Article

*Discovery Medicine* 2024; 36(186): 1441–1452
https://doi.org/10.24976/Discov.Med.202436186.134

# Gastrodin Ameliorates the Inflammation, Oxidative Stress, and Extracellular Matrix Degradation of IL-1$\beta$-Mediated Fibroblast-Like Synoviocytes via Suppressing the Gremlin-1/NF-$\kappa$B Pathway

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Published: 20 July 2024

**Background: Synovial inflammation plays a crucial role in osteoarthritis (OA). Gastrodin (GAS), an active ingredient derived from the Gastrodia elata Blume rhizome, possesses antioxidant and anti-inflammatory pharmacological effects. This research aimed to evaluate the function and molecular mechanism of GAS on human fibroblast-like synoviocytes of osteoarthritis (HFLS-OA) induced by interleukin (IL)-1$\beta$.**
**Methods: The impact of GAS on the viability of IL-1$\beta$-treated HFLS-OA cells was assessed using the cell counting kit-8 (CCK-8). Quantitative real-time reverse transcription PCR (qRT-PCR) was employed to detect changes in *IL-8*, *IL-6*, monocyte chemotactic protein-1 (*MCP-1*), tumor necrosis factor (*TNF*)-$\alpha$, and *Gremlin-1* mRNA expression in each group. Corresponding kits were utilized to measure the catalase (CAT) and superoxide dismutase (SOD) activities, as well as the nitric oxide (NO) level. Western blot analysis was conducted to examine the expression of extracellular matrix degradation-associated proteins and nuclear factor kappa-B (NF-$\kappa$B) pathway-correlated proteins in each group.**
**Results: GAS significantly promoted the proliferation of IL-1$\beta$-induced HFLS-OA cells and concurrently down-regulated *Gremlin-1* mRNA expression ($p < 0.05$). Through the down-regulation of *Gremlin-1* expression, GAS exhibited the following effects: decreased *IL-8*, *IL-6*, and *TNF*-$\alpha$ mRNA expression, as well as NO levels ($p < 0.05$); increased SOD and CAT activities ($p < 0.05$); down-regulated matrix metallopeptidase 13 (MMP-13) and MMP-1 protein expression levels ($p < 0.01$); and up-regulated collagen II protein expression level ($p < 0.01$) in IL-1$\beta$-treated HFLS-OA cells. Additionally, GAS decreased phospho-inhibitory kappa B (p-I$\kappa$B)/I$\kappa$B, phospho-inhibitory kappa B kinase (p-IKK)/IKK, and p-p65/p65 ratios in IL-1$\beta$-induced HFLS-OA cells by inhibiting Gremlin-1 expression ($p < 0.01$).**
**Conclusion: GAS demonstrates a positive impact on inflammation, oxidative stress, and extracellular matrix degradation in IL-1$\beta$-mediated HFLS-OA cells. This effect is achieved by suppressing Gremlin-1 expression and reducing NF-$\kappa$B pathway activity.**

**Keywords:** Gastrodin; human fibroblast-like synoviocytes of osteoarthritis (HFLS-OA) patients; NF-$\kappa$B pathway; inflammation; oxidative stress

## Introduction

Osteoarthritis (OA), a prevalent degenerative joint condition widely observed in the elderly population, exhibits an annually increasing incidence, significantly impacting the quality of life for sufferers and imposing a substantial disease burden on both society and families [1]. The major clinical manifestations of OA include articular cartilage degradation, synovitis, and subchondral osteosclerosis, contributing to its complex pathophysiology [2]. While previous studies have commonly acknowledged articular cartilage destruction, encompassing inflammation, oxidative stress, and extracellular matrix degradation, as the primary driver of OA progression [3], emerging evidence highlights the crucial role of synovial inflammation in this condition. Study utilizing magnetic resonance imaging and ultrasonography has revealed a link between synovial inflammation and OA progression [4]. Wang *et al*. [5] identified a positive association between knee effusion-synovitis and knee suffering, function, and stiffness scores in knee OA patients. Fibroblast-like synoviocytes (FLS), constituting a heterogeneous cell population in the synovium, play a pivotal role by producing substances that induce synovial tissue injury, including inflammatory mediators, oxygen free radicals, cytokines, and metalloproteinases [6].

Currently, the majority of OA drugs used in clinical practice primarily focus on regulating inflammation and alleviating pain [7]. However, these drugs not only lack a discernible improvement effect on joint injury and other symptoms but also present challenges such as adverse reactions, addiction, and uncertainty of efficacy [8]. Therefore, the urgent need for effective and safe OA treatment drugs persists to repair joint tissue and alleviate patient pain.

Inflammatory factors released by the inflamed synovium play a pivotal role in promoting joint injury associated with OA pathology [9]. Specifically, interleukin (IL)-1$\beta$ stimulates the synovium to generate pro-inflammatory factors such as cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), tumor necrosis factor (TNF)-$\alpha$, and IL-6. Moreover, IL-1$\beta$ promotes apoptosis by up-regulating the expression of apoptotic proteins [10]. Studies have elucidated the correlation between IL-1$\beta$-induced inflammation escalation and the activation of the nuclear factor kappa-B (NF-$\kappa$B) pathway. IL-1$\beta$ triggers the degradation of inhibitory kappa B $\alpha$ (I$\kappa$B$\alpha$) upon phosphorylation, facilitating the nuclear translocation of the transcription factor NF-$\kappa$B, ultimately mediating the secretion of inflammatory factors [11].

Additionally, fibroblast-like synoviocytes (FLS), serving as the primary effector cells for inflammatory factors in synovial tissue [12], have the capability to produce inflammation-related molecules such as IL-1$\beta$ and TNF-$\alpha$, thereby promoting inflammatory responses [10]. Through their responses to Toll-like receptor (TLR) ligands and inflammation-associated cytokines, FLS can also function as mediators in innate immune responses [13].

The extracellular matrix, primarily composed of collagen and proteoglycan, plays a crucial role in maintaining the biomechanical characteristics and functions of joints [14]. The degradation of the extracellular matrix is a key factor contributing to the deterioration of joint function in OA [15]. Sieghart *et al*. [16] reported that IL-1$\beta$ stimulation increased the expression of extracellular matrix-degrading enzymes in FLS, promoting extracellular matrix degradation and exacerbating joint injury.

Oxidative stress is implicated in synovial injury during OA progression. The excessive accumulation of oxygen-active substances induces inflammation, cell senescence, apoptosis, and further deterioration of joint function [17]. Acting as second messengers, excess oxygen radicals can promote OA by activating various signaling pathways, including NF-$\kappa$B, phosphatidylinositol-3-kinase (PI3K)/Protein Kinase B (AKT), and mitogen-activated protein kinase (MAPK) pathways [17]. Gremlin-1, a 184-amino acid protein, is also involved in OA pathogenesis. Gremlin-1 can facilitate extracellular matrix degradation and suppress chondrocyte proliferation by targeting bone morphogenetic protein (BMP) proteins [18]. Yi *et al*. [19] revealed a positive correlation between the levels of serum Gremlin-1 along with knee synovial fluid and knee injury in OA patients. Consequently, Gremlin-1 emerges as a potential target for OA treatment. However, the specific function and mechanism of Gremlin-1 in synovial inflammation remain unclear.

Gastrodin (GAS), a phenolic glycoside compound derived from the Gastrodia elata Blume rhizome, is known for its sedative and hypnotic effects, commonly employed in clinical treatment and prevention of conditions such as headache, dizziness, limb numbness and convulsions [20]. Beyond its sedative properties, GAS exhibits pharmacological activities including anti-inflammation, anti-oxidation and inhibition of apoptosis [20]. In a study by Liu *et al*. [21], GAS demonstrated its potential in ameliorating cerebral ischemic damage in rats by obstructing apoptosis and inflammation. Additionally, GAS has been found to exert renoprotective effects by modulating antioxidant systems such as glutathione (GSH), thioredoxin (Trx), and Nuclear factor erythroid 2-related factor 2 (Nrf2) [22]. Despite these known effects, the roles and associated mechanisms of GAS in synovial inflammation, particularly regarding the involvement of Gremlin-1, remain unknown. In the present study, we aimed to investigate the effects and related mechanisms of GAS in the human fibroblast-like synoviocytes of osteoarthritis (HFLS-OA) induced by interleukin (IL)-1$\beta$. Our research endeavors to provide a foundation for the potential use of GAS in the treatment of OA.

## Materials and Methods

### *Cell Culture*

HFLS-OA cells (408OA-05a) were sourced from Cell Applications (San Diego, CA, USA). These cells, derived from synovial tissue of OA patients, underwent verification for authenticity using Short Tandem Repeat (STR) analysis. Subsequently, the cells were cultured in Synovial Growth medium (Cell Applications, USA) within an incubator set at 37 °C and 5% $CO_2$ to facilitate continuous growth. Cells were tested free of mycoplasma contamination prior to the experiment.

### *Cell Transfection and Treatment*

Gastrodin (GAS), obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (S1318, purity $\geq$98%, Shanghai, China), was dissolved in phosphate buffer saline (PBS, Beyotime, Shanghai, China). To investigate the impact of GAS on HFLS-OA cells, various concentrations (100, 50, 10, 1, and 0 µM) of GAS were used to incubate HFLS-OA cells for 24 hours, and cell viability was assessed using the cell counting kit-8 (CCK-8) assay.

For cell seeding, cells in the logarithmic growth phase were digested and placed in a 6-well plate at a density of

$1 \times 10^5$ cells/well. Transfection was initiated when cell confluence reached 70%–80%. Both pcDNA3.1 blank vector and pcDNA3.1 *Gremlin-1* were synthesized by Shanghai GenePharma Co., Ltd (Shanghai, China). Cell transfection followed the protocols outlined in the Lipofectamine$^{TM}$ 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Post-transfection, cells were cultured in a $CO_2$ incubator at 37 °C.

Seven experimental groups were established: (1) Control group: HFLS-OA cells without any treatment; (2) IL-1$\beta$ group: HFLS-OA cells treated with 10 ng/mL IL-1$\beta$ for 48 hours to simulate *in vitro* inflammation [23]; (3) IL-1$\beta$ + GAS group: HFLS-OA cells incubated with 50 µM GAS for 24 hours and treated with 10 ng/mL IL-1$\beta$ for 48 hours; (4) IL-1$\beta$ + GAS + vector group: cells transfected with pcDNA3.1 blank vector, incubated with 50 µM GAS for 24 hours, and treated with 10 ng/mL IL-1$\beta$ for 24 hours; (5) IL-1$\beta$ + GAS + Gremlin-1 group: cells transfected with pcDNA3.1 Gremlin-1, incubated with 50 µM GAS for 24 hours, and treated with 10 ng/mL IL-1$\beta$ for 24 hours; (6) Vector group: cells transfected with pcDNA3.1 blank vector; (7) Gremlin-1 group: cells transfected with pcDNA3.1 *Gremlin-1*.

### Cell Counting Kit-8 (CCK-8)

HFLS-OA cells were seeded at a density of $1 \times 10^4$ cells/well in a 96-well plate and cultured until cell adherence. Following the CCK-8 kit operating instructions (CA1210, Solarbio, Beijing, China), cell viability was assessed after 24 hours of incubation with varying concentrations of GAS (0, 1, 10, 50, and 100 µM). Specifically, 10 µL of CCK-8 solution was added, and cells were further incubated for 2.5 hours at 37 °C in a $CO_2$ environment. Absorbance was measured at a wavelength of 450 nm using a microplate reader. For the IL-1$\beta$ + GAS and IL-1$\beta$ groups, a 24-hour incubation was conducted on the samples in 96-well plates with 10 ng/mL IL-1$\beta$ until cell adherence. Subsequently, the IL-1$\beta$ + GAS group was subjected to an additional 24-hour incubation with 50 µM GAS. Using the CCK-8 kit, cell viability was calculated using the formula: Cell viability (%) = $(OD_{24\,h} - OD_{0\,h})/OD_{0\,h} \times 100\%$.

### Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted from HFLS-OA cells using a total RNA extraction kit from Sigma (#83913, Louis, MO, USA). The concentration of the extracted RNA was determined using NanoDrop (Thermo Fisher Scientific, USA). Reverse transcription of cDNA was carried out using the obtained RNA following the instructions of the reverse transcription kit from Thermo (K1642, Waltham, MA, USA). Subsequently, the expression levels of the target genes in the cDNA were detected using the ABI 7500 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) with the Hieff ® qPCR SYBR Green Master Mix kit according to the handbook from Yeasen (#11201ES, Shanghai, China). The reaction program consisted of an initial step at 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 10 seconds, 55–60 °C for 20 seconds, and 72 °C for 20 seconds. The relative expression of the target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, with $\beta$-actin serving as an internal reference. The primer sequences utilized are presented in Table 1.

**Table 1. qRT-PCR primer sequences.**

| Target genes | Primer sequences (5′ to 3′) |
|---|---|
| *Gremlin-1* | F: TCATCAACCGCTTCTGTTACGGC<br>R: CAGAAGGAGCAGGACTGAAAGG |
| *IL-6* | F: AACCTGAACCTTCCAAAGATGG<br>R: TCTGGCTTGTTCCTCACTACT |
| *IL-8* | F: CATACTCCAAACCTTTCCACCCC<br>R: TCAGCCCTCTTCAAAAACTTCTCCA |
| *TNF-α* | F: GTGCTTGTTCCTCAGCCTCT<br>R: CACCCTTCTCCAGCTGGAAG |
| *MCP-1* | F: CAGCCAGATGCAATCAATGCC<br>R: TGGAATCCTGAACCCACTTCT |
| *β-actin* | F: AGCGAGCATCCCCCAAAGTT<br>R: GGGCACGAAGGCTCATCATT |

*IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *MCP-1*, monocyte chemotactic protein-1; qRT-PCR, quantitative real-time reverse transcription PCR.

### Superoxide Dismutase, Catalase and nitric oxide Detection

The culture medium of HFLS-OA cells underwent centrifugation at 2000 ×g for 10 minutes at 4 °C. Subsequently, the activities of catalase (CAT) and superoxide dismutase (SOD), along with the measurement of nitric oxide (NO) levels in the cell supernatant, were assessed following the operating guidelines of the kit from MLBIO (CAT, ml095173; SOD, ml092620; NO, ml076492. Shanghai, China).

### Lactate Dehydrogenase (LDH) Activity Assay

Lactate dehydrogenase (LDH), an enzyme presents in the cytoplasm that rapidly enters the cell culture medium when the plasma membrane is compromised, is commonly utilized to assess the toxicity of drugs to cells [24]. Following a 10-minute centrifugation of the cell culture medium at 2000 ×g at 4 °C, the supernatant was extracted. LDH activity in the supernatant was then measured in accordance with the operating instructions of the LDH kit from Solarbio (BC0680, Shanghai, China).

### Western Blot

Radio-immunoprecipitation assay (RIPA) lysate from Beyotime (China) was utilized to isolate total cellular pro-

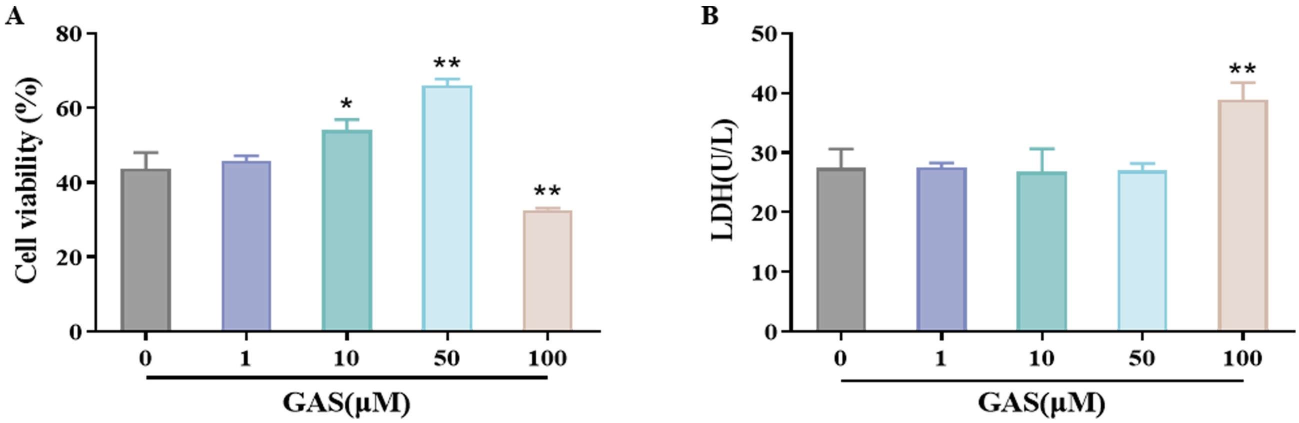


**Fig. 1. GAS promotes the proliferation of HFLS-OA cells.** (A) CCK-8 was adopted to detect the effect of GAS (gastrodin) at 0, 1, 10, 50 and 100 µM on the the cell viability of HFLS-OA. (B) The related kit was used to detect the effect of GAS at 0, 1, 10, 50 and 100 µM on the LDH activity of HFLS-OA cells. N = 3, *$p < 0.05$, **$p < 0.01$ vs. 0 µM. HFLS-OA, human fibroblast-like synoviocytes of osteoarthritis; GAS, gastrodin; CCK-8, cell counting kit-8; LDH, Lactate dehydrogenase.

tein, and the protein concentration was determined using a bicinchoninic acid assay (BCA) kit from Thermo (#23225, Waltham, MA, USA). The obtained protein was then boiled with loading buffer from Beyotime (P0015L, Shanghai, China). Subsequently, the protein sample was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred electrophoretically onto polyvinylidene difluoride membranes (PVDF, #1620177, Bio-Rad, Hercules, CA, USA). The membranes were blocked with 5% bovine serum albumin (BSA, SW3015, Solarbio, Beijing, China) for 1 hour, followed by an overnight incubation at 4 °C with primary antibodies. Primary antibodies were purchased from Abcam (Cambridge, MA, USA) and included matrix metallopeptidase (MMP)-1 (ab134184, 1:1000), p65 (ab32536, 1:1000), p-p65 (ab76302, 1:1000), inhibitory kappa B kinase (IKK) (ab32041, 1:1000), phospho-IKK (p-IKK) (ab38515, 1:1000), MMP-13 (ab39012, 1:5000), Collagen II (ab307674, 1:1000), Gremlin-1 (ab231065, 1:1000), and $\beta$-actin (ab8227, 1:1000). Primary antibodies inhibitory kappa B (I$\kappa$B) (9242, 1:1000) and phospho-inhibitory kappa B (p-I$\kappa$B) (2859, 1:1000) were obtained from Cell Signaling Technology (Danvers, MA, USA).

After three washes with Tris-Buffered saline containing Tween 20 (TBST, ST673, Beyotime, China), secondary antibodies (ab6721, 1:5000, Abcam, Cambridge, MA, USA) were applied for 2 hours at room temperature. Following another round of washing, enhanced chemiluminescence (ECL) luminescence agent (PE0010, Solarbio, Beijing, China) was applied uniformly to the membranes, and images were captured using a FliorchemHD2 imaging system. The grey values of protein bands were analyzed using Image J software (version 1.8.0, NIH, Bethesda, MD, United States). Finally, with $\beta$-actin as an internal control protein, the relative expression of the proteins was calculated.

### *Statistical Analysis*

Statistical analysis was performed using SPSS software (version 21.0, IBM, Armonk, NY, USA), and the graphical representation was generated using GraphPad software (version 8.3.0, La Jolla, CA, USA). *T*-test was employed for comparisons between two groups, while one-way analysis of variance (ANOVA) with post hoc Tukey test was utilized for comparisons among multiple groups. All results were presented as mean ± standard deviation (SD). A significance level of $p < 0.05$ was considered as the criterion for determining statistical significance.

## Results

### *Gastrodin Enhances the HFLS-OA Cell Proliferation*

To assess the impact of GAS on HFLS-OA cells, various GAS concentrations (0, 1, 10, 50, and 100 µM) were applied to stimulate the cells. The results indicated that GAS at 10 µM and 50 µM significantly increased the viability of HFLS-OA cells compared to the 0 µM group, while GAS at 100 µM significantly inhibited the viability of HFLS-OA cells ($p < 0.05$, Fig. 1A). Subsequently, the influence of GAS on LDH activity in the supernatant of HFLS-OA cells was examined using the relevant kit. The results showed no significant difference in LDH activity in cell supernatants among the 0 µM, 1 µM, 10 µM, and 50 µM groups, but LDH activity was significantly elevated in the 100 µM group ($p < 0.01$, Fig. 1B), indicating that the disruption of HFLS-OA cell membranes by 100 µM GAS resulted in the release of intracellular LDH. In summary, GAS at 10 µM and 50 µM was not toxic to HFLS-OA cells and even promoted cell proliferation. Therefore, GAS at 50 µM was selected for subsequent cell experiments.

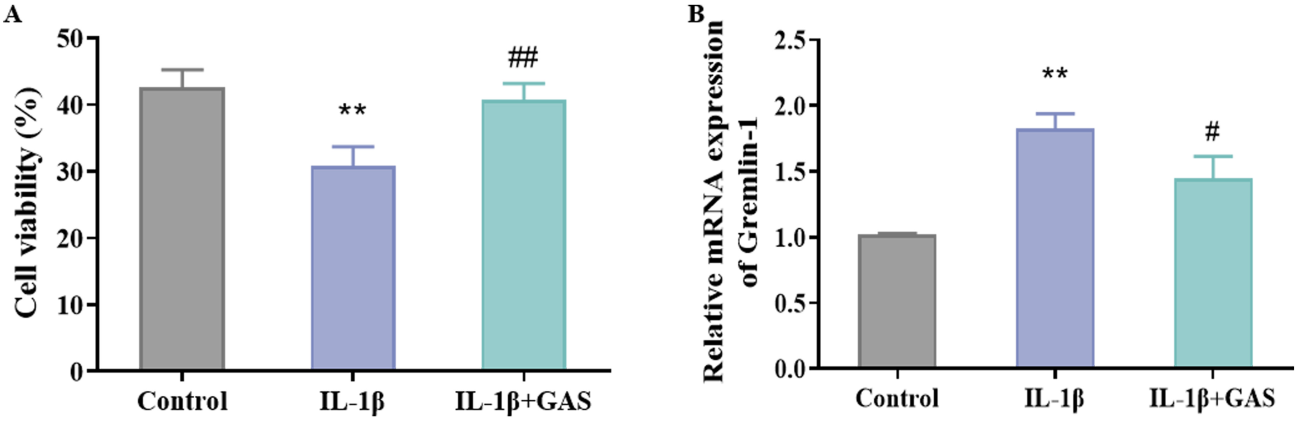


**Fig. 2. GAS promotes the proliferation of IL-1$\beta$-induced HFLS-OA and down-regulated the expression of *Gremlin-1*.** (A) CCK-8 detection of the cell viability of each group. (B) qRT-PCR for the *Gremlin-1* mRNA expression levels in the cells of each group. N = 3, **$p < 0.01$ vs. Control, #$p < 0.05$, ##$p < 0.01$ vs. IL-1$\beta$. qRT-PCR, quantitative real-time reverse transcription PCR; CCK-8, cell counting kit-8.

### *Gastrodin Promotes the Proliferation of IL-1$\beta$-Induced HFLS-OA Cells and Down-Regulates the Gremlin-1 Expression*

To assess the impact of GAS on synovial inflammation, HFLS-OA cells were treated with 10 ng/mL IL-1$\beta$ to establish an *in vitro* synovial inflammation model, and the effect of GAS on IL-1-induced HFLS-OA cell viability was examined. The results demonstrated that cell viability in the IL-1$\beta$ group was significantly lower compared to the Control group ($p < 0.01$). However, GAS reversed the inhibitory effect of IL-1$\beta$ on cell viability (Fig. 2A). Additionally, changes in *Gremlin-1* mRNA expression were analyzed by qRT-PCR to investigate whether *Gremlin-1* played a role in GAS effects on HFLS-OA cells. The qRT-PCR results indicated a substantial increase in *Gremlin-1* mRNA expression in the IL-1$\beta$ group compared to the Control group ($p < 0.01$). In the IL-1$\beta$ + GAS group, *Gremlin-1* mRNA expression was significantly lower than in the IL-1$\beta$ group ($p < 0.05$, Fig. 2B), suggesting a potential correlation between Gremlin-1 and the GAS-mediated promotion of IL-1$\beta$-induced HFLS-OA cell proliferation. Consequently, GAS promotes the proliferation of IL-1$\beta$-mediated HFLS-OA cells and down-regulates *Gremlin-1* expression.

### *Gastrodin Promotes the IL-1$\beta$-Mediated HFLS-OA Cell Proliferation by Inhibiting Gremlin-1*

The involvement of Gremlin-1 in the promotion of GAS on the proliferative activity of IL-1$\beta$-induced HFLS-OA cells is unclear. To investigate this, changes in cellular activity were assessed by transfecting pcDNA3.1 *Gremlin-1* into HFLS-OA cells to overexpress *Gremlin-1*. The qRT-PCR results showed a significant up-regulation of *Gremlin-1* mRNA expression in the *Gremlin-1* group compared to the Vector group (Fig. 3A, $p < 0.01$). In the IL-1$\beta$ + GAS + Gremlin-1 group, *Gremlin-1* mRNA expression was markedly increased relative to the IL-1$\beta$ + GAS + Vector group (Fig. 3B, $p < 0.01$), confirming successful up-regulation of *Gremlin-1* expression through pcDNA3.1 *Gremlin-1* transfection.

GAS treatment decreased Gremlin-1 protein expression compared to the IL-1$\beta$ group ($p < 0.01$). Meanwhile, Gremlin-1 protein expression was increased in the IL-1$\beta$ + GAS + Gremlin-1 group relative to the IL-1$\beta$ + GAS + Vector group ($p < 0.01$) (Fig. 3C). Furthermore, the CCK-8 assay illustrated that cell viability in the IL-1$\beta$ + GAS group was significantly increased compared to IL-1$\beta$; however, in the IL-1$\beta$ + GAS + Gremlin-1 group, viability was markedly decreased compared to the IL-1$\beta$ + GAS + Vector group (Fig. 3D, $p < 0.01$). It is reasonable to speculate that GAS promotes the proliferation of IL-1$\beta$-treated HFLS-OA cells by blocking Gremlin-1 expression.

### *Gastrodin Improves Inflammatory Responses in IL-1$\beta$-Handled HFLS-OA Cells through Curbing Gremlin-1 Expression*

Inflammatory factors secreted by synovial tissue contribute to joint injury associated with OA pathology [9]. To investigate the mechanism by which GAS affects inflammatory responses in IL-1$\beta$-treated HFLS-OA cells, we examined changes in the levels of inflammatory factors in the cells. Upon IL-1$\beta$ stimulation, the mRNA expression levels of monocyte chemotactic protein-1 (*MCP-1*), *IL-8*, *IL-6*, and *TNF-$\alpha$* in HFLS-OA cells were significantly increased compared to the Control group ($p < 0.01$), indicating that IL-1$\beta$ induced inflammation in HFLS-OA cells. The IL-1$\beta$ + GAS group exhibited significantly down-regulated *TNF-$\alpha$*, *IL-8*, *MCP-1*, and *IL-6* mRNA expression levels relative to the IL-1$\beta$ group ($p < 0.01$), suggesting that GAS alleviated inflammation in IL-1$\beta$-treated HFLS-OA cells. Furthermore, mRNA expression levels of *IL-6*, *IL-8*, *TNF-$\alpha$*, and *MCP-1* were much higher in the IL-1$\beta$ + GAS + Gremlin-1 group than those in the IL-1$\beta$ + GAS + Vector

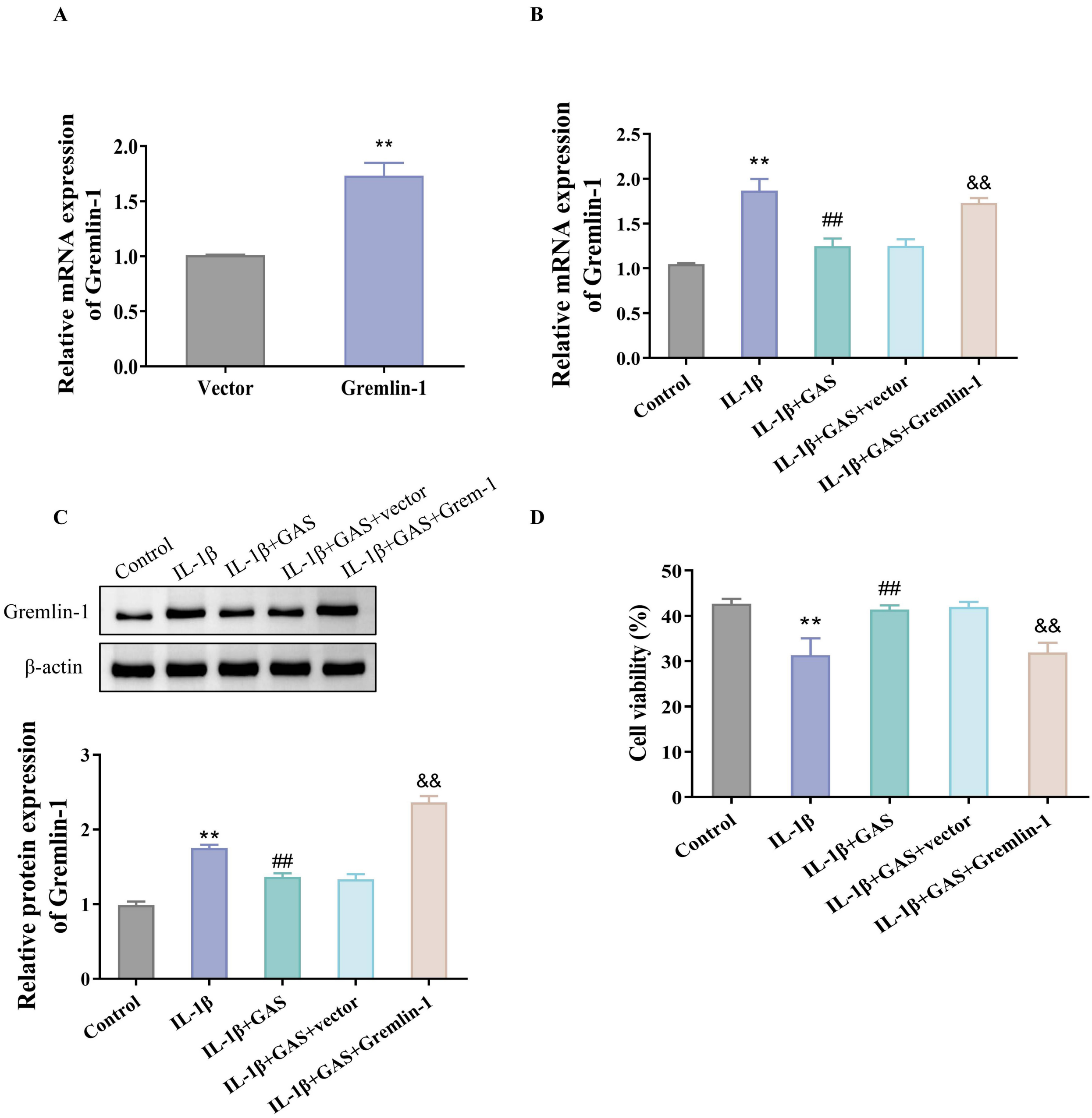


**Fig. 3. GAS promotes the proliferation of IL-1$\beta$-stimulated HFLS-OA cells via hindering Gremlin-1 expression.** (A) qRT-PCR results of the effect of transfection with pcDNA3.1 *Gremlin-1* on the *Gremlin-1* mRNA expression level in HFLS-OA cells, **$p < 0.01$, N = 3. (B) qRT-PCR results for the *Gremlin-1* mRNA expression level in each group, N = 3. (C) Western blot was adopted to detect the Gremlin-1 protein expression level in each group. (D) CCK-8 for the viability of each group. N = 3. **$p < 0.01$ vs. Control, ##$p < 0.01$ vs. IL-1$\beta$, &&$p < 0.01$ vs. IL-1$\beta$ + GAS + vector.

group ($p < 0.01$, Fig. 4). In summary, GAS ameliorates inflammatory responses in IL-1$\beta$-stimulated HFLS-OA cells by blocking Gremlin-1 expression.

### *Gastrodin Ameliorates the Oxidative Stress in IL-1β-Treated HFLS-OA Cells through Impeding Gremlin-1 Expression*

Oxidative stress induced by the accumulation of oxygen free radicals is also a significant factor in synovial injury in OA [17]. We assessed the influence of GAS on oxidative stress in HFLS-OA cells by measuring the activities of antioxidant enzymes CAT and SOD, as well as the NO level in cells. The results showed that the activities of CAT and SOD were significantly reduced ($p < 0.01$), and the NO level was notably up-regulated ($p < 0.05$) in the IL-1$\beta$ group compared to the Control group, indicating that IL-1$\beta$ led to oxidative stress in HFLS-OA cells. The IL-1$\beta$ + GAS group exhibited much higher SOD and CAT activi-

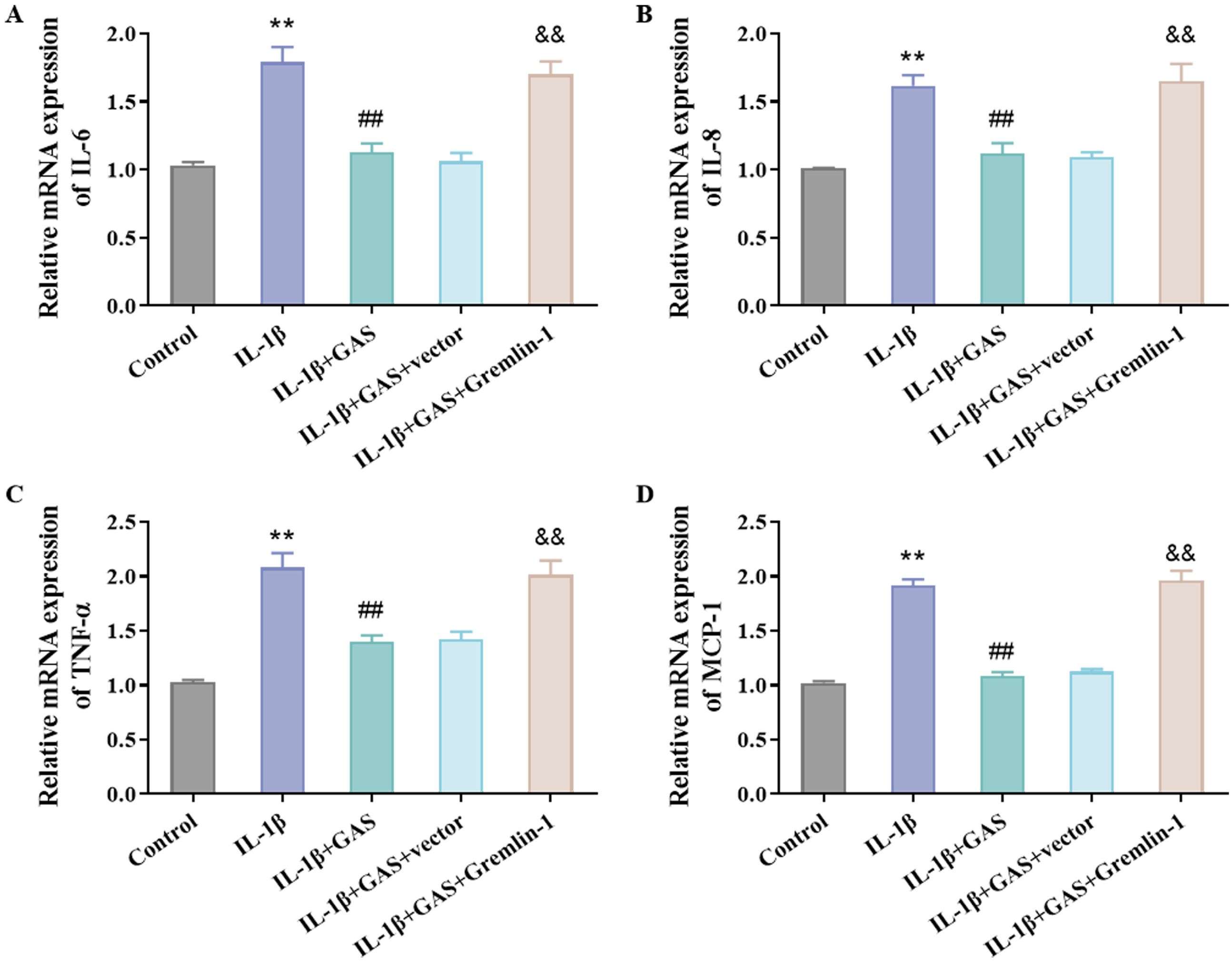


**Fig. 4. GAS ameliorates inflammation in HFLS-OA cells stimulated by IL-1$\beta$ through inhibiting Gremlin-1 expression.** qRT-PCR detection of the *IL-6* (A), *IL-8* (B), *TNF-$\alpha$* (C) and *MCP-1* (D) mRNA expression levels in each group of cells. N = 3, ##$p < 0.01$ vs. IL-1$\beta$, **$p < 0.01$ vs. Control, &&$p < 0.01$ vs. IL-1$\beta$ + GAS + vector.

ties but obviously lower NO level than the IL-1$\beta$ group ($p < 0.05$), implying that GAS stimulation could alleviate oxidative stress in IL-1$\beta$-induced HFLS-OA cells. In contrast to the IL-1$\beta$ + GAS + Vector group, the IL-1$\beta$ + GAS + Gremlin-1 group presented significantly diminished activities of SOD and CAT and noticeably raised NO level ($p < 0.05$, Fig. 5A–C) in cells. In summary, GAS ameliorates oxidative stress in IL-1$\beta$-treated HFLS-OA cells by inhibiting Gremlin-1 expression.

### *Gastrodin Attenuates the Extracellular Matrix Degradation in IL-1$\beta$-Treated HFLS-OA Cells via Inhibition of Gremlin-1 Expression*

Inflammatory factors and oxidative stress can stimulate extracellular matrix degradation, a major contributor to joint function deterioration [14,15]. To examine the effect of GAS on extracellular matrix degradation in HFLS-OA cells, the protein expression of extracellular matrix-degrading enzymes (MMP-13 and MMP-1) and collagen II was assessed using western blot. The western blot results are as follows: compared with the Control group, the protein expression levels of MMP-13 and MMP-1 increased significantly ($p < 0.01$), while collagen II protein expression decreased considerably ($p < 0.01$) in the IL-1$\beta$ group, indicating that IL-1$\beta$ promoted extracellular matrix degradation in HFLS-OA cells. Relative to the IL-1$\beta$ group, the levels of MMP-1 and MMP-13 in the IL-1$\beta$ + GAS group were significantly decreased ($p < 0.01$), while collagen II protein expression was substantially increased ($p < 0.01$), suggesting that GAS relieved IL-1$\beta$-mediated extracellular matrix degradation. In contrast to the IL-1$\beta$ + GAS + Vector group, the protein expression of MMP-1 and MMP-13 was significantly increased ($p < 0.01$), while collagen II protein expression was notably diminished ($p < 0.01$, Fig. 6A–D) in the IL-1$\beta$ + GAS + Gremlin-1 group cells, indicating that GAS alleviated extracellular matrix degradation in IL-1$\beta$-affected HFLS-OA cells by suppressing Gremlin-1 expression.

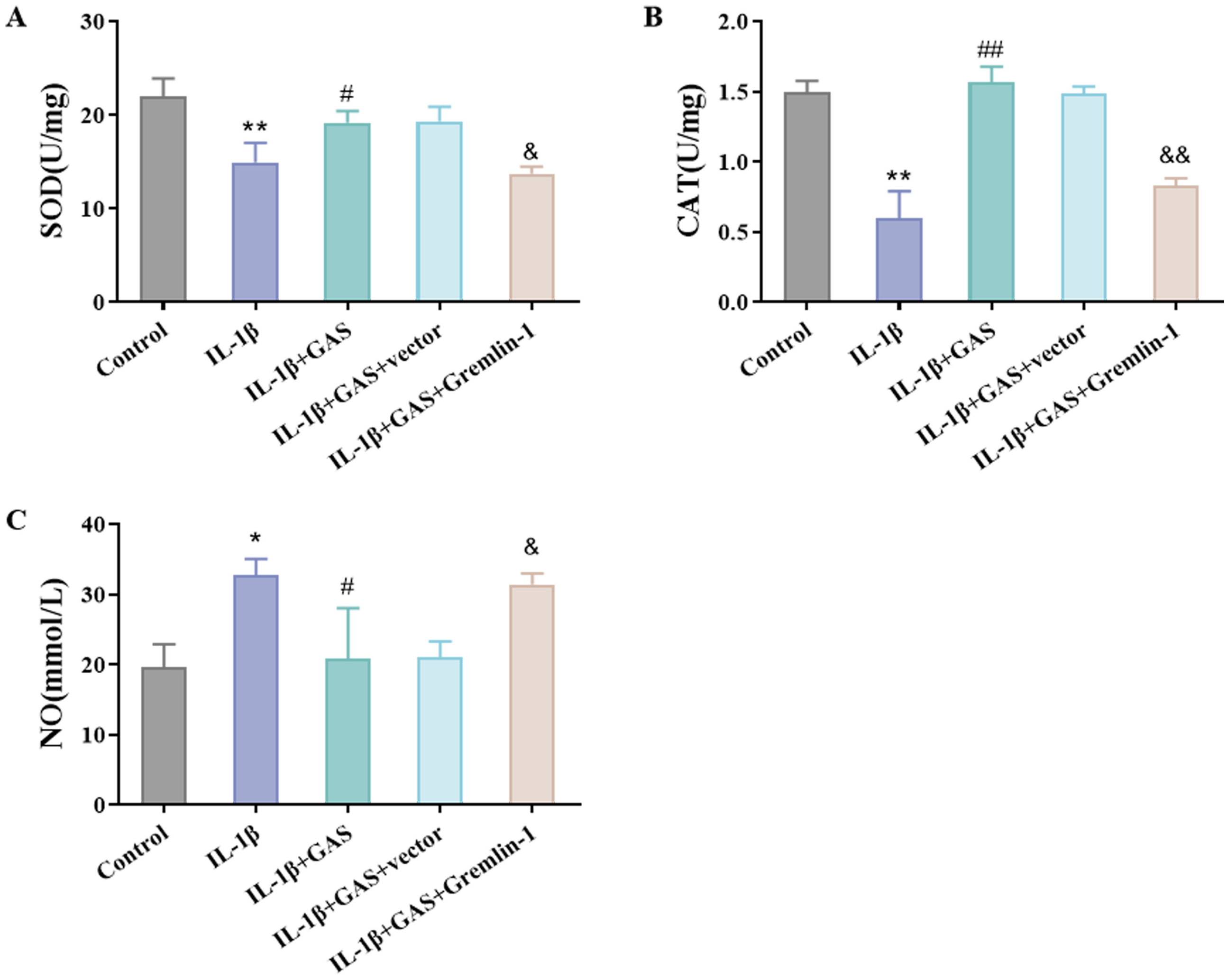


**Fig. 5. GAS ameliorates the oxidative stress in HFLS-OA cells mediated by IL-1$\beta$ through suppressing Gremlin-1 expression.** SOD activity (A), CAT activity (B) and NO level (C) were measured by corresponding kits. N = 3, *$p < 0.05$, **$p < 0.01$ vs. Control, #$p < 0.05$, ##$p < 0.01$ vs. IL-1$\beta$, &$p < 0.05$, &&$p < 0.01$ vs. IL-1$\beta$ + GAS + vector. SOD, superoxide dismutase; CAT, catalase; NO, nitric oxide.

### *Gastrodin Inhibits NF-κB Signaling Pathway Activity by Downregulating Gremlin-1 in IL-1β-Influenced HFLS-OA Cells*

NF-$\kappa$B signaling mediates various responses such as immunity, inflammation, and oxidative stress, and its abnormalities can lead to cartilage metabolism disorders, promoting OA progression [25]. To elucidate the involvement of GAS in OA via the NF-$\kappa$B signaling pathway, we examined the protein expression associated with the NF-$\kappa$B signaling pathway in cells using western blot. Briefly, the p-I$\kappa$B/I$\kappa$B, p-IKK/IKK, and p-p65/p65 ratios were significantly increased in the IL-1$\beta$ group compared to the Control group ($p < 0.01$), indicating the promotion of IL-1$\beta$ to IKK, I$\kappa$B, and p65 protein phosphorylation and the activation of the NF-$\kappa$B signaling pathway in HFLS-OA cells. The IL-1$\beta$ + GAS group exhibited much lower p-IKK/IKK, p-I$\kappa$B/I$\kappa$B, and p-p65/p65 ratios than the IL-1$\beta$ group ($p <$ 0.01), suggesting that GAS inhibited the phosphorylation of IKK, I$\kappa$B, and p65 proteins in IL-1$\beta$-impacted HFLS-OA cells, thereby blocking the NF-$\kappa$B signaling pathway. Additionally, the ratios of p-p65/p65, p-I$\kappa$B/I$\kappa$B, and p-IKK/IKK were significantly increased in the IL-1$\beta$ + GAS + Gremlin-1 group compared with the IL-1$\beta$ + GAS + Vector group ($p < 0.01$, Fig. 7A,B). Collectively, the inhibition of the NF-$\kappa$B signaling pathway in HFLS-OA cells upon IL-1$\beta$ mediation by GAS was achieved by downregulating Gremlin-1 expression.

## Discussion

OA, a prevalent degenerative joint disorder characterized by articular cartilage degeneration and joint pain, significantly impacts patients' quality of life [1]. Increasing evidence suggests the prominence of synovial inflammation in the development of OA [4,5]. Inflammation-promoting proteins such as TNF-$\alpha$, IL-1$\beta$, and IL-6 released from

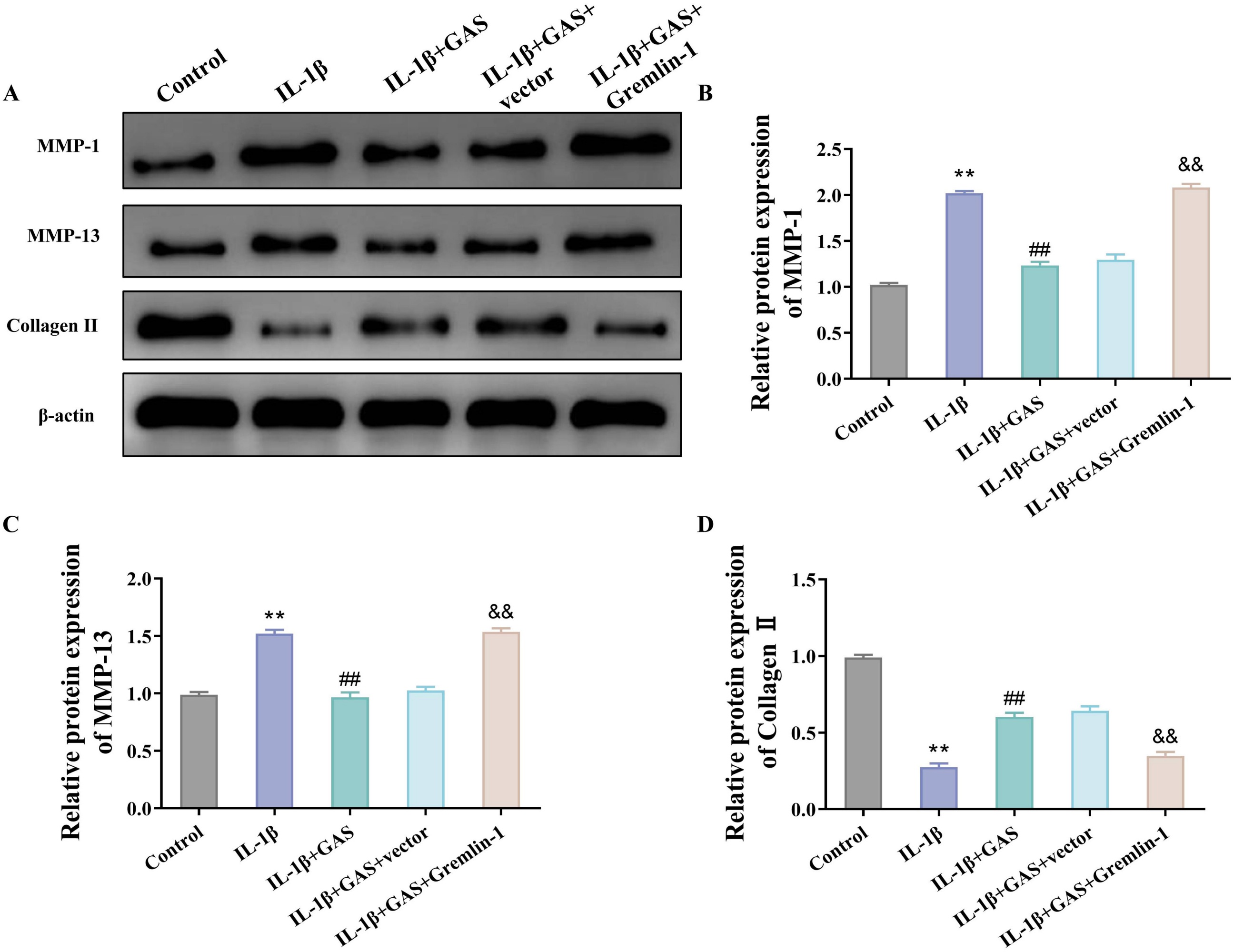


**Fig. 6. GAS attenuates the extracellular matrix degradation in IL-1$\beta$-induced HFLS-OA cells via reducing Gremlin-1 expression.** (A–D) Western blot detection of the changes of MMP-1, MMP-13 and collagen II proteins relative expression in each group of cells. N = 3, $**p < 0.01$ vs. Control, $\&\&p < 0.01$ vs. IL-1$\beta$ + GAS + vector, $\#\#p < 0.01$ vs. IL-1$\beta$. MMP, matrix metallopeptidase.

the synovium may contribute to cartilage lesions in OA. Among these, IL-1$\beta$ is a potent regulator of chondrocyte and synoviocyte catabolic processes and is commonly used to establish OA models [26]. FLS in synovial tissue actively participate in the chronic inflammatory response in OA. As OA progresses, FLS can proliferate, adhere to articular cartilage, and produce various cytokines and chemokines (TNF-$\alpha$, IL-6, and IL-1$\beta$) to attract other immune cells around articular cartilage, mediating humoral and cellular immunity in the body [27,28].

GAS, the main active component of Gastrodia elata Blume, exhibits pharmacological functions, including anti-inflammatory, sedative, analgesic, antioxidant, and anti-apoptotic activities on the cardiovascular system, immune system diseases, central nervous system, and digestive system [20,21]. GAS has been shown to ameliorate rat cerebral ischemic injury by inhibiting apoptosis and inflammatory response [21]. Lv *et al*. [29] reported that GAS reduced IL-1$\beta$, IL-6, and TNF levels in rat hepatitis tissues, effectively suppressing the inflammatory response. In this study, we observed that while GAS was not toxic to HFLS-OA cells at 50 µM, it exhibited toxicity at 100 µM. Therefore, GAS at 50 µM was used for subsequent *in vitro* experiments. Additionally, GAS was found to reverse the up-regulation of *IL-8*, *IL-6*, *MCP-1*, and *TNF-$\alpha$* mRNA expression in IL-1$\beta$-induced HFLS-OA cells, alleviating cellular inflammatory responses. This is consistent with the anti-inflammatory effects of GAS reported in previous studies.

Inflammatory cytokines released from damaged synovium not only encourage inflammatory responses but also contribute to oxidative stress and extracellular matrix degradation [30]. IL-1$\beta$, in particular, has been reported to mediate oxidative stress in cells, leading to damage to chondrocytes, degradation of the cartilage matrix, and the promotion of OA development [30]. Matrix metalloproteinases (MMPs) can be produced in response to inflammatory factors [31]. During extracellular matrix remodeling, MMP-1 significantly promotes the degradation of fibrillar collagen, while MMP-13 rapidly degrades collagen II [31]. The antioxidant pharmacological effects of GAS have been

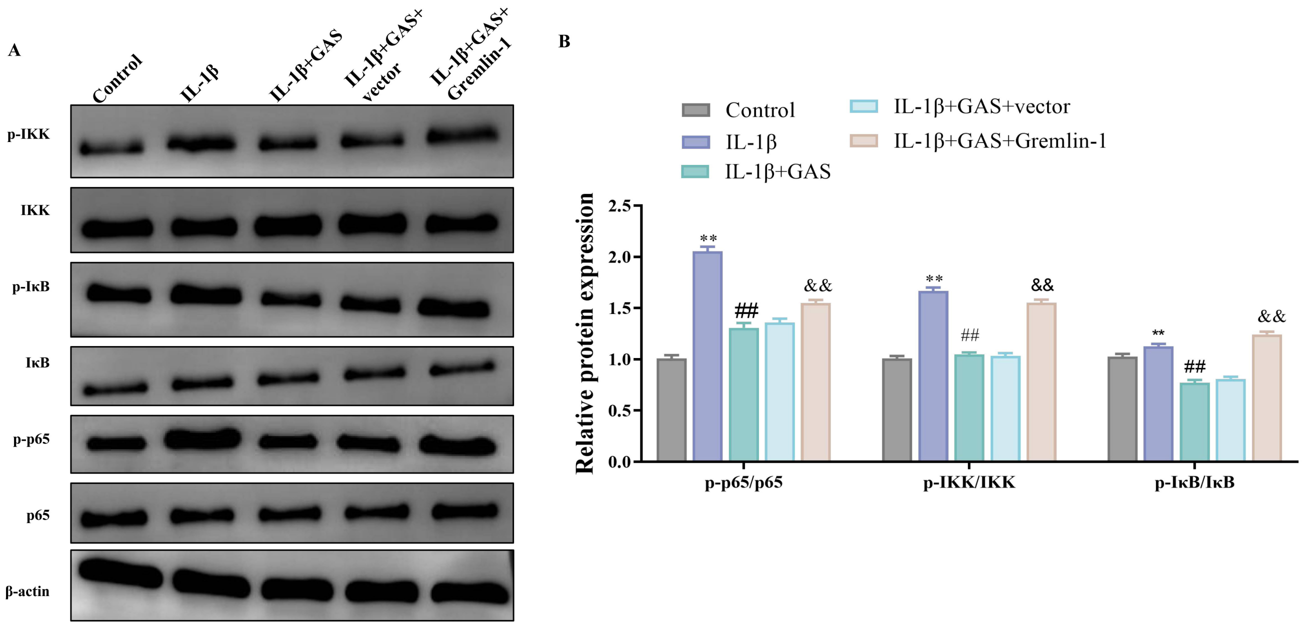


**Fig. 7. GAS inhibits nuclear factor kappa-B (NF-$\kappa$B) signaling pathway activity by down-regulating Gremlin-1 expression in IL-1$\beta$-induced HFLS-OA cells.** (A,B) The inhibitory kappa B kinase (IKK), p-IKK, phospho-inhibitory kappa B (p-I$\kappa$B), I$\kappa$B, p-p65 and p65 protein expression levels in HFLS-OA cells were assessed through western blot, the protein bands' gray values and then the ratios of p-p65/p65, p-IKK/IKK and p-I$\kappa$B/I$\kappa$B were calculated by Image J. N = 3, ##$p < 0.01$ vs. IL-1$\beta$, **$p < 0.01$ vs. Control, &&$p < 0.01$ vs. IL-1$\beta$ + GAS + Vector. NF-κB, nuclear factor kappa-B.

well documented. For instance, GAS administration has been shown to increase LDH and SOD activities, ameliorating oxidative stress in high glucose-induced MPC5 cells [32]. Additionally, GAS has been found to inhibit apoptosis and ameliorate IL-1$\beta$-induced extracellular matrix degradation and chondrocyte inflammation, as reported by Chen *et al*. [33]. In our study, GAS increased LDH and SOD activities, and decreased NO level in IL-1$\beta$-induced HFLS-OA cells, exhibiting antioxidant activity. Furthermore, GAS not only reversed the elevation of MMP-1 and MMP-13 protein expression and the down-regulation of collagen II protein expression in IL-1$\beta$-induced HFLS-OA cells but also alleviated extracellular matrix degradation, consistent with the findings of prior research.

Gremlin-1, a BMP antagonist belonging to the TGF-$\beta$ superfamily, plays a role in cartilage repair and bone formation. Gremlin-1 can suppress chondrocyte proliferation and promote extracellular matrix degradation by targeting BMP proteins [18]. Our study demonstrates that Gremlin-1 contributes to the way GAS affects HFLS-OA cells. *Gremlin-1* mRNA expression was down-regulated following GAS treatment compared to the IL-1$\beta$ group. Subsequently, we found that GAS alleviated cellular inflammation, oxidative stress, and extracellular matrix degradation in IL-1$\beta$-stimulated HFLS-OA cells by suppressing Gremlin-1 expression. Overall, Gremlin-1 might be a promising target for treating OA patients.

NF-$\kappa$B, a transcriptional activator abundant in eukaryotic cells, regulates numerous genes connected to immune, inflammatory, and oxidative stress responses [34]. NF-$\kappa$B serves as an oxidative stress sensor and a mediator of intracellular signal transduction for various inflammatory factors. Abnormalities in NF-$\kappa$B can lead to cartilage metabolism disorders and promote OA progression [25]. Resveratrol, a traditional Chinese medicine extract, has been shown to reduce IL-1$\beta$-stimulated oxidative stress and inflammation, relieving cartilage damage and chondrocyte issues in OA by inhibiting the NF-$\kappa$B pathway [35]. Hu *et al*. [36] found that saxagliptin could lower reactive oxygen species (ROS) generation, increase glutathione (GSH) expression in chondrocytes by down-regulating NF-$\kappa$B pathway signaling activity, and decrease the expression of extracellular matrix-degrading enzymes, thus alleviating chondrocyte oxidative stress and extracellular matrix degradation. According to our study, GAS reversed the increased ratios of p-I$\kappa$B/I$\kappa$B, p-IKK/IKK, and p-p65/p65 in IL-1$\beta$-induced HFLS-OA cells by inhibiting Gremlin-1 expression. This finding suggests that GAS attenuates the damage caused by IL-1$\beta$ to HFLS-OA cells by inhibiting IKK, I$\kappa$B, and p65 protein phosphorylation and preventing nuclear transcription factor NF-$\kappa$B activation. However, this study has some limitations, such as not thoroughly investigating how Gremlin-1 regulates the NF-$\kappa$B signaling pathway and the lack of relevant animal experiments to further verify the effectiveness of GAS in OA treatment and its impact on synovial inflammation.

## Conclusion

In summary, our study indicates that GAS promotes the proliferation of IL-1$\beta$-induced HFLS-OA cells while improving inflammation, oxidative stress, and extracellular matrix degradation. Additionally, GAS reduces NF-$\kappa$B pathway activity by inhibiting Gremlin-1 expression.

## Availability of Data and Materials

Data involved in the present work are available from the corresponding author upon request.

## Author Contributions

YH and XFD designed the research study. YH and XFD performed the research. ML, XYC, CHH, HYC and GJW provided help and advice on experiments. ML, XYC, CHH, HYC and GJW analyzed the data. YH, ML and XFD wrote the first draft. All authors contributed to significant 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

Not applicable.

## Acknowledgment

Not applicable.

## Funding

This work was supported by Research and Cultivation Fund project of Hainan Medical College: Study on the mechanism and application of Gremlin-1 gene in osteoarthritis after anterior cruciate ligament injury (HYPY2020014); Cultivation 530 Project of National Natural Science Foundation of China (General Project): Mechanism and application of NF-$\kappa$B-Gremlin-1 pathway in osteoarthritis after anterior cruciate ligament injury (2021MSXM10); Hainan Health industry scientific research project: Joint instability and mechanical loading contribute to the mechanism of osteoarthritis through the Gremlin-1 NF-$\kappa$B pathway (20A200467).

## Conflict of Interest

The authors declare no conflict of interest.

## References

[1] van den Bosch MHJ. Osteoarthritis year in review 2020: biology. Osteoarthritis and Cartilage. 2021; 29: 143–150.
[2] Loeser RF. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clinics in Geriatric Medicine. 2010; 26: 371–386.
[3] Yang L, Zhang J, Wang G. The effect of sodium hyaluronate treating knee osteoarthritis on synovial fluid interleukin -1$\beta$ and clinical treatment mechanism. Pakistan Journal of Pharmaceutical Sciences. 2015; 28: 407–410.
[4] Wang Y, Teichtahl AJ, Pelletier JP, Abram F, Wluka AE, Hussain SM, *et al.* Knee effusion volume assessed by magnetic resonance imaging and progression of knee osteoarthritis: data from the Osteoarthritis Initiative. Rheumatology. 2019; 58: 246–253.
[5] Wang X, Jin X, Blizzard L, Antony B, Han W, Zhu Z, *et al.* Associations Between Knee Effusion-synovitis and Joint Structural Changes in Patients with Knee Osteoarthritis. The Journal of Rheumatology. 2017; 44: 1644–1651.
[6] Maglaviceanu A, Wu B, Kapoor M. Fibroblast-like synoviocytes: Role in synovial fibrosis associated with osteoarthritis. Wound Repair and Regeneration. 2021; 29: 642–649.
[7] Furey J, Graziadei V, Pilkington I, Waterman J. The non-operative management of primary osteoarthritis. British Journal of Hospital Medicine. 2022; 83: 1–7.
[8] Li H, Lei M, Yu C, Lv Y, Song Y, Yang L. Mechano growth factor-E regulates apoptosis and inflammatory responses in fibroblast-like synoviocytes of knee osteoarthritis. International Orthopaedics. 2015; 39: 2503–2509.
[9] Sanchez-Lopez E, Coras R, Torres A, Lane NE, Guma M. Synovial inflammation in osteoarthritis progression. Nature Reviews. Rheumatology. 2022; 18: 258–275.
[10] Choi HM, Oh DH, Bang JS, Yang HI, Yoo MC, Kim KS. Differential effect of IL-1$\beta$ and TNF$\alpha$ on the production of IL-6, IL-8 and PGE2 in fibroblast-like synoviocytes and THP-1 macrophages. Rheumatology International. 2010; 30: 1025–1033.
[11] Hayden MS, Ghosh S. NF-κB in immunobiology. Cell Research. 2011; 21: 223–244.
[12] Liu BS, Janssen HLA, Boonstra A. IL-29 and IFN$\alpha$ differ in their ability to modulate IL-12 production by TLR-activated human macrophages and exhibit differential regulation of the IFN$\gamma$ receptor expression. Blood. 2011; 117: 2385–2395.
[13] Nair A, Kanda V, Bush-Joseph C, Verma N, Chubinskaya S, Mikecz K, *et al.* Synovial fluid from patients with early osteoarthritis modulates fibroblast-like synoviocyte responses to toll-like receptor 4 and toll-like receptor 2 ligands via soluble CD14. Arthritis and Rheumatism. 2012; 64: 2268–2277.
[14] Sarin JK, Nykänen O, Tiitu V, Mancini IAD, Brommer H, Visser J, *et al.* Arthroscopic Determination of Cartilage Proteoglycan Content and Collagen Network Structure with Near-Infrared Spectroscopy. Annals of Biomedical Engineering. 2019; 47: 1815–1826.
[15] Rahmati M, Nalesso G, Mobasheri A, Mozafari M. Aging and osteoarthritis: Central role of the extracellular matrix. Ageing Research Reviews. 2017; 40: 20–30.
[16] Sieghart D, Liszt M, Wanivenhaus A, Bröll H, Kiener H, Klösch B, *et al.* Hydrogen sulphide decreases IL-1$\beta$-induced activation of fibroblast-like synoviocytes from patients with osteoarthritis. Journal of Cellular and Molecular Medicine. 2015; 19: 187–197.
[17] Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. Biochimica et Biophysica Acta. 2016; 1862: 576–591.
[18] Pérez-Lozano ML, Sudre L, van Eegher S, Citadelle D, Pigenet A, Lafage-Proust MH, *et al.* Gremlin-1 and BMP-4 Overexpressed in Osteoarthritis Drive an Osteochondral-Remodeling Program in Osteoblasts and Hypertrophic Chondrocytes. International Journal of Molecular Sciences. 2022; 23: 2084.
[19] Yi J, Jin Q, Zhang B, Wu X, Ge D. Gremlin-1 Concentrations Are Correlated with the Severity of Knee Osteoarthritis. Medical Science Monitor. 2016; 22: 4062–4065.

[20] Liu Y, Gao J, Peng M, Meng H, Ma H, Cai P, *et al*. A Review on Central Nervous System Effects of Gastrodin. Frontiers in Pharmacology. 2018; 9: 24.

[21] Liu B, Li F, Shi J, Yang D, Deng Y, Gong Q. Gastrodin ameliorates subacute phase cerebral ischemia reperfusion injury by inhibiting inflammation and apoptosis in rats. Molecular Medicine Reports. 2016; 14: 4144–4152.

[22] Tian ZK, Zhang YJ, Feng ZJ, Jiang H, Cheng C, Sun JM, *et al*. Nephroprotective effect of gastrodin against lead-induced oxidative stress and inflammation in mice by the GSH, Trx, Nrf2 antioxidant system, and the HMGB1 pathway. Toxicology Research. 2021; 10: 249–263.

[23] Ungsudechachai T, Honsawek S, Jittikoon J, Udomsinprasert W. Clusterin exacerbates interleukin-1$\beta$-induced inflammation via suppressing PI3K/Akt pathway in human fibroblast-like synoviocytes of knee osteoarthritis. Scientific Reports. 2022; 12: 9963.

[24] Perche F, Patel NR, Torchilin VP. Accumulation and toxicity of antibody-targeted doxorubicin-loaded PEG-PE micelles in ovarian cancer cell spheroid model. Journal of Controlled Release. 2012; 164: 95–102.

[25] Lepetsos P, Papavassiliou KA, Papavassiliou AG. Redox and NF-κB signaling in osteoarthritis. Free Radical Biology & Medicine. 2019; 132: 90–100.

[26] Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators of Inflammation. 2014; 2014: 561459.

[27] Liu S, Cao C, Zhang Y, Liu G, Ren W, Ye Y, *et al*. PI3K/Akt inhibitor partly decreases TNF-$\alpha$-induced activation of fibroblast-like synoviocytes in osteoarthritis. Journal of Orthopaedic Surgery and Research. 2019; 14: 425.

[28] Laurent L, Anquetil F, Clavel C, Ndongo-Thiam N, Offer G, Miossec P, *et al*. IgM rheumatoid factor amplifies the inflammatory response of macrophages induced by the rheumatoid arthritis-specific immune complexes containing anticitrullinated protein antibodies. Annals of the Rheumatic Diseases. 2015; 74: 1425–1431.

[29] Lv H, Liu Y, Zhang B, Zheng Y, Ji H, Li S. The improvement effect of gastrodin on LPS/GalN-induced fulminant hepatitis via inhibiting inflammation and apoptosis and restoring autophagy. International Immunopharmacology. 2020; 85: 106627.

[30] Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. Free Radical Biology & Medicine. 2019; 132: 73–82.

[31] van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. Osteoarthritis and Cartilage. 2011; 19: 338–341.

[32] Huang L, Shao M, Zhu Y. Gastrodin inhibits high glucose-induced inflammation, oxidative stress and apoptosis in podocytes by activating the AMPK/Nrf2 signaling pathway. Experimental and Therapeutic Medicine. 2022; 23: 168.

[33] Chen J, Gu YT, Xie JJ, Wu CC, Xuan J, Guo WJ, *et al*. Gastrodin reduces IL-1$\beta$-induced apoptosis, inflammation, and matrix catabolism in osteoarthritis chondrocytes and attenuates rat cartilage degeneration in vivo. Biomedicine & Pharmacotherapy. 2018; 97: 642–651.

[34] Kanigur Sultuybek G, Soydas T, Yenmis G. NF-κB as the mediator of metformin's effect on ageing and ageing-related diseases. Clinical and Experimental Pharmacology & Physiology. 2019; 46: 413–422.

[35] Li W, Hu S, Chen X, Shi J. The Antioxidant Resveratrol Protects against Chondrocyte Apoptosis by Regulating the COX-2/NF-$\kappa$B Pathway in Created Temporomandibular Osteoarthritis. BioMed Research International. 2021; 2021: 9978651.

[36] Hu N, Gong X, Yin S, Li Q, Chen H, Li Y, *et al*. Saxagliptin suppresses degradation of type II collagen and aggrecan in primary human chondrocytes: a therapeutic implication in osteoarthritis. Artificial Cells, Nanomedicine, and Biotechnology. 2019; 47: 3239–3245.