT, GPx, TAC; reduced MDA, $P < 0.05$) and downregulated PKM_2 and KIM-1 mRNA expression ($P < 0.05$) compared to D-Cnt rats.

Conclusion: CeO_2NPs demonstrate protective effects against DN in this rat model by ameliorating hyperglycemia, dyslipidemia, oxidative stress, and renal injury marker expression. These findings suggest that CeO_2NPs may possess therapeutic potential for DN, warranting further investigation into their mechanisms and clinical applicability.

Keywords: Diabetic Nephropathy, Cerium Oxide Nanoparticles, Oxidative Stress, PKM_2, KIM-1

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Introduction

Diabetes mellitus (DM), a chronic metabolic disorder with a growing global prevalence, affects millions of individuals. Among the various types of diabetes, type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic beta cells and absolute insulin deficiency (1). The early onset and lifelong management of this disease have been associated with a high risk of chronic complications that significantly impact patients' quality of life. Among these debilitating complications, diabetic nephropathy (DN) is a progressive microvascular disorder that affects the kidneys and is recognized as a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide, particularly in individuals with T1D (2).

The pathophysiology of DN is complex and multifactorial, but chronic hyperglycemia plays a pivotal role in its initiation and progression. Hyperglycemia leads to a series of metabolic and hemodynamic disturbances in the kidney, including increased production of reactive oxygen species (ROS), resulting in the induction of oxidative stress (3). Oxidative stress is recognized as a crucial pathogenic mechanism in diabetic kidney damage, contributing to cellular injury, inflammation, and fibrosis (4). In addition to ROS overproduction, chronic hyperglycemia impairs the endogenous antioxidant defense system, exacerbating oxidative damage to various cellular and molecular structures in the kidney. ROS can directly damage lipids, proteins, and DNA, leading to cellular dysfunction, apoptosis, and necrosis. Beyond direct damage, oxidative stress plays a significant role in renal inflammation and fibrosis, both of which are major hallmarks of DN (5). ROS can activate inflammatory pathways, leading to increased production of pro-inflammatory cytokines and chemokines that further exacerbate kidney injury. Additionally, oxidative stress stimulates the production of extracellular matrix proteins, contributing to the accumulation of scar tissue (fibrosis) in the kidney, which impairs its normal function (6). Cerium oxide nanoparticles (CeO_2NPs), due to their unique antioxidant properties, have garnered increasing attention as potential therapeutic agents for diseases associated with oxidative stress (7). The ability of CeO_2NPs to cycle between Ce^{3+} and Ce^{4+} oxidation states allows them to catalytically scavenge a wide range of ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals (8). A key advantage of CeO_2NPs over traditional antioxidants is their stability and self-regenerative capability. Unlike small molecule antioxidants that are consumed after neutralizing ROS, CeO_2NPs can continuously cycle between their oxidation states, providing long-lasting antioxidant protection (9). This feature makes them particularly appealing for treating chronic diseases such as DN, which are characterized by persistent oxidative stress. To further elucidate the potential of CeO_2NPs in mitigating DN, investigating their impact on specific molecular markers of kidney damage is warranted.

Pyruvate kinase M2 (PKM_2), an enzyme critical for glycolysis and cellular metabolism, is increasingly recognized as a significant factor in the development of various diseases, including diabetic complications (10). In DN, changes in PKM_2 expression and activity correlate with the extent of kidney cell injury and disease progression. Specifically, PKM_2 activation under hyperglycemic conditions promotes the generation of ROS and inflammatory responses (11), suggesting that therapeutic interventions targeting PKM_2 activity may offer a clinically relevant approach to managing DN. Investigating the effect of CeO_2NPs on PKM_2 gene expression could provide new insights into how these nanoparticles impact renal metabolism in the context of diabetes. Kidney injury molecule-1 (KIM-1) is a well-established biomarker for kidney injury, particularly proximal tubular damage. Under normal conditions, KIM-1 expression in the kidney is very low, but it is markedly upregulated in response to various types of kidney injury, including ischemia, toxicity, and inflammation, all of which occur in DN (12). Elevated levels of KIM-1 in urine and kidney tissue are associated with the severity and progression of kidney disease in diabetic patients (13). Thus, KIM-1 is considered a sensitive and specific marker for assessing kidney damage in DN. Examining the effect of CeO_2NPs on KIM-1 gene expression could help determine whether these nanoparticles can mitigate kidney injury at the molecular level.

In this context, the present study aims to evaluate the impact of CeO_2NPs on a range of vital parameters in the DN rat model. With a specific focus on molecular mechanisms, the main objectives of this study were to determine the effects of CeO_2NPs on blood glucose levels, lipid profile, the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and total antioxidant capacity (TAC), malondialdehyde (MDA), and, innovatively, the expression of genes related to renal PKM_2 and KIM-1. The findings of this research could provide a novel understanding of the role of CeO_2NPs in modulating key pathways involved in the pathogenesis of DN and pave the way for the development of new therapeutic strategies.

Material and Methods

Animal

Eighteen male Wistar rats, weighing 200 ± 20 grams, were purchased from the Animal Breeding Center of Baqiyatallah University of Medical Sciences. The rats were kept in the central animal house to adapt to the new environment for five days. Throughout the experiment, the animals had free access to water and a

standard diet in a controlled room (temperature: 23 ± 2 °C; humidity: 50 ± 10%). Ethical approval was obtained from the Research Ethics Committee of Arak University of Medical Sciences (ethical approval number: IR.ARAKMU.REC.1398.020) and subsequently adhered to the principles of working with laboratory animals. After the adaptation period, the rats were prepared for diabetes induction. Following 12 hours of fasting, FBG was measured using a glucometer. Twelve rats were selected for diabetes induction via a single intraperitoneal injection of 55 mg/kg streptozotocin (STZ, Sigma Aldrich, USA) dissolved in citrate buffer solution (0.1 M, pH = 4.5). The remaining six rats were only injected with citrate buffer solution. After 72 hours, blood taken from the tail of rats was analyzed for FBG using a glucometer. Rats with FBG > 250 mg/dl were selected for treatment. Thus, three experimental groups containing six rats (each one) are as follows: 1) Normal group: rats received normal saline (N-Cnt), 2) Diabetic control group: diabetic rats received distilled water (D-Cnt), 3) Diabetic group: diabetic rats received 60 mg/kg CNPs (D-CeO2). CeO_2NPs was dissolved in the distilled water using a sonicator. Diabetic rats received CeO_2NPs through oral gavage for 35 consecutive days.

Cerium oxide nanoparticle synthesis

CeO_2NPs were synthesized in a stainless steel (S316L) reactor with a capacity of 100 cm^3, which can withstand temperatures and pressures up to 550 °C and 610 atm, respectively. First, 30 cm^3 of 0.1 M precursor solution with the desired concentration was prepared by dissolving a specific amount of cerium (III) nitrate hexahydrate in deionized water, then injected into the reactor. Second, the reactor vessel was placed in a furnace preheated to 500 °C. After a one-hour reaction, the reactor vessel was directly cooled with cold water. Subsequently, the nanoparticles were separated from the aqueous solution using a combination of centrifugation and water rinsing methods. Finally, the precipitated nanoparticles were washed three times with deionized water to remove unreacted precursors and dried at room temperature. Further details regarding the batch reactor and the nanoparticle synthesis procedure can be found in previous publications (14, 15).

Blood and kidney isolation

Rats were anesthetized using diethyl ether 24 hours after the final gavage. Blood was drawn from the heart and centrifuged at 3000 rpm to obtain serum for biochemical parameter analysis. Kidneys were also isolated and stored at -80 °C for RNA extraction and oxidative stress parameter analysis.

Biochemical parameters analysis

FBG and serum lipid profiles, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were determined using commercially available enzymatic kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's instructions. All measurements were performed in duplicate.

Antioxidant and lipid peroxidation assays

The activities of SOD, CAT, and GPx enzymes, as well as TAC and MDA levels in kidney tissue homogenates, were determined using commercially available assay kits (Teb Pazhouhan Razi (TPR), Tehran, Iran) following the manufacturers' protocols. Briefly, kidney tissues were homogenized in ice-cold buffer. The homogenates were then centrifuged, and the supernatant was used for the assays. SOD activity was assessed by measuring its ability to inhibit the reduction of nitroblue tetrazolium. CAT activity was determined by measuring the rate of hydrogen peroxide decomposition. GPx activity was measured by monitoring the reduction of hydrogen peroxide using glutathione as a substrate, coupled with the oxidation of NADPH. TAC was measured using the ferric reducing antioxidant power (FRAP) assay, quantifying the reduction of ferric ions to ferrous ions. MDA levels, an indicator of lipid peroxidation, were determined using the thiobarbituric acid reactive substances (TBARS) assay, in which MDA reacts with thiobarbituric acid to form a colored product measurable via spectrophotometry. Enzyme activities were expressed as units per milligram of protein, and TAC and MDA values were expressed as micromoles per milligram of protein. Protein concentration in the homogenates was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard.

RNA extraction and gene expression analysis

Total RNA was extracted from the frozen kidney tissues using a commercially available RNA extraction kit (Denazist's Kits Company, Mashhad, Iran) following the manufacturer's protocol. For cDNA synthesis, a total of 1 µg of RNA was reverse-transcribed using a commercially available cDNA synthesis kit (Denazist's Kits Company, Mashhad, Iran. Quantitative real-time PCR (qRT-PCR) was performed to determine the mRNA expression levels of PKM2 and KIM-1. using the LightCycler 96 system (Roche Diagnostics GmbH, Mannheim, Germany). β-actin was used as housekeeping genes. The comparative Ct (2-ΔΔCt) method was used to evaluate data. The primer sequences were as follows: PKM2, 5′- CGCCTGGACATTGACTCTG-3′ (forward) and 5′-GAAATTCAGCCGAGCCACATT-3′ (reverse); KIM-1, 5′-TGGCACTGTGACATCCTCAGA-3′ (forward) and 5′- GCAACGGACATGCCAACATA-3′ (reverse) and β-actin, 5′-GAGAAGATTTGGCACCACAC-3′ (forward) and 5′-CATCACAATGCCAGTGGTAC-3′ (reverse).

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test to determine significant differences between the experimental groups. The level of significance was set at $p < 0.05$. GraphPad Prism software (version 8) was used for statistical analysis.

Results

Effect of CeO_2NPs on FBG levels

FBG levels were significantly elevated in the D-Cnt group compared to the N-Cnt group at both Day 1 and Day 35 (Table 1). Following 35 days of treatment, the D-CeO_2 group, receiving CeO_2NPs, exhibited a significant reduction in FBG levels compared to the diabetic control group at Day 35 and also showed a significant reduction from their Day 1 levels (Table 1).

Effect of CeO_2NPs on serum lipid profile

The serum lipid profile was analyzed at the end of the 35-day treatment period. The results revealed significant alterations in the D-Cnt group compared to the N-Cnt group (Table 2). The levels of TC (180 ± 10 mg/dL), TG (155 ± 12 mg/dL), and LDL-C (110 ± 9 mg/dL) were significantly higher in the D-Cnt group compared to the N-Cnt group (TC: 90 ± 7 mg/dL, TG: 70 ± 6 mg/dL, LDL-C: 45 ± 5 mg/dL; $p < 0.001$ for all comparisons). Conversely, high-density lipoprotein cholesterol (HDL-C) levels (25 ± 3 mg/dL) were significantly lower in the D-Cnt group compared to the N-Cnt group (40 ± 4 mg/dL; $p < 0.001$). Treatment with CeO_2NPs (D-CeO_2 group) significantly improved the lipid profile, showing decreased levels of TC (120 ± 9 mg/dL), TG (90 ± 10 mg/dL), and LDL-C (70 ± 7 mg/dL), and increased levels of HDL-C (35 ± 3 mg/dL) compared to the D-Cnt group ($p < 0.001$ for TC, TG; $p < 0.01$ for LDL-C; $p < 0.05$ for HDL-C).

Effect of CeO_2NPs on antioxidant status

The D-Cnt group exhibited a significant decrease in the activities of antioxidant SOD (36 ± 93 vs. 100 ± 36 U/mg protein, $p < 0.001$), CAT (30 ± 3 vs 65 ± 6 U/mg protein, $p < 0.001$), and GPx (17 ± 9 vs 45 ± 86 U/mg protein, $p < 0.01$) in kidney tissue compared to the N-Cnt group (Figure 1 A-C). Treatment with CeONPs (D-CeONPs group) significantly increased the activities of SOD (68 ± 11 vs 36 ± 93 U/mg protein, $p < 0.01$), CAT (55 ± 5 vs 30 ± 3 U/mg protein, $p < 0.01$), and GPx (36 ± 76 vs 17 ± 9 U/mg protein, $p < 0.05$) compared to the D-Cnt group. Similarly, TAC in kidney tissue was significantly lower in the D-Cnt group (1.9 ± 0.2 µmol Trolox/mg protein, $p < 0.001$) compared to the N-Cnt group (5.4 ± 0.13 µmol Trolox/mg protein) (Figure 1D). Treatment with CeO_2NPs significantly increased TAC levels (3.4 ± 0.25 µmol Trolox/mg protein, $p < 0.01$) compared to the D-Cnt group. Conversely, MDA levels, a marker of lipid peroxidation, were significantly higher in the D-Cnt group (14 ± 1.0 µmol/mg protein, $p < 0.001$) compared to the N-Cnt group (4.6 ± 0.5 µmol/mg protein) (Figure 2D). Administration of CeO_2NPs resulted in a significant reduction in MDA levels (7.7 ± 0.8 µmol/mg protein, $p < 0.01$) compared to the D-Cnt group.

Effect of CeO_2NPs on mRNA expression of PKM_2 and KIM-1

The expression levels of both PKM_2 and KIM-1 were significantly upregulated in the D-Cnt group compared to the N-Cnt group (Figure 2 A-B). Specifically, PKM_2 mRNA expression was increased by 2.9 ± 0.3 fold

Table 1. FBG on Day 1 and Day 35 in All Experimental Groups (Mean ± SEM)

Group	Day 1	Day 35
N-Cnt	94 ± 12	96 ± 11
D-Cnt	290 ± 18 [a †]	285 ± 15 [a †]
D-CeO_2	288 ± 20 [a †]	160 ± 12 [b #]

Fasting blood glucose (FBG) levels on Day 1 (before treatment) and Day 35 (end of the treatment period) in the N-Cnt (normal control), D-Cnt (diabetic control), and D-CeO_2NPs (diabetic rats treated with cerium oxide nanoparticles CeO_2NPs) groups. [a] In comparison with N-Cnt; [b] In comparison with D-Cnt; [c]; p-value † <0.001; p-value # < 0.01. ANOVA and Bonferroni post hoc test statistical tests were used to analyze the data. Statistical significance was considered at $p < 0.05$.

Table 2. Serum lipid profile at day 35 in all experimental groups (Mean ± SEM)

Parameter (mg/dL)	N-Cnt (n=6)	D-Cnt (n=6)	D-CeO2 (n=6)
TC	90 ± 7	180 ± 10 [a †]	120 ± 9 [b †]
TG	70 ± 6	155 ± 12 [a †]	90 ± 10 [b †]
HDL-C	40 ± 4	25 ± 3 [a †]	35 ± 3 [b *]
LDL-C	45 ± 5	110 ± 9 [a †]	70 ± 7 [b #]

TC (Total Cholesterol), TG (Triglycerides), HDL-C (High-density lipoprotein cholesterol) and LDL-C (Low-density lipoprotein cholesterol) at the end of the treatment period (Day 35) in the N-Cnt (normal control), D-Cnt (diabetic control), and D-CeO_2NPs (diabetic rats treated with cerium oxide nanoparticles CeO_2NPs) groups. [a] In comparison with N-Cnt; [b] In comparison with D-Cnt; [c]; p-value † <0.001; p-value # < 0.01; *p < 0.05. ANOVA and Bonferroni post hoc test statistical tests were used to analyze the data. Statistical significance was considered at $p < 0.05$.

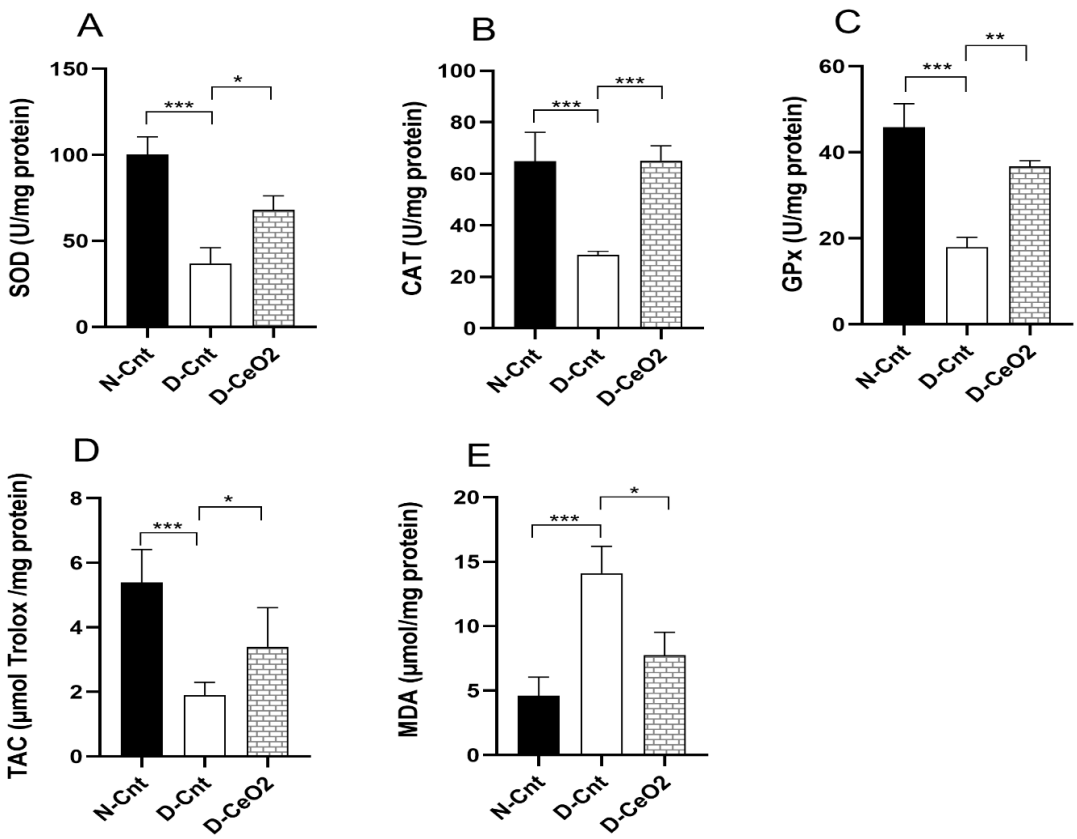

Figure 1. The mean ± SEM of **(A)** superoxide dismutase (SOD) activity, **(B)** catalase (CAT) activity, **(C)** glutathione peroxidase (GPx) activity, **(D)** total antioxidant capacity (TAC) and **(E)** malondialdehyde (MDA) levels in the kidney tissue of the N-Cnt (normal control), D-Cnt (diabetic control), and D-CeO_2 (diabetic rats treated with cerium oxide nanoparticles CeO_2NPs) groups at the end of the treatment period (Day 35). Statistical significance between groups is indicated by p-values within the text. Statistical significance was considered at $p < 0.05$ (*** P<0.001, ** P<0.01, * P<0.05).

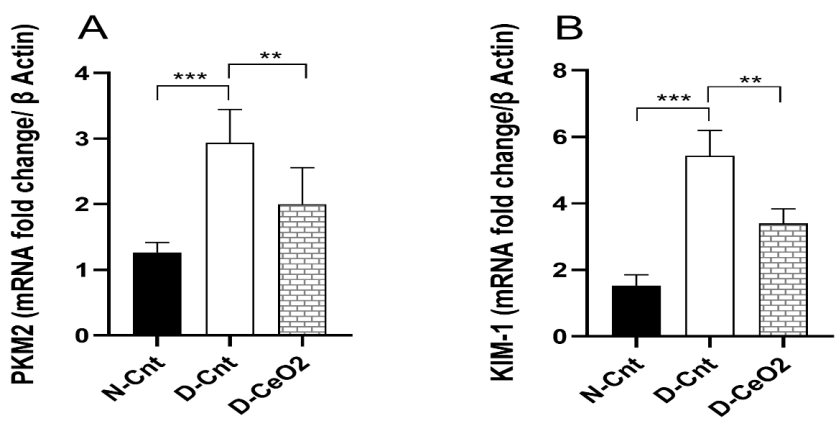

Figure 2. The mean ± SEM of relative mRNA expression levels of **(A)** pyruvate kinase M2 (PKM_2) and **(B)** kidney injury molecule-1 (KIM-1) in the kidney tissue of the N-Cnt (normal control), D-Cnt (diabetic control), and D-CeO_2 (diabetic rats treated with cerium oxide nanoparticles CeO_2NPs) groups at the end of the treatment period (Day 35). Gene expression levels are presented as fold change relative to the normal control group. Statistical significance between groups is indicated by p-values within the text. Statistical significance was considered at $p < 0.05$ (*** P<0.001, ** P<0.01)

change ($p < 0.01$), and KIM-1 mRNA expression was enhanced by 5.4 ± 0.5 fold change ($p < 0.001$) in the D-Cnt group relative to the N-Cnt group. Treatment with CeONPs (D-CeONPs group) resulted in a significant downregulation of both PKM_2 and KIM-1 mRNA expression compared to the D-Cnt group. PKM_2 mRNA expression was reduced to 2.1 ± 0.2 fold change ($p < 0.05$), and KIM-1 mRNA expression was reduced to 3.4 ± 0.3 fold change ($p < 0.01$) in the D-CeONPs group.

Discussion

This study investigates the therapeutic potential of CeO_2NPs in an STZ-induced rat model of DN. Our results demonstrate that CeO_2NP administration significantly attenuates the progression of DN, as evidenced by improved glycemic control, modulated lipid profiles, a substantial reduction in oxidative stress within kidney tissue, and favorable effects on key renal markers, PKM_2 and KIM-1.

In the present study, the D-Cnt group exhibited a significant increase in FBG levels compared to the N-Cnt group. This finding is consistent with the hallmark of STZ-induced experimental diabetes, which leads to the destruction of pancreatic β-cells and consequently hyperglycemia (16). Chronic elevation of blood glucose is a major driver in the development and progression of DN, contributing to kidney damage through mechanisms such as increased ROS production and the formation of advanced glycation end products (AGEs) (17). Notably, treatment with CeO_2NPs resulted in a significant reduction in FBG levels in the D-CeO_2 group compared to the D-Cnt group. This suggests that CeO_2NPs may have a beneficial effect on glycemic control in this diabetic model. Previous studies on the effect of CeO_2NPs on blood glucose have shown varied results. Some studies have reported a reduction in blood glucose levels, while others observed no significant effect. These discrepancies may be due to differences in diabetic models, CeO_2NP dosage, or other experimental factors (18, 19). The precise mechanisms by which CeO_2NPs affect glycemic control remain unclear. However, some evidence suggests that CeO_2NPs may lower blood glucose by improving β-cell function, increasing insulin secretion, or enhancing insulin sensitivity in peripheral tissues (20). The reduction in blood glucose with CeO_2NPs in our study supports the idea that these nanoparticles may possess metabolic benefits in addition to their renoprotective effects. Improved glycemic control is a critical goal in DN management, helping to slow disease progression and reduce the risk of complications (21). These findings warrant further investigation into the potential of CeO_2NPs as an adjunct therapy for DN.

In the present study, treatment with CeO_2NPs led to a significant improvement in the lipid profile of diabetic animals. Specifically, the levels of TC, TG, and LDL-C were decreased, while the level of HDL-C was increased. These findings are significant because dyslipidemia is a major risk factor for the progression of DN. Elevated TC, TG, and LDL-C, and reduced HDL-C contribute to lipid accumulation in the kidney parenchyma, increased inflammation, and worsening kidney damage (22). Studies have shown that CeO_2NPs can similarly improve lipid levels (19, 23). The exact mechanisms by which CeO_2NPs influence lipid metabolism are still not fully understood. However, some evidence suggests that CeO_2NPs may affect the activity of key enzymes involved in lipid synthesis and breakdown (24). Additionally, since oxidative stress and inflammation can disrupt lipid metabolism, the antioxidant and anti-inflammatory effects of CeO_2NPs may also contribute to the improved lipid profile (25). Overall, our findings suggest that CeO_2NPs, in addition to their renoprotective effects, may also possess metabolic benefits. Improving the lipid profile can help reduce the risk of cardiovascular complications associated with diabetes, as well as slow down the progression of DN. These results support further research into the potential of CeO_2NPs as a multi-targeted therapeutic agent for diabetic nephropathy.

Oxidative stress is unequivocally a central player in the pathogenesis of DN (26). In the present study, the D-Cnt group exhibited a marked reduction in the activity of key antioxidant enzymes, including SOD, CAT, and GPx, alongside a significant decline in TAC and a concurrent surge in MDA levels. The resultant overabundance of ROS not only directly damages essential cellular components such as lipids, proteins, and DNA, contributing to cellular dysfunction, apoptosis, and necrosis, but also acts as a potent driver of inflammation and fibrosis, the structural hallmarks of DN (27). Notably, the CeO_2NPs treatment effectively mitigated these diabetes-induced derangements in oxidative stress markers, restoring antioxidant enzyme activity, enhancing TAC, and suppressing MDA. These results are consistent with the results of other studies that have been conducted in other tissues and animal models (23, 28, 29). However, some studies have reported conflicting results regarding the effect of CeO_2NPs on oxidative stress (19). The reasons for these discrepancies may stem from variations in the administered nanoparticle dose, the specific tissue type examined, and the animal model employed. This capacity of CeO_2NPs to re-establish redox homeostasis within the kidney likely underlies their observed renoprotective effects, as the attenuation of oxidative stress interrupts a critical pathway driving both cellular damage and the progression of DN.

Emerging evidence suggests that PKM_2 is implicated in the pathogenesis of various diseases, including DN (30). In the context of DN, alterations in PKM_2 expression and activity have been observed (31). For instance, some studies have shown that PKM_2 activation may occur under hyperglycemic conditions and contribute to ROS production and inflammation (11, 32). Therefore, modulating PKM_2 activity could

be a potential therapeutic target for DN (33). KIM-1 is a key biomarker for proximal tubular damage in the kidney (34). Elevated KIM-1 levels in urine and kidney tissue correlate with the severity of kidney disease in diabetic patients, making it a sensitive and specific marker for assessing renal damage in this condition (35). In the present study, the expression of both PKM_2 and KIM-1 genes was significantly increased in the diabetic control group compared to the normal control group. This upregulation of PKM_2 and KIM-1 expression aligns with the findings of previous studies that demonstrated increased levels of these markers in response to renal injury under diabetic conditions (35–37). The increase in KIM-1 specifically indicates damage to proximal tubular cells, while alterations in PKM_2 expression reflect changes in cellular metabolism and inflammatory responses within the kidney (10, 39). Importantly, treatment with CeO_2NPs resulted in a significant reduction in the expression of both PKM_2 and KIM-1 genes compared to the D-Cnt group. This downregulation suggests that CeO_2NPs may attenuate kidney injury at the molecular level. The reduction in KIM-1 indicates decreased renal tubular cell damage, supporting the renoprotective effect of CeO_2NPs on kidney structure. The modulation of PKM_2 may reflect the impact of CeO_2NPs on renal cellular metabolism and the reduction of inflammatory responses. The precise mechanisms underlying these effects of CeO_2NPs on PKM_2 and KIM-1 gene expression are likely multifaceted. Based on current knowledge, no study has been conducted on the effects of CeO_2NPs on the gene expression of PKM_2 and KIM-1. Given the potent antioxidant and anti-inflammatory properties of CeO_2NPs, it is plausible that these nanoparticles indirectly normalize gene expression by reducing oxidative stress and inflammation in the kidney. Additionally, it is possible that CeO_2NPs exert direct effects on cellular signaling pathways involved in the regulation of PKM_2 and KIM-1 expression. Future research should further elucidate the precise molecular mechanisms by which CeO_2NPs exert their protective effects in DN.

Conclusion

In summary, the findings of this study demonstrate that CeO_2NPs provide significant protection against the progression of DN in an STZ-induced diabetic model. These beneficial effects appear to be mediated through a combination of potent antioxidant actions, favorable modulation of lipid metabolism, and beneficial interactions with cellular pathways involving key renal biomarkers such as PKM_2 and KIM-1. The ability of CeO_2NPs to catalytically scavenge ROS, coupled with their potential to influence metabolic and inflammatory processes, positions them as a promising multi-faceted therapeutic agent. Further rigorous investigation into their precise mechanisms of action, long-term safety, and efficacy in diverse and clinically relevant models is crucial to paving the way for their potential translation into effective clinical treatments for this debilitating and widespread diabetic complication.

Conflict of Interests

The authors declare no conflict of interest.

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Author Contributions

J.A, A.E, conducted the investigation and developed the methodology for the study. J.A & Z.S were responsible for validating the study methods and refining the methodology. J.A & N.R managed the software aspects of the study and performed data analysis. J.A& Z.S wrote and revised the drafting manuscript. Z.S played a crucial role in securing funding for the project and overseeing its administration.

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