Article

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Baicalin Reduces Renal Inflammation in Mesangial Proliferative Glomerulonephritis through Activation of Nrf2/ARE and PI3K/AKT Pathways

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Objective: Mesangial proliferative glomerulonephritis (MPGN) is a prevalent form of primary glomerulonephritis, distinguished by the proliferation of mesangial cells and the accompanying inflammatory response. Baicalin, the active ingredient in the Scutellaria baicalensis Georgi plant, has been observed to have a protective effect on the kidneys. However, its specific impact on MPGN has yet to be studied widely. Hence, this study aimed to investigate the effect on MPGN and the underlying mechanisms of Baicalin.

Methods: Thirty-six Sprague-Dawley (SD) rats, aged 6 to 8 weeks, were randomly allocated into different subgroups: control, model, benazepril, and three baicalin subgroups (low, medium, and high dose), each consisting of six rats. The concentrations of 24-hour urinary protein, blood urea nitrogen (BUN), serum creatinine (SCr), triglycerides (TG), total cholesterol (TC), interleukins (IL-1α, IL-2, IL-10), and interferon-γ (IFN-γ) were measured with biochemistry. The pathological alterations in the renal tissue were examined using Hematoxylin and Eosin (HE) along with Periodic Acid-Schiff (PAS) staining. Concurrently, the extent of apoptosis was evaluated using TdT-mediated dUTP nick end labeling (TUNEL) staining. *In vitro*, mesangial cells were exposed to 30 μg/mL lipopolysaccharide for 24 h, with or without varying concentrations of baicalin (10, 20, 40 μM). MTT assay was applied to estimate cell activity, flow cytometry to evaluate the cell cycle, and 5-ethynyl-2-deoxyuridine (EdU) detection to measure cell proliferation. IL-1α, IL-2, IL-10, and IFN-γ concentrations in the cell supernatant were assayed with biochemistry. Furthermore, the expression of apoptosis-related proteins, concluding BCL2-Associated X (Bax), Bcl-2, NOD-like receptor thermal protein domain associated protein 3 (NLRP3), and caspase-1, NF-E2-related factor 2/antioxidant response element (Nrf2/ARE) pathway-related proteins (Nrf2 and HO-1), and phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) pathway-related proteins (p-PI3K, PI3K, p-AKT, and AKT) in both the renal tissue and cell supernatant were measured.

Results: Baicalin treatment significantly reduced the 24-hour urinary protein, serum levels of BUN, SCr, TG, TC, IL-1α, IL-2, IL-10, and IFN-γ *in vivo* experiments. Baicalin treatment also improved the pathological condition of renal tissue and decreased the occurrence of apoptosis. *In vitro*, findings confirmed that baicalin inhabits the proliferation of mesangial cells triggered by Lipopolysaccharide (LPS), induces a G1 phase cell cycle arrest, and reduces the concentrations of IL-1α, IL-2, IL-10, and IFN-γ. Baicalin also decreased the ratios of p-PI3K/PI3K and p-AKT/AKT while enhancing the levels of Nrf2 and HO-1 in both renal tissue and cell supernatant.

Conclusions: Baicalin can mitigate MPGN by impeding the proliferation and inflammation of mesangial cells by activating Nrf2/ARE and PI3K/AKT pathways.

Keywords: baicalin; mesangial proliferative glomerulonephritis; Inflammatory response; Nrf2/ARE pathway; PI3K/AKT pathway

Introduction

Mesangial proliferative glomerulonephritis (MPGN) is a glomerular disease primarily characterized by diffused Mesangial cell proliferation and extracellular matrix deposition [1]. These abnormally proliferated Mesangial cells release inflammatory mediators, typically resulting in interstitial fibrosis and glomerular sclerosis. These conditions often progress to end-stage renal disease [2,3]. Despite studies identifying MPGN as a leading cause of end-stage renal disease, its exact pathogenesis has not been fully elucidated [4]. Currently, there is no single effective drug treatment for MPGN. A multi-faceted approach is required, incorporating corticosteroids, immunosuppressants, antiplatelet, and antilipidemic drugs. Interventions to inhibit inflammation, Mesangial cell proliferation, and extracellular matrix deposition are considered primary strategies to delay the progression of glomerulonephritis [5–7].

Baicalin, a flavonoid extracted from Scutellaria baicalensis Georgi, boasts potent biological activities, including anti-inflammatory, antioxidant, and anti-tumor effects [8–10]. Observations have been made regarding its

impact on renal function in patients with diabetic nephropathy. These studies suggest that baicalin can potentially improve renal function and slow the progression of diabetic nephropathy, primarily through anti-inflammatory, antioxidative mechanisms [11]. In a study involving rats with renal ischemia-reperfusion injury, baicalin mitigated renal injury and enhanced renal function by inhibiting pro-inflammatory response and preventing mitochondrial-mediated apoptosis [12]. Yet, the number of studies examining the effects of baicalin on MPGN still needs to be increased.

In animal models, evidence has shown the protective effects of natural nuclear factor-erythroid-2-related factor (Nrf2) activators on oxidative stress, inflammation, and kidney disease. The absence of Nrf2 has been observed to intensify these pathways, resulting in autoimmune glomerulonephritis [13]. The activation of the Nrf2/antioxidant response element (ARE) pathway promotes the transcription of downstream antioxidant genes and curbs the upregulation of inflammatory fibrosis factors. This effectively delays the progression of diabetic renal fibrosis [14]. In parallel, phosphoinositide-3-kinase (PI3K), an important regulator of various signal transduction pathways, is believed to play a role in cellular inflammation and oxidative stress [15,16]. Li *et al.* [17] established a connection between the PI3K/AKT/mTOR signal pathway and mesangial cell apoptosis and proliferation, suggesting its involvement in the MPGN process. Therefore, for Nrf2/ARE pathway and PI3K/AKT pathway, we explored the potential mechanism of baicalin in treating MPGN.

In this study, we examined the MPGN rats induced by anti Thy1.1 monoclonal antibody and glomerular mesangial cells treated with Lipopolysaccharide (LPS) to probe the potential impact of baicalin on MPGN.

Materials and Methods

Animal

We obtained thirty-six healthy, specific-pathogen-free (SPF) adult male Sprague-Dawley (SD) rats, weighing between 200 g and 220 g and aged 6 to 8 weeks, from Beijing Baosco Biomedical Technology Co., Ltd.

Construction and Grouping of MPGN Rat Model

SD rats were randomly assigned into six subgroups: control, model, benazepril, and low, medium, and high-dose baicalin groups (n = 6/subgroup). All subgroups, except for the control, were used to establish the MPGN rat model by injecting 2.5 mg/kg of anti-Thy1.1 monoclonal antibody (batch number: 471585, Beyotime, Shanghai, China) into the tail vein, as per the established methodology [18]. After establishing the MPGN model, the baicalin groups received daily oral doses of baicalin (50 mg/kg, 75 mg/kg, and 100 mg/kg, batch number: 572667, Sigma-Aldrich, purity 95%, St. Louis, MO, USA). The benazepril group was administered a daily oral dose of 50 mg/kg benazepril (batch number: 20186365, Beijing Novartis Pharmaceutical Co., Ltd., Beijing, China). The rats in the control and model subgroups received an equivalent volume of normal saline daily for 4 weeks. During this period, the rats' 24-h urine was collected, and blood samples were drawn from the tail vein and stored post-centrifugation. Following the last dose administration, the rats were anesthetized using 3% pentobarbital sodium (batch number: P3761, 30 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) to allow for kidney collection. The rats were then euthanized using 150~200 mg/kg of pentobarbital sodium.

Twenty-Four Hour Urinary Protein and Biochemical Analysis

Bradford Protein Assay Kit (batch number: PC0010, Solarbio, Beijing, China) was applied to determine urinary protein concentration. The levels of blood urea nitrogen (BUN), serum creatinine (SCr), triglycerides (TG), and total cholesterol (TC) in serum were measured, using a BUN assay kit (batch number: MAK006, Sigma-Aldrich, St. Louis, MO, USA), a SCr assay kit (batch number: MAK080, Sigma-Aldrich, St. Louis, MO, USA), a TG assay kit (batch number: F001-1-1, Shanghai Koaibo Biology, China), and a TC assay kit (batch number: A111-1-1, Shanghai Koaibo Biology, China) respectively. Additionally, we used assay kits for Interleukin (IL)-1α (batch number: H001), IL-2 (batch number: H003), IL-10 (batch number: H009-1-2), and interferon-γ (IFN-γ, batch number: H025), all procured from Shanghai Koaibo Biology, China.

Hematoxylin and Eosin (HE) and Periodic Acid-Schiff (PAS) Staining of Kidney Tissue

Renal tissue sections of 4 μm thickness were prepared using standard procedures and dewaxed until water-clear. They were then stained either with hematoxylin-eosin (batch number: C0105S, Biyuntian, China) or periodic acid-Schiff (batch number: G1281, PAS, Biyuntian, China). The pathological changes of the kidney were observed under a microscope (CX31, batch number: KB0801003, Olympus, Tokyo, Japan).

TdT-Mediated dUTP Nick End Labeling (TUNEL) Staining

Dyeing was performed following the TUNEL kit (batch number: S7101, Sigma-Aldrich, St. Louis, MO, USA) instructions, it was observed under a microscope (batch number: DM1000, Leica, Wetzlar, Germany), and images were captured.

Cell Culture and Grouping

Human mesangial cells at the fifth passage were purchased from the Procell Life Science & Technology Co., Ltd. (Wuhan, China). Upon receipt, we confirmed the

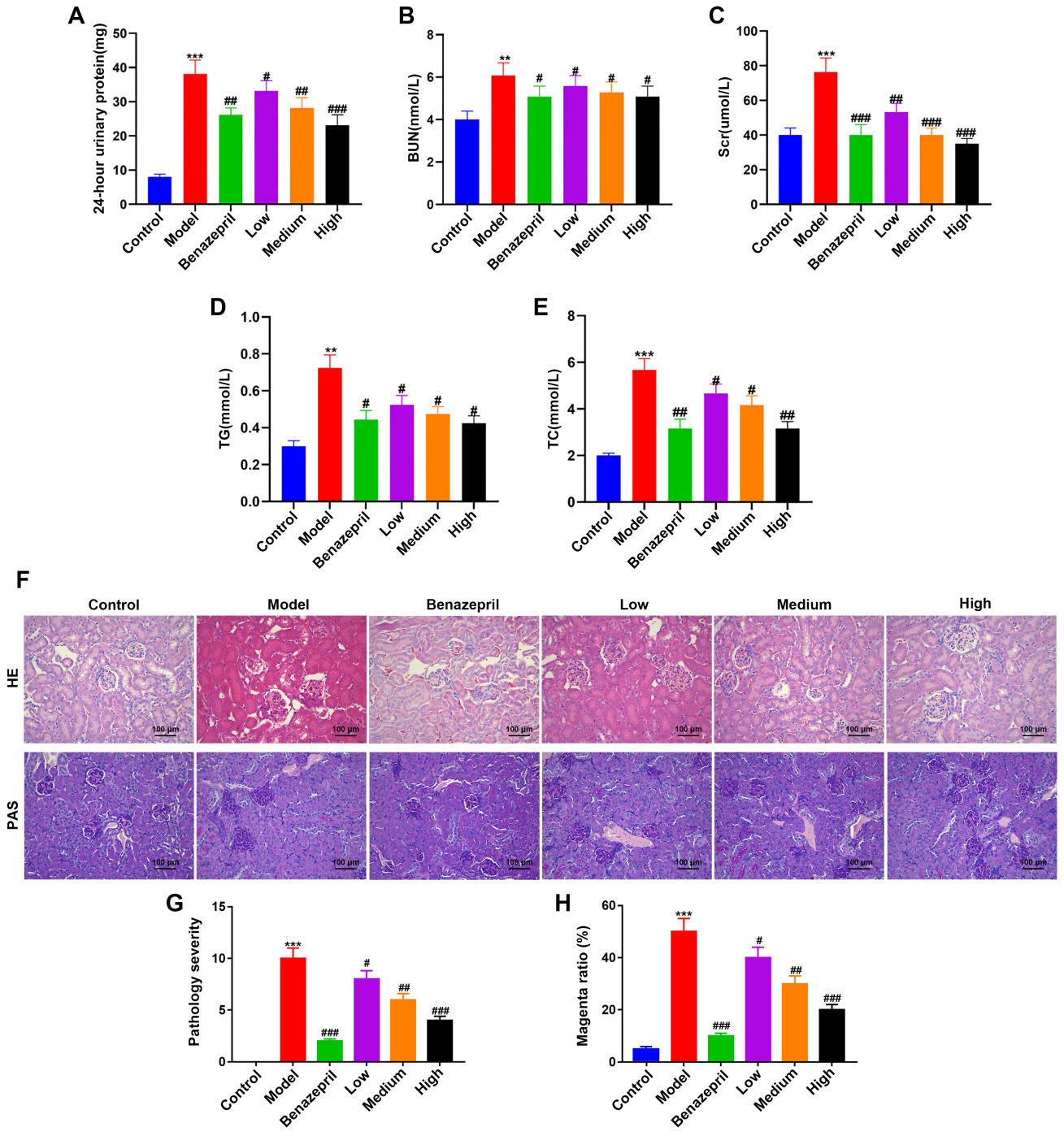

Fig. 1. Baicalin ameliorated renal pathological injury in MPGN model rats. (A) 24 h urinary protein content (n = 6). (B–E) Serum concentrations of BUN, SCr, TG, and TC (n = 6). (F–H) Renal tissue sections were obtained and stained with HE and PAS (n = 6). The images of glomeruli and tubules were obtained under an optical microscope (200 × magnification) (scale = 100 μm). $^{**}p < 0.01$, $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus model subgroup.

cell line's origin and checked for misidentification using an approved DNA-based method. We cross-referenced the cell line name with the database of misidentified Cell Lines maintained by the International Committee for Cell Line Accreditation (ICLAC). The genetic characteristics of cell lines were established using short tandem repeat (STR) profiling, which also confirmed the absence of cross-contamination. Furthermore, the mycoplasma tests were conducted to ensure the cells were free from contamination. The cells were cultured in 10% fetal bovine serum-Dulbecco's modification of Eagle's medium Dulbecco (FBS-DMEM) at 37 °C with a 5% CO_2 environment. Cells were treated with 30 μg/mL LPS (batch number: L5293, Sigma-Aldrich, St. Louis, MO, USA) for 24

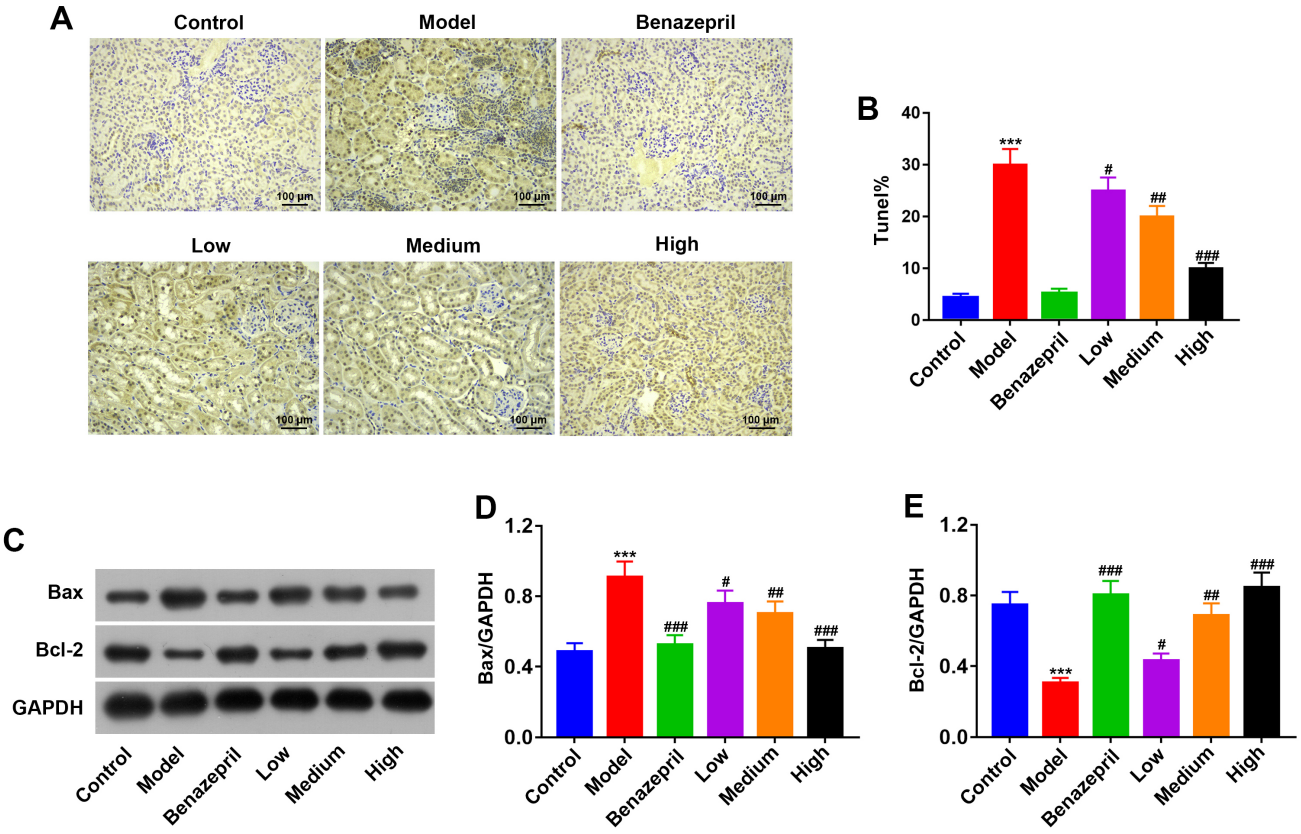

Fig. 2. Baicalin ameliorated renal apoptosis in MPGN model rats. (A,B) Renal apoptosis was detected by TUNEL (n = 6). The brown granules in the nucleus were apoptosis-positive cells (400 × magnification, scale = 100 μm). (C–E) Detection of apoptosis-related proteins Bax and Bcl-2 by Western blot (n = 6). $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus model subgroup.

h with or without baicalin, at concentrations of 10, 20, and 40 μM. The LPS dose for the *in vitro* experiment was determined based on a previous study [2].

MTT Detection

The cells in logarithmic phase were collected and the concentration of the cell suspension was adjusted. The cells were inoculated into 96-well plates at a density of 5×10^3 cells per well, allocating 180 μL to each well. We set up three replicate wells for each subgroup. The cells were cultured overnight in a 37 °C, 5% CO_2 incubator to facilitate cell adhesion to the well walls. Following this, the culture plate was removed and added 20 μL MTT (5 mg/mL, batch number: C1736, Bioswamp, Wuhan, China) to each well, continuing the culture for an additional 4 hours. After removing the culture medium, 150 μL dimethyl sulfoxide (DMSO) (batch number: D2650, Sigma-Aldrich, St. Louis, MO, USA) was added to each well, shaking at a low speed for 10 minutes. Lastly, the optical density (OD) value was measured at a wavelength of 490 nm, using an enzyme labeling instrument (batch number: AMR-100, Allsheng, Hangzhou, China).

Flow Cytometry Detection

The cells of each subgroup were collected and centrifuged for 5 min. The supernatant was discarded, washed twice with pre-cooled phosphate belanced solution (PBS) containing 75% ethanol, and fixed in the refrigerator at –20 °C for more than 24 h. After the sample was centrifuged at 700 ×g for 5 minutes, the supernatant was discarded. The remaining material was washed twice with pre-chilled PBS. 100 uL RNase A solution at a concentration of 1 mg/mL were added, followed by 400 uL Propidium Iodide (PI) solution with a concentration of 50 μg/mL (Solarbio, batch number: C0080, Beijing, China). It was incubated at 4 °C for 10 min without light. We utilized flow cytometry (BD Accuri C6 Plus, batch number: C6 Plus, San Jose, CA, USA) to assess the cell cycle stages.

5-ethynyl-2-deoxyuridine (EdU)

About 6×10^3 cells in each subgroup were collected and inoculated into a 96-well plate. Each well was treated with 50 μM EdU solution (Diluted with Minimum Essential Medium (MEM) medium 1:1000, RiboBio, batch number: C10310-1, Guangzhou, China) for 2 h. Cell fixation, Apollo staining, and DNA staining were performed follow-

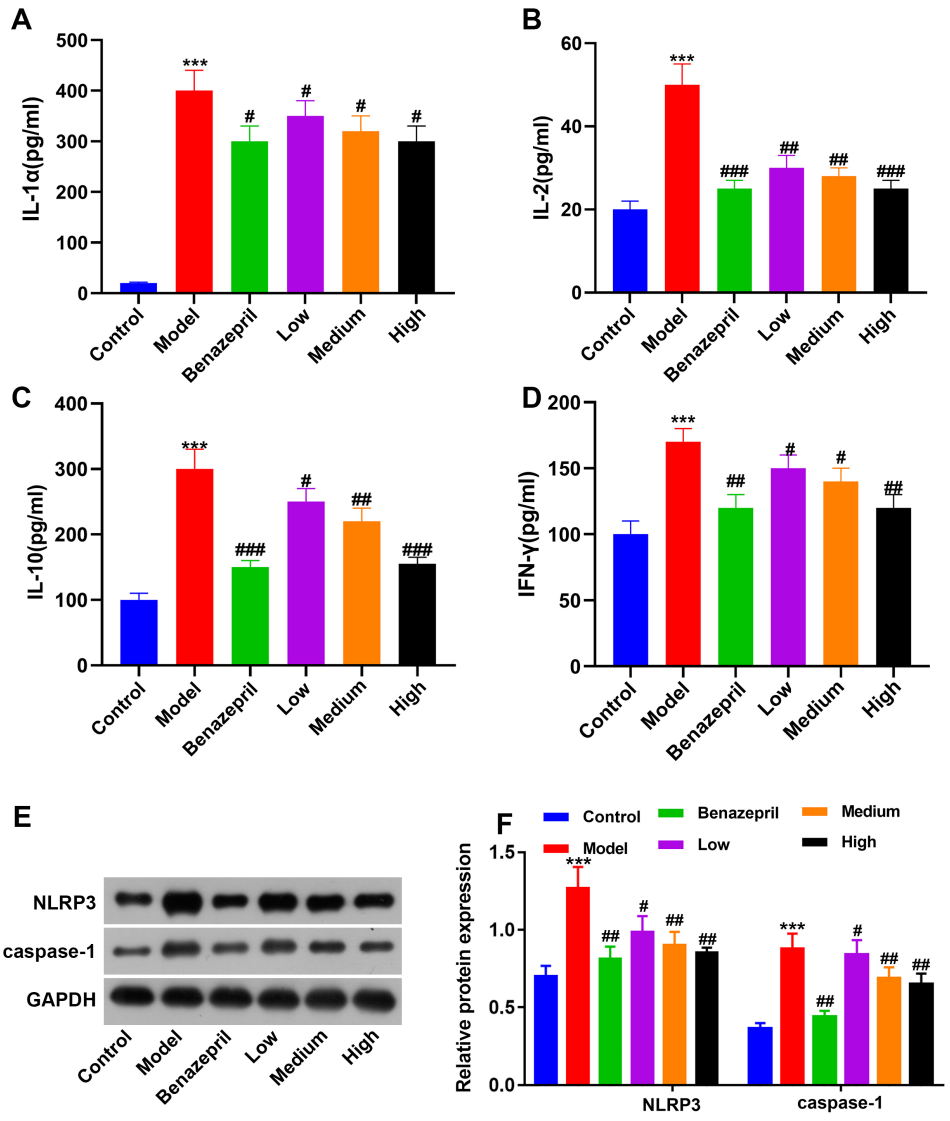

Fig. 3. Baicalin prevented inflammation in MPGN model rats. The concentrations of (A) IL-1 α, (B) IL-2, (C) IL-10, and (D) IFN- γ were detected according to the requirements of the kit (n = 6). (E,F) The apoptosis-related proteins NLRP3 and caspase-1 were detected by western blot (n = 6). $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus model subgroup.

ing the protocol provided by the EdU cell proliferation test kit. We then visualized and captured images of the cells using a fluorescence microscope (ECLIPSE Ts2, batch number: Ts2/Ts2-FL, Nikon, Tokyo, Japan).

Western Blot

A radioimmunoprecipitation assay lysis buffer (Solarbio, R0030, Beijing, China) was used to extract total protein, which was then quantified using a bicinchninic acid (BCA) assay kit (Solarbio, PC0020, Beijing, China). 20 μg of the extracted protein was transferred to polyvinylidene fluoride membranes (Millipore, IPVH00010, MA, USA). The membranes were blocked overnight at 4 °C using 5% skim milk powder (Solarbio, D8340, Beijing, China) and incubated with primary antibodies for 12 hours at a concentration of 1:1000. The antibodies used targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5174), BCL2-Associated X (Bax) (2772), Bcl-2 (15071), NOD-like receptor thermal protein domain associated protein 3 (NLRP3) (15101), caspase-1 (83383), p-PI3K (17366), PI3K (4255), p-AKT (4060), AKT (9272), Nrf2 (33649), and HO-1 (43966), all obtained from CST (MA, USA). After primary antibody incubation, goat anti-rabbit IgG secondary antibody (1:1000, Solarbio, K1034G-AF594, Beijing, China) was applied for 1 hour. GAPDH served as the endogenous control. Finally, the grayscale values of each protein were analyzed using Image J software v1.5.24 (NIH, Bethesda, MD, USA).

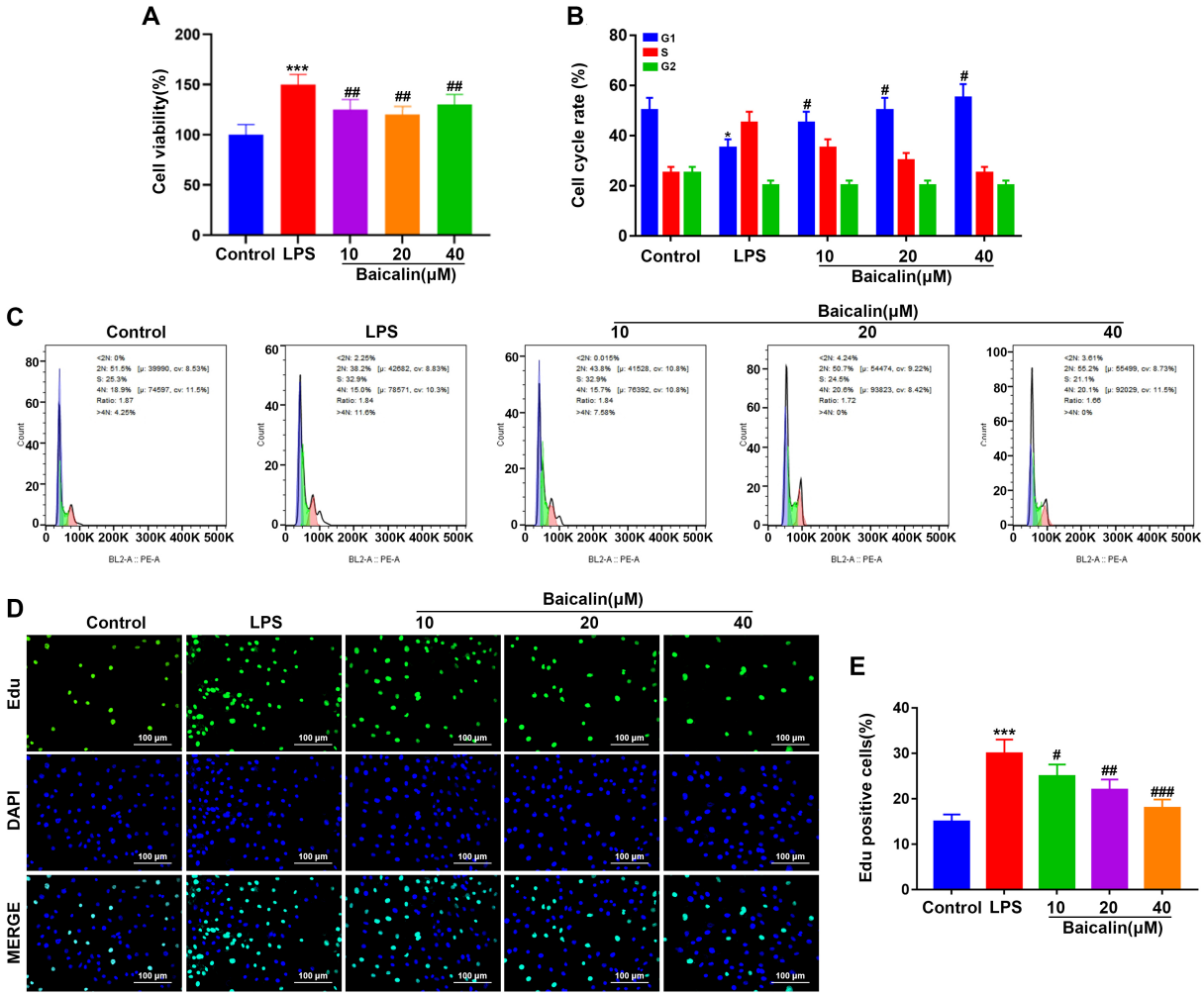

Fig. 4. Baicalin inhibits Mesangial cell proliferation and affects the cell cycle. (A) Cell viability was appraised via MTT. (B,C) Flow cytometry was applied to detect the cell cycle. (D,E) Determination of Mesangial cell proliferation by EdU assay. $^{*}p < 0.05$, $^{***}p < 0.001$, versus control subgroup (200 × magnification, scale = 100 μ m). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus LPS subgroup. The sample size: n = 3.

Statistical Analysis

SPSS 18.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Data were presented as the mean ± SD. One-way Analysis of Variance (ANOVA) was applied for difference comparison among subgroups, $p < 0.05$ represented a significant difference.

Results

Baicalin Ameliorated Renal Pathological Injury in MPGN Model Rats.

To evaluate the therapeutic effects of baicalin on Thy1.1 monoclonal antibody-induced MPGN model rats, we assessed 24-hour urinary protein and serum levels of BUN, SCr, TG, and TC. The baicalin treatment significantly reduced the 24-hour urinary protein concentration compared to the model subgroup (Fig. 1A). Concurrently, the baicalin decreased the f serum levels of BUN and SCr, demonstrating dose-dependent improvement in renal function (Fig. 1B,C). Furthermore, the serum concentrations of TG and TC-treated subgroup in the baicalin were significantly lower than those in the model subgroup (Fig. 1D,E). The control subgroup displayed normal glomerular and renal tubular morphology and structure, as observed through HE and PAS staining. In contrast, the model subgroup showed substantial kidney pathology. The HE staining revealed glomerular deformation, renal tubule dilatation, constriction of glomerular space, and indistinct boundaries between components. PAS staining identified glomerular sclerosis and an expansion of the glomerular basement membrane. However, kidney tissue pathology was noticeably alleviated in baicalin-treated rats (Fig. 1F–H). The positive drug control subgroup treated with benazepril showed similar protection to baicalin on MPGN versus the model subgroup.

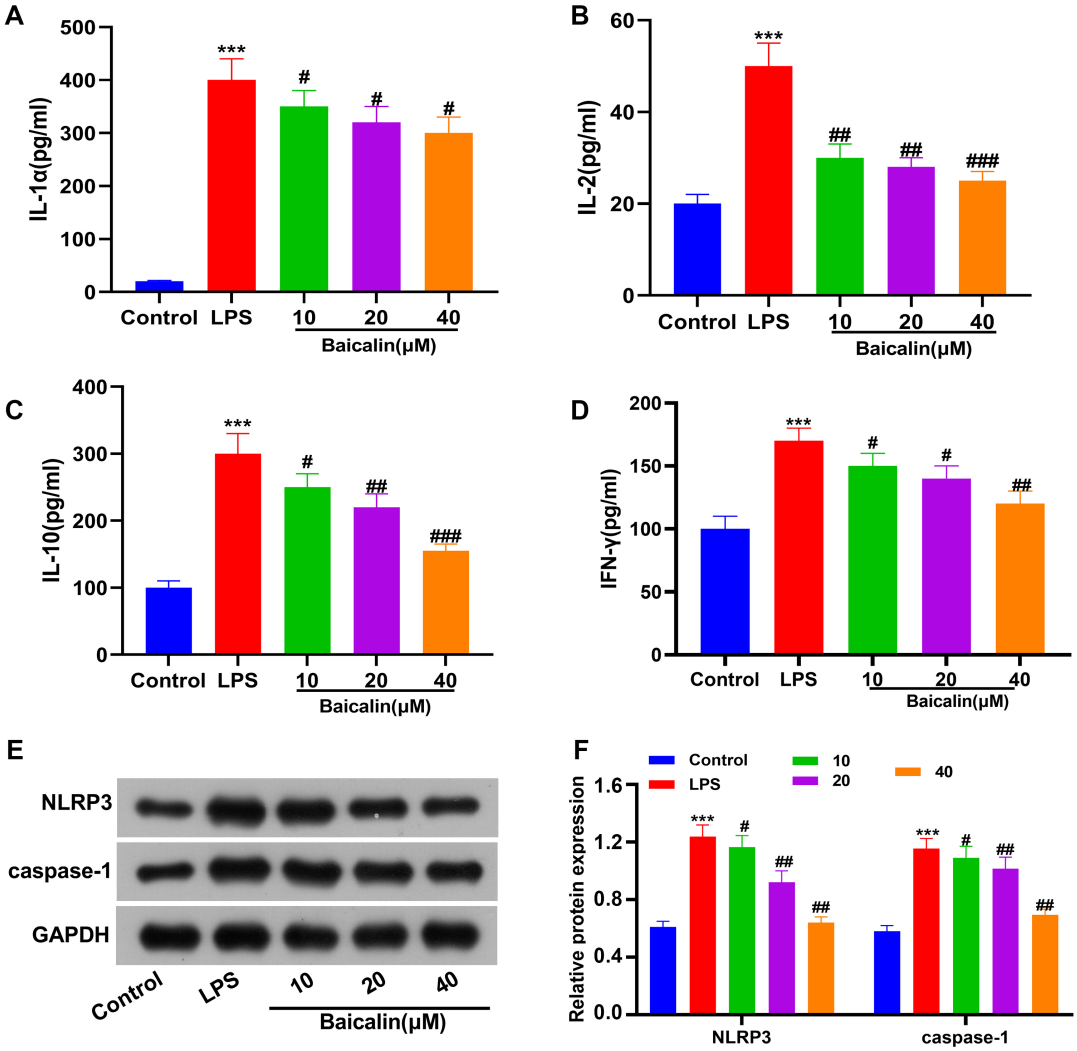

Fig. 5. Baicalin ameliorated inflammatory response in Mesangial cell model. The concentrations of (A–D) IL-1α, IL-2, IL-10, and IFN-γ were appraised. (E,F) Detection of NLRP3 and caspase-1. $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus LPS subgroup. The sample size: n = 3.

Baicalin Ameliorated Renal Cell Apoptosis in MPGN Model Rats

As evidenced by TUNEL staining, the model subgroup of rats exhibited a significant increase in the number of apoptotic cells in the kidney compared to the control subgroup, indicating an elevated apoptosis rate. The baicalin intervention and benazepril subgroup displayed significantly reduced renal cell apoptosis, with the rate of apoptosis diminishing in a dose-dependent fashion compared to the model subgroup (Fig. 2A,B). In addition, a marked increase in Bax concentration was exhibited, whereas the Bcl-2 concentration was significantly reduced compared to the control subgroup. In contrast, the baicalin-treated and the benazepril subgroups showed a considerable decrease in Bax concentrations and a notable increase in Bcl-2 concentrations compared to the model subgroup (Fig. 2C–E).

Baicalin Prevented Inflammation in MPGN Model Rats

MPGN is a common pathological type of primary glomerulonephritis with an increased inflammatory reaction. Versus the model subgroup, the serum concentrations of IL-1α, IL-2, IL-10, and IFN-γ in the baicalin and benazepril subgroups decreased significantly, implying an alleviating outcome on inflammatory reaction (Fig. 3A–D). Western blot analysis revealed that both baicalin and benazepril interventions resulted in reduced concentrations of NLRP3 and caspase-1 versus the model subgroup (Fig. 3E,F).

Baicalin Inhibited Mesangial Cell Proliferation and Affected Cell Cycle

To assess the effect of baicalin on LPS-induced mesangial cells in a laboratory setting, we treated these cells

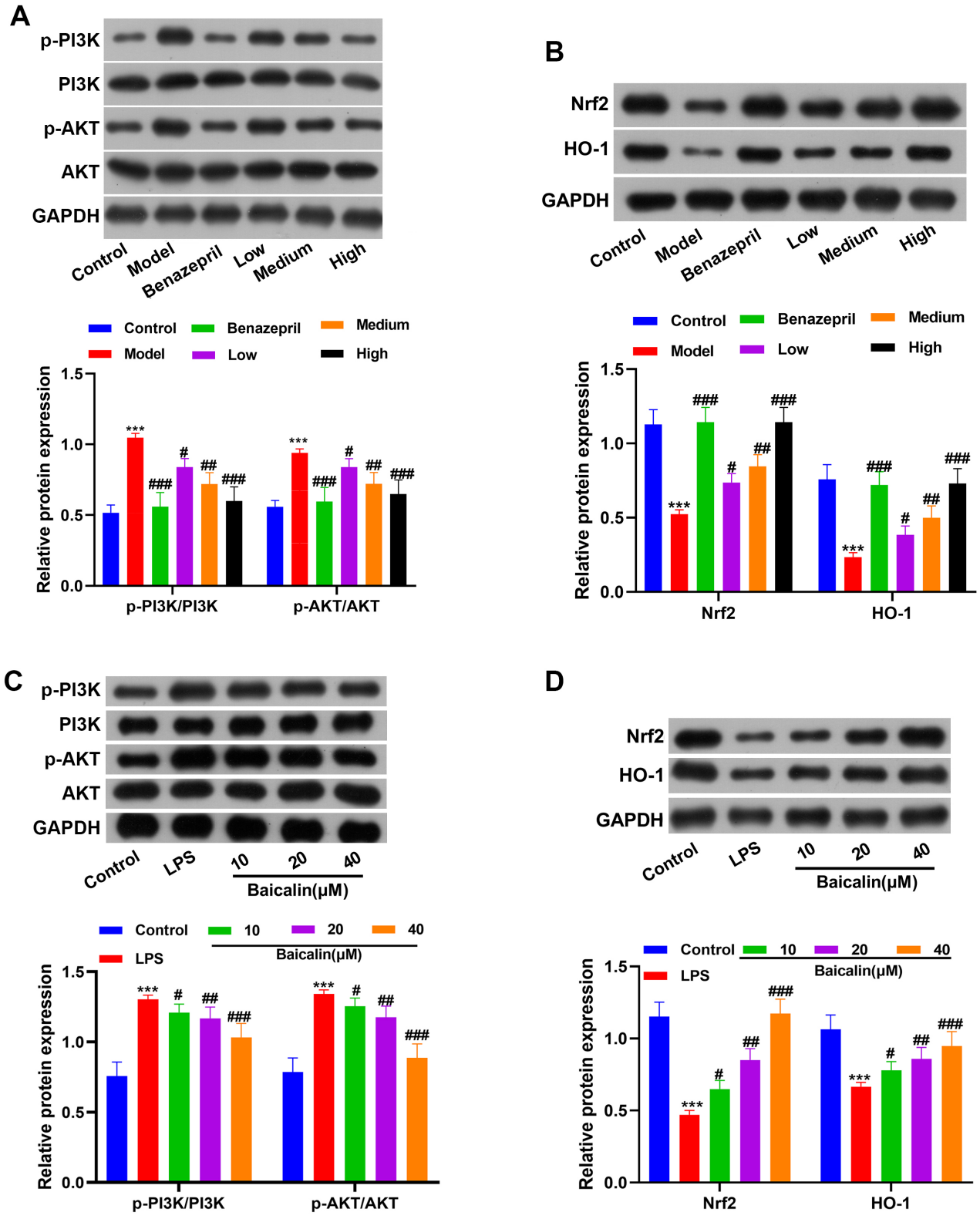

Fig. 6. Baicalin mediated by Nrf2/ARE and PI3K/AKT pathways. (A,B) The valuation of p-PI3K, PI3K, p-AKT, and AKT concentrations via western blot. $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus model subgroup (n = 6). (C,D) The valuation of p-PI3K, PI3K, p-AKT, and AKT concentrations via western blot. $^{***}p < 0.001$, versus control subgroup. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, versus LPS subgroup. The sample size: n = 3.

with various concentrations of baicalin for 24 hours following LPS incubation. The MTT assay revealed a significant increase in mesangial cell vitality in the LPS group compared to the control group, whereas baicalin was able to inhibits mesangial cell proliferation versus LPS subgroup (Fig. 4A). Cell cycle analysis showed that LPS decreased the percentage of cells in the G1 phase, suggesting it accelerates cell cycle progression. In contrast, treatment with baicalin resulted in G1 phase cell arrest compared to the LPS group (Fig. 4B,C). As confirmed by EdU staining, the rate of EdU-positive cells increased in the LPS group but was significantly reduced in the baicalin-treated group (Fig. 4D,E).

Baicalin Ameliorated Inflammatory Response in Mesangial Cell Model

The levels of IL-1α, IL-2, IL-10, and IFN-γ in the supernatant were significantly elevated in the LPS-treated group versus the control subgroup. IL-1α, IL-2, IL-10, and IFN-γ levels in the cell supernatant significantly decreased in a dose-dependent manner in the baicalin-treated group versus the LPS subgroup (Fig. 5A–D). As confirmed through western blot, NLRP3, and caspase-1 proteins were significantly elevated in the LPS subgroup compared to the control subgroup. However, these protein levels markedly decreased in the baicalin-treated group versus the LPS subgroup (Fig. 5E,F).

Baicalin Mediated Nrf2/ARE and PI3K/AKT Pathways

To elucidate the molecular mechanisms underlying baicalin's anti-proliferative and anti-inflammatory effects on MPGN, the concentrations of the Nrf2/ARE pathway and PI3K/AKT pathway-related proteins *in vivo* and *in vitro* were examined. The findings confirmed that baicalin significantly reduced the concentrations of phosphorylated PI3K (p-PI3K) and phosphorylated AKT (p-AKT) while simultaneously increasing the concentrations of Nrf2 and HO-1 in rat kidney tissue and LPS-induced mesangial cells (as shown in Fig. 6A–D).

Discussion

Baicalin, a flavonoid derived from the root of Scutellaria baicalensis, is recognized for its anti-inflammatory properties. This compound has demonstrated the potential to mitigate LPS-induced inflammation [19]. Zhang *et al.* [20] reported that baicalin exhibits anti-inflammatory and cytoprotective effects and can modulate autophagy by suppressing Toll-likereceptor4 (TLR4) activity during Salmonella typhimurium infection. Furthermore, Wang *et al.* [21] have validated that baicalin could inhibit apoptosis in the liver and kidney tissues in a rat model of preeclampsia, thereby suggesting its potential as a therapeutic agent for preventing liver and kidney damage. Shi *et al.*'s [22] findings revealed that baicalin could mitigate acute renal injury caused by *in vitro* circulatory failure or cardiac arrest by decreasing oxidative stress and inflammation in renal tissue. The current study confirmed that baicalin exhibits a protective effect in rats with MPGN, reducing renal inflammation and the prevalence of apoptosis in renal tissue. *In vitro*, cellular models have shown that baicalin can reduce LPS-induced mesangial cell activity and inflammation.

MPGN is fundamentally characterized by glomerular cell proliferation and/or mesangial extracellular matrix deposition. An escalation in mesangial cell proliferation may increase albuminuria, TG, and TC, and decrease serum albumin [23]. In addition to albuminuria, BUN and SCr may rise due to impaired excretion following renal injury, leading to elevated serum concentrations of these substances [24]. In this study, the MPGN rat model was generated via anti-Thy1.1 monoclonal antibody induction and subsequently treated with varying concentrations of baicalin. The results demonstrated an increased levels of 24-h urinary protein, serum levels of BUN, SCr, TG, and TC in the model subgroup, along with glomerular deformation, sclerosis, mesangial dilatation of the glomerular basement, renal tubule dilatation, and glomerular sac space stenosis versus the control subgroup. These findings suggested that renal tissue and function in the MPGN rats were compromised, consistent with previous studies [18]. Baicalin intervention significantly decreased the 24-h urinary protein levels of BUN, SCr, TG, and TC and reduced the pathological injury in the renal tissue. Moreover, baicalin reduced the prevalence of apoptosis in renal tissue.

The progression of MPGN is significantly influenced by inflammation. Studies have shown that inflammatory immune cells, specifically T lymphocytes and macrophages, are involved in the MPGN's pathological process. These macrophages secrete cytokines such as TNF-α, IFN-γ, and IL-12 at increased levels, highlighting the importance of inflammation in glomerular injury [18]. Our results indicate that the levels of IL-1α, IL-2, IL-10, and IFN-γ are significantly higher in the serum of MPGN model rats. However, baicalin reduces these concentrations, thereby attenuating the inflammatory reaction. We also established a LPS-induced mesangial cell model, demonstrating that baicalin could inhibit LPS-induced mesangial cell proliferation and decrease the levels of IL-1α, IL-2, IL-10, and IFN-γ in the supernatant, which was also consistent with previous studies [2].

The Nrf2/ARE pathway, a vital cellular defense system against oxidative stress, has garnered considerable attention due to its association with chronic glomerulonephritis [25]. Previous research suggests that regulating the Nrf2 signal pathway can reduce oxidative stress, thereby improving membranous glomerulonephritis [26]. Huang *et al.*'s research [4] underscored the significant role of the Nrf2/ARE pathway in the pathogenesis of experimental anti-Thy1.1 MPGN. The proliferation of glomerular mesan-

gial cells and deposition of the extracellular matrix is significantly regulated by the PI3K/AKT signaling pathway. Shreds of evidence show that p-AKT may contribute to the initiation and progression of Mesangial hyperplasia in the anti-Thy-1 glomerulonephritis model [27–30]. Our study further demonstrated that baicalin significantly reduces p-PI3K/PI3K and p-AKT/AKT levels, increasing the Nrf2 and HO-1 levels in rat kidney tissue and LPS-induced Mesangial cells. This suggests that the Nrf2/ARE and PI3K/AKT pathways could be the potential mechanisms of baicalin in the treatment of MPGN.

Conclusions

In conclusion, this study establishes that baicalin significantly mitigates MPGN by curbing both inflammation and Mesangial cell proliferation in MPGN model rats and *in vitro* cell models, acting through Nrf2/ARE and PI3K/AKT pathways. These findings lay a noteworthy experimental foundation for understanding baicalin's mechanisms in MPGN intervention, potentially paving the way for innovative therapeutic strategies.

Abbreviations

MPGN, Mesangial proliferative glomerulonephritis; BUN, blood urea nitrogen; SCr, serum creatinine; TG, triglycerides; TC, total cholesterol; IL, interleukin; IFN-γ, interferon-γ.

Availability of Data and Materials

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

Author Contributions

JYX designed the research study. XYN performed the research. DPL, YC provided help and advice on the experiments. YQS analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All experimental protocols of this study were approved by Beijing Baiaosike Biomedical Technology Co., Ltd. ethics committee; (No): MDL 2022-08-04-22.

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Conflict of Interest

The authors declare no conflict of interest.

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