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Renoprotective mechanisms of celastrol in high glucose-mediated HK-2 cell injury through inhibition of the PI3K/Akt/NF-κB signalling pathway

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ABSTRACT

Hyperglycemia-induced inflammation and fibrosis in renal tubular epithelial cells are critical factors driving the progression of diabetic nephropathy (DN). Celastrol, a bioactive compound derived from *Tripterygium wilfordii Hook.F*, is recognized for its anti-inflammatory and anti-fibrotic properties. This study aimed to investigate the renoprotective effects of celastrol against high glucose (HG)-induced damage in human kidney 2 (HK-2) cells. Briefly, HK-2 cells were exposed to high glucose and treated with celastrol. Cell viability and apoptosis were evaluated using CCK-8 assay kit and flow cytometry, respectively. The pro-inflammatory cytokines, oxidative stress markers, and fibrotic-related proteins were measured using ELISA and immunoblotting. To further confirm the mechanistic actions of celastrol, the PI3K/Akt/NF-κB pathway was examined, and HG-treated cells were co-incubated with the NF-κB inhibitor bortezomib. Our result revealed that celastrol at the moderate concentration of 50 nM mitigated HG-induced toxicity, suggesting an optimal therapeutic window. Celastrol improved cell viability and reduced apoptosis in HG-treated HK-2 cells. It significantly decreased levels of inflammatory cytokines such as IL-6, TNF-α, IL-1β, and MCP-1, while enhancing antioxidant activities of GSH-Px and SOD, and lowering MDA levels, indicating diminished oxidative stress. Mechanistically, these renoprotective effects of celastrol partly attributed via inhibition of the PI3K/Akt/NF-κB signalling pathway, as blocking NF-κB signalling by bortezomib resulted in similar inhibitory effects against inflammation and fibrosis. Collectively, celastrol acts as a renoprotective agent against renal inflammation, oxidative stress, and fibrosis, partly through the inhibition of the PI3K/Akt/NF-κB pathway, offering potential therapeutic benefits against hyperglycemia-induced renal injury in DN.

1. Introduction

Diabetic nephropathy (DN) is among the microvascular complications of diabetes mellitus and recognized as one of the leading causes of end-stage renal disorder [1,2]. Renal tubular epithelial cells, which reside in the tubulointerstitial space, play a crucial role in kidney recovery following injury [3,4]. In response to injury caused by chronic hyperglycemia, tubular epithelial cells undergo abnormal phenotypic changes, leading to the persistent production and secretion of various humoral mediators and cytokines, which in turn drive interstitial inflammation and fibrosis [5,6]. Such damage to renal tubular epithelial cells, characterized by inflammation and fibrosis, plays a crucial role in the pathophysiological mechanism of DN [7,8]. Therefore, gaining a deeper understanding of the underlying mechanisms that drive inflammation and fibrosis in renal tubular epithelial cells is of great interest for developing effective therapeutics to halt the progression of DN.

Fibrosis, apoptosis, and oxidative stress are critical factors that accelerate the inflammatory response, particularly in the context of DN [9]. Numerous studies emphasize the role of these factors as key drivers of kidney injury progression. For example, renal fibrosis, which is characterized by excessive deposition of extracellular matrix components, promotes the infiltration of inflammatory cells and the release of pro-inflammatory cytokines, further exacerbating renal damage [9,10]. The oxidative stress induced by hyperglycemia leads to the

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overproduction of reactive oxygen species (ROS), which damages cellular components such as lipids, proteins, and nucleic acids. This, in turn, exacerbates endothelial dysfunction and inflammation [11]. Additionally, hyperglycemia activates endogenous apoptotic pathways, resulting in endothelial cell death and further promoting inflammation and fibrosis in renal tissue [12]. These interconnected processes collectively contribute to the progressive nature of DN. Targeting fibrosis, apoptosis, and oxidative stress may therefore provide effective therapeutic strategies to mitigate inflammation and renal damage in DN.

The PI3K/Akt signaling pathway plays a pivotal role in regulating key cellular processes, including glucose metabolism, cell survival, growth, and protein synthesis [13]. This pathway is essential for maintaining cellular homeostasis and responds to various stresses, such as oxidative damage [14,15]. NF-κB, a critical downstream effector of the PI3K/Akt pathway [16], serves as a central regulator of immune and inflammatory responses. It influences numerous metabolic and immune processes, driving inflammation and contributing to tissue damage. In the context of DN, hyperglycemia-induced activation of the PI3K/Akt/NF-κB pathway has been implicated in oxidative stress, apoptosis, and renal inflammation, all of which exacerbate kidney injury and dysfunction [17,18]. Given its central role in these pathological processes, targeting the PI3K/Akt/NF-κB pathway to modulate oxidative stress, apoptosis, and inflammation presents a promising therapeutic strategy to alleviate renal tubular damage and slow the progression of DN.

Several compounds have been investigated for their potential therapeutic effects in DN, one of which is celastrol. Numerous studies have demonstrated that celastrol possesses significant anti-inflammatory, antioxidant, anti-apoptotic, and renal protective properties, showing potential therapeutic effects in DN [19,20]. In high-glucose-induced renal cell injury models, celastrol exerts renal protective effects by modulating inflammation and cellular damage [21]. Additionally, in early-stage diabetic nephropathy rat models, celastrol alleviates renal injury and inhibits glomerular basement membrane thickening through modulation of the PI3K/Akt pathway [22]. However, these studies still have limitations in directly elucidating the role of celastrol in alleviating inflammatory injury in HK-2 cells. While existing reports suggest that celastrol can mitigate renal inflammation and damage in diabetic rats [23], the specific mechanism by which it exerts its effects through the PI3K/Akt/NF-κB signaling pathway in high-glucose-induced HK-2 cell injury remains insufficiently studied.

Therefore, the aim of this study is to confirm the protective effects of celastrol on high-glucose-induced inflammatory injury in human renal tubular epithelial cells (HK-2 cells), and to determine whether its action occurs through inhibition of the PI3K/Akt/NF-κB signaling pathway. This study will provide new evidence for further elucidating the therapeutic potential of celastrol in diabetic nephropathy and lay the foundation for developing intervention strategies targeting renal tubular epithelial cell inflammation.

2. Methods

2.1. Cell culture and treatment

Human kidney 2 (HK-2) cells were directly purchased from Shanghai Binsui Biotechnology Co., Ltd. (Shanghai, China). Briefly, cells were cultured with Dulbecco's modified Eagle's medium (DMEM)/F12 (no. 11320033, Gibco, NY, USA) supplemented with 10 % fetal bovine serum (no. 10270-106, Gibco, NY, USA) and 1 % penicillin/streptomycin (no. 15070063, ThermoFisher, Grand Island, NY, USA) in a 5 % CO_2 incubator at 37 °C. Cells in the logarithmic growth phase were seeded into 6-well plates at a density of 3×10^4 cells/well (100 μL) and then assigned into the following groups: negative control (NC, normal glucose), high glucose (HG), HG + cel H (treatment with high glucose and high-concentration [100 nM] celastrol), HG + cel M (treatment with high glucose and moderate-concentration [50 nM] celastrol), HG + cel L (treatment with high glucose and low-concentration [20 nM] celastrol), inhibition NF-κB (treatment with high glucose and the NF-κB inhibitor bortezomib [Abcam, Cambridge, UK]) and inhibition NC (control of the inhibition NF-κB group). For HG treatment, 50 mmol/L glucose was added to the growth medium. Celastrol purchased from Sigma was dissolved in dimethyl sulfoxide (DMSO) (no. 102510041, Sigma-Aldrich Corporation, Missouri, USA.)(as a vehicle) and freshly diluted to the desired concentration before use. Cells were treated with the above concentrations (high, moderate and low) of celastrol for 24 h.

2.2. Cell counting kit-8 (CCK-8) assay

CKK-8 assay was utilized to measure the viability of HK-2 cells. Briefly, HK-2 cells in the logarithmic growth phase were seeded into 96-well plates at a density of 4×10^3 cells/well, and 3 wells were included in each group. After the cells were cultured for 24 h, 10 μL CCK-8 reagent (no. F25, SciBioCold, Beijing, China) was added to each well and incubated with the cells for 1 h. Thereafter, the absorbance of each well at 450 nm was measured.

2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to detect the relative levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and monocyte chemoattractant protein-1 (MCP-1) in HK-2 cells. Briefly, HK-2 cells were cultured for 24 h and the culture medium was harvested for centrifugation at 1000×*g* for 5 min. The supernatant was then transferred into an Eppendorf tube. The protocols of IL-6, TNF-α, IL-1β (no. SEA079Hu, SEA133Hu, SEA563Hu, Wuhan USCN Business Co.,Ltd., Wuhan, China) and MCP-1(no. JL19334, Jianglaibio, Shanghai, China) ELISA kit were performed according to the kit instructions: blank wells, standard wells and sample wells to be tested were set on the ELISA plate. After sealing, the plate was incubated at 37 °C for 90 min and then the liquid was discarded. Then, the plate was washed 5 times in washing solution. Except for the blank wells, 100 μL enzyme conjugate diluent was added into each well of the remaining wells and incubated at 37 °C for 15 min. Subsequently, 100 μL stop solution was added to each well and the optical density value was measured at 450 nm with a microplate reader.

2.4. Quantification of oxidative stress-related markers

HK-2 cells cultured for 24 h were harvested and lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime). Cell lysates were centrifuged at 1000×*g* for 5 min to separate the supernatant, which was then collected. Protein concentrations were quantified using the BCA Protein Assay Kit (Nanjing JianCheng Bioengineering Institute). Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels were determined in accordance with the manufacturer's instrument of kits (no.A005-1, BC5165 and A003-1, Nanjing Jiancheng Bioengineering Institute, China).

2.5. Flow cytometry

Flow cytometry was employed to assess and quantify the apoptotic cell population. HK-2 cells were plated into 96-well plates at a density of 4×10^3 cells per well for 48 h under standard growth conditions to ensure adequate cell attachment and proliferation. Cell-growth medium was then replaced with DMEM. The cells were incubated for 20 min without light exposure, after which the cells were washed with phosphate-buffered saline (PBS) buffer. The cells were subsequently detached with 0.25 % trypsin-ethylenediaminetetraacetic acid (EDTA) solution, harvested and stained with fluorescent-labeled primary antibodies for 1 h at 4 °C. The apoptotic cell population was analyzed using a Guava EasyCyte Mini flow cytometer (BD, NJ, USA).

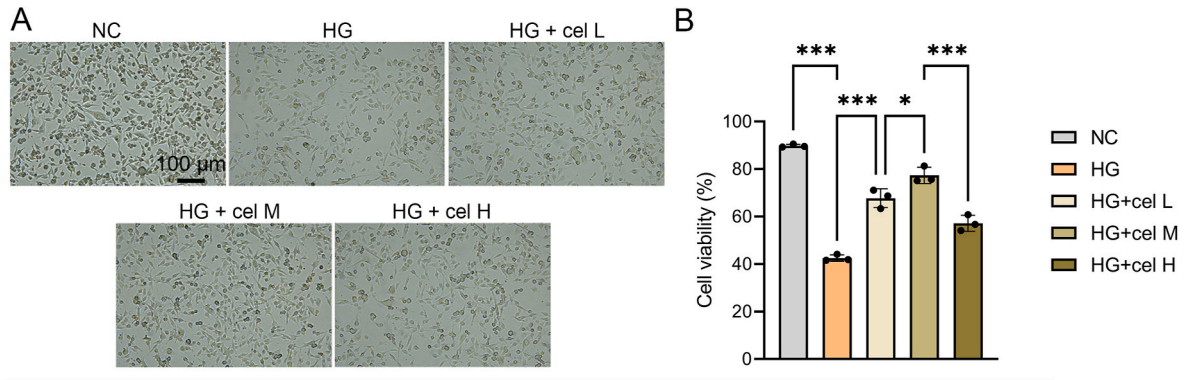

Fig. 1. Celastrol exhibited protective properties to attenuate glucotoxicity effects in HK-2 cells. **A.** The morphology of HK-2 cells from each group was assessed using an inverted microscope. **B.** Cell viability was measured by CKK-8 assay; *$p < 0.05$, ***$p < 0.001$. HK-2 cells were exposed to high glucose and treated with low-, moderate-, and high-dose celastrol. NC: negative control, HG: high glucose, cel: celastrol, L: low, M: moderate, H: high.

2.6. Immunoblotting

Total protein was extracted from HK-2 cells on ice and the protein concentration was quantified by bicinchoninic acid assay (BCA) method. Samples (30 μg protein) were loaded onto sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, fractioned through electrophoresis, and transferred onto nitrocellulose membranes. The membrane was blocked for 2 h and incubated overnight at 4 °C with diluted primary antibodies against α-SMA (no. AF1032, 1:500, Affinity Biosciences, Shanghai, China), E-cadherin (no. BF0219, 1:2000, Affinity Biosciences, Shanghai, China), PI3K (no. AF6241, 1:1500, Affinity Biosciences, Shanghai, China), p-PI3K (no. AF3241, 1:1000, Affinity Biosciences, Shanghai, China), AKT (no. AF0836, 1:1000, Affinity Biosciences, Shanghai, China), p-AKT (no.

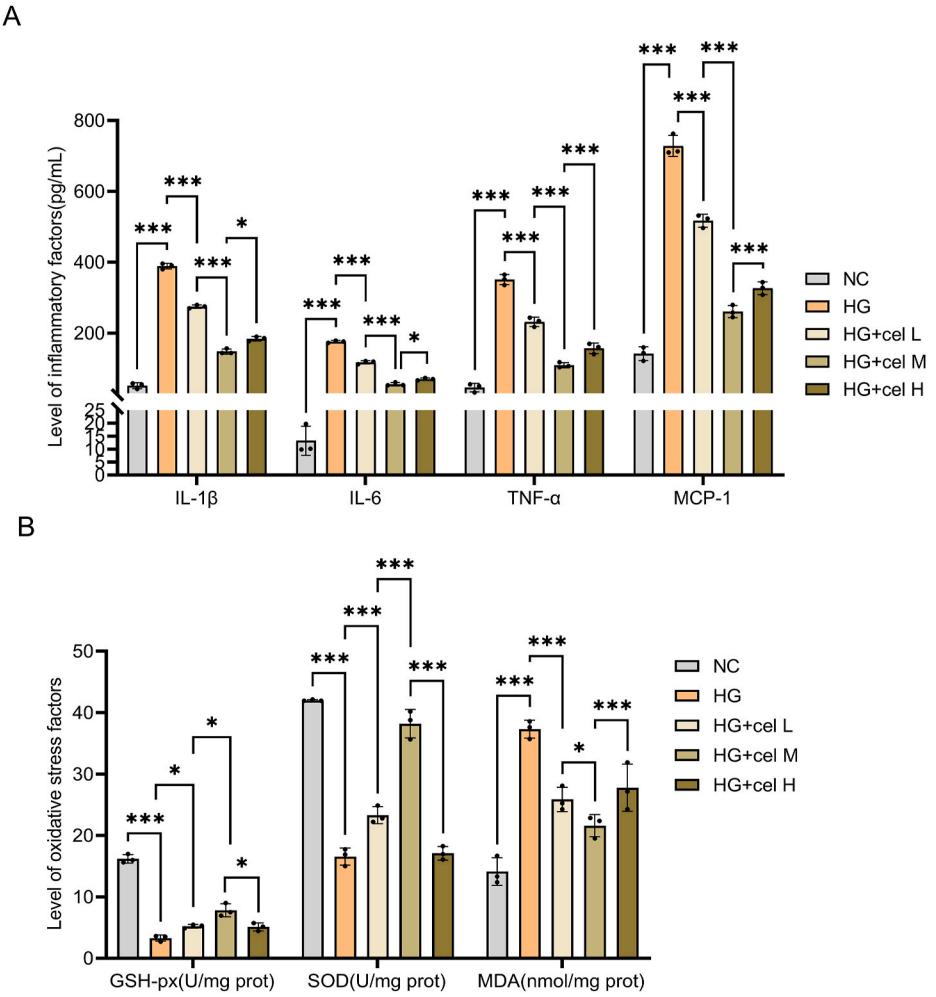

Fig. 2. Celastrol suppressed inflammatory response and oxidative stress in HG-treated HK-2 cells. **A.** The relative levels of pro-inflammatory cytokines including IL-6, TNF-α, IL-1β and MCP-1 in the culture supernatant were measured by ELISA. **B.** Determination of GSH-Px and SOD activities and MDA levels in each group. *$p < 0.05$, ***$p < 0.001$.

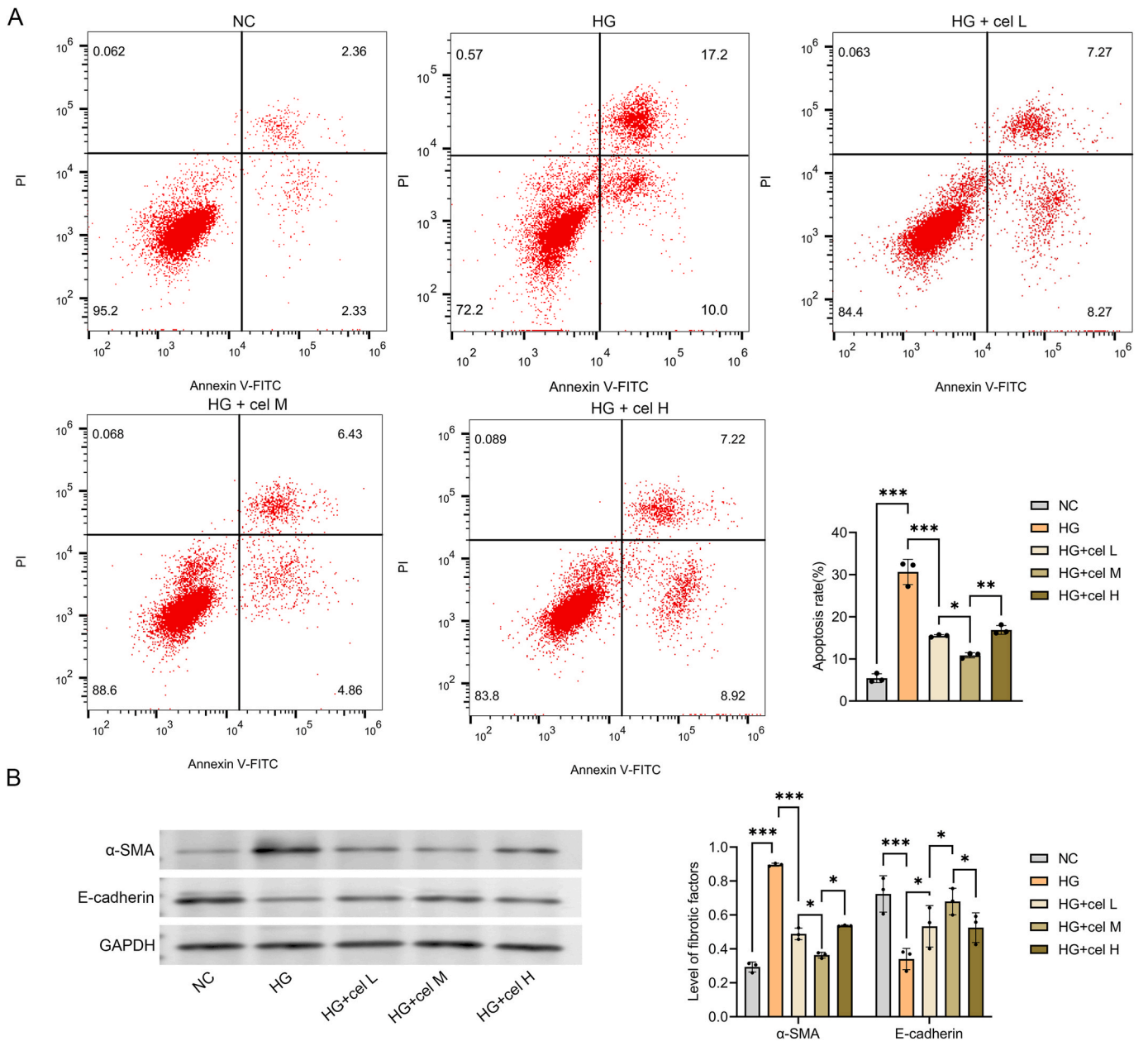

Fig. 3. Celastrol alleviated apoptosis and fibrosis of HG-treated HK-2 cells. **A.** The cell apoptosis analysis was measured by flow cytometry. **B.** The protein expression of α-SMA and E-cadherin was measured using immunoblotting. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

AF0832, 1:1000, Affinity Biosciences, Shanghai, China), NF-κB (no. AF5006, 1:1000, Affinity Biosciences, Shanghai, China), p-NF-κB (no. AF2006, 1:1000, Affinity Biosciences, Shanghai, China), and GAPDH (no. AF7021, 1:5000, Affinity Biosciences, Shanghai, China). In the following day, the membrane was further incubated with a secondary IgG antibody (no. GB23303, 1:10000, Servicebio Technology Co., Ltd, Wuhan, China)) at room temperature for 2 h. Protein bands were visualized using enhanced chemiluminescence reagent (no. M2301, HaiGene, Heilongjiang, China). Finally, the protein bands were viewed with a gel imaging system and then analyzed with the ImageJ software.

2.7. Statistical analysis

Statistical analysis was performed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard deviation. A one-way analysis of variance (ANOVA) was used to compare data across multiple groups, followed by the Newman–Keuls or Student–Newman–Keuls (SNK) test for pairwise comparisons. For comparisons between two groups, a two-tailed unpaired *t*-test was performed. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Celastrol remarkably improved the viability of HG-treated HK-2 cells

To investigate the effect of Celastrol on the viability of HG-treated HK-2 cells, we observed cell morphology and proliferation under a microscope. Normal HK-2 cells were shown to be densely arranged with a "cobblestone paving" shape, while HG-exposed HK-2 cells displayed a long spindle shape with enlarged intercellular spaces. These lesions were significantly alleviated in response to celastrol, of which, the moderate-dose celastrol showed superior effects to low- and high-dose celastrol (Fig. 1A). CCK-8 assays revealed that viability was reduced in HG-treated HK-2 cells, but this was significantly improved upon Celastrol

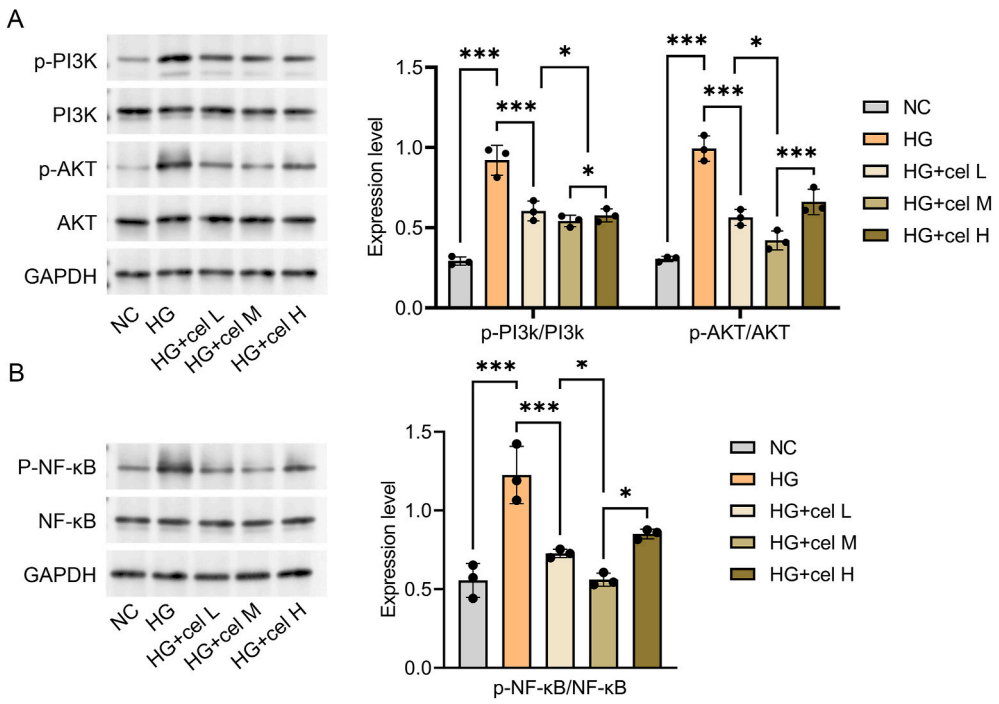

Fig. 4. Celastrol inhibited the PI3K/Akt/NF-κB pathway in HG-treated HK-2 cells. **A.** The relative expression of PI3K, AKT, p-PI3K and p-AKT proteins were measured using immunoblotting. **B.** The relative expression of NF-κB and p-NF-κB proteins were determined. *$p < 0.05$, ***$p < 0.001$.

treatment, with the moderate dose demonstrating the most pronounced effect ($p < 0.05$, Fig. 1B).These results suggest an optimal therapeutic window for Celastrol in protecting against high glucose-induced cell damage.

3.2. Celastrol attenuated inflammatory responses and oxidative stress in HG-treated HK-2 cells

IL-6, TNF-α, IL-1β, and MCP-1 levels were increased in the culture supernatant of HG-exposed HK-2 cells relative to normal HK-2 cells ($p < 0.001$, Fig. 2A). Moreover, HG-exposed HK-2 cells exhibited lower GSH-Px and SOD activities and higher MDA levels than normal cells ($p < 0.001$, Fig. 2B). These effects were significantly improved following celastrol treatment ($p < 0.001$), with the moderate concentration showing greater efficacy compared to both the low and high doses.

3.3. Celastrol alleviated apoptosis and fibrosis in HG-treated HK-2 cells

The flow cytometry analysis revealed a significant increase in cell apoptosis in HG-induced HK-2 cells compared to normal cells. ($p < 0.001$, Fig. 3A). Moreover, in HG-treated HK-2 cells, the expression of the fibrosis-related proteins, α-SMA showed a significant increase, while E-cadherin expression was downregulated compared to normal cells ($p < 0.001$). Celastrol treatment reversed these changes, with the moderate concentration demonstrating greater efficacy compared to low and high concentrations. ($p < 0.05$, Fig. 3B).

3.4. Celastrol inhibited the PI3K/Akt/NF-κB pathway in HG-treated HK-2 cells

The inhibition of the PI3K/AKT/NF-κB pathway contributes to alleviating kidney tubular epithelial injury in DN [21]. Of note, it was identified that celastrol could induce apoptosis of cancer cells via inactivating the PI3K/Akt/NF-κB pathway [22]. Our previous reports demonstrated that the beneficial effects of celastrol on insulin resistance, obesity and inflammatory response are mainly regulated through the PI3K/Akt/NF-κB pathway [23–27]. Considering this, we further hypothesized that celastrol may also alleviate HG-induced injury in HK-2 cells by regulating this pathway. The phosphorylation levels of PI3K, AKT and NF-κB were significantly elevated in HG-treated HK-2 cells compared to normal cells ($p < 0.001$, Fig. 4A). Treatment with celastrol effectively reduced this HG-induced PI3K/Akt/NF-κB activation, with the moderate concentration showing superior efficacy compared to low and high concentrations. ($p < 0.05$, Fig. 4A and B).

3.5. Celastrol and the NF-κB inhibitor, bortezomib exhibit comparable renoprotective effects against glucotoxicity-mediated renal cell injury

To further verify the mechanistic actions of celastrol in alleviating HG-mediated renal injury via the PI3K/Akt/NF-κB pathway, we co-incubated HG-treated HK-2 cells with the NF-κB inhibitor bortezomib. Treatment with bortezomib in the HG-treated HK-2 cells increased cell viability, diminished the levels of pro-inflammatory cytokines IL-6, TNF-α, IL-1β, and MCP-1, attenuated oxidative stress and decreased cell apoptosis and fibrosis ($p < 0.05$, Fig. 5A–E).

4. Discussion

Glomerulosclerosis, renal tubulointerstitial inflammation, and fibrosis are key pathological events in the progression of DN [24]. Hyperglycemia, advanced glycation end products (AGEs), reactive oxygen species (ROS), and inflammatory mediators contribute to kidney damage through the activation of various signaling pathways [25,26]. However, in the early stages of DN, renal tubular damage often occurs first, and some patients may exhibit clinical features such as proteinuria even when there are no significant structural abnormalities in the glomerulus [27,28]. Therefore, inflammatory injury and fibrosis of the renal tubules have become core elements of early pathological changes in DN [29–31]. While extensive research has shown that Celastrol has therapeutic effects in DN, most studies focus on changes in glomerular structure and function [32,33]. The protective effects and mechanisms of celastrol on renal tubular epithelial cells under high glucose conditions remain insufficiently explored. Our results demonstrate that Celastrol significantly alleviates high glucose-induced inflammation,

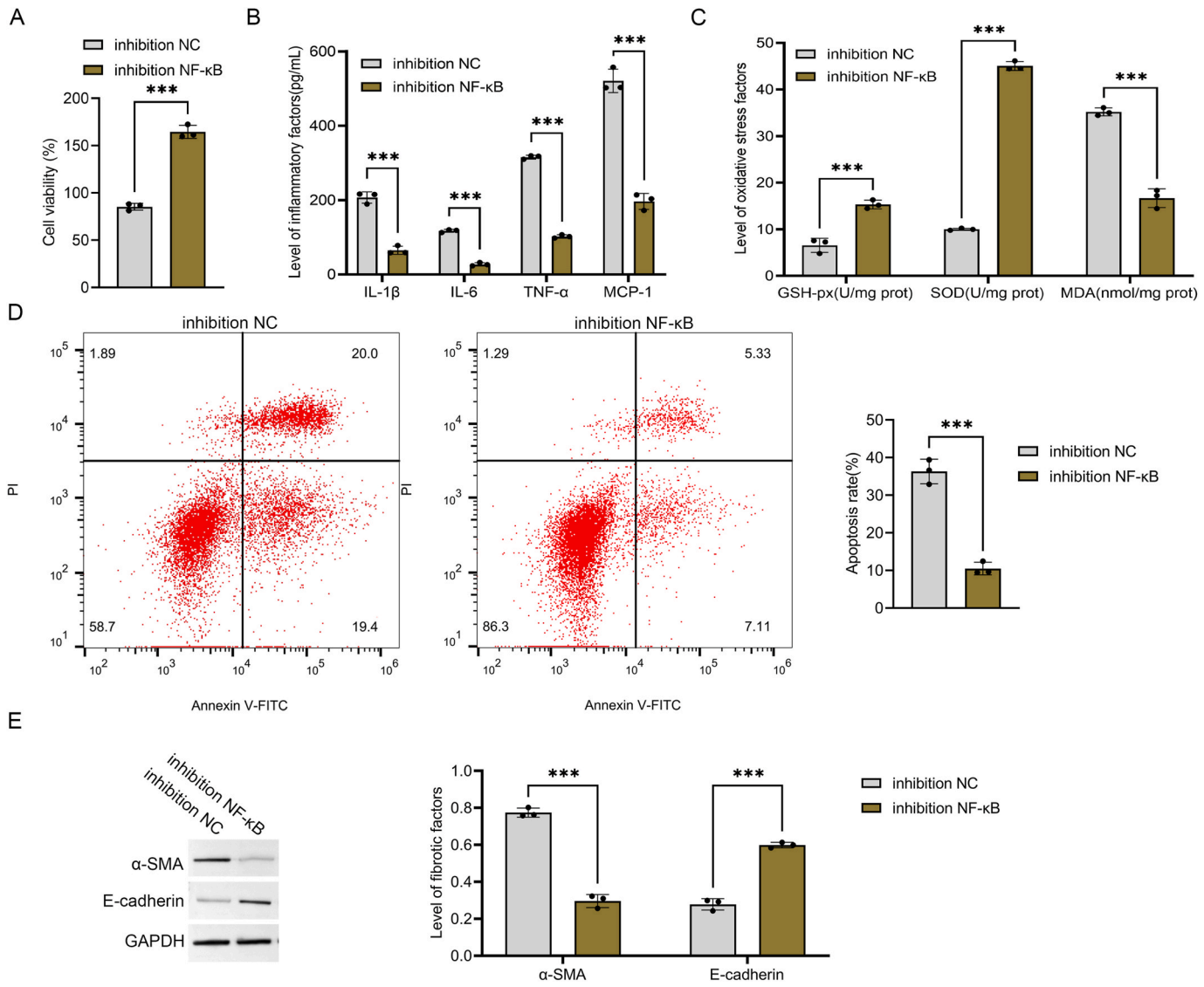

Fig. 5. HG-treated HK-2 cells were incubated with bortezomib. Inhibition of the NF-κB pathway by bortezomib resulted in similar beneficial effects as celastrol, further reducing inflammation and fibrosis in HG-treated HK-2 cells. **A.** The cell viability analysis of HK-2 cells was carried out using the CKK-8 assay kit. **B.** The relative levels of pro-inflammatory cytokines IL-6, TNF-α, IL-1β and MCP-1 were measured using ELISA. **C.** The quantification of oxidative stress-related markers including GSH-Px and SOD and MDA levels following bortezomib treatment. **D.** The apoptosis rate of HG-mediated HK-2 cells injury was measured by flow cytometry. **E.** The relative expression of α-SMA and E-cadherin proteins in HG-stimulated HK-2 cells. $*p < 0.05$, $***p < 0.001$. NC: negative control.

oxidative stress, apoptosis, and fibrosis in HK-2 cells by inhibiting the PI3K/Akt/NF-κB signaling pathway, revealing its unique protective mechanism in renal tubular epithelial cells (Fig. 6).

HG-induced oxidative stress and inflammation are major mechanisms in the onset and progression of DN [34]. Under hyperglycemic conditions, excessive generation of ROS and the release of pro-inflammatory mediators exacerbate damage to renal tubular epithelial cells [35]. This process promotes apoptosis, inflammation, and fibrosis, accelerating the decline in kidney function. Previous studies have highlighted the critical role of pro-inflammatory cytokines such as IL-6, TNF-α, IL-1β, and MCP-1 in the pathogenesis of diabetic nephropathy. These cytokines enhance the inflammatory response, driving the pathological progression of DN [36]. Our study found that Celastrol suppressed the levels of these pro-inflammatory mediators in a dose-dependent manner. Moreover, Celastrol not only exerts anti-inflammatory effects but also effectively alleviates oxidative stress. In HG-treated HK-2 cells, Celastrol increased the activity of key antioxidant enzymes such as GSH-Px and SOD, while reducing the levels of MDA, indicating its significant protective effects against oxidative stress. MDA, a product of lipid peroxidation, is a classic marker of oxidative stress [37]. Our findings further confirm the protective role of Celastrol in alleviating high glucose-induced oxidative stress and inflammation, providing new experimental evidence for its potential therapeutic use in diabetic nephropathy.

Furthermore, the renoprotective effects of celastrol on apoptosis and fibrosis in HG-treated HK-2 cells were significantly observed, as these detrimental effects were reversed by celastrol treatment. Cisplatin-induced apoptosis in renal tubular cells was significantly attenuated in vitro following treatment with celastrol [38]. A growing body of recent reports has highlighted the potent anti-fibrotic effects of celastrol in a range of metabolic and inflammation-related diseases [39,40]. Moreover, celastrol treatment has been shown to mitigate renal fibrosis induced by unilateral ureteral obstruction in mice [41]. This is evidenced by a significant reduction in tubular injury, collagen deposition, and the expression of pro-fibrotic proteins such as fibronectin (FN), collagen I (Col I), and α-SMA [42].These findings suggest that celastrol may offer a promising therapeutic strategy for mitigating renal fibrosis in the context of metabolic diseases, including DN.

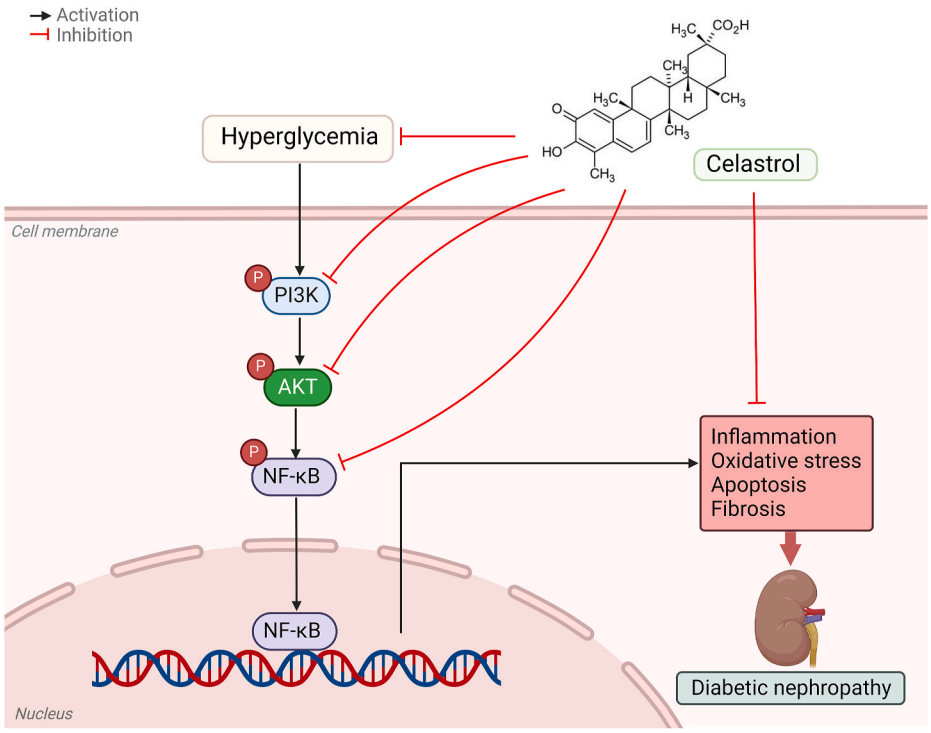

Fig. 6. The proposed mechanistic targets of celastrol in alleviating hyperglycemia-induced renal damage. Under hyperglycemic conditions, the PI3K/Akt/NF-κB signaling pathway is activated, leading to increased inflammation, oxidative stress, apoptosis, and fibrosis, all of which contribute to the progression of hyperglycemia-induced DN. Celastrol inhibits the activation of the PI3K/Akt/NF-κB pathway, thereby mitigating various detrimental processes associated with renal damage, thus highlighting its potential as a therapeutic agent for hyperglycemia-mediated metabolic dysfunctions, including diabetic nephropathy (DN). Created with BioRender.com.

The excessive activation of the PI3K/Akt/NF-κB signaling pathway plays a crucial role in the pathogenesis and progression of DN [43]. We postulated that excessive activation of the PI3K/Akt/NF-κB pathway by high glucose levels leads to increased inflammation, oxidative stress, and fibrosis in renal tubular cells. This overactivation causes cellular damage, promoting renal injury and accelerating the progression of DN. The present study contributes to the body of knowledge by demonstrating that celastrol can mitigate these detrimental effects, offering potential protection against the progression of hyperglycemia-induced DN. Our results are aligned with a previous study [44], which showed that celastrol treatment significantly reduced the expression of phosphorylated PI3K, Akt, IKKα/β, IκBα, and NF-κB subunit p65 in human osteosarcoma U–2OS cells. A study by Sha et al. indicated that celastrol was capable of inhibiting the activation of the PI3K/Akt/NF-κB pathways in gastric cancer cells [45]. Consistent with these, a previous report demonstrated that curcumin (a potent inhibitor of NF-κB activation) mitigates renal interstitial fibrosis and inflammation by inhibiting the TLR4/NF-κB and PI3K/Akt pathways [46]. This was evidenced by the reduced expression of vimentin and α-SMA in TGF-β1-induced HK-2 cells, as well as decreased levels of IL-6, IL-1β, and TNF-α in LPS-induced HK-2 cells. Moreover, wogonin, a natural flavonoid isolated from the traditional Chinese herb *Scutellaria baicalensis*, has been shown to attenuate tubulointerstitial fibrosis and renal tubular cell injury in diabetic nephropathy by inhibiting autophagy and inflammation through the PI3K/Akt/NF-κB pathway [47], suggesting that inhibition of the PI3K/Akt/NF-κB pathway could be a promising therapeutic strategy for mitigating renal injury in DN.

Despite the positive results obtained in this study, there are several limitations that need to be acknowledged. Firstly, our previous studies have confirmed the attributive properties of celastrol as an NF-κB inhibitor [48,49]. In our study, bortezomib was used as a control group to verify that celastrol exerts its renal protective effects through the inhibition of the PI3K/Akt/NF-κB pathway. However, this study was conducted exclusively in vitro using the HK-2 renal tubular epithelial cell line derived from normal adult male kidneys, which may not fully replicate the complex physiological interactions in vivo. Furthermore, the activation of the PI3K/Akt/NF-κB pathway involves multiple signaling molecules and regulatory mechanisms, and its complexity warrants further investigation. Additionally, the long-term safety, bioavailability, and therapeutic efficacy of celastrol will need to be thoroughly evaluated in clinical trials.

5. Conclusion

In summary, our findings demonstrate that celastrol exerts in vitro renoprotective properties against hyperglycemia-mediated inflammation, oxidative stress and fibrosis in HG-treated HK-2 cells. Mechanistically, we further validated that the underlying mechanistic actions of celastrol against these detrimental occasions induced by glucotoxicity are partly attributed by suppressing the PI3K/Akt/NF-κB pathway. Hence, our findings offer valuable insights into the potential utility of celastrol as a therapeutic agent against hyperglycemia-induced renal injury, contributing to the management of DN and its associated complications.

CRediT authorship contribution statement

Xiaojuan Wang: Writing – original draft, Methodology, Investigation, Data curation. **Mohamad Hafizi Abu Bakar:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Mohd Asyraf Kassim:** Writing – review & editing, Resources, Conceptualization. **Khairul Anuar Shariff:** Writing – review & editing, Resources, Formal analysis, Conceptualization. **Mohamad Norisham Mohamad Rosdi:** Writing – review & editing, Validation, Conceptualization.

Consent to participate

Not applicable.

Consent to publish

All authors agreed to submit the manuscript.

Ethical approval

Not applicable.

Data and code availability

Data will be made available upon request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2025.101928.

Data availability

Data will be made available on request.

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