Original Research

Epigallocatechin-3-gallate ameliorates renal endoplasmic reticulum stress-mediated inflammation in type 2 diabetic rats

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Impact Statement

Diabetes mellitus is one of the most prevalent chronic metabolic disorders, which is usually accompanied by various vascular complications, including nephropathy. Diabetic nephropathy is one of the leading causes of end-stage renal failure, which causes high mortality in diabetic patients. Sustained hyperglycemia usually induces a renal endoplasmic reticulum (ER) stress response. Unregulated ER stress promotes the overactivation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome and subsequently induces cell pyroptosis. Accumulating evidence has manifested that epigallocatechin-3-gallate exhibits anti-ER stress and anti-inflammatory properties, however, it is still unknown whether epigallocatechin-3-gallate could confer renoprotective activity via repressing NLRP3 inflammasome by suppressing ER stress in type 2 diabetes. The current study demonstrated that epigallocatechin-3-gallate treatment could repress the overactivation of NLRP3 inflammasome via suppressing ER stress and promoting renal functional recovery. ER stress-induced NLRP3 inflammasome overactivation might be an essential target for epigallocatechin-3-gallate treatment in diabetic patients.

Abstract

Epigallocatechin-3-gallate (EGCG), an essential polyphenolic constituent found in tea leaves, possesses various potent biological activities. This research was undertaken to investigate the impact of EGCG against endoplasmic reticulum (ER) stress-mediated inflammation and to clarify the underlying molecular mechanism in type 2 diabetic kidneys. The male rats were randomized into four groups: normal, diabetic, low-dose EGCG, and high-dose EGCG. In type 2 diabetic rats, hyperglycemia and hyperlipidemia noticeably caused renal structural damage and dysfunction and aggravated ER stress. Meanwhile, sustained ER stress activated the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome and then upregulated the contents of inflammatory cytokines in the diabetic kidney. Following supplementation with 40 mg/kg and 80 mg/kg EGCG, hyperglycemia, hyperlipidemia, and renal histopathological alterations and dysfunction were noticeably ameliorated; renal ER stress, NLRP3 inflammasome, and inflammatory response were markedly repressed in the EGCG treatment groups. In summary, the current study highlighted the renoprotective effects of EGCG in type 2 diabetes and its mechanisms are mainly associated with the repression of ER stress-mediated NLRP3 inflammasome overactivation.

Keywords: Epigallocatechin-3-gallate, diabetic nephropathy, rat, endoplasmic reticulum stress, NOD-like receptor family pyrin domain containing 3 inflammasome, inflammation

Experimental Biology and Medicine **2022; 247: 1410–1419. DOI: 10.1177/15353702221106479**

Introduction

Diabetes mellitus is one of the most prevalent chronic metabolic disorders with sustained hyperglycemia, which is generally accompanied by many vascular complications, including neuropathy, retinopathy, cardiomyopathy, and nephropathy.[1] Diabetic nephropathy (DN) is not only a pivotal factor in end-stage renal failure but also a crucial cause of high mortality in patients with diabetes.[2] Approximately 40% of type 2 diabetic patients eventually develop DN. DN has become a severe threat to diabetic patients' health.[3] Although the pathogenesis of DN is complicated and not fully elucidated, it is deemed that oxidative stress, endoplasmic reticulum (ER) stress, and inflammatory response are the major causes of the occurrence and progression of DN.[4–6]

The ER is an important cellular specialized organelle, which plays a key role in the synthesis, folding, and structural maturation of proteins in the eukaryotic cells.[7] Diverse adverse stimuli such as hyperglycemia, lipid overaccumulation, and advanced glycation end products (AGEs) cause the excessive aggregation of misfolded proteins in ER lumen, leading to the ER stress that is first mediated by the unfolded protein response (UPR).[8,9] Under ER stress conditions, three UPR sensors, including inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase RNA-like ER kinase (PERK), are activated to restore ER homeostasis.[10] However, unregulated ER stress causes cellular dysfunction and inflammation, ultimately resulting in cell injury and death.[11] In recent years, extensive research has shown that ER stress can mediate the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome overactivation.[12] The NLRP3 inflammasome is a multiprotein complex that can participate in inflammatory reactions and induce pyroptotic cell death.[13] A previous study reported that the NLRP3 inflammasome-induced inflammatory response occurs not only in immune cells but also in tubular epithelial cells in the diabetic kidney.[14] Furthermore, another experimental study demonstrated that ER stress-mediated NLRP3 inflammasome overactivation plays a vital role in angiotensin II-induced human renal proximal tubular cell injury.[15]

Epigallocatechin-3-gallate (EGCG) is an essential bioactive polyphenolic constituent found in green tea leaves.[16] A growing body of evidence has shown that EGCG exhibits low toxicity and diverse bioactivities, such as antioxidative, anti-inflammatory, anti-cancer, and antifibrotic properties.[17] A previous study has demonstrated that EGCG exerts its renoprotective effect in high-fat food and streptozotocin-induced diabetic rats by suppressing fibrosis and apoptosis.[18] Moreover, EGCG can alleviate high glucose-induced podocyte apoptosis by suppressing ER stress.[19] However, in type 2 diabetic kidneys, whether EGCG inhibits the overactivation of the NLRP3 inflammasome via repressing ER stress has not yet been determined. Therefore, the aim of the current research was to elucidate the protective effects of EGCG on the kidney by evaluating the ER stress-mediated NLRP3 inflammasome pathway in type 2 diabetic rats.

Materials and methods

Experimental animals

The animal procedures were performed in strict accordance with the Guidelines for the Use of Laboratory Animals of Hefei Normal University. Thirty-two healthy male Sprague-Dawley rats (age, 6–7 weeks; bodyweight, 160–200 g) were provided by the Experimental Animal Center of Anhui Medical University. All the rats were maintained in a monitored laboratory environment under the specific temperature of (23 ± 1°C), the humidity of (55 ± 5%) with an alternating 12 h light and 12 h dark cycle, and fed with drinking water and food *ad libitum*.

Induction of type 2 diabetes

The experimental animals were acclimatized for a week and then randomly assigned into 4 experimental groups: (1) normal control (NC) group; (2) DN group; (3) DN + low dose EGCG (LE) group; and (4) DN + high dose EGCG (HE) group. Each group consisted of 8 rats. The rats allocated to the NC group received a standard laboratory rodent food, whereas the rats assigned to the other groups received a high-fat food (consisting of 0.5% pig bile salt, 10% lard, 15% white sugar, 2% cholesterol, and 72.5% standard rodent food) throughout the whole experiment. After 4 weeks of high-fat food feeding, these obese rats were fasted overnight and intraperitoneally (i.p.) injected with 30 mg/kg streptozotocin (Sigma-Aldrich, St. Louis, MO, USA) prepared in ice-cold citrate buffer, twice a week, while the rats allocated to the NC group were fasted and injected, i.p., with citrate buffer. After three days of streptozotocin injection, fasting blood glucose (FBG) levels from the tail veins were detected using a handheld glucometer (Johnson, USA). An animal with a tail FBG level beyond 11.1 mmol/L was considered type 2 diabetic.[20] Subsequently, rats assigned to the LE and HE groups were daily gavaged with 40 mg/kg and 80 mg/kg EGCG (purity ⩾ 95%, Sigma-Aldrich), respectively, freshly dissolved in 0.9% saline.[21] Rats assigned to the NC and DN groups were daily gavaged with a similar volume of 0.9% saline over the same time period. EGCG treatment lasted for 8 weeks.

Assessment of renal function

After 8 weeks of treatment with EGCG, rats were kept in metabolic cages to collect urine every 24 h. The urine volume was recorded, and the urine protein content was measured using an assay kit (Jiancheng Biotechnology, Nanjing, China). Then, the overnight-fasted rats were weighed and injected, i.p., with 2% pentobarbital sodium at a dosage of 45 mg/kg to induce anesthesia. The blood samples from the abdominal aorta were gathered to obtain serum, and their bilateral kidneys were harvested immediately.

Serum biochemical assays

The levels of FBG, blood urea nitrogen (BUN), glycated serum protein (GSP), serum creatinine (sCr), triglycerides, total cholesterol (T-CHO) in serum were measured using corresponding commercial kits (Jiancheng Biotechnology). Serum AGEs were measured by an ELISA kit (Cusabio Biotechnology, Wuhan, China).

Histopathological assessments

One part of the fresh left kidney was dissected, fastened in a solution of 4% paraformaldehyde, and fixed in a paraffin block. The paraffin sections were made into serial slices at a thickness of 4 μm and subjected to periodic acid-Schiff (PAS) reagents (Servicebio Biotechnology, Wuhan, China). Slices were imaged using a CaseViewer digital slide scanner.

The renal ultrastructure was observed as per the detailed method.[22] In brief, the right fresh kidneys were dissected into 1 mm × 1 mm × 1 mm cubes and fixed in a solution of 2.5% glutaraldehyde. Subsequently, these renal tissues were post-fixed in 1% osmium tetroxide and embedded in Epon812. Renal tissues were cut into 70 nm thick ultrathin sections and stained with uranyl acetate and lead citrate. These ultrathin

Table 1. The primer sequences used in this study.

Gene	Primer sequence	Length (bp)
IRE1	Forward: 5′-GACGGACAGAATACACCATCA-3′ Reverse: 5′-TCTTGTAGTCCACGTCATCCTC-3′	114
ATF6	Forward: 5′-GGTGTATTACGCTTCGCCTG-3′ Reverse: 5′-TTGTGGTCTTGTTATGGGTGGT-3′	134
PERK	Forward: 5′-CCAAGCTGTACATGAGCCCAGA-3′ Reverse: 5′-TTTCTGAGTGAACAGTGGTGGAAAC-3′	179
GRP78	Forward: 5′-CGCGCTCGATACTGGCTGTGACTA-3′ Reverse: 5′-TTCATCTTGCCGGCGCTGTG-3′	168
CHOP	Forward: 5′-GTGTTCCAGAAGGAAGTGCA-3′ Reverse: 5′-CTCCTGCAGATCCTCATACC-3′	150
GAPDH	Forward: 5′-ACTCAGAAGACTGTGGATGG-3′ Reverse: 5′-TACATTGGGGGTAGGAACAC-3′	163

IRE1: inositol-requiring enzyme 1; ATF6: activating transcription factor 6; PERK: protein kinase RNA-like ER kinase; GRP78: glucose-regulated protein 78; CHOP: C/EBP homologous protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

sections were imaged by a JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan).

Measurement of inflammatory factors

The kidney tissue sample (100 mg) was ground in liquid nitrogen and then homogenized in nine-fold ice-cold 0.9% saline. After centrifugation at 3000 × g for 20 min, the supernatant liquid was gathered, and then the protein quantification was finished using a bicinchoninic acid kit (Beyotime Biotechnology, Shanghai, China). Subsequently, renal tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, interleukin (IL)-1β, and IL-18 were measured as per the instruction manuals of corresponding ELISA kits (Cusabio Biotechnology).

Immunohistochemical (IHC) assessment

The IHC assessment was conducted in accordance with the detailed method.[13] Briefly, the kidney slices mounted in wax were deparaffinized and rehydrated. Following incubation with hydrogen peroxide and bovine serum albumin, these renal slices were subjected to anti-kidney injury molecule 1 (KIM1; Boster Biotechnology, Wuhan, China) and IRE1 (Proteintech Biotechnology, Wuhan, China) primary antibodies. After that, the renal slices were subjected to a secondary antibody (Boster Biotechnology). The results of the IHC assessment were imaged using CaseViewer. The semi-quantitative analysis was calculated to determine the expression of KIM1 and IRE1 in renal tissue using Image-Pro Plus software.

Real-time PCR assessment

Total RNA in renal tissue was extracted using Beyozol reagent (Beyotime Biotechnology). Forward and reverse primers for IRE1, ATF6, glucose-regulated protein 78 (GRP78), PERK, C/EBP homologous protein (CHOP), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were described in Table 1, and the GAPDH gene was used as the housekeeping gene. The real-time PCR assessment was performed using a TB Green Premix Ex TaqII assay kit (Takara, Dalian, China). Ultimately, the relative quantification of mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Kidney tissue samples (80 mg) were homogenized in 800 μL of RIPA Lysis Solution containing protease inhibitors (Beyotime Biotechnology). Following centrifugation at 3000 × g for 20 min, the supernatant was separated to calculate the total protein content, mixed with the loading buffer (v: v = 4: 1), and then boiled at 100°C for 5 min. Next, SDS-PAGE was utilized to separate the proteins at a concentration of 60 μg. The isolated protein was electrically transferred onto a PVDF membrane. After that, all the membranes were immersed in 5% non-fat milk powder in tris-buffered saline containing 0.1% Tween-20 (TBST) for 2 h and then incubated for 16 h at 4°C with the corresponding primary antibodies: IRE1, X box-binding protein (XBP) 1 (Abcam, Cambridge, UK), NLRP3 (Boster Biotechnology), caspase-1 (p20) (Boster Biotechnology), and GAPDH (Boster Biotechnology). The next day, these membranes were covered with secondary antibodies. Finally, these membranes were treated using an enhanced chemiluminescence substrate, and all the images were obtained using an imaging instrument (Tanon, Shanghai, China).

Statistical analysis

Statistical data was analyzed using GraphPad 5.0 (GraphPad Software, USA). The quantitative results were presented as means with their standard deviation (SD) for each group. Statistical comparisons were subjected to a one-way analysis of variance followed by the Newman-Keuls test. The values of $P < 0.05$ were considered to have a significant difference.

Results

EGCG reduced blood glucose and lipid parameters

The effect of EGCG treatment on blood glucose and lipids was measured after 8 weeks of gavage treatment. As shown in Figure 1, FBG, GSP, triglycerides, T-CHO, and AGEs in the DN group were markedly higher than those in the NC group, indicating that hyperglycemia and hyperlipidemia existed in type 2 diabetic rats. These indices were significantly ameliorated after supplementation with 40 mg/kg and 80 mg/kg EGCG in the LE and HE groups. In particular,

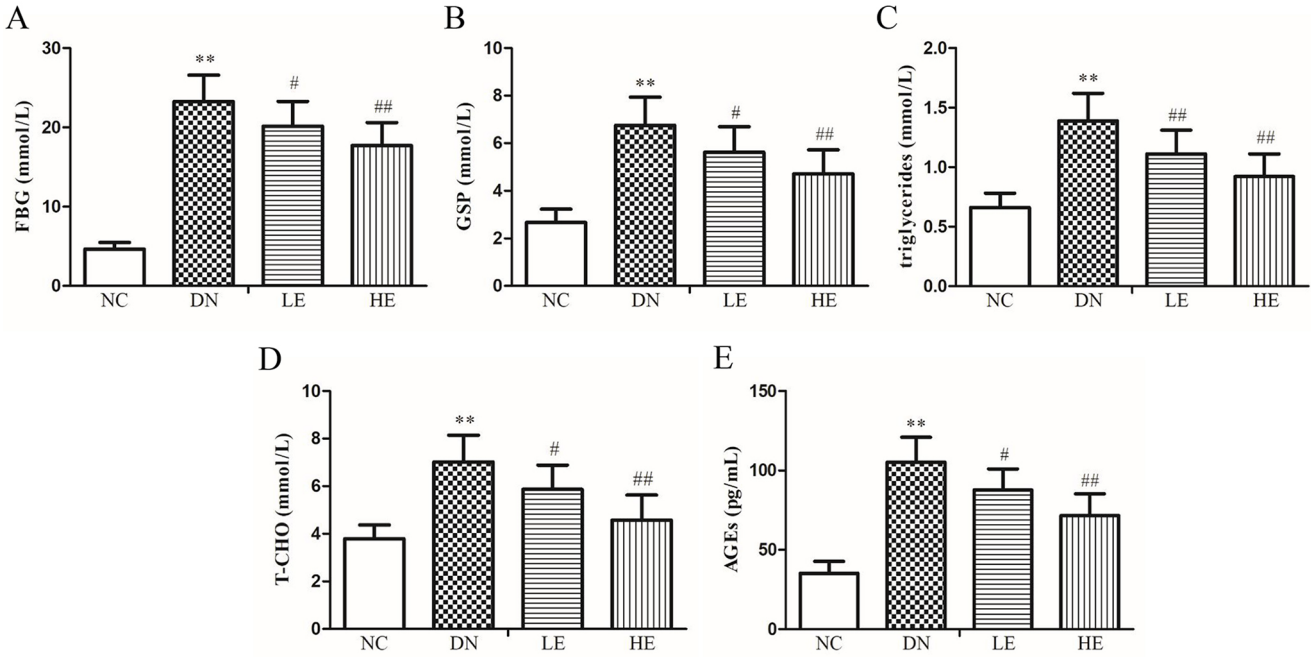

Figure 1. Effects of EGCG treatment on blood glucose and lipid parameters in type 2 diabetic rats: (A) FBG, (B) GSP, (C) triglycerides, (D) T-CHO, and (E) AGEs. All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with DN group.

the amelioration effect of 80 mg/kg EGCG was better than that derived from the lower, indicating that EGCG treatment could attenuate hyperglycemia and hyperlipidemia in type 2 diabetic rats.

EGCG promoted renal functional recovery

As shown in Figure 2, the volume of 24 h urine, 24 h urine protein, sCr, and BUN distinctly increased in the DN group compared with the NC group, indicating that renal injury and dysfunction were exacerbated in type 2 diabetic rats. Renal function was markedly improved in the LE and HE groups after EGCG treatment. Moreover, the results in the HE group were closer to those in the NC group than the low-dose EGCG treatment, indicating that EGCG promoted renal functional recovery in type 2 diabetic rats.

Effects of EGCG on renal histological changes

The PAS staining of kidneys was assessed in all groups (Figure 3(A)). In the NC group, the glomerular structure of the kidney was normal, and inflammatory cell infiltration was rare. In the DN group, the glomerular mesangial matrix was increased perceptibly, the glomerular cavity was narrowed, and inflammatory cells were infiltrated in the kidney. Following treatment with EGCG, renal pathological changes were slightly ameliorated in the LE group. The pathological changes and inflammatory cell infiltration in the HE group were ameliorated in kidney tissue.

Electron microscopy (Figure 3(B)) displayed that the glomerular basement membrane (GBM) was homogeneous with the same thickness. The foot processes of podocytes were distributed evenly in the renal tissue in the NC group. Conversely, the thickness of GBM increased obviously, the fusion of foot processes aggravated, and even the foot processes were disappeared in the DN group. On treatment with EGCG, the thickened GBM and foot processes fusion were alleviated in kidney tissue, and the improvement effect in the HE group was better than that in the LE group, indicating that EGCG exhibited a beneficial effect on renal pathological alterations in type 2 diabetic rats.

EGCG decreased renal KIM1 and IRE1 expression

According to the results of the IHC assessment (Figure 4), sustained hyperglycemia elevated the protein expression levels of KIM1 and IRE1 in the kidneys of diabetic rats. After treating diabetic rats with different doses of EGCG, the expression of KIM1 and IRE1 declined in renal tissues, which further demonstrated that EGCG exerted a beneficial impact on type 2 diabetic kidneys.

EGCG attenuated renal ER stress

The real-time PCR assay was utilized to examine the mRNA expression of renal ER stress-related proteins. As shown in Figure 5, the mRNA expression levels of renal IRE1, ATF6, PERK, GRP78, and CHOP in diabetic rats were markedly higher than those in normal rats, demonstrating that ER stress was aggravated in the diabetic kidney. In contrast to the DN group, EGCG treatment markedly reduced the mRNA expression levels of renal ER stress markers, especially in the HE group. These findings showed that EGCG treatment noticeably alleviated renal ER stress in type 2 diabetic rats.

EGCG reduced renal pro-inflammatory cytokines

The levels of renal pro-inflammatory cytokines, including TNF-α, MCP-1, IL-1β, and IL-18, reflect the inflammatory

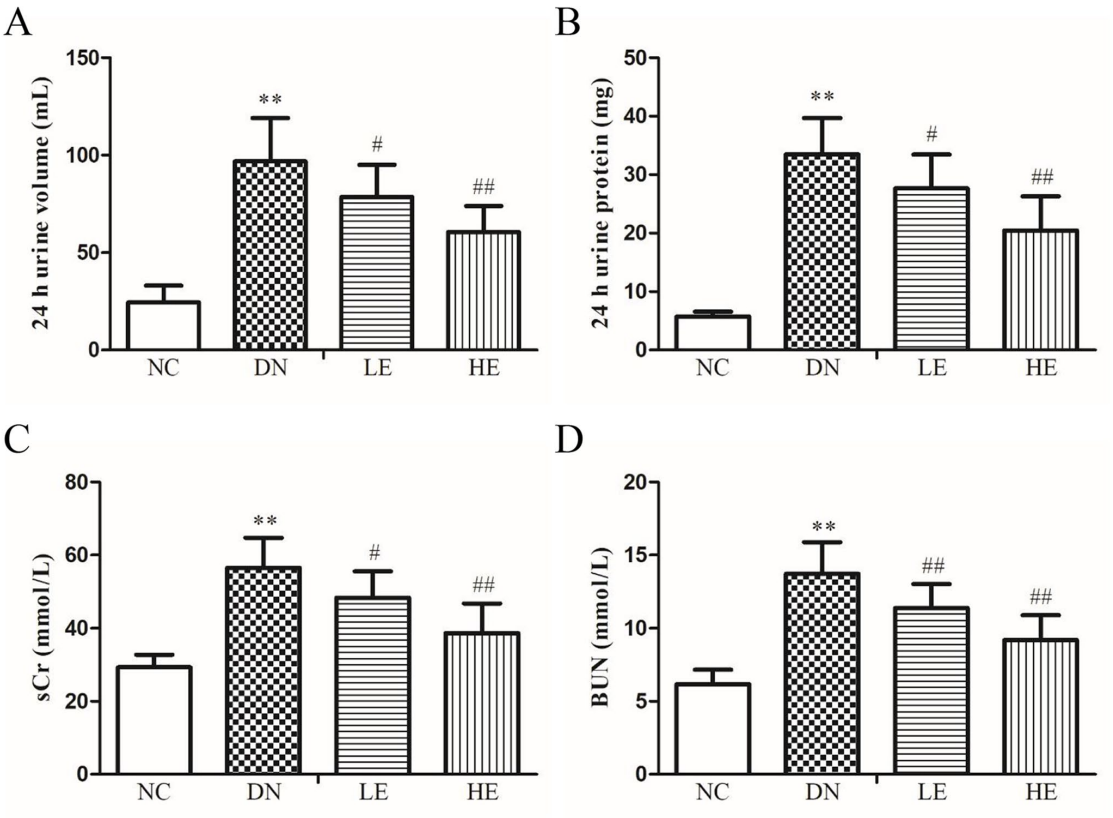

Figure 2. Effects of EGCG treatment on renal functional indices: (A) 24 h urine volume, (B) 24 h urine protein, (C) sCr, and (D) BUN. All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with DN group.

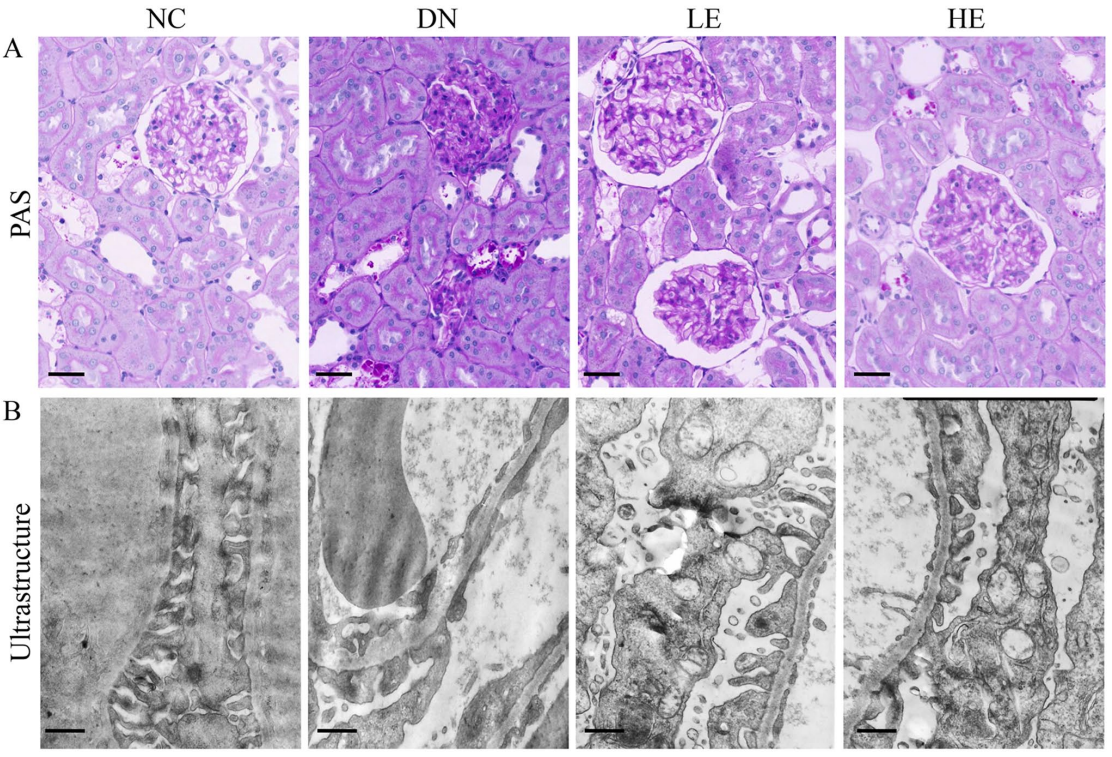

Figure 3. Effects of EGCG treatment on pathological changes in kidney tissue. (A) PAS staining. Scale bar: 30 μm. (B) Ultrastructure. Scale bar: 1 μm. (A color version of this figure is available in the online journal.)

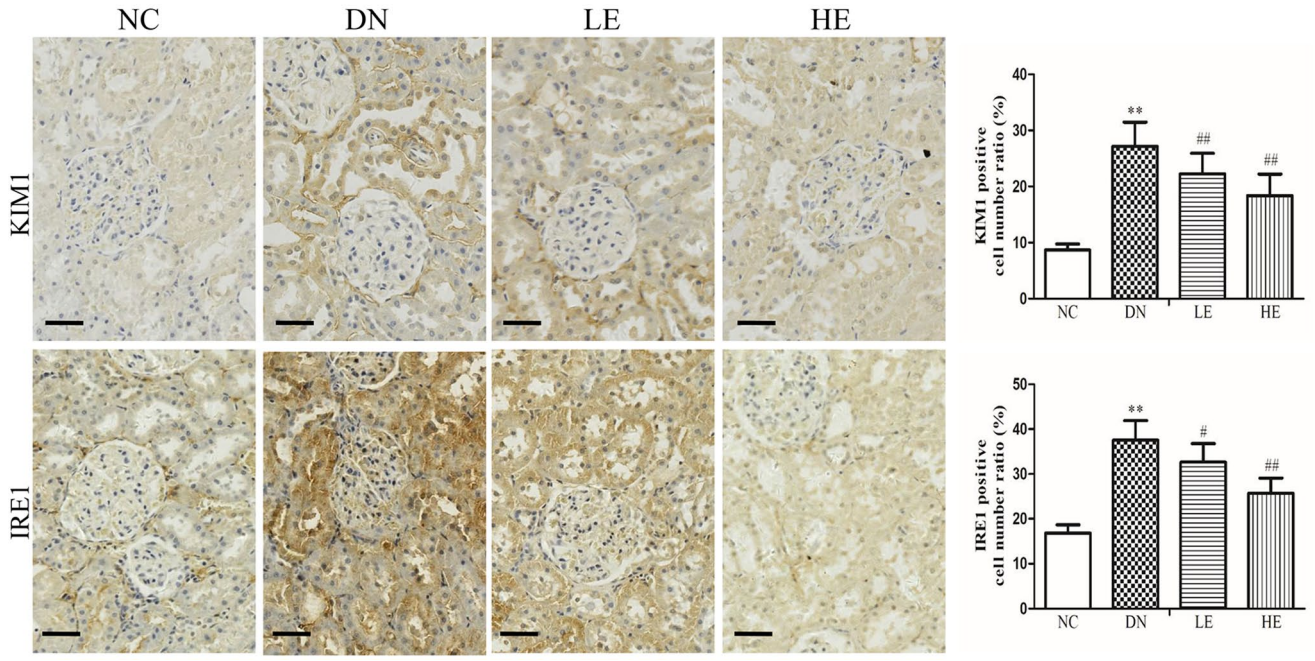

Figure 4. Effects of EGCG treatment on KIM1 and IRE1 expression in kidney tissue. (A) Expression of renal KIM1 in IHC assay. (B) Expression of renal IRE1 in IHC assay. Scale bar: 50 μm. All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with the NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with the DN group. (A color version of this figure is available in the online journal.)

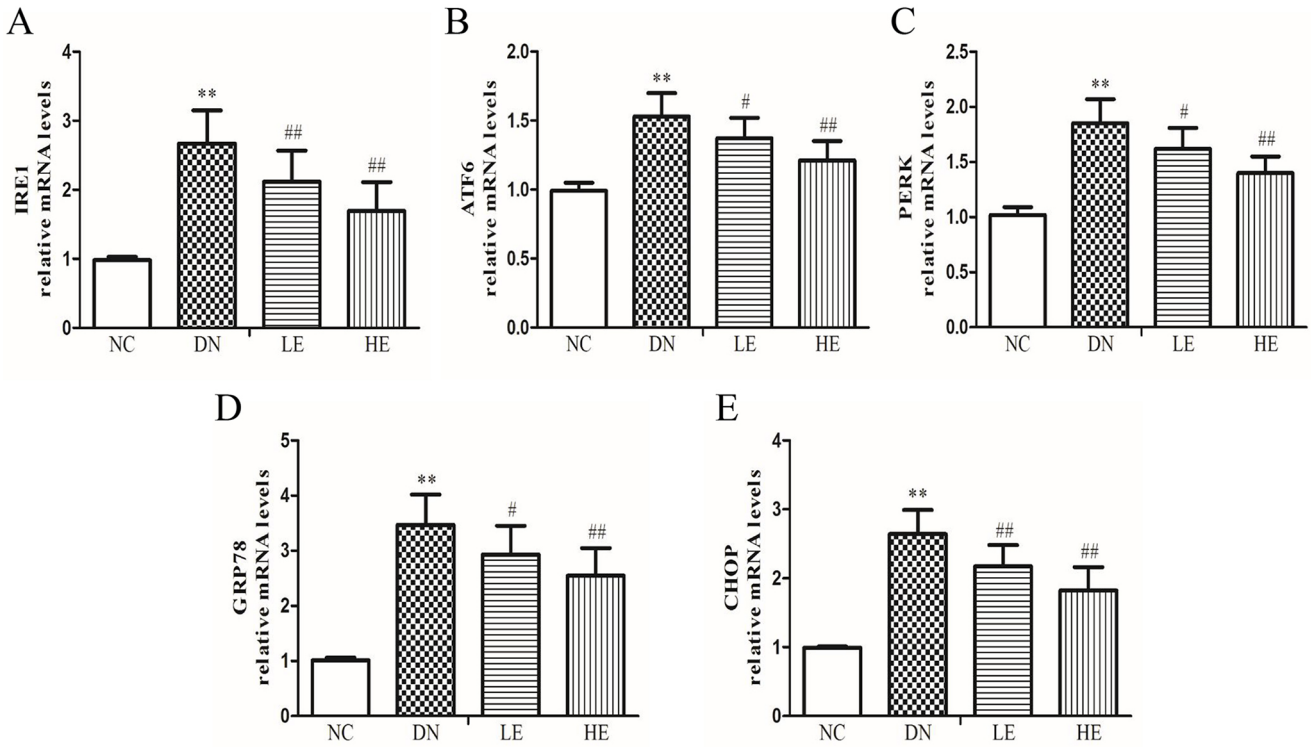

Figure 5. EGCG treatment decreases the mRNA expression of ER stress markers in kidney tissue of diabetic rats: (A) IRE1, (B) ATF6, (C) PERK, (D) GRP78, and (E) CHOP.
All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with DN group.

state of the kidney. In the DN group, the contents of renal pro-inflammatory cytokines were markedly elevated. After treatment with 40 mg/kg and 80 mg/kg of EGCG, the contents of renal pro-inflammatory cytokines were decreased. Moreover, the amelioration effect in the HE group was better than that in the LE group, which revealed that EGCG possessed an anti-inflammatory property in the diabetic kidney (Figure 6).

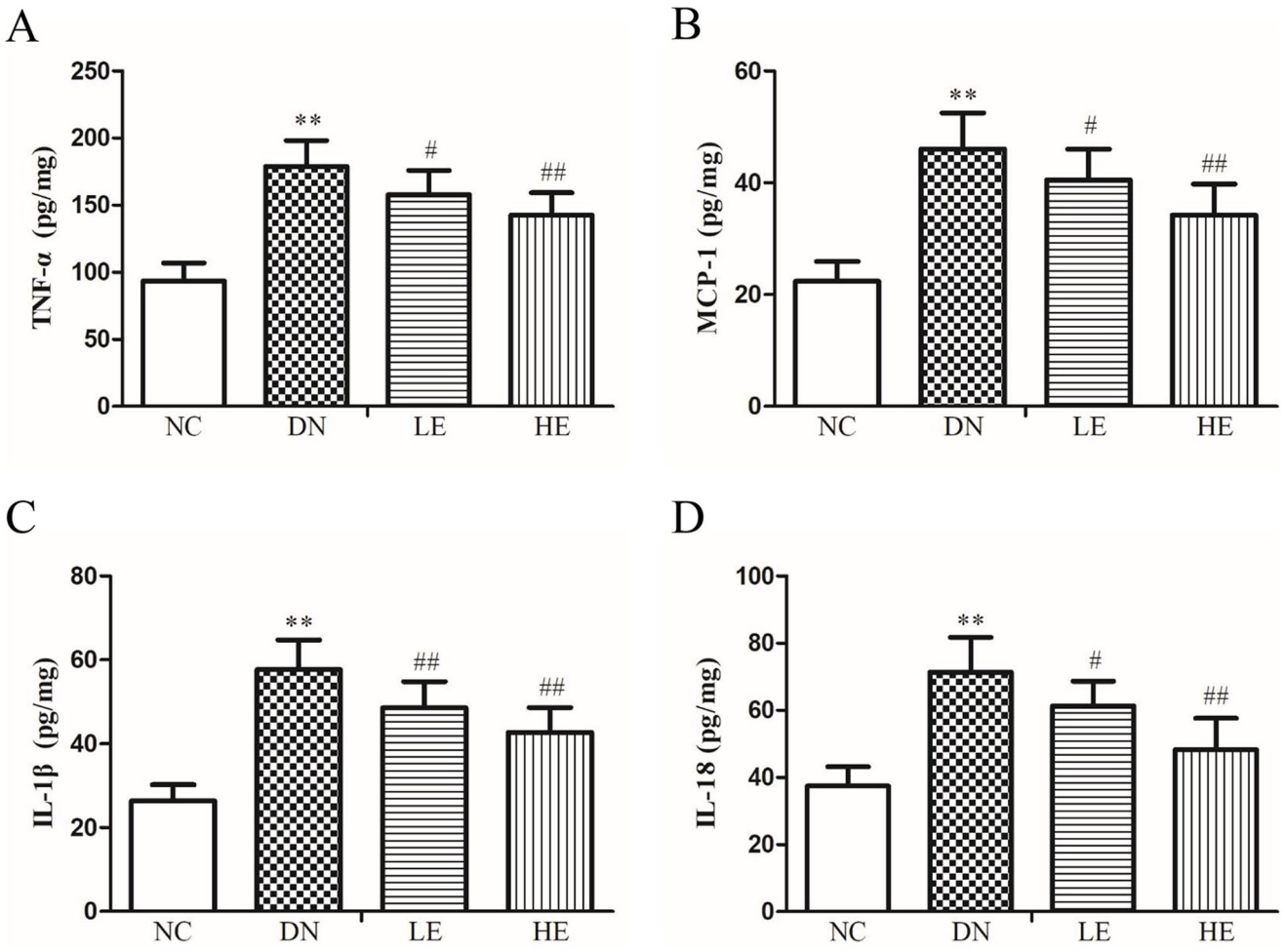

Figure 6. EGCG treatment downregulates renal inflammatory cytokines in type 2 diabetic rats: (A) TNF-α, (B) MCP-1, (C) IL-1β, and (D) IL-18. All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with DN group.

Effects of EGCG on renal IRE1/XBP1 and NLRP3 inflammasome

As shown in Figure 7, diabetes increased the protein expression levels of renal IRE1, XBP1, NLRP3, and caspase-1, confirming that renal ER stress was aggravated and the NLRP3 inflammasome was activated. On treatment with 40 mg/kg and 80 mg/kg EGCG, ER stress and NLRP3 inflammasome were repressed in the EGCG treatment groups. These findings demonstrated that EGCG could repress the overactivation of the renal NLRP3 inflammasome via suppressing ER stress.

Discussion

DN is not only one of the microangiopathies caused by diabetes but also the most prominent factor in end-stage renal disease.[23] The pathological alterations of DN are well characterized by increases in glomerular filtration rate and microalbuminuria, glomerular mesangial matrix expansion, GBM thickening, and interstitial fibrosis. FBG and GSP are commonly used to assess blood glucose levels, while triglycerides and T-CHO reflect the levels of blood lipids in type 2 diabetes.[24] Growing studies suggest that elevated AGEs levels, caused by a glucose-induced metabolic disorder in type 2 diabetes, are a crucial player in the progression of kidney disease.[25] In the current work, our experimental data showed that in the DN group, FBG, GSP, triglycerides, T-CHO, and AGEs were higher than those in the normal rats, indicating that the model of type 2 diabetic rats was successfully established.

Meanwhile, renal function-related indices and pathological observations demonstrated that renal excretory function and architecture were damaged in diabetic rats. KIM1 is often utilized to assess renal injury in acute and chronic renal diseases.[26] Our IHC results indicated that the KIM1 expression level was highly upregulated in the diabetic kidney, demonstrating that type 2 diabetes causes renal injury and dysfunction. After ingestion with 40 mg/kg and 80 mg/kg EGCG, hyperglycemia, hyperlipidemia, AGEs, renal pathological changes, excretory dysfunction, and KIM1 protein expression were ameliorated in the LE and HE groups. Although the blood glucose levels improved in the EGCG treatment groups, they were much higher than 11.1 mmol/L and were still in a hyperglycemic state. Therefore, we speculated that EGCG had a direct protective effect on the kidneys in type 2 diabetic rats.

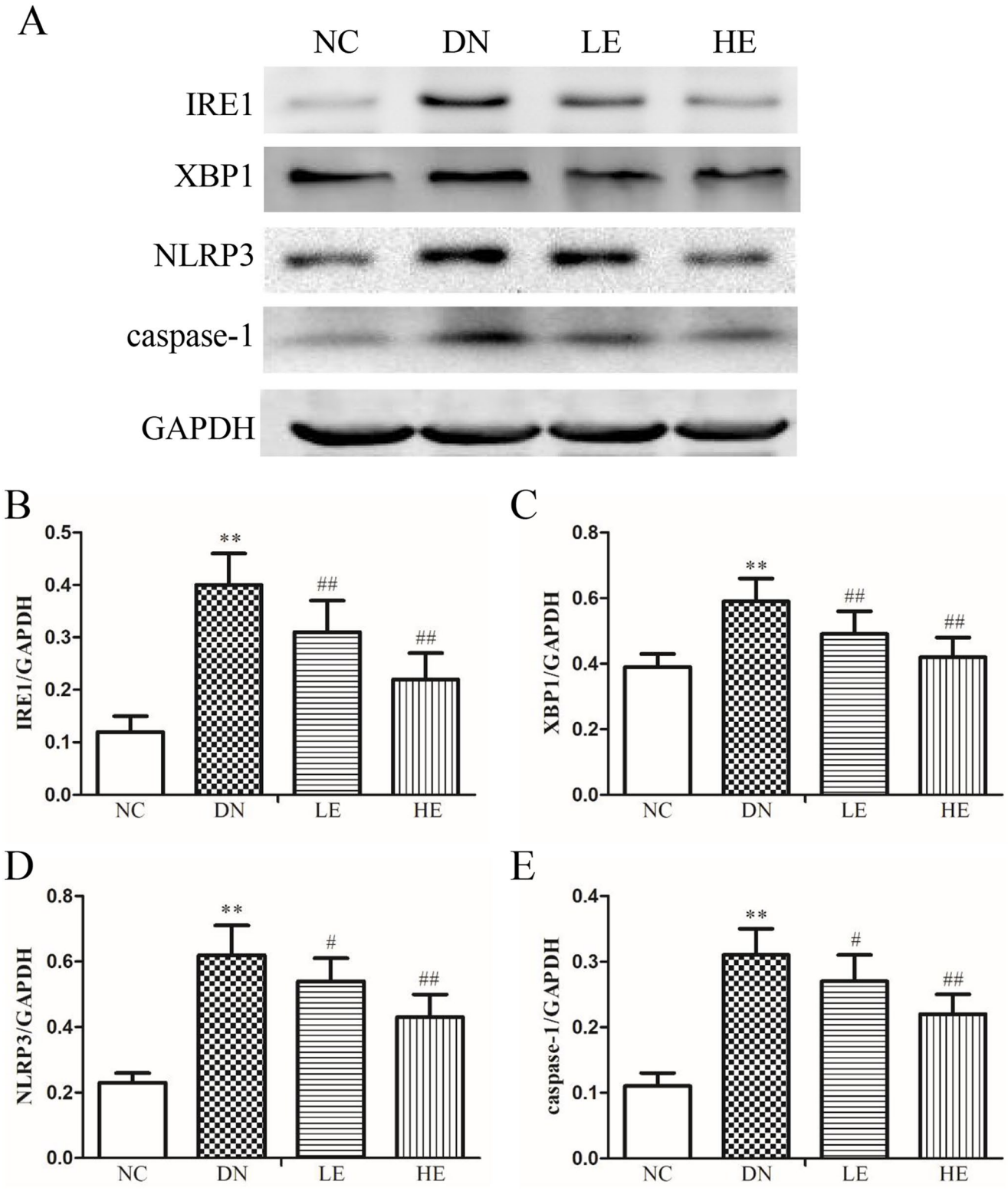

Figure 7. Effects of EGCG treatment on IRE1/NLRP3 inflammasome expression in kidney tissue of each group. (A) Western blotting results for renal IRE1, XBP1, NLRP3, and caspase-1 (p20). (B) to (E) Relative expression of IRE1, XBP1, NLRP3, and caspase-1 (p20) proteins in kidney tissue by western blot assay. GAPDH was used as the housekeeping protein.
All values were presented as the mean ± SD. $^{**}P < 0.01$, when compared with NC group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with DN group.

EGCG, one of the primary bioactive ingredients of green tea, has significant biological activities such as anti-oxidation, anti-inflammation, and anti-fibrosis in various tissues and cells.[27] Recent research has demonstrated that EGCG has a positive impact on reducing chronic metabolism conditions such as obesity and diabetes.[28] In the current work, we referred to the relevant literature and conducted preliminary experiments to determine the doses of EGCG at 40 mg/kg

and 80 mg/kg.[18,21] Our experimental results demonstrated that EGCG had hypoglycemic and hypolipidemic effects in diabetic rats, and the improvement effect of 80 mg/kg EGCG was better than that derived from the lower EGCG dose, as evidenced by the improvement of FBG, GSP, triglycerides, T-CHO, and AGEs. In addition, previous research reported that EGCG exerted a renoprotective effect against fibrosis and apoptosis in diabetic rats.[18] Recently, Xiang *et al.*[19] reported that EGCG exhibited an anti-ER stress effect on podocyte injury caused by high glucose. However, there is no report about the impact of EGCG on ER stress in the diabetic kidney. Thus, we hoped to elucidate this effect and its molecular mechanism *in vivo*.

ER stress has been recognized as one of the prime factors involved in the occurrence and development of DN. In recent research, it has been pointed out that many attributes of DN, including hyperlipidemia, hyperglycemia, and elevated AGEs, can trigger ER stress in kidney cells.[29] In the human or murine model of kidney diseases, UPR sensors, including IRE1, ATF6, and PERK, were highly expressed in tubular and glomeruli cells. In addition to the UPR sensors, GRP78 and CHOP are also considered to be classic markers of ER stress.[30] GRP78 is a key regulator of ER stress bound to UPR sensors. Unregulated ER stress leads to over-expression of GRP78 and CHOP; the latter is an important pro-apoptotic transcription factor.[31] Under hyperglycemia conditions, irremediable ER stress was aggravated in kidney tissue, as evidenced by the increased expression levels of IRE1, ATF6, PERK, GRP78, and CHOP. Our data showed that, in contrast to the normal rats, renal ER stress markers were highly expressed in diabetic rats, which further demonstrated that renal ER stress was aggravated in type 2 diabetes. Chronic treatment with EGCG for 8 weeks, the mRNA expression levels of ER stress markers were reduced significantly in kidney tissue, indicating the beneficial impact of EGCG against renal ER stress in type 2 diabetic animals.

DN is also tightly related to NLRP3 inflammasome-induced inflammation.[32] Previous evidence showed that IRE1, one of three UPR sensors, is also a salient activator for the NLRP3 inflammasome involved in renal injury.[33] IRE1 is an ER transmembrane sensor that senses the initiation of ER stress and then regulates the XBP1 expression to trigger downstream pathways to resistance ER stress, such as the UPR pathway, nuclear factor κB pathway, and the NLRP3 inflammasome.[34,35] The NLRP3 inflammasome is considered a potential therapeutic target to ameliorate renal inflammation and mainly comprises NLRP3 and procaspase-1.[36,37] On the one hand, an activated NLRP3 inflammasome acts as a high-molecular-weight platform for promoting the cleavage of the procaspase-1 and generation of bioactive IL-1β and IL-18, exacerbating the inflammatory responses and tissue damages.[38] On the other hand, activated renal NLRP3 inflammasome upregulates KIM1 expression and aggravates tubule cell injury.[39] The current work showed that compared with the normal kidney, the concentrations of renal pro-inflammatory cytokines and the IRE1, XBP1, NLRP3, and caspase-1 (p20) expression were noticeably elevated in the diabetic kidney, which confirmed that unregulated ER stress could mediate overactivation of the NLRP3 inflammasome and, then, the inflammatory reaction and renal injury were aggravated in the diabetic rats. Recently, Bao and Peng[40] found that EGCG had anti-inflammatory properties in chronic kidney disease. Jhang *et al.*[41] also reported that EGCG exhibited anti-inflammatory effects by inhibiting the activation of the NLRP3 inflammasome in mice with gouty arthritis. In our study, after supplementation with EGCG, the expression of ER stress markers and the NLRP3 inflammasome noticeably declined, confirming that EGCG had an anti-inflammatory effect on the kidney mainly through repression of the ER stress and NLRP3 inflammasome in type 2 diabetes.

In conclusion, EGCG exhibited markedly renoprotective effects in type 2 diabetes and its molecular mechanism was associated with the repression of NLRP3 inflammasome overactivation mediated by ER stress. These findings indicate that EGCG offers promising therapeutic potential for the treatment of DN in the future.

AUTHORS' CONTRIBUTIONS

RY, JC, and QJ designed the research. RY, SM, XY, and QJ ran the experiments. RY, XY, and JC analyzed data and wrote the manuscript. JC, QJ, and SM revised the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Natural Science Research Project of Hefei Normal University (grant number: 2021KJZD25); the Natural Science Research Project of Bengbu Medical College (grant number: 2020byzd033); the Natural Science Research Project of Anhui Educational Committee (grant numbers: KJ2021A0697, KJ2021A0922); the Anhui Provincial Natural Science Foundation (grant number: 2008085MC65); and the Research Activities of Postdoctoral Researchers Foundation of Anhui Province (grant number: 2020B470), China.

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(Received March 31, 2022, Accepted May 19, 2022)