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Research Article

Curcumin Attenuates Ferroptosis and Ameliorates Erectile Function in Diabetic Rats by Activating Nrf2/HO-1 Pathway

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Previous studies have shown that curcumin has a therapeutic effect on diabetic erectile dysfunction (ED), but the exact mechanism of action remains unclear. In order to ascertain the involvement of ferroptosis in diabetic ED and explore the underlying mechanism by which curcumin mitigates ferroptosis in penile endothelial cells under conditions of high-glucose stimulation, we conducted an investigation utilizing a rat model of diabetes induced by a high-fat diet and streptozotocin injection. The 40 male Sprague–Dawley rats were randomly divided into four groups: blank control, diabetes control, ferroptosis inhibitor treatment, and curcumin treatment. After 8 weeks, the erectile function of all rats was evaluated through electrical stimulation of the cavernous nerve. Histological and molecular changes of the cavernous body were analyzed using western blot and immunohistochemistry. Penile endothelial cells were then treated with appropriate concentrations of high glucose and curcumin to explore the mechanism of nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling in curcumin's ability to alleviate ferroptosis in endothelial cells stimulated by high glucose. According to research, diabetic rats experience a decrease in erectile function, damaged endothelial cells, and ferroptosis in tissues. However, curcumin treatment has been shown to be effective in improving erectile function and related tissue and molecular changes in diabetic rats. Furthermore, in vitro experiments have confirmed that curcumin can inhibit the occurrence of ferroptosis in penile endothelial cells that are stimulated by high glucose through Nrf2/HO-1 signaling. Curcumin has been found to improve the occurrence of penile ED in diabetic penile endothelial cells. This is achieved by inhibiting the level of ferroptosis, and the mechanism behind this improvement may be linked to the upregulation of the expression level of Nrf2/HO-1.

1. Introduction

Erectile dysfunction (ED) is a prevalent complication of diabetes mellitus (DM), significantly affecting the quality of life of diabetic men [1]. The incidence of ED in diabetic patients increases with the duration of diabetes. The pathogenesis of diabetic ED is complex, involving changes in the central nervous system, peripheral nervous system [2], and endothelial function. Currently, oral phosphodiesterase type 5 inhibitors are the primary drugs used to treat ED. However, their therapeutic impact on diabetic ED patients is somewhat restricted [3]. As a result, it is crucial to enhance our comprehension of the molecular mechanisms that contribute to the development of diabetic ED.

DM is a chronic metabolic disease that is characterized by insulin resistance and impaired glucose homeostasis. Research indicates that diabetes-induced endothelial dysfunction plays a significant role in the development of macrovascular and microvascular complications in diabetes [4]. This dysfunction is primarily characterized by oxidative stress [5], reduced nitric oxide (NO) release [6], increased production of inflammatory factors [7], and an imbalance between endothelial damage and repair [8]. Ferroptosis is a cell death program that occurs due to iron-dependent lipid

peroxidation [9]. This process has been found to be linked with inflammation and oxidative stress in various diseases, such as cardiovascular and cerebrovascular ischemia-reperfusion injury [10, 11], diabetic nephropathy [12], and diabetic osteoporosis [13]. The cysteine/glutamate antiporter X_c^- system, also known as xCT, is responsible for providing the necessary raw materials for glutathione synthesis [14]. However, the compound erastin has the ability to inhibit xCT, leading to a reduction in glutathione synthesis [15]. This disruption of intracellular redox homeostasis triggers ferroptosis. Glutathione peroxidase 4 (GPX4) is another important player in ferroptosis, as it is involved in the glutathione-dependent catalytic reduction of lipid peroxides [16]. Ferrostatin-1 is a selective inhibitor of erastin-induced ferroptosis. By reducing oxidative stress and lipid peroxidation in renal tubules, it can effectively reduce the occurrence of renal tubular ferroptosis and improve fibrosis in diabetic mice. These findings suggest that studying the potential involvement of ferroptosis in endothelial dysfunction could be a meaningful avenue of research in diabetic ED.

Turmeric-derived polyphenol derivative, curcumin, has been found to be effective in treating cardiovascular diseases. It has been found to improve aortic sclerosis by decreasing the production of peroxides and increasing the expression of the antioxidant manganese-containing superoxide dismutase [17]. Furthermore, in experiments involving a high-fat diet, curcumin has been observed to reduce the increase of vascular oxidative stress levels caused by the diet [18]. Heme oxygenase-1 (HO-1) is an enzyme that breaks down heme due to intracellular oxidative stress and provides cytoprotective effects such as antioxidant and anti-inflammatory properties in various tissues, including cardiovascular tissues [19]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that plays a crucial role in the antioxidant pathway of cells. It directly promotes the expression of HO-1 and thus reduces oxidative stress, making it an important factor in maintaining cellular health [20]. Previous studies have shown that water-soluble derivatives of curcumin can enhance erectile function in diabetic rats by stimulating the HO-1 enzyme [21]. However, the exact mechanism remains unclear. In this study, we aimed to test the hypothesis that curcumin improves erectile function in diabetic ED by reducing ferroptosis and oxidative stress levels in the cavernous body and increasing Nrf2 levels in a type 2 diabetic rats model induced by streptozotocin (STZ).

2. Materials and Methods

2.1. Animals. All animal experiments were approved by the Ethical Committee for Experimental Animal Welfare of Nanjing University of Chinese Medicine. Forty 8-week-old male Sprague–Dawley rats, weighing 250–300 g, from Shanghai Slake Laboratory Animal Co. LTD. (Shanghai, China) were included in this study. After 1 week of adaptive feeding, 30 rats were fed with a high-fat diet for 8 weeks, then the rats were intraperitoneally injected with freshly prepared STZ (30 mg/kg; Sigma-Aldrich, USA) and repeated 3 days later. The rest of the rats were only treated with citrate/phosphate buffer, which served as controls. Three days after the second injection, blood samples were collected from the tail vein to measure the random blood glucose of the rats, and the rats with blood glucose greater than 16.7 mmol/L were considered diabetic rats. These rats were randomly divided into the diabetic control group, the ferrostatin-1 treatment group, and the curcumin treatment group. The ferrostatin-1 treatment group was daily intraperitoneal injected with 1 mg/kg ferrostatin-1 (MedChemExpress, China), and the curcumin treatment group was daily given 30 mg/kg curcumin (Shanghai yuanye Bio-Technology, China) by gavage. After 8 weeks of continuous treatment, peak intracavernous pressure (ICP) was measured, and penile tissue was harvested from all rats.

2.2. Erectile Function Evaluation. The erectile function of the rats was evaluated by measuring the ICP and ICP/mean systemic arterial pressure (MAP) ratio as described previously by our group [22]. Briefly, under isopentane inhalation anesthesia, pelvic ganglia and cavernous nerves on either side of the prostate were exposed by transabdominal midline dissection. A heparinized needle was inserted into the penile sponge and connected to an RM6240E/EC multichannel biological signal acquisition and processing system (Chengdu Implement Company, Chengdu, China). Pressure changes within the penis were detected over a period of 1 min with electrical stimulation at 5 V, with at least a 5-min interval between stimuli. The MAP of the rats was monitored by cannulation of the left internal carotid artery. After ICP/MAP assessment, rats were euthanized, and penis specimens were collected and stored separately in 4% paraformaldehyde and −80°C freezer.

2.3. Cell Treatment. The primary cultured rat penile endothelial cells (CCECs) were purchased from Procell Life Science & Technology Co. Ltd. (CP-R133, Wuhan, China), and the cells were cultured in Endothelial Cell Medium (ScienCell, USA) containing 5% fetal bovine serum, 1% endothelial cell growth supplement and 1% double antibody, and placed in an incubator at 37°C and 5% CO_2 cultivated in. After the cells underwent resuscitation passage, we used the second or third passage of rat cavernous endothelial cells for subsequent experiments. We used different concentrations of high glucose or curcumin to culture the cells for 24 hr. According to the Cell Counting Kit-8 (CCK-8; Beyotime, Nantong, China) experiment, the effect of the drug concentration on the cell viability under the concentration gradient was evaluated, and we adopted the glucose concentration of 60 mM and the curcumin concentration of 30 μM as the intervention dose of the subsequent cell experiment. Nrf2 siRNA was biosynthesized by Keygen Biotech (Nanjing, China), and Lipofectamine 2,000 was used for siRNA transfection.

2.4. Immunohistochemical Staining. Tissues were fixed overnight using 4% paraformaldehyde for immunohistochemical staining experiments. After deparaffinization and rehydration of sections, endogenous peroxidase activity was quenched with 0.3% H_2O_2 for 10 min, and tissue sections were blocked with 3% bovine serum albumin for another 30 min. Sections were then incubated with primary antibodies against GPX4

Table 1: Primers sequences for determination of mRNA expression levels.

Gene	Forward primer	Reverse primer
Slc7a11	5′-TGAATGCCTTGTCTGCTTTG-3′	5′-GAATTGCAGGGAACTGTGGT-3′
GPX4	5′-TCAGCAAGATCTGCGTGAAC-3′	5′-GGGGCAGGTCCTTCTCTATC-3′
Nrf2	5′-TGCCCACATTCCCAAACAAG-3′	5′-GCTATCGAGTGACTGAGCCT-3′
HO-1	5′-CGCCTCCAGAGTTTCCGCCTCCAAC-3′	5′-ATAGACTGGGTTCTGCTTGTTTCGC-3′
GAPDH	5′-AACGGATTTGGTCGTATTGGG-3′	5′-CCTGGAAGATGGTGATGGGAT-3′

Note: Slc7a11 = solute carrier family 7 member 11; GPX4 = glutathione peroxidase 4; Nrf2 = nuclear factor erythroid 2-related factor 2; HO-1 = heme oxygenase-1.

(1 : 100; Abcam, Cambridge, MA, USA) and eNOS (1 : 100; Abcam, Cambridge, MA, USA) overnight at 4°C. Sections were further incubated with goat anti-rabbit secondary antibody for 2 hr at room temperature. Finally, sections were counterstained with hematoxylin. Image J software (Bethesda, Maryland, USA) was used to analyze the areas of GPX4 and eNOS staining in the cavernous tissue. Six sections were analyzed for each penile sample, and the average result was calculated for data analysis.

2.5. Western Blot. Protein was extracted from penile cavernous tissue and CCECs using radioimmunoprecipitation assay lysis buffer (Beyotime, Nantong, China) and protease inhibitors. The samples were lysed on ice at 4°C for 20 min and then centrifuged to extract the supernatant. The protein concentration was determined by the bicinchoninic acid protein assay (BCA) method (Epizyme, Shanghai, China). Sample loading was performed according to the total protein content of the determined samples determined by BCA. Protein bands were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis system and then transferred to polyvinylidene fluoride membranes. After blocking with 5% skim milk at room temperature for 1 hr, and incubated with the following primary antibodies overnight at 4°C: eNOS (1 : 1,000; Abcam, Cambridge, USA), vWF (1 : 1,000; Santa Cruz Biotechnology, USA), GPX4 (1 : 1,000; Abcam, Cambridge, USA), xCT (1 : 1,000; Abcam, Cambridge, USA), malondialdehyde (MDA) (1 : 1,000; Abcam, Cambridge, USA), 4-hydroxynonenal (4HNE) (1 : 1,000; Abcam, Cambridge, USA), HO-1 (1 : 1,000; Abcam, Cambridge, USA), Nrf2 (1 : 1,000; Cell Signaling, Danvers, USA), GAPDH (1 : 1,000; Cell Signaling, Danvers, USA) and β-actin (1 : 1,000; Cell Signaling, Danvers, USA). Anti-rabbit IgG (H + L) and mouse IgG (H + L) antibodies (1 : 1,000; Epizyme, Shanghai, China) were used as secondary antibodies. Protein bands were visualized using an enhanced chemiluminescence chemiluminescence system and analyzed using Quantity One software (Bio-Rad, Hercules, CA, USA).

2.6. Reverse Transcription-Polymerase Chain Reaction. Total RNA was extracted from treated CCECs using MolPure® Cell/Tissue Total RNA Kit (YEASEN, USA) according to the manufacturer's instructions. The purity and concentration of extracted RNA were assessed using NanoPhotometer-N60 (IMPLEN, Germany). Subsequently, RNA was reverse transcribed into cDNA using One Step RT-qPCR SYBR Green Kit (YEASEN, USA), and real-time quantitative PCR reaction assays were performed. The relative expression levels for each transcript in each group were calculated using the $2^{-\Delta\Delta CT}$ method. The primers used for real-time PCR are listed in Table 1.

2.7. Statistical Analyses. The data were analyzed using GraphPad Prism 9.0 Software (San Diego, CA, USA) and were presented as mean ± standard error of the mean. To perform multiple comparisons, we used one-way analysis of variance, followed by post hoc comparisons using the least significant difference test. P value less than 0.05 was considered statistically significant.

3. Results

3.1. Metabolic Parameters. To evaluate the effectiveness of the diabetes model and the impact of drug intervention on the diabetes model, we conducted regular monitoring of body weight and blood glucose levels in rats after modeling. The results showed that at the beginning of the modeling, there were no significant differences in body weight between the groups of rats. At the end of the experiment, the body weight of the diabetes model rats was significantly lower than that of the normal rats, but there was no significant difference in body weight between the drug intervention group and the diabetes control group (Figure 1(a)). In addition, the blood glucose level of the diabetes rats was significantly higher than that of the normal rats at the start of the modeling, and it remained elevated until the end of the experiment. There was no significant effect of drug intervention on the blood glucose levels of the diabetes rats compared to the diabetes control group (Figure 1(b)).

3.2. Curcumin Restores Erectile Function in Diabetic Rats. The assessment of erectile function was performed using the ratio of ICP to MAP in penile cavernous manometry (Figure 2). Results demonstrated a significant reduction in erectile function among diabetic rats when compared with normal rats. However, treatment with ferrostatin-1, a ferroptosis inhibitor, improved the erectile function of diabetic rats significantly. Additionally, the administration of curcumin also showed improvement in the erectile function of diabetic rats in comparison to untreated diabetic rats.

3.3. Curcumin Protects against Endothelial Injury of Corpus Cavernosum in Diabetic Rats. The study evaluated the content of endothelial cells in rat penis using a western blot experiment (Figures 3(a) and 3(c)). Our findings indicated that diabetic rats had a significantly lower number of endothelial cells compared to normal rats. However, after treatment with ferrostatin-1 or curcumin, the number of

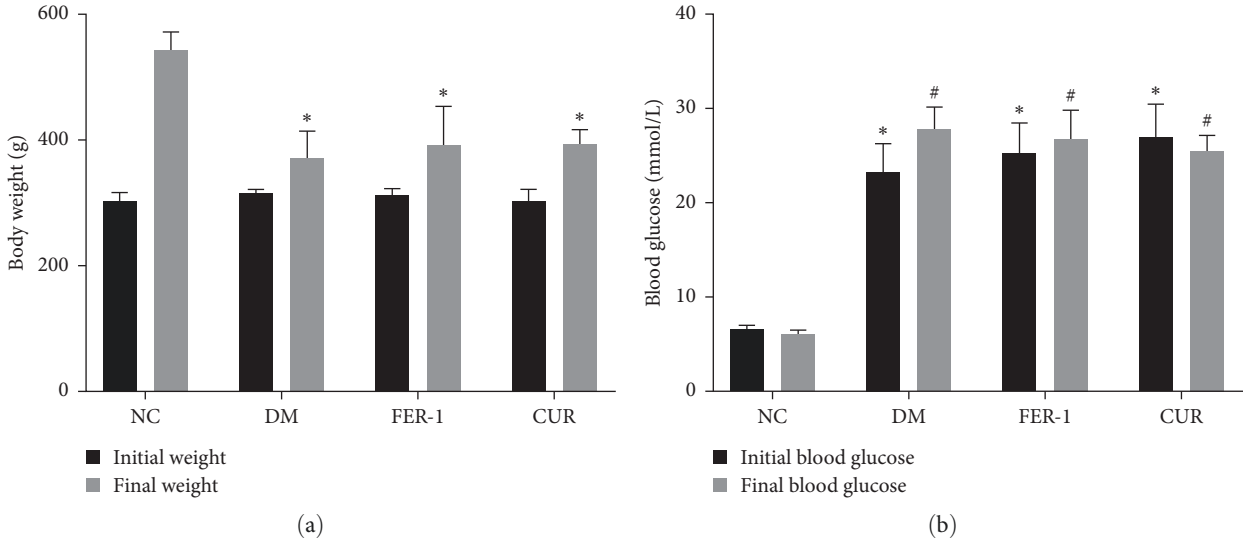

Figure 1: Metabolic parameters in rats. Body weight (a) and blood glucose level (b) of experimental rats before and after drug treatment in each group. Data were presented as mean ± SD from four animals per group. $^{*}P < 0.0001$ compared with the NC group in the initial stage. $^{\#}P < 0.0001$ compared with the NC group in the final stage. NC = normal control rats; DM = rats with diabetes mellitus; FER-1 = diabetic rats treated with ferrostatin-1; CUR = diabetic rats treated with curcumin.

endothelial cells in diabetic rats increased. The study evaluated endothelial nitric oxide synthase (eNOS) as an indicator of endothelial function in the corpus cavernosum using western blot (Figures 3(a) and 3(b)) and immunohistochemistry (Figures 3(d) and 3(e)). The results indicated a significant decrease in the expression level of eNOS in the penile tissue of diabetic rats compared to normal rats. However, after administering ferrostatin-1 treatment, the eNOS level was partially restored, and curcumin had a similar effect.

3.4. Curcumin Inhibits Ferroptosis in Diabetic Rat Penile Tissue. GPX4 and xCT are two marker proteins that play a crucial role in the occurrence of ferroptosis. The expression levels of GPX4 and xCT in the penile tissues of diabetic rats were significantly reduced, as shown by western blot analysis (Figure 4(a)–4(c)). However, after ferrostatin-1 intervention, the occurrence of ferroptosis in the penile tissues was significantly improved. Additionally, curcumin intervention was also found to effectively restore GPX4 and xCT expression levels. In addition, we conducted a quantitative analysis of GPX4 expression levels in tissue using immunohistochemical staining (Figures 4(d) and 4(e)). Our results showed a decrease in GPX4 expression in diabetic rats; however, after intervention with ferrostatin-1 or curcumin, there was a significant increase in expression levels compared to the control group. These findings suggest that ferrostatin-1 and curcumin may have potential therapeutic effects on diabetic rats through the regulation of GPX4 expression.

3.5. Curcumin Improves Oxidative Stress in Diabetic Rat Penile Tissue by Activating Nrf2/HO-1 Signaling Pathway. MDA and 4HNE are oxidation products after free radicals act on lipid peroxidation, reflecting the degree of lipid peroxidation in the body. In this study, we investigated the effects of ferrostatin-1 and curcumin on lipid peroxidation in the penis tissues of diabetic rats (Figure 5(a)–5(c)). Western blot analysis revealed a significant increase in the expression levels of MDA and 4HNE in the penis tissues of diabetic rats. However, after treatment with ferrostatin-1 and curcumin, the expression levels of MDA and 4HNE were significantly decreased, indicating a reduction in lipid peroxidation.

To investigate the potential involvement of Nrf2/HO-1 in the amelioration of penile tissue damage by curcumin, the expression levels of Nrf2 and HO-1 were evaluated using western blot assay (Figure 5(d)–5(f)). The findings revealed that the levels of Nrf2 and HO-1 in the penile tissues of diabetic rats were significantly lower than those of normal control rats. However, following an 8-week treatment with curcumin, the levels of Nrf2 and HO-1 were significantly elevated compared to the diabetic group.

3.6. Curcumin Inhibits Ferroptosis in Penile Endothelial Cells Stimulated by High Glucose. To investigate whether the protective effect of curcumin on penile erectile function is mediated by endothelial cells, commercial rat penile cells were used in follow-up experiments. The CCK-8 test shows curcumin therapy partially restored the endothelial cell vitality stimulated by high glucose (Figure 6(a)). The mRNA expression levels of GPX4 and xCT in the cells were evaluated 24 hr after treatment with high glucose or curcumin. The results demonstrated that the expressions of GPX4 and xCT in endothelial cells were significantly reduced after exposure to high glucose; however, their expression levels were restored after curcumin treatment (Figures 6(b) and 6(c)). To assess the level of intracellular oxidation, western blotting was conducted to detect the expression level of MDA (Figure 6(d)–6(e)). The results revealed that the MDA expression in penile endothelial cells induced by high glucose was significantly increased. Meanwhile, the intracellular MDA level was ameliorated after curcumin treatment.

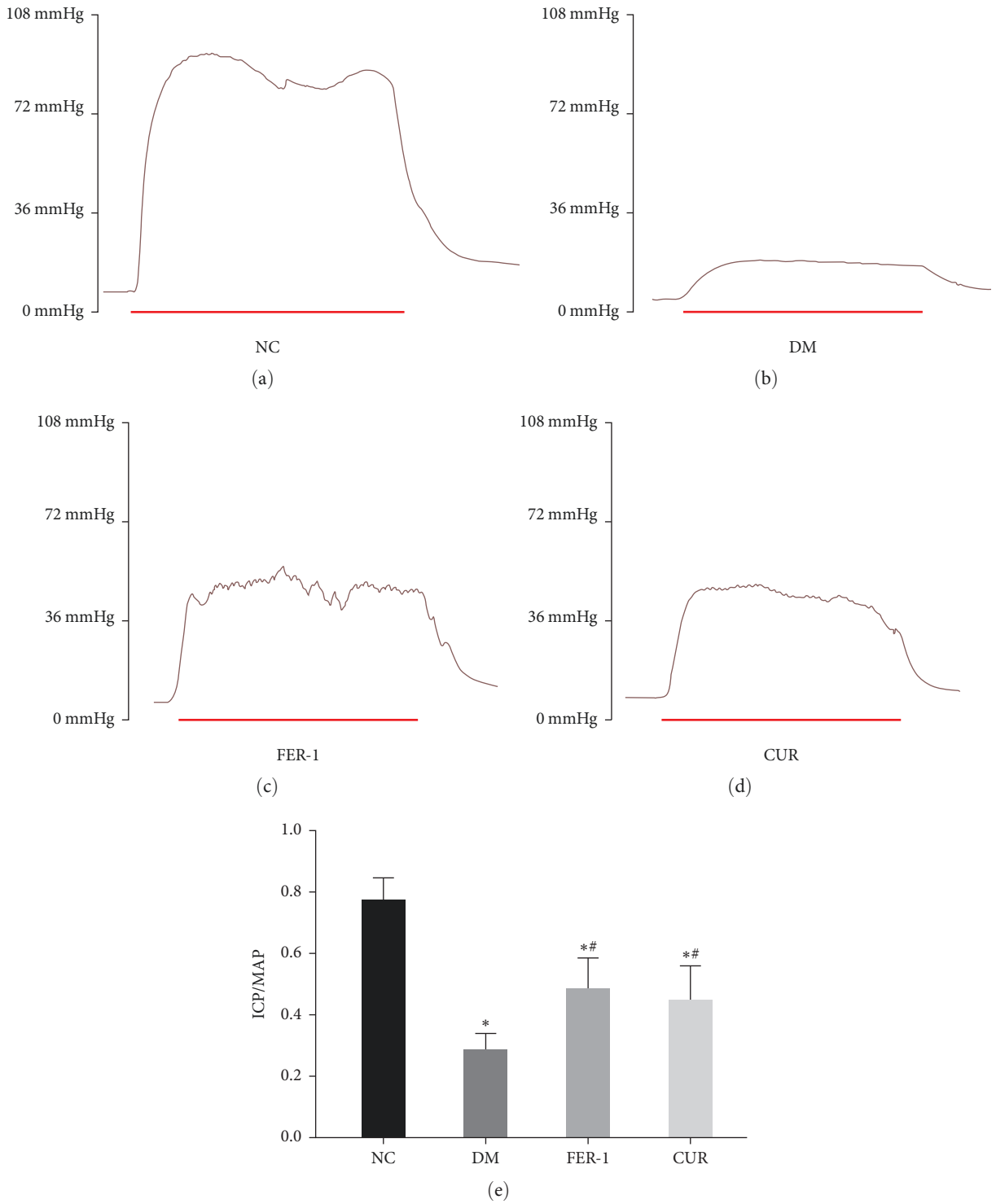

Figure 2: Evaluation of erectile function in each group. Representative traces of ICP with stimulation at 5.0 V for 1 min in the NC group (a), DM group (b), FER-1 group (c), CUR group (d), and the ICP/MAP (e) in each group. Data were presented as mean ± SD from four animals per group. $^{*}P<0.005$ compared with the NC group. $^{\#}P<0.05$ compared with the DM group. ICP/MAP: peak intracavernous pressure/mean system arterial pressure. NC = normal control rats; DM = rats with diabetes mellitus; FER-1 = diabetic rats treated with ferrostatin-1; CUR = diabetic rats treated with curcumin.

3.7. Curcumin Inhibits Ferroptosis of Penile Endothelial Cells by Regulating the Expression of Nrf2 and HO-1. After 24 hr of high-glucose stimulation, the expression of Nrf2 and HO-1 in penile endothelial cells was found to be decreased by western blotting and qPCR compared to the control group (Figure 7(a)–7(c)). Cotreatment with curcumin partially alleviated the downregulation of Nrf2 and HO-1 induced by high glucose. To investigate whether curcumin can regulate

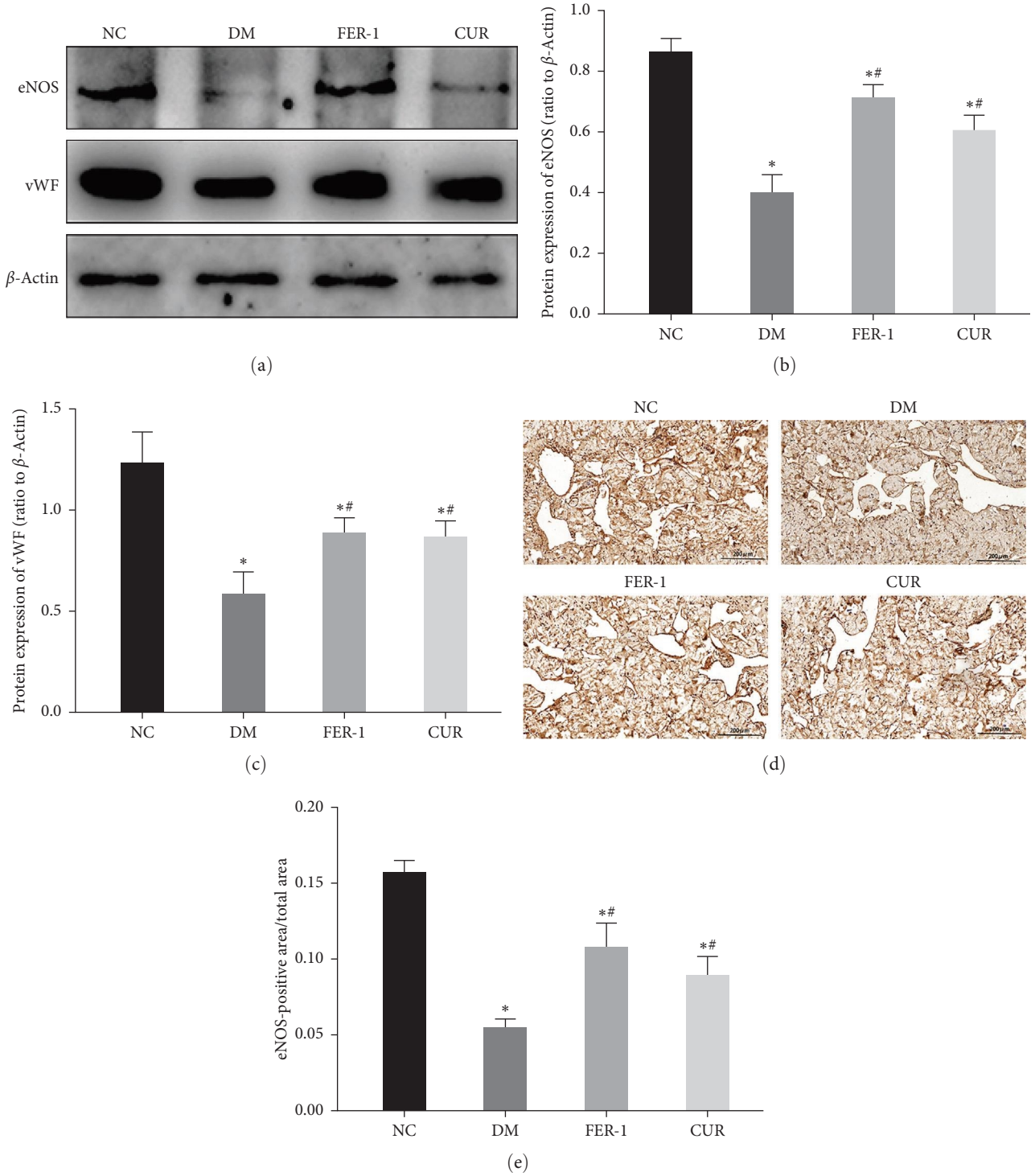

FIGURE 3: Endothelial contents in the corpus cavernosum. Representative stripes of western blot (a), statistical analysis of eNOS (b), vWF (c), immunohistochemical results (d), and statistical analysis of eNOS (e) in penile tissues of NC group, DM group, FER-1 group, and CUR group, scale bars = 200 μm. Data were presented as mean ± SD from three animals per group. $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the DM group. NC = normal control rats; DM = rats with diabetes mellitus; FER-1 = diabetic rats treated with ferrostatin-1; CUR = diabetic rats treated with curcumin.

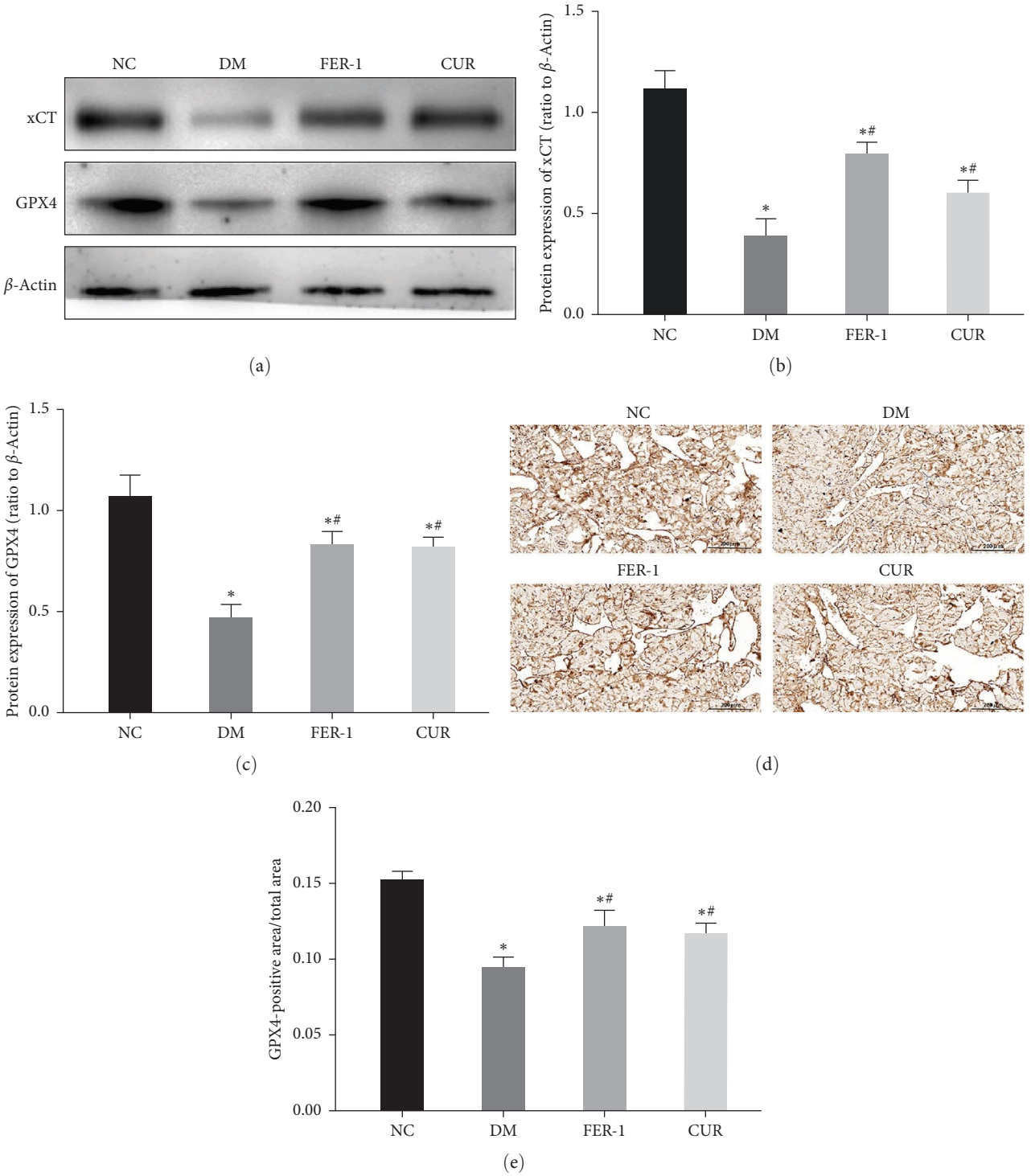

FIGURE 4: Expression of GPX4 and xCT in rat penile tissues. Representative stripes of western blot (a), statistical analysis of xCT (b), GPX4 (c), immunohistochemical results (d), and statistical analysis of GPX4 (e) in penile tissues of NC group, DM group, FER-1 group, and CUR group, scale bars = 200 μm. Data were presented as mean ± SD from three animals per group. $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the DM group. NC = normal control rats; DM = rats with diabetes mellitus; FER-1 = diabetic rats treated with ferrostatin-1; CUR = diabetic rats treated with curcumin.

Nrf2 expression and improve ferroptosis in penile endothelial cells, siRNA transfection experiments targeting Nrf2 were conducted. Western blotting experiments showed that Nrf2 siRNA transfection exacerbated the high-glucose-induced decrease in GPX4 and xCT expression, which was significantly restored by curcumin treatment (Figures 7(e), 7(g), and 7(h)). In addition, Nrf2 siRNA transfection exacerbated oxidative stress damage and cell viability reduction

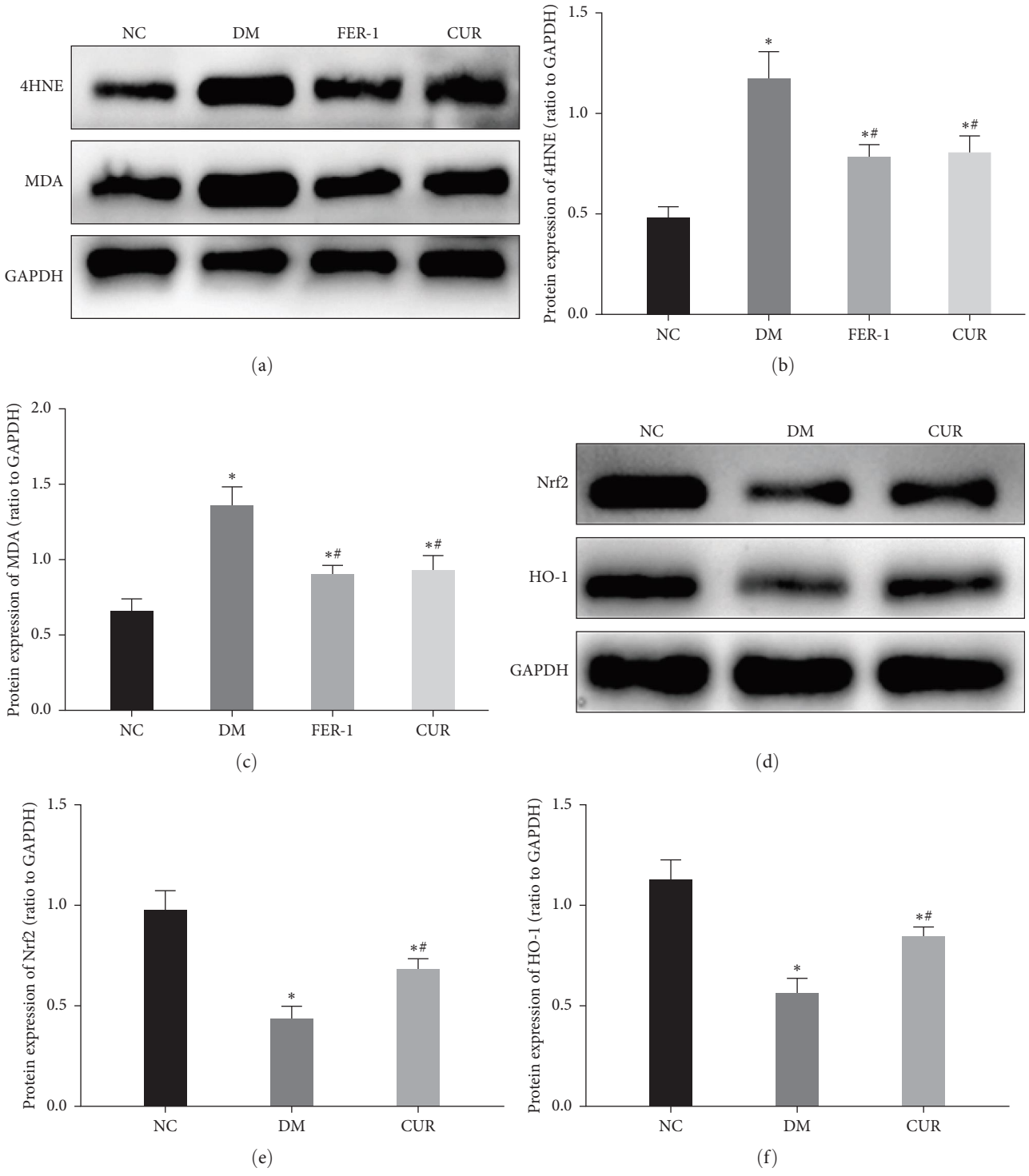

Figure 5: The levels of oxidative stress and protein content of Nrf2 and HO-1 in the penis tissue. Representative stripes of western blot (a, d), statistical analysis of 4HNE (b), MDA (c), Nrf2 (e), and HO-1 (f). Data were presented as mean ± SD from three animals per group. $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the DM group. NC = normal control rats; DM = rats with diabetes mellitus; FER-1 = diabetic rats treated with ferrostatin-1; CUR = diabetic rats treated with curcumin.

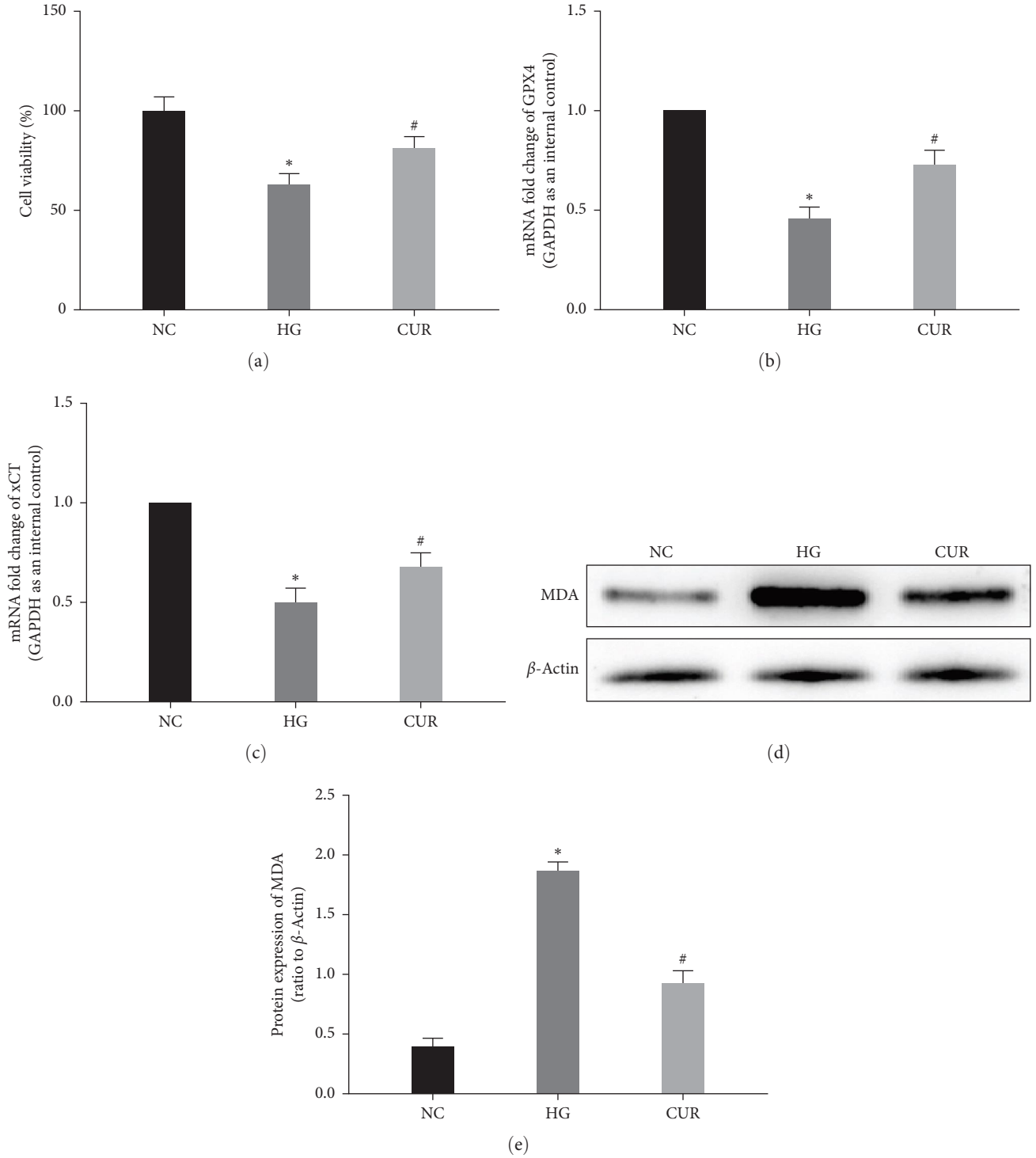

Figure 6: Curcumin inhibits ferroptosis in penile endothelial cells stimulated by high glucose. CCECs were exposed to 60 mM glucose for 24 hr. After glucose treatment, CCECs were treated with DMSO or 30 μM curcumin for 24 hr. CCK-8 assay for evaluating cell viability (a). Realtime PCR analyses of GPX4 (b) and xCT (c) genes in CCECs. Representative stripes of western blot (d) and statistical analysis of MDA (e). Data were presented as mean $\pm$ SD from three samples per group. $^{*}P < 0.05$ compared with the NC group. $^{\#}P < 0.05$ compared with the HG group. NC = black control; HG = CCECs treated with glucose; CUR = CCECs treated with glucose and curcumin.

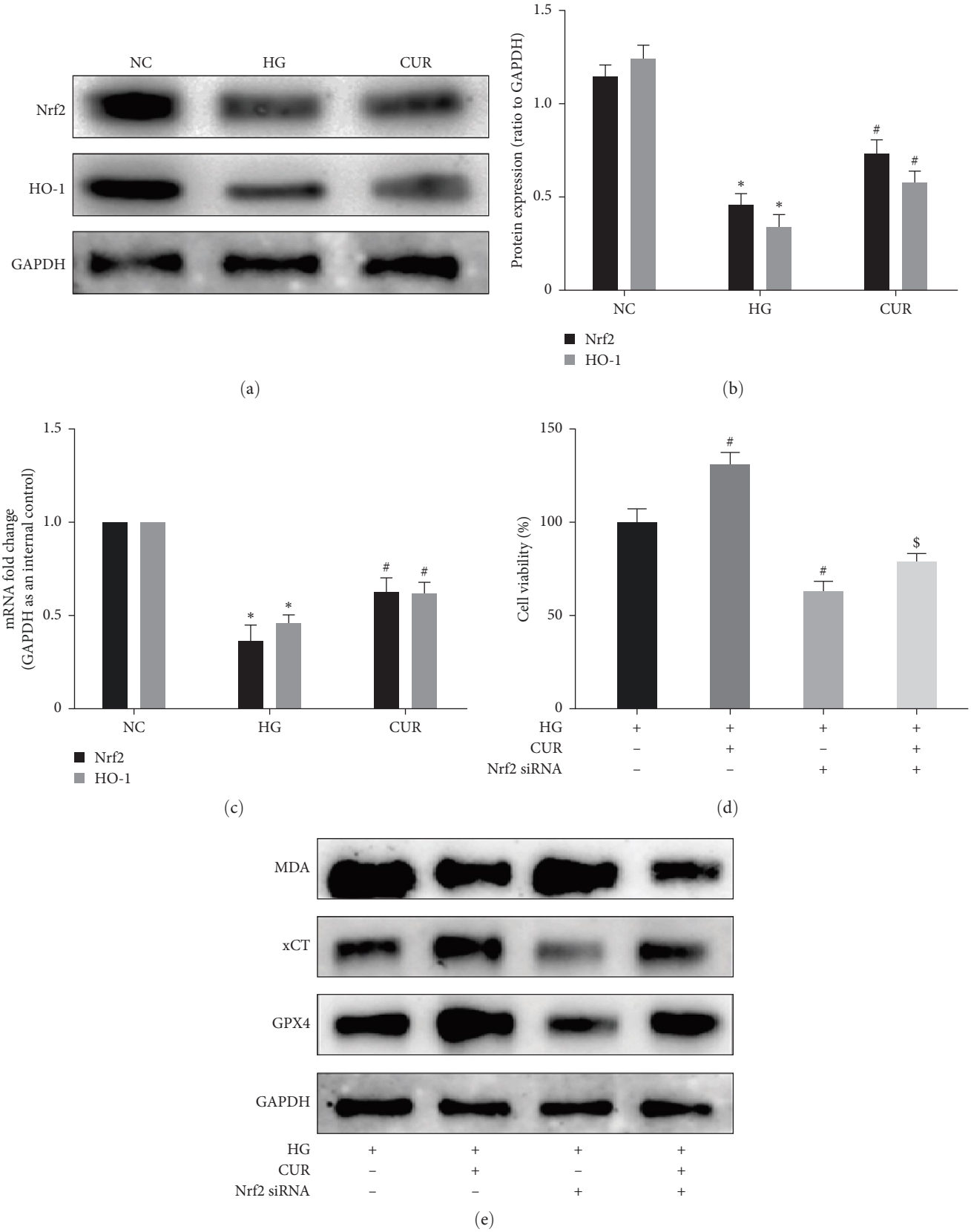

Figure 7: Continued.

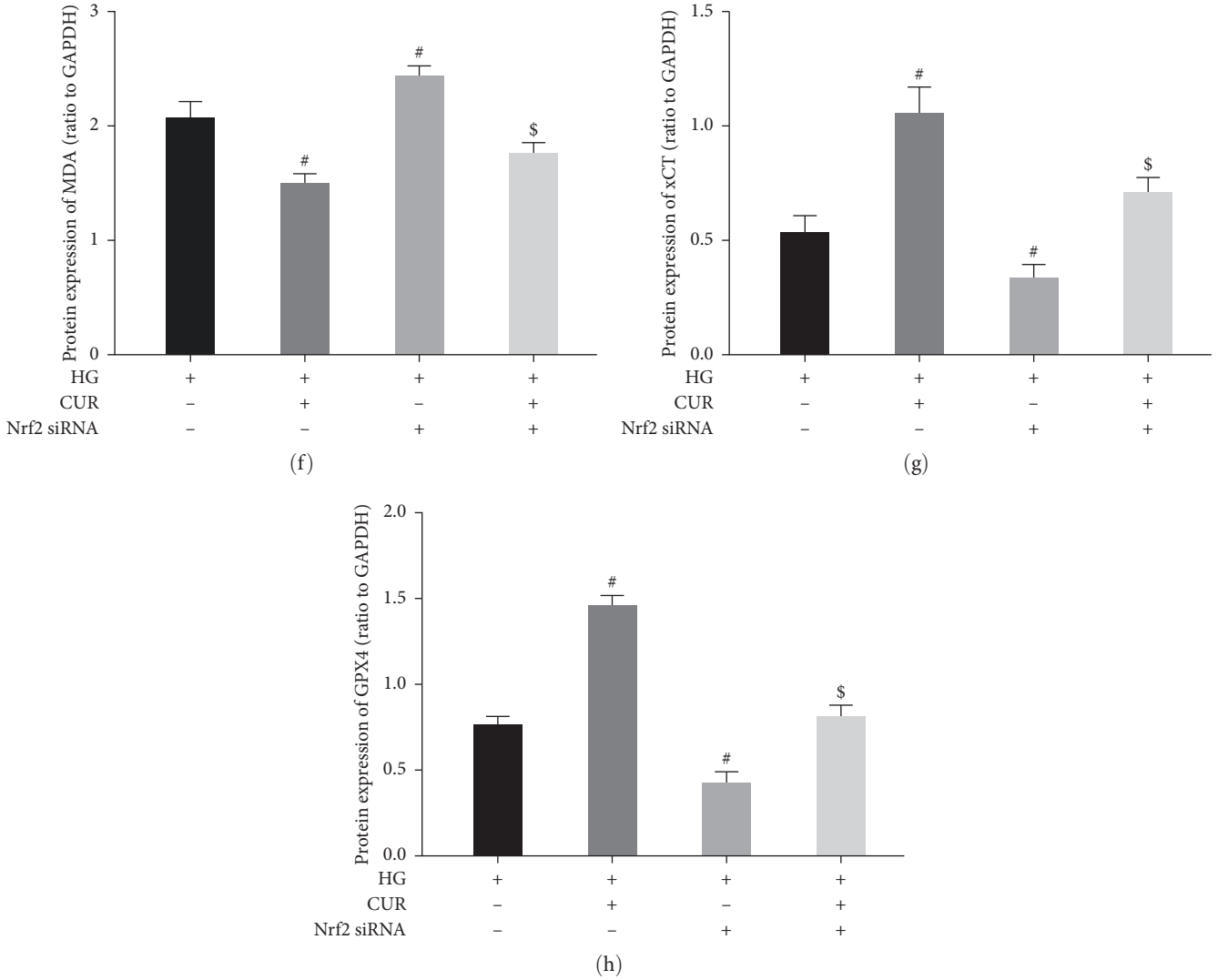

Figure 7: Curcumin inhibits ferroptosis in penile endothelial cells via Nrf2/HO-1 signaling. CCECs were exposed to 60 mM glucose for 24 hr. After glucose treatment, CCECs were treated with DMSO or 30 μM curcumin for 24 hr. Representative stripes of western blot (a) and statistical analysis of Nrf2 and HO-1 (b). Realtime PCR analyses of Nrf2 and HO-1 (c) genes in CCECs. CCECs were stably transfected with Nrf2 siRNA and then exposed to 60 mM glucose for 24 hr. After glucose treatment, CCECs were treated with DMSO or 30 μM curcumin for 24 hr. CCK-8 assay for evaluating cell viability (d). Representative stripes of western blot (e), statistical analysis of MDA (f), xCT (g), and GPX4 (h) in CCECs. Data were presented as mean ± SD from three samples per group. $^{*}P<0.05$ compared with the NC group. $^{\#}P<0.05$ compared with the HG group. $^{\$}P<0.05$ compared with the Nrf2 siRNA group. NC = black control; HG = CCECs treated with glucose; CUR = CCECs treated with glucose and curcumin; Nrf2 siRNA = CCECs were stably transfected with Nrf2 siRNA.

induced by high glucose stimulation, while curcumin significantly improved oxidative stress damage and cell viability (Figures 7(d) and 7(f)).

4. Discussion

DM is a chronic metabolic disease that results from continuous exposure to high levels of glucose and glycation end products. This leads to the accumulation of reactive oxygen species (ROS) and enhanced oxidative stress in tissues, which can seriously disrupt the homeostasis of vascular injury and repair. Research has indicated that penile cavernous vessels can serve as a marker for systemic vascular conditions in men, and ED may be an early indication of systemic vascular disease in those with diabetes [23]. Type 1 diabetes is a rare form of diabetes, accounting for only 1% of diabetic patients in China. The main cause of type 1 diabetes is a significant reduction or complete lack of insulin production. In contrast, type 2 DM is much more prevalent and is typically characterized by insulin resistance or insufficient insulin secretion over time. The type 1 diabetes rat model is commonly used in animal experiments because it only requires a large dose of STZ injection to simulate absolute insulin deficiency [24]. However, in the present study, we induced insulin resistance through a long-term high-fat diet and combined it with insulin deficiency induced by multiple intraperitoneal injections of low doses of STZ [25]. This approach allows for a more comprehensive understanding of the effects of both insulin

deficiency and insulin resistance, which are both important factors in the development of type 2 diabetes. Sex remains a crucial aspect of physiological needs for middle-aged and older men. However, diabetic ED patients often experience organic penile tissue lesions, and the current first-line drugs have limited treatment efficacy. As such, it is imperative to gain a comprehensive understanding of the pathogenesis of diabetic ED and develop new targeted drugs to address this issue. In recent years, curcumin has gained popularity in the treatment of vascular diseases due to its anti-inflammatory and antioxidative properties [26]. Our findings align with previous studies that have shown curcumin's ability to effectively reduce tissue oxidative stress levels and protect the erectile function of diabetic rats.

Ferroptosis is a recently identified form of cell death that is triggered by iron-dependence and lipid peroxidation. Unlike apoptosis, necrosis, and autophagy inhibitors, ferroptosis is not affected by them [27]. One of the essential molecules in the process of ferroptosis is glutathione (GSH). GSH plays a crucial role in reducing harmful lipid peroxides produced by cell metabolism to harmless lipid alcohols due to its strong reducing ability [28]. Cystine is necessary for the synthesis of glutathione, and it is transported from extracellular to intracellular by xCT, which is composed of Slc3a2 and Slc7a11 [29]. When GSH depletion occurs, it is oxidized to oxidized glutathione (GSSH) by GPX4, which reduces harmful lipid peroxides. Consequently, xCT and GPX4 are two significant markers in the development of ferroptosis. Ferroptosis is believed to be a contributing factor to the development of diabetes-related complications. In a high glucose environment, the expression of GPX4 can be inhibited, leading to the accumulation of ROS in tissues and subsequent cell damage via ferroptosis [30]. However, it is currently unclear whether curcumin can protect penile endothelial cells through this mechanism. In our study, we discovered that the erectile function of diabetic rats was significantly diminished. Additionally, we observed a significant decrease in the expression levels of the system and GPX4, while the levels of MDA and 4HNE were notably elevated in penile tissues. However, we found that intervention with a ferroptosis inhibitor can reverse the protein expression of these molecules and alleviate the ED experienced by diabetic rats to a certain extent. This intervention has the same therapeutic effect as the ferroptosis inhibitor, ferrostatin-1.

Curcumin, a natural polyphenol, has been found to effectively reduce diabetes-induced endothelial dysfunction by inhibiting oxidative stress. This is achieved by reducing the amount of ROS and superoxide aggregation while also increasing the activity of HO-1 [26]. Nrf2, a transcription factor, plays a crucial role in regulating antioxidant effects. By directly activating HO-1 signaling, Nrf2 can effectively counteract oxidative stress and prevent pathological damage in tissues, thereby acting as an antioxidant. The Nrf2/HO-1 signaling pathway plays a crucial role in the protective effect of Fraxetin against ferroptosis in myocardial infarction [31]. Similarly, melatonin has been shown to reduce ferroptosis in diabetic osteoporosis through the Nrf2/HO-1 signaling pathway, thereby improving the osteogenic ability of osteoblasts [32]. This study confirms previous reports that high glucose levels inhibit Nrf2/HO-1 signaling, leading to increased oxidative stress and ferroptosis in diabetic penile tissues. Simultaneously, curcumin intervention has the ability to partially restore the level of Nrf2/HO-1 signaling, which ultimately inhibits the onset of ferroptosis.

This experiment has several limitations. First, in modeling type 2 diabetes, the diabetic model rats were fed a high-fat diet to induce insulin resistance, while the normal control group was fed a normal diet. As a result, the potential impact of a short-term high-fat diet on the experimental results was not accounted for. Second, while the anti-inflammatory and antioxidative properties of curcumin are well-established, its intragastric bioavailability remains low, and methods for enhancing its bioavailability require further exploration.

5. Conclusions

The findings of this study suggest that ferroptosis plays a role in the development of ED and causes damage to endothelial cells. Furthermore, the results indicate that curcumin can enhance the erectile function of diabetic rats by inhibiting ferroptosis in the corpus cavernosum. The specific mechanism by which this occurs may involve the regulation of the Nrf2/HO-1 pathway.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Yuehui Jiang and Siyan Xing contributed equally to this work.

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