Article

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Experimental Study of EGCG on Endoplasmic Reticulum Stress-Induced Renal Apoptosis in T2DM Rats

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Background: Diabetic nephropathy (DN), a severe complication of diabetes, is one of the primary causes of chronic kidney disease (CKD) in China. Epigallocatechin gallate (EGCG), a natural compound found in tea leaves, exhibits both preventive and therapeutic properties against several diseases, including cancer, obesity, diabetes, and cardiovascular diseases. Therefore, this study aimed to investigate the protective effects of EGCG on renal tissue apoptosis induced by endoplasmic reticulum stress in rats with type 2 diabetes mellitus (T2DM).

Methods: The kidney tissues of Wistar rats were collected and analyzed following treatment with Streptozotocin (STZ) and EGCG. Moreover, fasting blood glucose (FBG), fasting insulin (FINS), urea nitrogen (UREA), and creatinine (CREA) were assessed using corresponding enzyme-linked immunosorbent assay (ELISA) kits. Similarly, the apoptosis index (AI) was examined utilizing terminal deoxynucleotidyl transferase-mediated dUTP Nick end labeling (TUNEL). Furthermore, the expression levels of C/EBP homologous protein (*CHOP*) and phosphorylated c-Jun N-terminal kinase (*p-JNK*) were determined in each group of rats by immunohistochemistry and Western blot analysis. Additionally, the mRNA transcription levels of *CHOP* and *JNK* were assessed using real-time fluorescent quantitative polymerase chain reaction (RT-qPCR).

Results: Compared to the normal control group (NOR), the levels of FBG, FINS, UREA, CREA, and apoptosis index (AI) were elevated ($p < 0.0001$) in the diabetic model control group (MOR). Furthermore, the mean optical density values of *CHOP* and *p-JNK*, the mRNA and protein expression levels of CHOP, the protein expression levels of p-JNK, and the mRNA expression levels of *JNK* were significantly increased in the experimental group ($p < 0.0001$). Additionally, compared to the MOR group, the levels of FBG, FINS, UREA, CREA, and AI were significantly alleviated in both the EGCG low-dose group (EGCG1) and EGCG high-dose group (EGCG2) ($p < 0.0001$). Similarly, the average optical density values of *CHOP* and *p-JNK*, the mRNA and protein expression levels of CHOP, the protein expression level of p-JNK, and the mRNA expression level of *JNK* were significantly decreased ($p < 0.01$).

Conclusions: These findings indicate that EGCG reduces renal injury and cell apoptosis in diabetic rats by inhibiting the expression of endoplasmic reticulum stress-related proteins, such as CHOP and JNK.

Keywords: type 2 diabetes mellitus; diabetic nephropathy; endoplasmic reticulum stress; apoptosis; Epigallocatechin gallate

Introduction

Diabetes mellitus (DM) is a common metabolic disease characterized by chronic hyperglycemia, often accompanied by lipid and protein metabolism disorders. It gives rise to various complications involving the cardiovascular system, cerebrovascular system, eyes, and kidneys. Diabetic nephropathy (DN) is a severe complication of diabetes and a leading cause of chronic kidney disease (CKD) in China [1]. The current strategies to prevent and treat diabetic nephropathy primarily focus on regulating blood glucose and blood pressure levels, inhibiting the activation of the renin-angiotensin system (RAS), and using renal replacement therapies such as hemodialysis or kidney transplantation in the advanced stage of DN [2]. However, hemodialysis can lead to cardiovascular and cerebrovascular events, while renal transplantation is expensive and may induce rejection reactions, thus limiting its efficacy in addressing complications [3]. Hence, the development of effective prevention and treatment strategies for diabetic kidney disease is crucial.

Apoptosis has been identified as a critical factor contributing to the progression of DN from its initial to more advanced stages [4–6]. Endoplasmic reticulum stress-

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induced apoptosis is closely associated with the occurrence and development of DN. The endoplasmic reticulum (ER) is a membrane-bound organelle found in eukaryotic cells, playing a significant role in regulating apoptosis. Excessive or prolonged ER-stress leads to the activation of pro-apoptotic transcription factors such as c-Jun N-terminal kinase (*JNK*) and C/EBP homologous protein (*CHOP*), triggering the apoptosis pathway in cells [7,8]. Therefore, targeting ER-stress-induced apoptosis is a potential approach for both preventing and treating DM.

Epigallocatechin gallate (EGCG) is a catechin compound predominantly found in tea and is a crucial component of tea polyphenols. EGCG exhibits both preventive and therapeutic properties against several diseases such as cancer, obesity, diabetes, and cardiovascular diseases. Previous studies have demonstrated the ability of EGCG to reduce cell apoptosis in cardiomyocytes, nerve cells, and renal podocytes [9,10]. The underlying mechanism involves the ability of EGCG to reduce the levels of endoplasmic reticulum stress marker proteins such as Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), 78-kDa glucose-regulated protein (GRP78), and Caspase-12 [11,12]. Additionally, research has demonstrated that EGCG mitigates hepatocyte apoptosis in diabetic rats by decreasing the expression levels of ER-stress-related proteins PERK and GRP78 [13]. However, there is a scarcity of research investigating the effect of EGCG on apoptosis in the diabetic kidney and the proteins CHOP and JNK.

This study aimed to investigate the beneficial impacts of EGCG on the kidneys in diabetic rats. We established a diabetic rat model and assessed changes in renal function, cell apoptosis, and the expression levels of ER-stress-related apoptosis proteins, CHOP, and JNK. The protective mechanism of EGCG might be associated with the modulation of ER-stress-induced apoptosis. This study holds promise for providing novel insights into the clinical treatment of diabetes and its associated complications. Additionally, it serves as an experimental basis for developing natural products, aiming at reducing ER-stress-induced apoptosis.

Materials and Methods

Reagents and Antibodies

Epigallocatechin gallate (EGCG) (#SE8120) was obtained from Beijing Solebo in Beijing, China. Streptozotocin (STZ) was purchased from Sigma in St. Louis, MO, USA (#CV900890). The blood glucose tester (#GA-3) was obtained from Changsha Sano Technology Co., Ltd. in Changsha, China. The insulin detection kit was obtained from North Biotechnology Institute in Beijing, China (#20162400316). The *in-situ* apoptosis kit was purchased from Roche in Basel, Switzerland (#11684817910). Anti-rabbit monoclonal antibody phosphorylated JNK (p-JNK) (#bs-1640R, 1:400 for Western blot (WB) and 1:100 for immunohistochemistry (IHC)) was purchased from Boorson Biotechnology Co., Ltd., Beijing, China. Anti-rabbit monoclonal antibody CHOP (#bs-20669R, 1:500 for WB and 1:100 for IHC) was purchased from Boorson Biotechnology Co., Ltd., Beijing, China. The anti-rabbit monoclonal antibody JNK used for WB was purchased from Boorson Biotechnology Co., Ltd., Beijing, China (#bs-2590R, 1:500 dilution), and the anti-mouse monoclonal antibody glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used for WB was purchased from Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China (#TA-08, 1:1000 dilution).

Establishment of Animal Models

The diabetes mellitus type 2 (T2DM) rat model was established using previously established methods [13]. Male Wistar rats (n = 32), weighing 180–200 g, were obtained from the Animal Center of North Sichuan Medical College, China (SCXK, CHUAN, 2018-018). Eight rats were selected using a random number table method and assigned as a normal control group (NOR). The remaining 24 rats were fed with a high-fat diet for 4 weeks and then injected intraperitoneally with STZ (25 $mg \cdot kg^{-1}$). Those with fasting blood glucose (FBG) levels $\geq$11.1 $mmol \cdot L^{-1}$ were considered T2DM rat models. Moreover, fasting blood glucose (FBG), fasting insulin (FINS), urea nitrogen (UREA), and creatinine (CREA) levels were determined following the 8th week of treatment.

Furthermore, they were divided into three groups utilizing the random number table method (n = 8 per group): the diabetic model control group (MOR), the EGCG low-dose group (EGCG1, Intragastric administration of 20 $mg \cdot kg^{-1} \cdot d^{-1}$), and the EGCG high-dose group (EGCG2, Intragastric administration of 50 $mg \cdot kg^{-1} \cdot d^{-1}$). The NOR group received the same volume of normal saline. Meanwhile, the rats were regularly assessed for FBG and FINS levels during the feeding period. After 8 weeks of intragastric administration of EGCG, the rats in each group underwent a 12-hour fasting, followed by collection of their blood plasma and serum samples through cardiac puncture and stored at –80 °C for subsequent analysis. After anesthesia with 1% pentobarbital, rats in each group were euthanized using the spinal dislocation method, and kidney tissue was quickly extracted. Some tissue sections were preserved for immunohistochemistry, while the remaining tissues were frozen in liquid nitrogen for further analysis.

Blood Index Detection

The levels of fasting blood glucose (FBG) and fasting insulin (FINS), before and after treatment, were determined using corresponding enzyme-linked immunosorbent assay (ELISA) kits coupled with an automatic biochemical analyzer. Moreover, UREA and creatinine (CREA) levels were observed to assess renal function, as well as to examine the changes in these indicators following EGCG intervention.

Detection of Renal Tissue Apoptosis Using TUNEL

Renal cell apoptosis was evaluated employing the terminal deoxynucleotidyl transferase-mediated dUTP Nick end labeling (TUNEL) method. The cells were treated with TUNEL reagent and brownish-yellow nuclei were observed under the light microscope. Apoptosis index (AI), referring to the percentage of apoptotic cells in 5 fields observed under high magnification (400×), was assessed as follows: AI = (number of apoptotic cells in kidney/total number of kidney cells) × 100%.

Immunohistochemical Detection of CHOP and P-JNK Protein Expression

Kidney tissues smaller than 0.5 × 0.5 × 0.1 cm were washed with a phosphate buffer saline (PBS) solution, fixed with 4% paraformaldehyde, and embedded in paraffin. The paraffin-embedded tissues were cut into sections and subsequently underwent an array of processes, including dewaxing, antigen retrieval, blocking, incubation with primary and secondary antibodies (1:1000), color development, counterstaining, and sealing. Positive expression of CHOP and p-JNK proteins appeared as brownish-yellow staining in the cytoplasm. The results were analyzed by assessing the optical density values. Moreover, Image J software (System version MAC OS13, Software version 1.52a, NIH, Bethesda, MD, USA) was used to determine both the integrated option density (IOD) value and the area value of each image. Subsequently, the mean density value was calculated as follows: the mean density = IOD/area. However, the mean density was calculated by observing five random areas for each sample.

WB Detection of CHOP and P-JNK Protein Expression in Rat Kidney Tissues

Approximately 50 mg of tissue fragments were collected and washed 2–3 times with pre-cooled phosphate buffer saline (PBS). Subsequently, 200 μL of lysis buffer was added to each tube along with Phenylmethanesulfonyl fluoride (PMSF) at 1:100. After tissue disruption using a homogenizer, the protein content of each sample was quantified utilizing a Bicinchoninic Acid (BCA) kit. The proteins were separated through Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and subsequently transferred onto a vinylidene difluoride (PVDF) membrane at an initial voltage of 80 V for 30 minutes followed by a constant voltage of 100 V for one hour. The membranes were then blocked with 5% skim milk powder at room temperature for one hour. Meanwhile, the primary antibodies (CHOP, p-JNK, JNK, and GAPDH) were diluted in a sealing solution and incubated overnight with the membranes at 4 °C. The next day, membranes were washed and incubated with a secondary antibody (1:1000 dilution) at room temperature for one hour in the dark. The membranes were washed three times with 1× TBST, exposed, and washed again. The gray values of the protein bands were assessed using Image J software.

Detection of CHOP and JNK mRNA in Kidney Tissues Using RT-PCR

Total RNA was extracted from the kidney tissue, quantified, and subsequently reverse-transcribed into cDNA. The reaction conditions for reverse transcription were as follows: initial incubation at 37 ℃ for 50 minutes followed by reaction at 70 °C for 10 minutes. The resulting cDNA was stored at –20 °C for subsequent analysis. The amplification process consisted of a cycle at 95 ℃ for 10 minutes, followed by denaturation at 95 ℃ for 15 seconds and annealing/elongation at 60 ℃ for 60 seconds, for a total of 45 cycles. Polymerase chain reaction (PCR) was performed using the following reaction system: 10 μL of 2× UltraSYBR Mixture, 0.4 μL of each primer (10 μM), 2 μL of template cDNA, and 20 μL of total reaction volume. The relative mRNA expression was assessed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) were as follows: *CHOP* (F: 5′-GTTGGCATCACCTCCTGTCT-3′; R: 5′-CCCTCTCCTTTGGTCTACCC-3′); *JNK* (F: 5′-CGGCCGTGGTGAATATTTGG-3′; R: 5′-ATTCTGAAATGGCCGGCTGA-3′); glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (F: 5′-TGGAGTCTACTGGCGTCTT-3′; R: 5′-TGTCATATTTCTCGTGGTTCA-3′).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9 software (System version MAC OS13, Software version 9.5.1, Graphpad Software, San Diego, CA, USA). The data were presented as the mean ± standard deviation ($\bar{x} \pm s$). The analysis of variance (ANOVA) was used for comparisons among multiple groups. The MOR group was used as the specific control group, and Dunnett's multiple comparisons were used to examine significant differences between each group and the specific control group. A p-value of less than 0.05 was considered statistically significant.

Results

Changes in Glucose Metabolism Following EGCG Intervention in Rats

The T2DM model rats exhibited typical symptoms of diabetes mellitus, including messy and dull hair, decreased activity, and a progressive decrease in body weight. The fasting blood glucose (FBG) and fasting insulin (FINS) levels were determined as indicators of glucose metabolism. We observed that the levels of FBG and FINS were significantly higher in the MOR group compared to the NOR group ($p < 0.0001$). However, following EGCG intervention, FBG and FINS levels significantly decreased in a dose-dependent manner ($p < 0.0001$). The changes in glucose metabolism following EGCG treatment are given in Table 1.

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Table 1. Changes in FBG, FINS, UREA, and CREA in rats after EGCG intervention ($\bar{x} \pm s$, n = 8).

Group	FBG (mmol/L)	FINS (mIU/L)	UREA (μmol/L)	CREA (μmol/L)
NOR (n = 8)	6.24 ± 0.62	16.36 ± 2.38	4.81 ± 0.42	13.22 ± 2.65
MOR (n = 8)	20.53 ± 3.66****	22.32 ± 3.74****	12.12 ± 1.14****	50.14 ± 5.29****
EGCG1 (n = 8)	15. 78 ± 2.62$^{\#\#\#\#}$	19.72 ± 3.26$^{\#\#\#\#}$	8.13 ± 0.31$^{\#\#\#\#}$	27.35 ± 2.52$^{\#\#\#\#}$
EGCG2 (n = 8)	13.06 ± 2.34$^{\#\#\#\#}$	15.51 ± 1.93$^{\#\#\#\#}$	6.36 ± 0.61$^{\#\#\#\#}$	18.21 ± 3.61$^{\#\#\#\#}$

FBG, fasting blood glucose; FINS, fasting insulin; UREA, urea nitrogen; CREA, creatinine; EGCG, Epigallocatechin gallate; NOR, normal control group; MOR, diabetic model control group; EGCG1, EGCG low-dose group; EGCG2, EGCG high-dose group. ****$p < 0.0001$, compared to the NOR; $^{\#\#\#\#}p < 0.0001$, compared to the MOR.

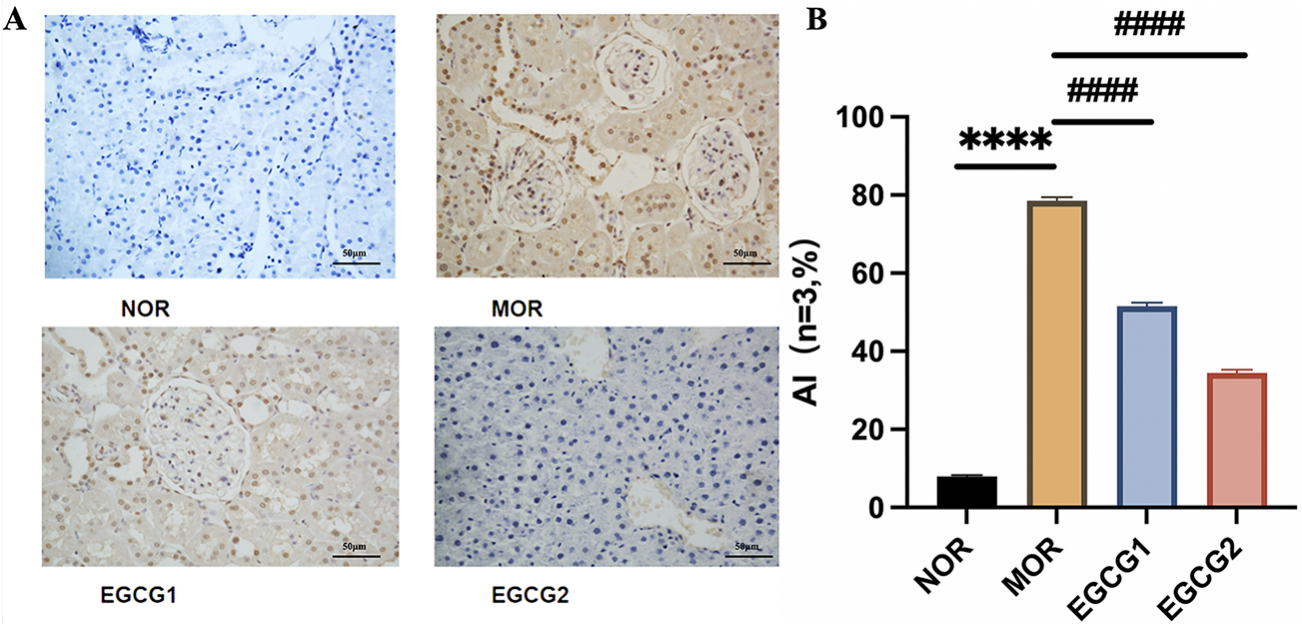

Fig. 1. Detection of renal cell apoptosis in each group using the TUNEL method (n = 3). (A) Under light microscopy using the *in-situ* terminal deoxynucleotidyl transferase-mediated dUTP Nick end labeling (TUNEL) method, normal nuclei appeared blue-purple, while apoptotic nuclei appeared brown. (B) Comparison of the apoptotic index among all groups. ****$p < 0.0001$, compared to the NOR group; $^{\#\#\#\#}p < 0.001$, compared to the MOR group. NOR, normal control group; MOR, diabetic model control group; EGCG1, EGCG low-dose group; EGCG2, EGCG high-dose group.

Changes in Renal Function Following EGCG Intervention in Rats

We assessed renal function in the rats by measuring urea nitrogen (UREA) and creatinine (CREA) levels, where increased levels are indicative of renal function damage. We observed that the levels of UREA and CREA were higher in the MOR group compared to the NOR group ($p < 0.0001$). However, after EGCG intervention, there was a significant decrease in the levels of UREA and CREA ($p < 0.0001$), indicating the protective impact of EGCG on renal function in type 2 diabetic rats. The changes in renal function after EGCG treatment are shown in Table 1.

Impact of EGCG on Renal Apoptosis

Renal cell apoptosis was examined in each group of rats using the TUNEL method. The findings revealed that normal nuclei appeared blue-purple, while apoptotic nuclei appeared brown. We did not observe significant apoptosis in the NOR group. However, in the MOR group, the apoptotic index (AI) was significantly higher compared to the NOR group, increasing from 8% to 79%. Moreover, in the intervention groups EGCG1 and EGCG2, the AI decreased to 52% and 35%, respectively, indicating a significant difference compared to the MOR group ($p < 0.001$). The degree of renal cell apoptosis in each group of rats is depicted in Fig. 1.

Effect of EGCG on CHOP and P-JNK Protein Expression in Diabetic Rats as Detected by Immunohistochemistry

As shown in Fig. 2, CHOP and p-JNK proteins were primarily expressed in the cytoplasm of kidney cells, evidenced by the brownish-yellow granules in the cytoplasm of positive cells. The mean optical density of CHOP and

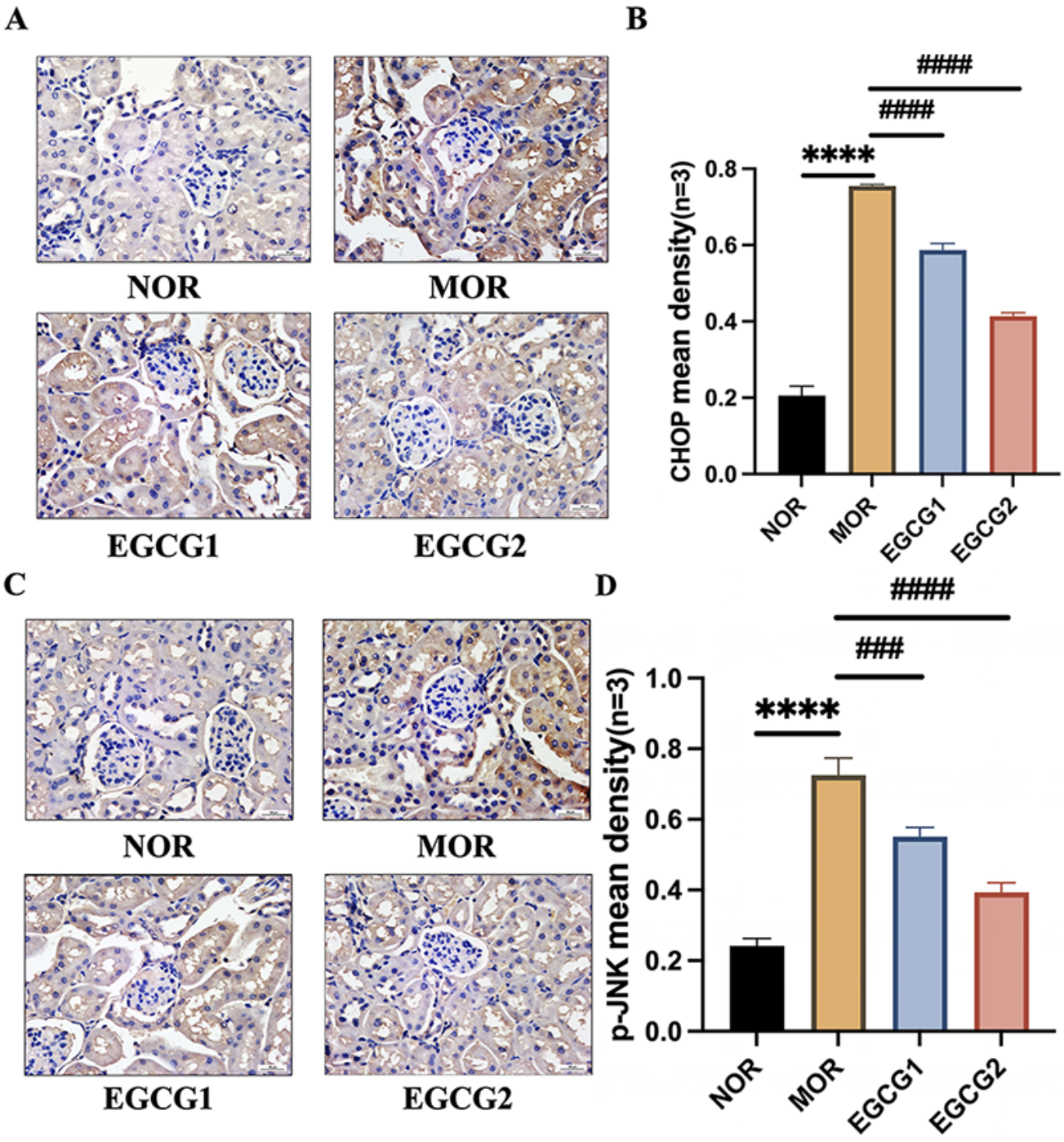

Fig. 2. Immunohistochemical examination of *CHOP* and *p-JNK* mean optical density in renal tissues of diabetic rats in each group (n = 3). (A) *CHOP* expression in renal tissue sections of each group. (B) Comparison of *CHOP*'s mean optical density in renal tissue sections of each group. (C) *p-JNK* expression in renal tissue sections of each group. (D) Comparison of *p-JNK*'s mean optical density in renal tissue sections of each group. ****$p < 0.0001$, compared to the NOR group; $^{###}p < 0.001$, compared to the MOR group; $^{####}p < 0.0001$ compared to the MOR group. NOR, normal control group; MOR, diabetic model control group; EGCG1, EGCG low-dose group; EGCG2, EGCG high-dose group; CHOP, C/EBP homologous protein; *p-JNK*, phosphorylated c-Jun N-terminal kinase.

p-JNK proteins was significantly higher in the MOR group compared to the NOR group ($p < 0.0001$). However, the average optical density values of CHOP and p-JNK proteins were significantly lower in both the EGCG1 and EGCG2 groups compared to the MOR group ($p < 0.0001$).

The Expression Levels of CHOP and P-JNK Proteins in Kidney Tissues Using WB Analysis

We found that the expression levels of p-JNK and CHOP proteins were significantly higher in the MOR group compared to the NOR group ($p < 0.0001$). However, when

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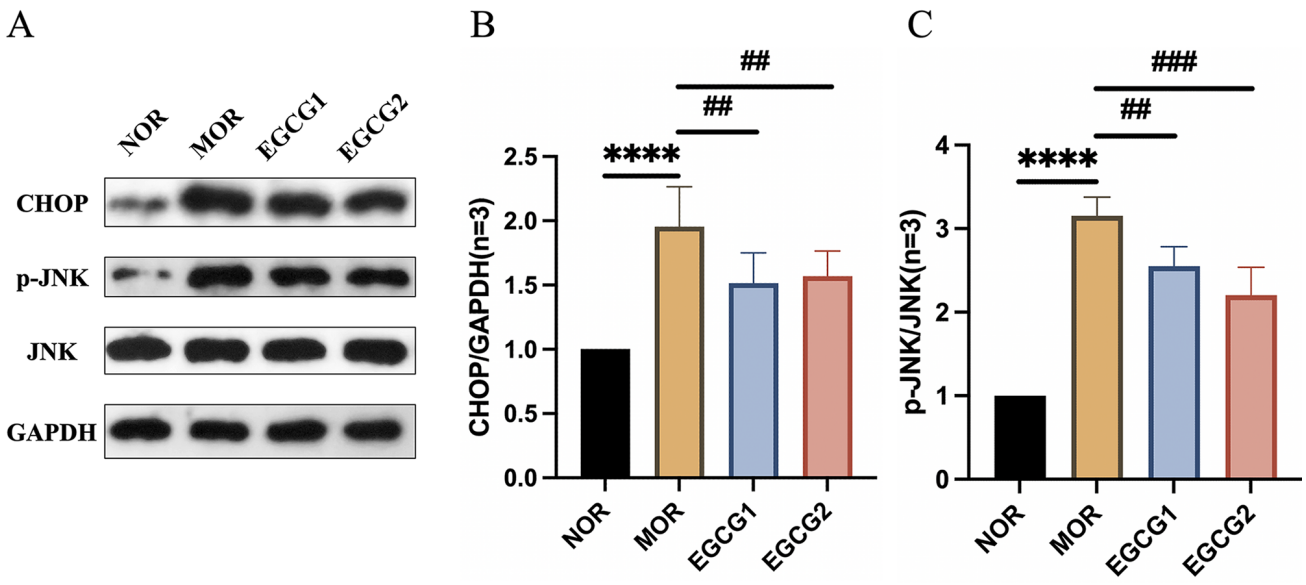

Fig. 3. The expression levels of ER-stress proteins CHOP and p-JNK in the kidneys of rats in each group (n = 3) . (A) Western blot (WB) was used to detect the protein expression levels of CHOP, p-JNK, and JNK in each group, which were normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (B) Bar chart shows the statistical results of CHOP/GAPDH. (C) The bar chart shows the statistical results of p-JNK/JNK. ****$p < 0.0001$, compared to the NOR group; $^{\#\#}p < 0.01$, compared to the MOR group; $^{\#\#\#}p < 0.001$, compared to the MOR group. NOR, normal control group; MOR, diabetic model control group; EGCG1, EGCG low-dose group; EGCG2, EGCG high-dose group; ER, endoplasmic reticulum.

compared to the MOR group, the protein expression levels of p-JNK ($p = 0.0087$, 0.0002) and CHOP ($p = 0.0062$, 0.0023) were significantly reduced in both the EGCG1 and EGCG2 groups. The expression levels of p-JNK and CHOP proteins are shown in Fig. 3.

The Expression of CHOP and JNK mRNAs in the Kidney was Assessed Using RT-qPCR

RT-qPCR results showed that the mRNA expression levels of *JNK* and *CHOP* were significantly higher in the MOR group compared to the NOR group ($p < 0.0001$). Whereas their expression levels were substantially lower in the kidney tissues of both the EGCG1 and EGCG2 groups compared to the MOR group ($p < 0.0001$). These findings are illustrated in Fig. 4.

Discussion

Diabetic nephropathy (DN) is a severe complication of diabetes, ranking among the primary causes of chronic kidney disease (CKD) in China. It is characterized by proteinuria and a progressive decline in glomerular filtration rate (GFR) [2]. The primary underlying factor involves reduced podocyte function and number [14]. In our study, the MOR group exhibited higher levels of FBG, FINS, CREA, and UREA compared to the NOR group, indicating abnormal GFR and impaired kidney function in T2DM rats. However, following EGCG intervention, the levels of FBG, FINS, CREA, and UREA significantly decreased in diabetic rats, suggesting that EGCG can mitigate kidney function injury to some extent in T2DM rats. Currently, the exact pathogenesis of diabetic nephropathy remains incompletely understood. However, oxidative stress and autophagy in kidney cells are recognized as major contributors [15]. Additionally, research has found that Endoplasmic reticulum stress (ERS) plays a pivotal role in the progression of diabetic nephropathy [16]. The endoplasmic reticulum plays a crucial role in cellular activity including protein synthesis and transportation. Upon stimulation by various factors, misfolded or unfolded proteins accumulate within their lumen, resulting in an imbalance in calcium ion concentration and triggering the unfolded protein response (UPR). This response involves the activation of three transmembrane proteins: Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), inositol requiring enzyme 1 (IRE1), and transcription activator factor 6 (ATF6). During the compensation process of the UPR, Endoplasmic reticulum stress (ERS) provides protective effects. However, as stimulus intensity further escalates, signal transduction pathways such as JNK, CHOP, Caspase-12 are activated, resulting in apoptosis induction [17]. In this study, TUNEL results revealed elevated AI in the kidney tissue of MOR rats compared to the NOR rats. However, after EGCG intervention, AI levels decreased, indicating that EGCG can reduce apoptosis in kidney cells of T2DM rats to a certain extent.

Furthermore, CHOP serves as a crucial signaling molecule in various apoptosis pathways induced by ERS.

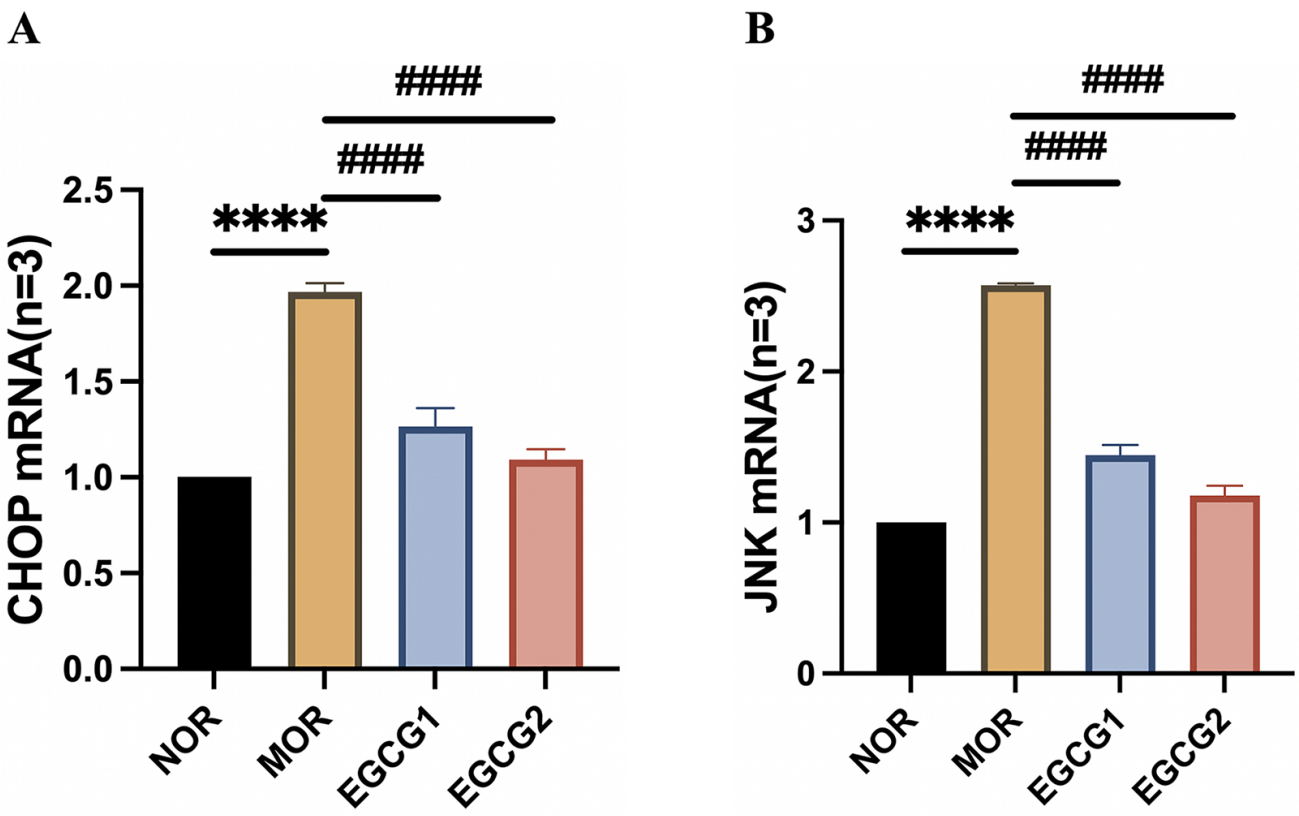

Fig. 4. The expression levels of *CHOP* and *JNK* mRNA in the kidney were detected through RT-qPCR (n = 3). (A) The relative expression of *JNK* mRNA. (B) The relative expression of *CHOP* mRNA. ****$p < 0.0001$, compared to the NOR group; $^{\#\#\#\#}p < 0.0001$, compared to the MOR group. RT-qPCR, real-time fluorescent quantitative polymerase chain reaction; NOR, normal control group; MOR, diabetic model control group; EGCG1, EGCG low-dose group; EGCG2, EGCG high-dose group.

When the UPR is compromised, PERK, ATF6, and IRE1 can activate CHOP, thereby inducing apoptosis. Specifically, PERK triggers the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) through oligomerization and autophosphorylation, resulting in increased expression levels of ATF4 and CHOP [18]. ATF6 undergoes translocation to the Golgi apparatus and undergoes proteolytic cleavage by S1P and S2P enzymes before being transported to the nucleus, where it activates the downstream CHOP pathway [19]. Additionally, IRE1 synergistically activates CHOP by activating downstream p38 mitogen-activated protein kinase (MAPK) and JNK pathways [20]. Several studies have found the role of CHOP in the apoptosis of renal podocytes, mesangial cells, and endothelial cells [21–24]. In this study, we confirmed through immunohistochemistry along with WB and RT-qPCR analyses that both protein and mRNA expression levels of CHOP were significantly higher in the kidney tissues of diabetic rats compared to NOR rats. This finding aligns with previous results. However, following EGCG intervention, the expression level of CHOP decreased in kidney tissues, indicating that EGCG can downregulate its expression level. Previous research has demonstrated that EGCG can inhibit ERS-mediated apoptosis in retinal pigment epithelial cells by downregulating the expression level of CHOP [25]. Our earlier study has confirmed that EGCG reduces hepatocyte apoptosis in T2DM rats, which correlates with decreased levels of ERS proteins such as PERK and GRP78 [13]. Upon activation by UPR pathway proteins such as PERK, ATF6, and IRE1, the active form of CHOP regulates downstream B-Cell Leukemia/Lymphoma-2 (BCL-2) family proteins, including BCL-2 and BCL-2-associated X protein (BAX) [26]. This eventually induces the release of cytochrome c (Cyt-C) and apoptosis-inducing factor (AIF), leading to apoptosis [27]. Subsequent investigations are needed to explore the effect of EGCG on the expression of CHOP's upstream factors and downstream BCL-2 family proteins in the kidney tissue of T2DM rats.

JNK is an important member of the MAPK family. In response to ERS, IRE1 can recruit the receptor for activation of apoptosis signal-regulated kinase 1/MAP3K5 (ASK1), TRAF2, and its downstream target JNK [26]. Additionally, research has indicated that JNK activation could increase the expression level of BAX, decrease the expression level of BCL-2, and induce apoptosis [28]. Furthermore, it has been reported that JNK plays a crucial role in apoptosis during diabetic nephropathy [29,30]. In the present study, using immunohistochemistry, WB, and RT-

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qPCR, we found that the expression levels of p-JNK protein and *JNK* mRNA were significantly higher in the kidney tissues of the MOR group compared to the NOR group. However, the expression levels of p-JNK protein and *JNK* mRNA were decreased following EGCG intervention, indicating that EGCG could down-regulate the expression level of p-JNK in renal tissues. Moreover, it has been indicated that EGCG can reduce myocardial fibrosis and enhance cardiac function in diabetic rats through the JNK pathway [31]. Similarly, another study demonstrated that EGCG could reduce myocardial injury in ischemia-reperfusion rats by reducing the level of JNK [32]. Furthermore, EGCG has been found to prevent insulin resistance and improve glucose metabolism by downregulating the expression level of JNK in the liver of rats [33]. Currently, the effects of EGCG on JNK primarily focus on the anti-inflammatory and anti-growth effects on cancer cells [34,35]. Furthermore, subsequent investigation should elucidate the effects of EGCG on the expression of downstream apoptosis pathways of the UPR such as Caspase-12, BCL-2, BAX, and Cyt-C in T2DM rat kidney cells.

EGCG is a natural product derived from tea and possesses a wide range of effects including anti-inflammatory, anti-cancer, and metabolic diseases [36,37]. In the treatment of diabetic nephropathy, EGCG exhibits efficacy in reducing kidney tissue injury in diabetic rats by reducing ERS-related proteins [12]. Our previous study revealed the protective role of EGCG in T2DM rat liver cells, where it decreases the expression levels of ERS-related proteins such as PERK and GRP78 [13]. The outcomes of this study demonstrated that EGCG intervention significantly reduces the levels of FBG, FINS, UREA, and CREA in the serum of T2DM rats. Additionally, it reduces apoptosis of kidney tissue cells and alleviates the expression of CHOP, both at protein and mRNA levels, as well as p-JNK protein and *JNK* mRNA in kidney tissue. These findings indicate that EGCG can improve the renal function in diabetic rats. However, further studies, involving the role of the BCL-2 family and downstream cytokines such as Cyt-C, are warranted to elucidate the underlying mechanisms of EGCG on renal function in T2DM rats.

Conclusions

In summary, this study revealed that EGCG alleviates diabetes symptoms and reduces renal dysfunction in T2DM rats by regulating the expression of *CHOP* and *JNK* in renal tissue.

Availability of Data and Materials

All experimental data included in this study can be obtained by contacting the corresponding author if needed.

Author Contributions

Experimental studies designed by ZL and QZ. ZL, DM, JG, YZ, JK, RH conducted the study. QZ provided help and advice on the experiment. XJ analyzed the data. All authors contributed to the editorial changes to the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The animal experiments involved in this study have been reviewed and approved by the Medical Ethics Committee of North Sichuan Medical College, Approval number: NSMC Ethical Animal Review [2022]043.

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Conflict of Interest

The authors declare no conflict of interest.

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