Sinigrin attenuates angiotensin II-induced kidney injury by inactivating nuclear factor-κB and extracellular signal-regulated kinase signaling *in vivo* and *in vitro*

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Received April 2, 2021; Accepted June 2, 2021

DOI: 10.3892/ijmm.2021.4994

Abstract. The present study investigated the function of sinigrin in angiotensin II (Ang II)-induced renal damage. The results demonstrated that systolic blood pressure (SBP) and diastolic blood pressure (DBP) were increased in Ang II-challenged rats, and sinigrin treatment inhibited their increase. The levels of blood urea nitrogen (BUN) and serum creatinine (SCR) were increased by Ang II in the rats, and these were reversed by sinigrin in a dose-dependent manner. In addition, the Ang II-induced elevation of urinary protein levels was inhibited by sinigrin treatment. Glomerular basement membrane thickness and ECM degradation markers, such as collagen I, collagen IV and fibronectin, were suppressed by sinigrin in the Ang II-challenged rats. Moreover, the levels of inflammatory regulators, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), were reduced following sinigrin treatment of the Ang II-challenged rats and in Ang II-exposed proximal tubule epithelial cells. Furthermore, the superoxide dismutase (SOD) and catalase (CAT) levels were downregulated, whereas the malondialdehyde (MDA) levels were upregulated by Ang II; these effects were reversed by sinigrin treatment *in vivo* and *in vitro*. Mechanistically, sinigrin inhibited the Ang II-induced phosphorylation of ERK, p65 and IκBα. Thus, sinigrin attenuated Ang II-induced renal injury by inactivating ERK and NF-κB signaling. Sinigrin may thus prove to be a potential candidate for the treatment of hypertension-induced kidney damage.

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Key words: hypertension, kidney damage, sinigrin, ERK, NF-κB

Introduction

Hypertension is a prevalent cardiovascular disorder worldwide, and the kidneys have been identified as primary target organs in the pathophysiology of hypertension (1,2). Hypertensive renal injury is characterized by inflammation, the excessive accumulation of extracellular matrix (ECM) and renal tubular injury (3). The renin-angiotensin system (RAS) system performs crucial functions during hypertension-induced kidney injury development, which is regulated by an essential factor of the RAS, namely angiotensin II (Ang II) (4,5). Ang II can promote the release of inflammation-related factors, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), leading to small renal artery endothelial cell damage and kidney vascular remodeling (6,7). In addition, Ang II enhances the accumulation of ECM in smooth vascular cells, tubular epithelial cells and glomerular mesangial cells, causing kidney glomerular sclerosis and parenchyma fibrosis (3). However, the mechanisms of Ang II-induced kidney damage are complex and unclear.

Previous studies have suggested that various critical signaling pathways participate in Ang II-induced kidney damage, including the nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase (ERK) pathways (8,9). The NF-κB and ERK signaling pathways have been reported to mediate the expression of inflammatory and apoptotic mediators (10). Wu *et al* (11) indicated that NF-κB signaling plays an important role in Ang II-induced hypertensive renal damage. Additionally, previous studies have confirmed that the inhibition of NF-κB or ERK signaling can attenuate the progression of acute renal damage (10,12). Therefore, the NF-κB and ERK signaling pathways may be considered as targets for the treatment of hypertension-induced renal injury.

Sinigrin ($C_{10}H_{16}KNO_9S_2$) is a natural compound obtained from cruciferous vegetables, including cauliflower, broccoli, and cabbage, and is usually used for the treatment of multiple disorders in combination with other herbs. Sinigrin has been shown to suppress adipocyte differentiation through the ERK signaling pathway (13). Furthermore, sinigrin can exert potent anti-atherogenic effects by disrupting the NF-κB and MAPK pathways (14). Sinigrin can also significantly inhibit inflammatory responses by suppressing the NF-κB/MAPK pathway or NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in macrophages (15). However, the effects of sinigrin on Ang II-induced renal injury and the related molecular mechanisms remain unclear.

Thus, the present study investigated the effects of sinigrin on Ang II-induced kidney damage. The findings presented herein demonstrate that sinigrin can protect against toward Ang II-induced renal injury by inactivating NF-κB and ERK signaling *in vivo* and *in vitro*.

Materials and methods

Induction of hypertension in rats via Ang II infusion. To evaluate the function of sinigrin in the modulation of hypertension-induced kidney damage, a hypertensive rat model was constructed using Ang II. Briefly, Sprague-Dawley rats (n=10 per group, weighing 200-225 g, male, SiPeiFu Biotechnology Co., Ltd.) were subcutaneously administered Ang II (Sigma-Aldrich; Merck KGaA) for 28 days via low-dose Ang II infusion from osmotic minipumps (model 2002, ALZET, DURECT Corporation) in the dorsum of the neck under anesthesia (pentobarbital sodium, 50 mg/kg i.p.). Beginning from the first day of Ang II pump implantation, sinigrin (#85440, Sigma-Aldrich; Merck KGaA) was administered at the indicated doses to the rats once daily via oral gavage and continued for 28 days. The rats were assigned to 5 groups as follows: The sham-operated (sham) group (n=10, infused with normal saline and orally administered normal saline); Ang II group (n=10, infused with Ang II and orally administered normal saline); Ang II + sinigrin (10 mg/kg) group (n=10, infused with Ang II and orally administered 10 mg/kg sinigrin); Ang II + sinigrin (20 mg/kg) group (n=10, infused with Ang II and orally administered 20 mg/kg sinigrin); Ang II + sinigrin (40 mg/kg) group (n=10, infused with Ang II and orally administered 40 mg/kg sinigrin). At the end of the treatment period, the rats were housed in individual metabolic cages for 24 h, and provided with water and food *ad libitum*, and urine samples were obtained from the rats. Prior to sacrifice by cervical dislocation, the rats were anesthetized by pentobarbital sodium (50 mg/kg i.p.) and blood samples (5 ml) were harvested from the abdominal aorta for one time. Subsequently, the kidneys were excised and collected; one part was placed in 10% formalin and embedded in paraffin for histopathological analysis, and the other was snap-frozen in liquid nitrogen for protein evaluation. All procedures and experimental protocols were approved by the Animal Ethics Committee of the Third Affiliated Hospital of Shandong First Medical University (no. LL202001003).

Measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP). SBP and DBP were recorded and analyzed using the CODA Monitor (Kent Scientific) with a tail-cuff non-invasive method according to the instructions of the manufacturer. Prior to the measurement, the rats were placed in the holder for 5 min, and the SBP and DBP were recorded three times for each rat at 0, 7, 14, 21 and 28 days of Ang II infusion.

Analysis of kidney damage markers. The levels of serum creatinine (SCR) and blood urea nitrogen (BUN) were measured using an automatic biochemical analyzer (Beckman Coulter, Inc.) as described in a previous study (16). The levels of urinary protein were examined using a BCA Protein Assay kit (#P0010, Beyotime Institute of Biotechnology).

Histological analysis of kidney tissues. For the histological analysis of kidney tissues, the kidneys were perfused with physiological salt solution, treated with formalin (10%) and longitudinally sectioned, followed by fixation in formalin (10%) overnight. Subsequently, the kidney tissues were paraffin-embedded and sectioned as previously described (17). Glomerular basement membrane thickness was analyzed by periodic acid-Schiff (PAS) staining (Beyotime Institute of Biotechnology). Briefly, the sections were treated with periodic acid at room temperature for 5 min, and then incubated with Schiff at room temperature for 15 min.

Cells and cell culture. Human HK-2 proximal tubule epithelial cell lines were maintained at the Central Laboratory of the Third Affiliated Hospital of Shandong First Medical University and incubated at 37˚C with 5% CO_2 in Dulbecco's modified Eagle medium (Cytiva) containing fetal bovine serum (15%, Gibco; Thermo Fisher Scientific, Inc.), streptomycin (0.1 mg/ml; Beijing Solarbio Science & Technology Co., Ltd.) and penicillin (100 units/ml; Beijing Solarbio Science & Technology Co., Ltd.). To induce HK-2 cell injury, the cells were exposed to Ang II (Sigma-Aldrich; Merck KGaA) at a concentration of 1 μM. Subsequently, the cells were treated with sinigrin at1, 10 and 100 μg/ml.

MTT assays. The viability of the HK-2 cells was evaluated by MTT assays. In brief, ~$2x10^4$ cells were plated in 96-well plates and incubated for 12 h at room temperature. Following the indicated treatments, the cells were mixed with MTT solution (5 mg/ml, 10 μl) and incubated for 4 h at room temperature. The medium was then removed, and DMSO (150 μl) was added to the cells. Cell viability was determined by measuring the absorbance at 570 nm using an ELISA browser (Bio-Tek EL 800; BioTek Instruments, Inc.).

Analysis of cell apoptosis. HK-2 cells (~$2x10^5$) were plated in 6-well plates. Cell apoptosis was assessed using the Annexin V-FITC Apoptosis Detection kit (#6592, Cell Signaling Technology, Inc.) according to the manufacturer's instructions. In brief, ~$2x10^6$ cells collected and washed cells were resuspended in binding buffer, dyed with propidium iodide at 25˚C, and subjected to flow cytometric analysis (FACSCalibur; BD Biosciences).

Analysis of oxidative stress-related enzymes. Oxidative stress-related enzymes, including superoxide dismutase

(SOD), malondialdehyde (MDA) and catalase (CAT), were analyzed *in vivo* and *in vitro*. The activity of SOD was measured using a SOD assay kit (#706003; Cayman Chemical Company). The activity of SOD was analyzed using a microplate reader (BioTek Instruments, Inc.) at 450 nm. The activity of MDA was measured using a MDA assay kit (#700870; Cayman Chemical Company). The activity of MDA was analyzed using a microplate reader (BioTek Instruments, Inc.) at 405±414 nm. The activity of CAT was measured using a CAT assay kit (#707002, Cayman Chemical Company). The activity of CAT was analyzed using a microplate reader (BioTek Instruments, Inc.) at 340 nm.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). First-strand cDNA was manufactured using the PrimeScript™ II 1st Strand cDNA Synthesis kit (#6210A; Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. The qPCR assays were performed using Premix Ex Taq SYBR-Green (#RR820A; Takara Biotechnology Co., Ltd.). The primer sequences were as follows: TNF-α forward, 5'-CCCAGG CAGTCAGATCATCTTC-3' and reverse, 5'-GCTTGA GGGTTTGCTACAACATG-3'; IL-6 forward, 5'-TCAGGA AATTTGCCTATTGAAAATTT-3' and reverse, 5'-GCT TTGTCTTTCTTGTTATCTTTTAAGTTGT-3'; monocyte chemoattractant protein-1 (MCP-1) forward, 5'-CATAGC AGCCACCTTCATTCC-3' and reverse, 5'-TCTCCTTGG CCACAATGGTC-3'; GAPDH forward, 5'-AACGGATTT GGTCGTATTGGG-3' and reverse, 5'-CCTGGAAGA TGGTGATGGGAT-3'. The RT-qPCR reaction conditions were as follows: 95˚C, 10 sec (denaturation); 55˚C, 30 sec (annealing); 72˚C, 30 sec (extension) for 40 cycles. The relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (18).

Western blot analysis. Total protein was isolated from the cells using RIPA buffer (Cell Signaling Technology, Inc.) and quantified using the BCA Protein Quantification kit (Abbkine Scientific Co., Ltd.). Protein samples were subjected to 10% SDS-PAGE and transferred to PVDF membranes (EMD Millipore), followed by blocking with 5% skimmed milk at room temperature and incubating with primary antibodies at 4˚C overnight. The horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5,000, cat. no. BA1039; Wuhan Boster Biological Technology, Ltd.) were used to incubate the membranes for 1 h at room temperature, followed by visualization by using a chemiluminescence detection kit (Beyotime Institute of Biotechnology, Inc.). The primary antibodies used in the present study were the following: Collagen I (#ab260043, 1:500; Abcam), collagen IV (1:500, cat. no. ab236640; Abcam), fibronectin (1:1,000, cat. no. ab268020; Abcam), ERK (1:500, cat. no. 4695; Cell Signaling Technology, Inc.), p65 (1:1,000, cat. no. 8242; Cell Signaling Technology, Inc.), IκB α (1:500, cat. no. 8412; Cell Signaling Technology, Inc.), p-ERK (1:300, cat. no. 4370; Cell Signaling Technology, Inc.), p-p65 (1:500, cat. no. 3033; Cell Signaling Technology, Inc.), p-IκBα (1:300, cat. no. 2859; Cell Signaling Technology, Inc.) and GAPDH (1:2,000, cat. no. ab181602; Abcam). The density of the protein bands was quantified using ImageJ software (version V1.8.0; National Institutes of Health).

Statistical analysis. Data are expressed as the mean ± SD, and statistical analysis was conducted using GraphPad Prism 7 (GraphPad Software, Inc.). One-way ANOVA followed by Tukey's post hoc test was used to assess the differences between the groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sinigrin alleviates Ang II-induced kidney dysfunction in vivo. To investigate the role of sinigrin in the modulation of hypertension-induced kidney damage, a spontaneously hypertensive rat model was constructed using Ang II. It was observed that SBP and DBP were increased in the Ang II-challenged rats, and sinigrin treatment attenuated this increase in a dose-dependent manner (Fig. 1A and B). The levels of BUN and SCR were enhanced by Ang II in the rats, and sinigrin reversed this effect in a dose-dependent manner (Fig. 1C and D). Moreover, the Ang II-induced increase in urinary protein levels was inhibited by sinigrin treatment in the rats (Fig. 1E). These results suggest that sinigrin may alleviate Ang II-induced kidney dysfunction *in vivo*.

Sinigrin attenuates Ang II-induced kidney injury in vivo. The present study then explored the influence of sinigrin on Ang II-induced kidney injury in a rat model. The results revealed that renal glomerular basement membrane (GBM) thickness was increased in the Ang II-challenged rats, which was reduced by treatment with sinigrin in a dose-dependent manner (Fig. 2A). Moreover, the expression of fibronectin, collagen IV and I was increased by Ang II in the rats, and sinigrin reversed this effect in a dose-dependent manner (Fig. 2B-E). These results indicate that sinigrin may alleviate Ang II-induced kidney injury *in vivo*.

Sinigrin reduces Ang II-induced inflammation and oxidative stress in vivo. Given that oxidative stress and inflammatory processes are involved in hypertension-related kidney damage, the present study further explored the effects of sinigrin on associated markers in the rat model. It was found that the expression of TNF-α, IL-6 and MCP-1 was upregulated by Ang II and treatment with sinigrin attenuated this upregulation in the rats (Fig. 3A-D). Moreover, the levels of SOD and CAT were decreased, whereas the levels of MDA were increased in the Ang II-challenged rats; these effects were reversed by treatment with sinigrin (Fig. 3E-G). Taken together, the results suggest that sinigrin may reduce Ang II-induced inflammation and oxidative stress *in vivo*.

Sinigrin inhibits ERK and NF-κB signaling in the Ang II-induced spontaneously hypertensive rat model. The present study then investigated the underlying mechanisms of sinigrin-mediated hypertensive kidney

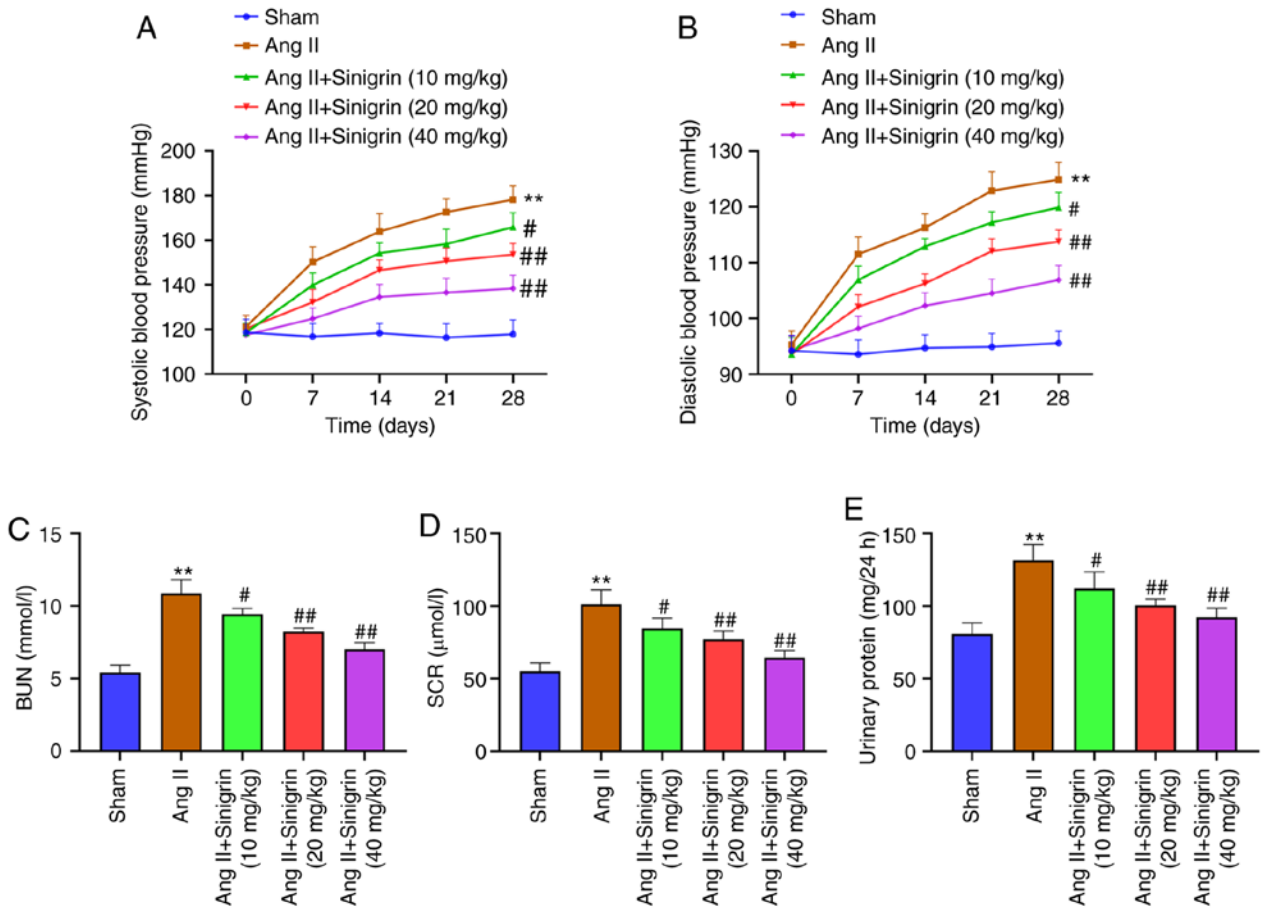

Figure 1. Sinigrin alleviates Ang II-induced kidney dysfunction *in vivo*. (A-E) The hypertensive rat model was constructed by challenge with Ang II and the rats were then treated with sinigrin at the indicated doses. (A and B) SBP and DBP were recorded and analyzed. (C and D) BUN and SCR were tested by automatic biochemical analyzer. (E) The levels of urinary protein were measured using a urinary protein measurement kit. **P<0.01 vs. sham group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; serum creatinine.

damage. Notably, the phosphorylation of ERK, p65 and IκBα was stimulated in the Ang II-challenged rats; however, this was reduced by sinigrin treatment (Fig. 4), indicating that sinigrin may attenuate hypertension-induced kidney damage by inactivating ERK and NF-κB signaling *in vivo*.

Sinigrin reduces Ang II-induced HK-2 cell injury in vitro. Ang II is a vasoconstrictive peptide that modulates blood pressure homeostasis and can cause hypertensive renal inflammation in renal tubular epithelial cells (19,20). Therefore, in the present study, the Ang II-induced injury model using HK-2 cells, a human renal tubular epithelial cell line, was used to determine the function of sinigrin *in vitro*. HK-2 cells were treated with sinigrin at the indicated concentrations, which did not exhibit cytotoxicity to the cells (Fig. 5A). The viability of the Ang II-exposed HK-2 cells was reduced however, and this was attenuated by by sinigrin treatment in a concentration-dependent manner (Fig. 5B). Sinigrin also reversed the Ang II-induced apoptosis of HK-2 cells in a concentration-dependent manner (Fig. 5C). In addition, the levels of SOD and CAT were reduced, whereas the levels of MDA were enhanced the Ang II-exposed HK-2 cells; these effects reversed by treatment with sinigrin (Fig. 5D-F). Taken together, these results suggest that sinigrin attenuates Ang II-induced HK-2 cell injury *in vitro*.

Sinigrin alleviates Ang II-induced inflammation and ECM degradation in HK-2 cells. It was demonstrated that the expression of TNF-α, IL-6 and MCP-1 was upregulated by Ang II in the HK-2 cells, and treatment with sinigrin abrogated this effect (Fig. 6A-C). Furthermore, the expression of fibronectin, collagen IV and I was elevated by Ang II in the cells, and this increase was reversed by sinigrin treatment in a concentration-dependent manner (Fig. 6D-G). These results indicate that sinigrin may alleviate Ang II-induced inflammation and ECM degradation in HK-2 cells.

Sinigrin inactivates ERK and NF-κB signaling in Ang II-exposed HK-2 cells. The results revealed that the phosphorylation of ERK, p65 and IκBα was enhanced in Ang II-exposed HK-2 cells, and sinigrin treatment reduced this effect in a concentration-dependent manner (Fig. 7). Therefore, sinigrin may attenuate hypertension-induced kidney damage by inactivating ERK and NF-κB signaling *in vitro*.

Discussion

Kidney damage is a prevailing complication of hypertension, which induces severe inflammatory response, oxidative stress and ECM degradation (21). Sinigrin is a natural compound

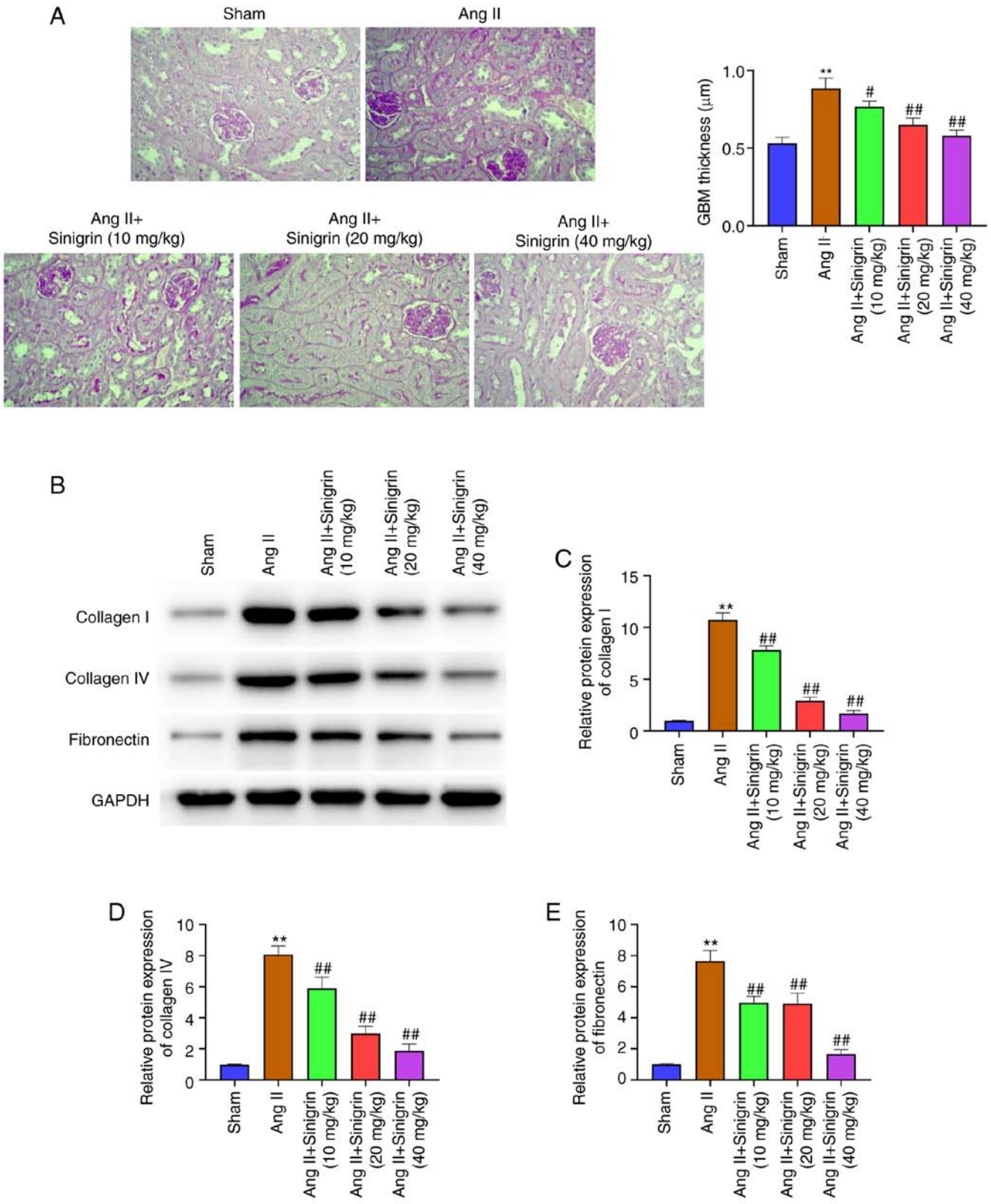

Figure 2. Sinigrin attenuates Ang II-induced kidney injury *in vivo*. (A-E) The hypertensive rat model was constructed by challenge with Ang II and the rats were then treated with sinigrin at the indicated doses. (A) GBM thickness was analyzed by periodic acid-Schiff (PAS) staining. (B-E) The expression of collagen I, collagen IV, fibronectin and GAPDH was examined by western blot analysis and the results were quantified using ImageJ software. **P<0.01 vs. sham group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II; GBM, glomerular basement membrane.

extracted from cruciferous vegetables, which has anti-inflammatory and antioxidant activities (15,22). Nevertheless, the effects of sinigrin on hypertension-induced kidney damage remain elusive. In the present study, it was found that sinigrin attenuated Ang II-induced renal injury by inactivating ERK and NF-κB signaling *in vivo* and *in vitro*.

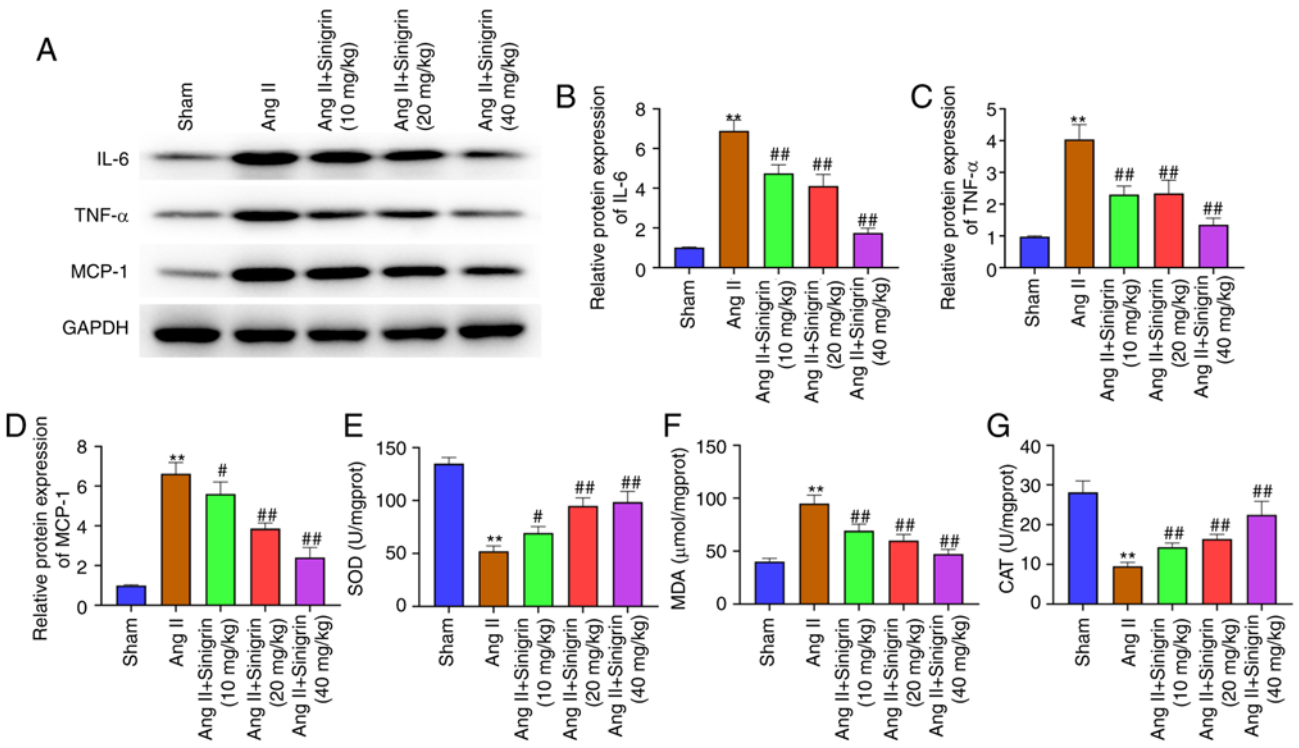

Figure 3. Sinigrin attenuates Ang II-induced inflammation and oxidative stress *in vivo*. (A-G) The hypertensive rat model was constructed by challenge with Ang II and the rats were then treated with sinigrin at the indicated doses. (A-D) The expression of TNF-α, IL-6, MCP-1, and GAPDH was measured by western blot analysis and the results were quantified using ImageJ software. (E-G) The levels of MDA, SOD, and CAT were determined in the rats. **P<0.01 vs. sham group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

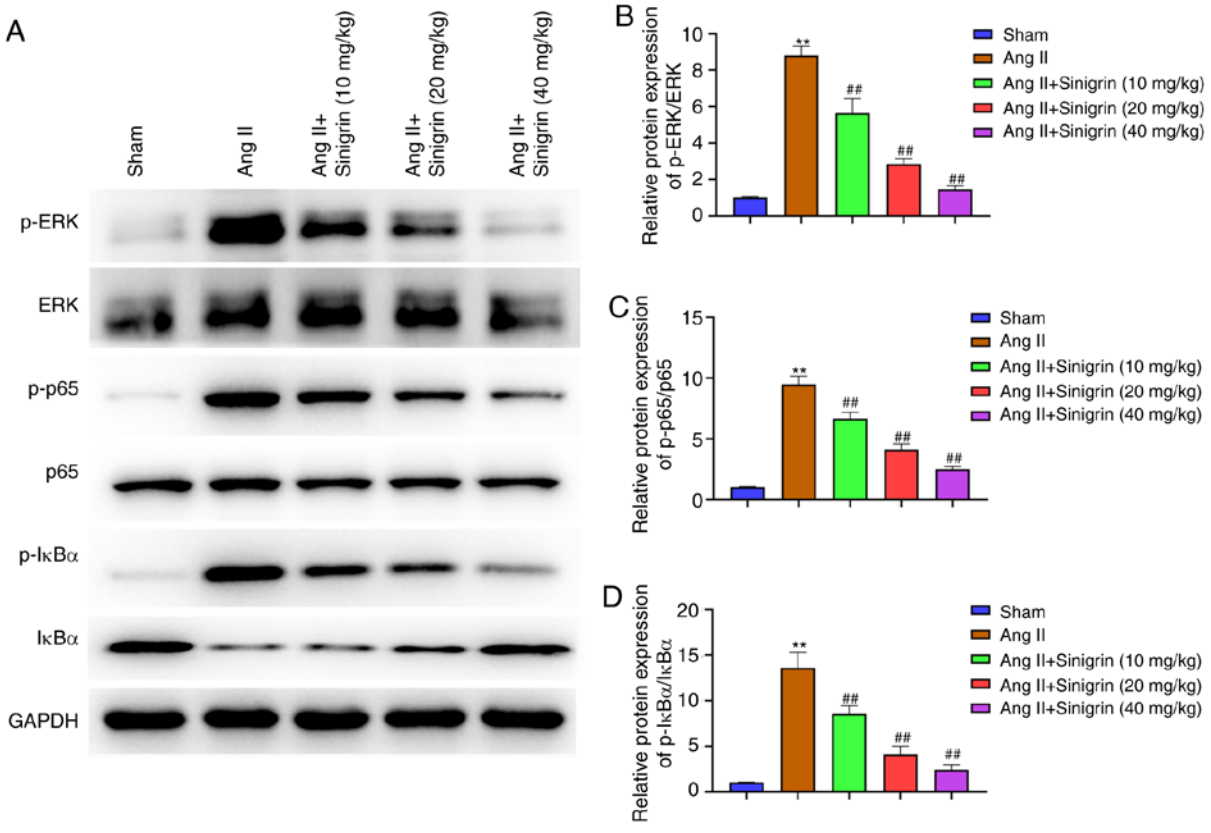

Figure 4. Sinigrin inhibits ERK and NF-κB signaling in the Ang II-induced spontaneously hypertensive rat model. (A-D) The hypertensive rat model was constructed by Ang II and treated with sinigrin as the indicated doses. (A-D) The phosphorylation of ERK, p65, IκBα, and the expression levels of ERK, p65, IκBα, and GAPDH were determined by western blot analysis and the results were quantified using ImageJ software. **P<0.01 vs. sham group; $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II.

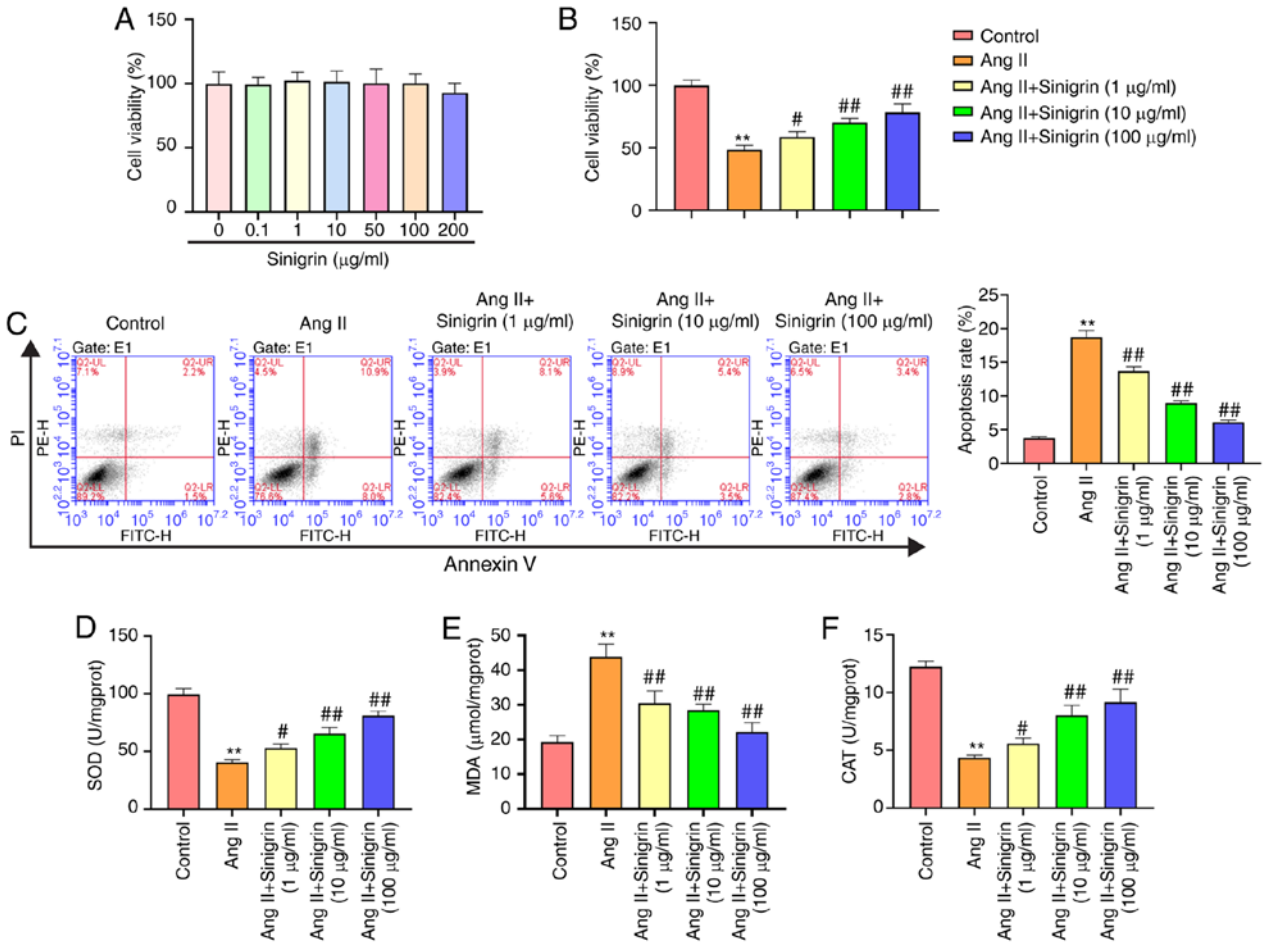

Figure 5. Sinigrin reduces Ang II-induced HK-2 cell injury *in vitro*. (A) HK-2 cells were treated with sinigrin at the indicated concentrations. (B-F) The Ang II-treated HK-2 cells were treated with sinigrin at the indicated concentrations. (B) The viability of the cells was analyzed by MTT assays. (C) The apoptosis of the cells was examined by flow cytometric analysis. (D-F) The levels of MDA, SOD and CAT were determined in the cells. **P<0.01 vs. control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

With the emergence of natural compounds in plants for the treatment of various disorders, several natural compounds have been identified to regulate hypertension-induced kidney damage. Brazilian red propolis has been reported to alleviate hypertension-induced kidney damage (23). Boldine can inhibit hypertension-related renal injury by repressing TGF-β expression (24). Moreover, previous studies have demonstrated that sinigrin can inhibit inflammatory responses. Sinigrin has been reported to exhibit anti-inflammatory and neuroprotective activities by inhibiting NF-κB/TNF-α signaling (25). Sinigrin can suppress inflammatory mediator production by reducing NF-κB/MAPK signaling or NLRP3 inflammatory activation in macrophages (15). Sinigrin has also been demonstrated to exhibit antioxidant activity in previous studies (22,26). In addition, it has been found that sinigrin can regulate cell viability and apoptosis under pathological conditions (27). In the present study, it was demonstrated that sinigrin was able to alleviate Ang II-induced kidney dysfunction, kidney injury, inflammation and oxidative stress *in vivo* and *in vitro*. The findings demonstrated the critical role of sinigrin in the inhibition of hypertension-related renal damage, and shed light on a novel effect of sinigrin on Ang II-induced renal injury.

ERK and NF-κB signaling contributes to the progression of hypertension-induced kidney damage, and the inhibition of ERK and NF-κB signaling can attenuate the adverse effects of hypertension-related renal injury. It has been reported that quercetin can ameliorate sodium fluoride-induced hypertension by reducing oxidative stress through ERK/PPARγ signaling modulation (28). Angiotensin has been found to attenuate hypertension-related renal fibrosis by inhibiting mTOR/ERK signaling in an apolipoprotein E-deficient mouse model (29). Nebivolol can inhibit profibrotic and pro-oxidant responses during the vascular remodeling of renovascular hypertension by regulating ERK signaling (30). Exercise training can relieve hypertension by targeting NF-κB signaling in the hypothalamic paraventricular nucleus (31). Rutin has been reported to ameliorate sodium fluoride-induced hypertension by regulating NF-κB/Nrf2 signaling in rats (32). The present study revealed that the phosphorylation of ERK, p65 and IκBα was stimulated by Ang II, and sinigrin treatment reduced this effect in a concentration-dependent manner *in vivo* and *in vitro*. This finding suggests that sinigrin may inactivate ERK and NF-κB signaling.

In conclusion, the present study demonstrated that sinigrin alleviated Ang II-induced renal injury by inactivating ERK and NF-κB signaling. Sinigrin may thus be a potential candidate for the treatment of hypertension-induced kidney damage.

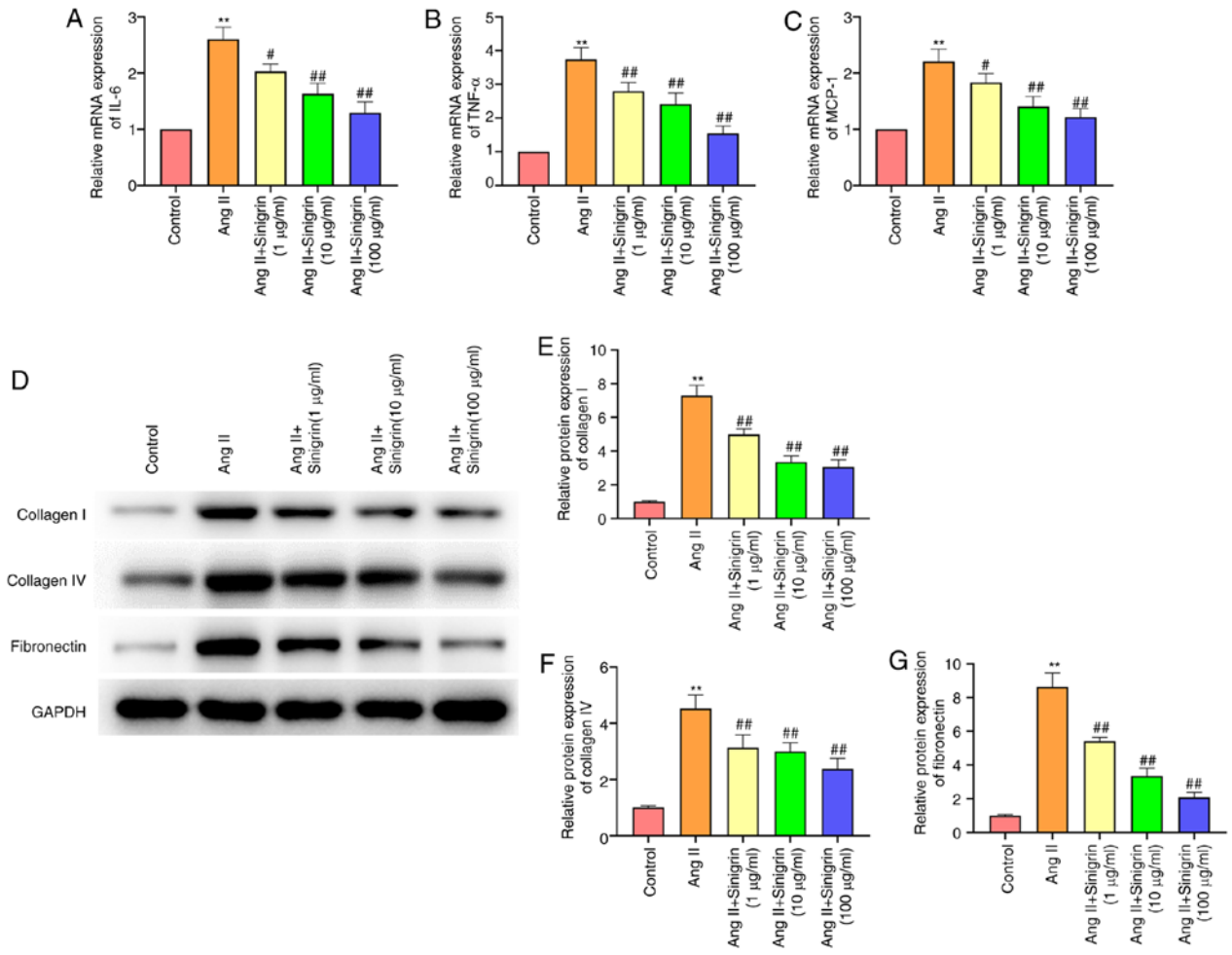

Figure 6. Sinigrin relieves Ang II-induced inflammation and extracellular matrix (ECM) degradation in HK-2 cells. (A-G) Ang II-treated HK-2 cells were treated with sinigrin at the indicated concentrations. (A-C) The expression of TNF-α, IL-6, MCP-1 and GAPDH was measured by RT-qPCR in the cells. (D-G) The expression of collagen I, collagen IV, fibronectin and GAPDH was examined by western blot analysis and the results were quantified using ImageJ software. **P<0.01 vs. control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II; TNF-α, tumor necrosis factor α; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1.

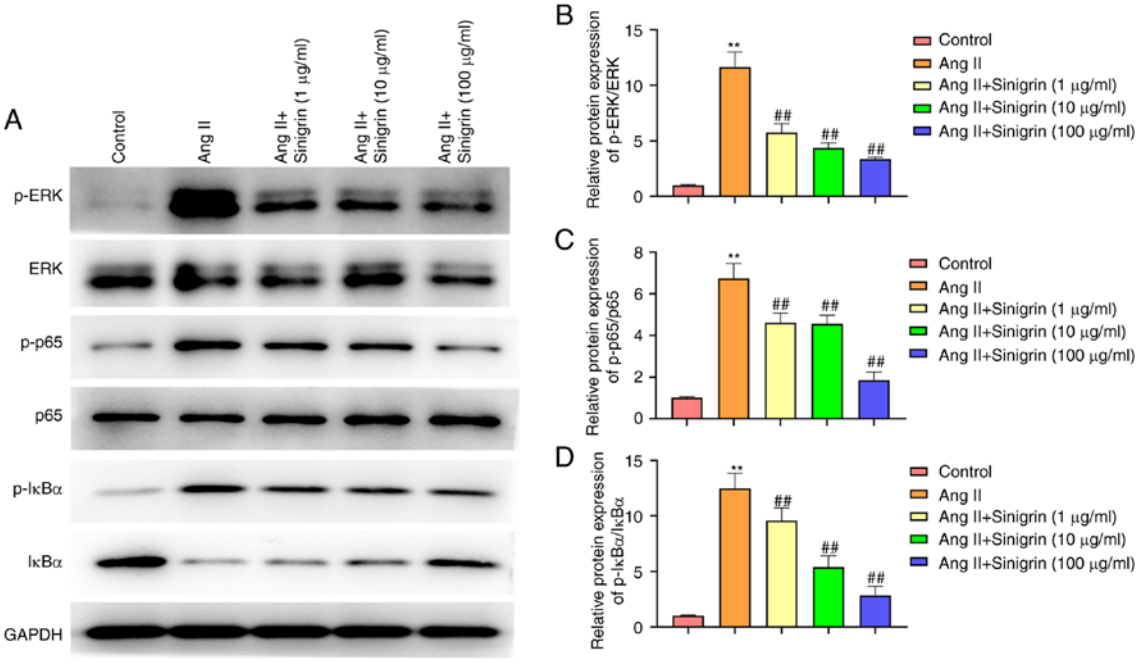

Figure 7. Sinigrin inactivates ERK and NF-κB signaling in Ang II-treated HK-2 cells. (A-D) Ang II-treated HK-2 cells were treated with sinigrin at the indicated concentrations. The phosphorylation of ERK, p65, IκBα, and the expression levels of ERK, p65, IκBα, and GAPDH were examined by western blot analysis and the results were quantified using ImageJ software. **P<0.01 vs. control group; $^{\#\#}$P<0.01 vs. Ang II group. Ang II, angiotensin II.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CC made substantial contributions to conception and design, and revised the manuscript. XY, YH and LT performed the research. WC and YW analyzed the data. LT wrote and revised the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The experimental protocol of the present study was performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by The Third Affiliated Hospital of Shandong First Medical University (Affiliated Hospital of Shandong Academy of Medical Sciences) (no. LL202001003).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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