# Paeonol Ameliorates Inflammation and Cartilage of Chondrocytes in Osteoarthritis by Upregulating SIRT1

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**Research**

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# Abstract

**Objective:** To explore the possible role of paeonol on chondrocyte inflammation and cartilage protection in osteoarthritis (OA).

**Methods:** Primary chondrocytes were isolated from rat stifle joints, and were identified through toluidine blue staining and immunofluorescence staining of type II collagen. The chondrocytes were transfected with sh-SIRT1 or/and paeonol (0, 20, 50, 100, 200, 1000 mg/L) before OA modeling induced by IL-1β. ELISA determined the expressions of TNF-α, IL-17 and IL-6, and apoptotic rate was examined by flow cytometry. qRT-PCR and Western blot quantified the expressions of MMP-1, MMP-3, MMP-13, TIMP-1, cleaved-caspase-3, Bax, Bcl-2, and the proteins related to NF-κB pathway.

**Results:** Increases in TNF-α, IL-17, IL-6, MMP-1, MMP-3 and MMP-13 and decrease in TIMP-1 were found in IL-1β stimulated chondrocytes. The apoptotic rate as well as the expressions of cleaved-caspase-3 and Bax was up-regulated, and Bcl-2 expression was suppressed in response to IL-1β treatment. NF-κB pathway was activated in IL-1β-stimulated chondrocytes. Paeonol enhanced SIRT1 expression to inactivate NF-κB pathway, thus ameliorating the secretion of inflammatory cytokines, extracellular matrix degradation and chondrocyte apoptosis.

**Conclusion:** Paeonol inhibits IL-1β induced inflammation and extracellular matrix degradation in chondrocytes through up-regulating SIRT1 and suppressing NF-κB pathway.


# Introduction

Osteoarthritis (OA), a chronic disease which impacts the lives of millions of people worldwide, primarily induces disability, joint stiffness, and pain [1]. The pathogenesis of OA, featured by extracellular matrix (ECM) degradation and cell stress, is primarily induced by micro- and macro-injuries that activate maladaptive repair responses, such as pro-inflammatory pathways of innate immunity [2]. Chondrocytes synthesize and secrete components of the ECM infrastructure as well as various enzymes responsible for matrix-degradation, aggrecan-degradation and hydrolysis, which consequently facilitates the degradation and elimination of denatured and dysfunctional ECM proteins, thus altering ECM structure[3]. IL-1β is generally applied for OA modeling for it can elicit matrix metalloproteinases (MMPs) and nitric oxide (NO) in chondrocytes, among which MMP-3 and MMP-13 act as the two most important collagenases involved in the degradation of the cartilage matrix in OA [4]. Tissue inhibitors of metalloproteinases (TIMPs), including TIMP-1 and TIMP-2, were generated to neutralize the physiological activities of MMPs [5]. The existing therapy of OA includes interventions that relief the symptoms only, and the only definite treatment for OA is joint arthroplasty, which is expensive and requires revision in 10 ~ 15 years [6]. Inhibition of chondrocyte inflammation and ECM degradation could be a promising strategy to blunt OA progression.

Paeonol is the mainly effective compound in Paeonia lactiflora Pallas, Cynanchum paniculatum, and Paeonia suffruticosa Andr [7]. Its protective role in hepatocytes has been documented against oxidative

stress and inflammation, and hepatocyte apoptosis in LPS/d-GalN-induced acute liver failure (ALF) in mice with both NF-κB and MARK signaling pathways involved [8]. More importantly, paeonol has been demonstrated to inhibit TNF-α and IL-6 expressions released in chondrocytes after IL-1β treatment [7], whereas, the precise mechanism has yet to be developed. SIRT1 has been denoted to be involved in the development of OA [9–11]. In addition, SIRT expression was decreased in degenerated cartilage, and SIRT1 inhibition in chondrocytes elicits hypertrophy and cartilage matrix degradation [12]. More importantly, paeonol up-regulated expression and nucleus accumulation of SIRT1 in high-glucose-induced glomerular mesangial cells (GMCs) [13]. So far, it remains much to be seen how paeonol regulates OA development and whether it implicates in OA through mediating SIRT1.

In the present study, we examined the effects of paeonol on inflammation and ECM degradation in IL-1β-stimulated chondrocytes from rat joints. Moreover, we analyzed the precise mechanism underlying the regulation of paeonol on OA is associated with SIRT1 and NF-κB signaling pathway.

## Materials And Methods

# Isolation and culture of chondrocytes

Specific pathogen free (SPF) Wistar rats (n = 5), weighting 180 ~ 200 g, were obtained from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China). The rats were anesthetized with 50 mg/kg of pentobarbital sodium and then killed by cervical dislocation. The mice were immersed in 75% ethanol with their bilateral stifle joints collected under sterilized conditions. The articular cartilage was isolated and cut into pieces (1 mm × 1 mm × 1 mm), followed by washing with PBS. The pieces were then centrifuged at 800 r/min for 5 min prior to digestion by 0.25% pancreatin at 37℃ for 1 h. After the supernatant was removed, the cartilage was digested by 0.04% type II collagenase containing 5% FBS at 37℃ overnight using water bath. Then the cartilage was filtered using a 200 mesh screen before centrifuged at 800 r/min for 5 min. After the supernatant was discarded, the cells were washed and then were incubated with DMEM/F12 (Gibco, Grand Island, NY, USA) containing 10% FBS and 1% double-antibody at 37℃ under 5% $CO_2$. All experiments were in accordance with the guidance for the care and use of laboratory animals.

# Identification of chondrocytes

When the cells grew over 80% of the slides, the cells were washed with PBS for twice and fixed with 4% paraformaldehyde for 20 min at room temperature. Then toluidine blue staining and immunofluorescence staining of type II collagen were performed for identification of chondrocytes. Toluidine blue staining: The cells were subjected to staining by 1% toluidine blue for 30 min, dehydration by gradient alcohol and sealing by neutral balsam before observation under a microscope. Immunofluorescence staining: The cells were successively incubated with 3% $H_2O_2$ for 10 min and with goat serum at room temperature for 15 min. COL2A1 antibody (sc-52658, 1:100, Santa Cruz, CA, USA) was added for incubation at 4℃ overnight, and then FITC-labeled secondary antibody was incubated with the chondrocytes for 1 h. The

excessive secondary antibody was removed by PBS wash, after which fluorescent quencher was added. The chondrocytes were visualized and photographed under a fluorescence microscope.

# Treatment of chondrocytes

The powder of paeonol (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) was dissolved in DMSO to prepare for 1.0 g/L mother solution. The mother solution was maintained at room temperature and was diluted into the appropriate concentration before the performance of following experiments. Paeonol (0, 20, 50, 100, 200 and 1,000 mg/L) was used to treat the chondrocytes for 24 h before the toxicity of chondrocytes was measured by MTT. Then the PBS-dissolved IL-1β (10 ng/ml, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) reagent was applied to induce OA model in chondrocytes for 24 h.

# Cell transfection

Sh-SIRT1 (2 μg) and sh-NC (2 μg) were synthesized by GenePharma (Shanghai, China), and transfection was conducted using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) for 24 h in accordance with the manufacturer's protocols.

# MTT

The chondrocytes were inoculated in 96-well plates (3,000 cells/well), and paeonol (0, 20, 50, 100, 200 and 1,000 mg/L) was added into each well. Each group has three repeated wells. The chondrocytes were cultured in an incubator at 37℃ under 5% $CO_2$ for 24 h, followed by incubation with MTT (5 mg/ml) dissolved by 10 μl of DMSO for 4 h. Then the OD value was examined at 570 nm.

# Flow cytometry

After corresponding treatment, the concentration of chondrocytes in all groups were adjusted to $10^5$ cell/ml. Suspension (3 ml) of each sample was collected in centrifuge tubes (10 ml) and centrifuged at 500 r/min for 5 min, after which the culture solution was removed. Then the samples were washed with PBS and centrifuged at 500 r/min for 5 min. After that, the supernatant was discarded, and the cells were re-suspended using 100 μl of binding buffer. Annexin V-FITC (5 μl) and PI (5 μl) were mixed and incubated with the cells in dark for 15 min. The fluorescence of FITC and PI was detected by flow cytometer to analyze the apoptotic rate of chondrocytes. The detection on each group was performed in three times.

# ELASA

The expressions of TNF-α, IL-17 and IL-6 were detected using ELISA kit (R&D Systems, MN, USA), and all procedures were measured according to the protocols.

# qRT-PCR

TRIZOL was applied to extract the total RNAs (Invitrogen, Carlsbad, CA, USA), and RNAs were reversely transcribed using reverse transcription kit (TaKaRa, Tokyo, Japan) according to the instruction. The

expressions of RNAs were examined using LightCycler 480 (Roche, Indianapolis, IN, USA) and the reaction conditions were performed in accordance with the directions of fluorescence quantitative PCR kit (SYBR Green Mix, Roche Diagnostics, Indianapolis, IN). The thermal cycle parameters were as follows: 95℃ for 5 s; 95℃ for 5 s, 60℃ for 10 s, 72℃ for 10 s (total of 45 cycles); and finally extension at 72℃ for 5 min. Each reaction was performed in triplication. GAPDH was used for normalization. Data were analyzed using $2^{-\Delta\Delta Ct}$ and ΔΔCt was expressed as $(Ct_{\text{target gene}} - Ct_{\text{internal control}})_{\text{experimental group}} - (Ct_{\text{target gene}} - Ct_{\text{internal control}})_{\text{control group}}$. The primers of all genes and their internal controls were shown in Table 1.

Table 1
Primer sequences of all genes.

| Name of primer | Sequences |
|---|---|
| SIRT1-F | GCAGGTAGTTCCTCGGTGTC |
| SIRT1 -R | CACAACTCAGCACGCAGAAC |
| MMP-1-F | GGGGGAAGTACCTTGACTGC |
| MMP-1-R | GTTGTCGGTCCACGTCTCAT |
| MMP-3-F | CTACCTCACCACGACCCCTA |
| MMP-3-R | CCCTTGTCTTGCCCAGAGAG |
| MMP-13-F | GGACTCACTGTTGGTCCCTG |
| MMP-13-R | GGATTCCCGCAAGAGTCACA |
| TIMP-1-F | GCCTCTCTTACAGGCCGTTT |
| TIMP-1-R | AGCAGGGCTCAGATTATGCC |
| GAPDH-F | CTCTCCTGTCCGGGAGTGTA |
| GAPDH-R | GTGATTAGGCCCACAGCCTT |
| Note: F, forward; R, reverse. | |

# Western blot

Total proteins were extracted using RIPA lysis buffer (Beyotime, Shanghai, China), and then were quantified by BCA kit (Beyotime, Shanghai, China). The proteins were heated with the loading buffer for denaturation through boiling water bath for 3 min. Electrophoresis was used for protein separation at 80 V for 30 min and then were switched to 120 V for 1 ~ 2 h. The proteins were subjected to membrane transferring through ice bath at 300 mA for 60 min. After that, the membranes were sealed with blocking buffer at room temperature for 60 min or at 4℃ overnight. The primary antibody for GAPDH (ab181602,

1:10000), SIRT1 (ab110304, 1:1000), MMP-1 (ab137332, 1:1000), MMP-3 (ab52915, 1:1000), MMP-13 (ab39012, 1:3000), TIMP-1 (ab61224, 1:1000), total-caspase-3 (ab13847, 1:500), cleaved-caspase-3 (ab49822, 1:500), Bax (ab32503, 1:1000), Bcl-2 (ab196495, 1:1000), IκBα (ab32518, 1:1000), p-IκBα (ab133462, 1:1000), p65 (ab16502, 1:2000),or p-p65 (ab86299, 1:2000) (all from Abcam, Cambridge, MA, USA) was incubated with the membranes at room temperature for 1 h, after which the secondary antibody was added and incubated at room temperature for 1 h. Finally, the membranes were detected after color development.

# Statistical analysis

GraphPad prism7 was applied for statistical analysis, and all data were represented as average ± standard deviation (average ± SD). Difference between two groups was assessed through *T* test, and comparisons among multiple groups were measured using One-way analysis of variance with Dunnett's multiple comparisons test as post hoc test. *P* values of less than 0.5 were regarded as statistical significant.

## Results

# Identification of primary chondrocytes

The extracted cells were subjected to toluidine blue staining and immunofluorescence staining of type II collagen. Toluidine blue staining suggested that the extracted cells were blue-violet (Fig. 1A), implying the generation of aggrecan in these cells. According to immunofluorescence staining of type II collagen, the extracted cells were full with green fluorescence-represented type II collagen in the cytoplasm (Fig. 1B). All these results indicated that the extracted cells were chondrocytes.

# Effect of paeonol on cartilage and inflammation in IL-1β-stimulated chondrocytes

Paeonol (0, 20, 50, 100, 200, 1000 mg/L) was used to treat chondrocytes for 24 h before cell viability was detected by MTT assay. The cell toxicity experiments disclosed that paeonol (concentration less than 200 mg/L) had no toxic effect on chondrocytes, and chondrocyte viability was significantly decreased when chondrocytes were treated with paeonol at the concentration of 1,000 mg/L (Fig. 2A, $P < 0.05$).

After pre-treatment of paeonol (0, 20, 50, 100, 200 mg/L) for 24 h, the chondrocytes were stimulated by 10 ng/ml of IL-1β for 24 h. In IL-1β group, the levels of TNF-α, IL-17 and IL-6 were notably elevated when compared with Control group (Fig. 2B, $P < 0.05$). Compared with IL-1β group, the expressions of above inflammatory cytokines were inhibited by paeonol pre-treatment in a dose dependent manner (Fig. 2B, $P < 0.05$).

qRT-PCR and Western blot unraveled increases in the expressions of MMP-1, MMP-3 and MMP-13 and a decline in TIMP-1 expression in IL-1β group, compared to Control group (Fig. 2C-D, $P < 0.05$). Paeonol

treatment hampered the expressions of MMP-1, MMP-3 and MMP-13, and upregulated TIMP-1 expression in a dose dependent manner in comparison with IL-1β group (Fig. 2C-D, $P < 0.05$).

Flow cytometry uncovered that apoptotic rate in IL-1β group was marked higher than that in Control group, while paeonol treatment decreased apoptotic rate of chondrocytes in a dose dependent manner (Fig. 2E, $P < 0.05$). In addition, the expressions of cleaved-caspase-3 and Bax in IL-1β group were much higher than those in Control group, and Bcl-2 expression was downregulated in IL-1β group, compared with Control group (Fig. 2F, $P < 0.05$). Paeonol acted as a potent inhibitor of cleaved-caspase-3 and Bax, and upregulated Bcl-2 expression in a dose dependent manner (Fig. 2F, $P < 0.05$).

Aforementioned results confirmed that IL-1β treatment could activate inflammation in chondrocytes, and encourage degradation of extracellular matrix and chondrocyte apoptosis. More importantly, paeonol could impair IL-1β-triggered inflammation, extracellular matrix degradation and chondrocyte apoptosis in a dose dependent manner.

# Paeonol regulates inflammation in chondrocytes and protects cartilage through SIRT1

The expressions of SIRT1 in chondrocytes treated with paeonol (0, 20, 50, 100, 200 mg/L) and IL-1β (10 ng/ml) were assessed by qRT-PCR and Western blot. In IL-1β group, chondrocytes had lower expression of SIRT1 than in Control group, and paeonol rescued SIRT1 expression in a dose dependent manner (Fig. 3A-B, $P < 0.05$), suggesting the important role of SIRT1 in paeonol regulating chondrocyte inflammation and extracellular matrix degradation.

Then the chondrocytes were transfected with sh-SIRT1 or sh-NC before paeonol (200 mg/L) and IL-1β treatment. qRT-PCR and Western blot suggested the markedly decreased SIRT1 expression in chondrocytes after sh-SIRT1, compared with sh-NC group (Fig. 3C-D, $P < 0.05$).

After transfection of sh-SIRT1, the expressions of TNF-α, IL-17 and IL-6 was higher than those in sh-NC + Paeonol group (Fig. 3E, $P < 0.05$). In sh-SIRT1 + Paeonol group, the levels of MMP-1, MMP-3 and MMP-13 were increased in chondrocyte, and TIMP-1 expression was decreased when compared with sh-NC + Paeonol group (Fig. 3F-G, $P < 0.05$). Transfection of sh-SIRT1 strengthened the apoptotic rate of chondrocytes, expressions of cleaved-caspase-3 and Bax in addition to suppressing Bcl-2 expression, compared with sh-NC + Paeonol group (Fig. 3I, $P < 0.05$). Taken together, transfection of sh-SIRT1 could partially abolish the effect of paeonol on inflammation in chondrocytes and degradation of extracellular matrix, and paeonol acted its protective effects on chondrocytes through upregulating SIRT1.

# Paeonol inhibits NF-κB signaling pathway

Compared with Control group, the expressions of p-IκBa and p-p65 were increased in IL-1β group, but downregulated by paeonol in a dose dependent manner in comparison to IL-1β group (Fig. 4A-B, $P < 0.05$). Moreover, transfection of sh-SIRT1 significantly increased the expressions of p-IκBa and p-p65 in chondrocytes, compared with sh-NC-Paeonol group (Fig. 4A-B, $P < 0.05$). These results elucidated that IL-

1β treatment could activate NF-κB signaling pathway in chondrocytes, and paeonol could inhibit the activation of NF-κB signaling pathway through upregulating SIRT1 in a dose dependent manner.

# Discussion

OA is a multifactorial disorder characterized by low-grade, chronic inflammatory response, resulting in interactions between immune system and factors including local tissue damage and metabolic dysfunction [14]. Therefore, how to inhibit the release of inflammatory cytokines and block cellular signaling pathways is regarded as an attractive option for the management and treatment of OA. Collected evidence in present study supported that paeonol alleviated chondrocyte inflammation and ECM degradation induced by IL-1βvia enhancing SIRT1 expression. Our results indicated that NF-κB signaling pathway is activated in inflammatory chondrocytes, while whose activation can be suppressed by paeonol through regulating SIRT1.

Paeonol is the main component isolated from the root bark of paeonia suffruticosa, which has been reported to have pharmacological effects on inflammation and pain-related indication in diseases including OA [15]. However, little is known regarding the precise mechanism underlying the anti-inflammatory effect of paeonol on OA. Here, chondrocytes from the stifle joint of rats were isolated and stimulated with IL-1β to explore the potential effects and mechanism of paeonol treatment in OA. We confirmed paeonol has no toxicity on chondrocytes through MTT assay, which showed that paeonol (less than 200 mg/L) had little toxicity on chondrocytes. Additionally, the expressions of TNF-α, IL-6 and IL-17 were markedly increased in IL-1β-treated chondrocytes. The inflammatory mediators such as IL-1β trigger the expressions of inflammatory factors such as TNF-α, leading to the enhanced secretion of IL-6 and IL-17 [16]. Moreover, paeonol treatment could suppress the expressions of inflammatory cytokines in a dose dependent manner. MMP-3 can cleave multiple ECM including aggrecan [17]. Both MMP-3 and MMP-13 are responsible for the digestion of type II collagen by causing the triple helix to unwind and inducing cleavage at the P4-P11’ site [18]. In OA, the increased expressions of MMP-1, MMP-3, MMP-13 and decreased expression of TIMP-1 was found [19]. Consistently, the expressions of MMP-1, MMP-3 and MMP-13 were facilitated, and the expression of TIMP-1 was inhibited in IL-1β-stimulated chondrocytes. After paeonol treatment, MMP-1, MMP-3 and MMP-13 expressions were attenuated and TIMP-1 expression was elevated. In addition, the apoptotic rate of chondrocytes was increased by IL-1β stimulation and ameliorated by paeonol treatment. Taken together, paeonol is able to protect chondrocytes against inflammation, ECM degradation and cell apoptosis.

SIRT has been elucidated to exert its anti-inflammatory effect and regulate ECM in OA [20], and overexpression of SIRT1 in human chondrocytes leads to repression of MMP-3, MMP-8 and MMP-13 expressions [21]. Herein, we found that SIRT1 expression was hampered in the chondrocytes treated with IL-1β, while paeonol treatment could dose-dependently increase SIRT expression. Silence of SIRT1 enhanced the chondrocyte inflammation, apoptosis and ECM degradation, and the positive effects of paeonol were attenuated by SIRT1 silence. Therefore, we concluded that paeonol upregulated SIRT to implicate in OA development. A former study demonstrated that NF-κB signaling pathway has been

activated in OA [22]. In monosodium urate (MSU)-induced arthritis (MIA), paeonol has been reported to reduce the expressions of TNF-α, IL-1β and IL-6 through inhibiting NF-κB-mediated proinflammatory cytokine production [23]. The implication of NF-κB signaling pathway in paeonol attenuating inflammatory response and apoptosis in IL-1β-stimulated chondrocytes was explored. In our study, NF-κB signaling pathway was activated by IL-1β in chondrocytes, and paeonol treatment reduced the expressions of proteins related to NF-κB signaling pathway. However, the inhibition of paeonol on NF-κB signaling pathway was neutralized by SIRT1 silence. P65, an indicator of NF-κB signaling pathway exists in the cytoplasm, and when activated by inflammatory cytokines such as IL-1β, p65 is phosphorylated and translocates into the nucleus, further leading to increases in the expressions of multiple inflammation-related genes like MMPs and IL-6 [24, 25]. Interestingly, paeonol has been demonstrated to inhibit the phosphorylation of p65, thus inactivating NF-κB signaling pathway [26]. Accordingly, this study declared that paeonol dose-dependently enhanced SIRT1 expression to inactivate NF-κB signaling pathway.

Our results confirm the potential of paeonol as a candidate for OA drugs by virtue of its ability to suppress chondrocyte inflammation and ECM degradation through upregulating SIRT1 and inactivating NF-κB signaling pathway.

# Declarations


### Acknowledgement

All authors have no financial or personal relationships with other people or organizations that could inappropriately influence this work.


### Author contributions

SP conceived the ideas. LY designed the experiments. SP performed the experiments. LY analyzed the data. SP and JJQ provided critical materials. SP and LY wrote the manuscript. JJQ supervised the study. All the authors have read and approved the final version for publication.

### Compliance with ethical standard

### Declare of interest

The author declares that they have nothing worth disclosure.

### Ethical approval and informed consent to participate

The experimental scheme was authorized by the Committee of Experimental Animals of Shanxi Bethune Hospital. All procedures were in compliance with the Guide for the Care and Use of Laboratory Animals.

### Informed consent

Informed consent was obtained from all individual participants included in the study.

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# Figures

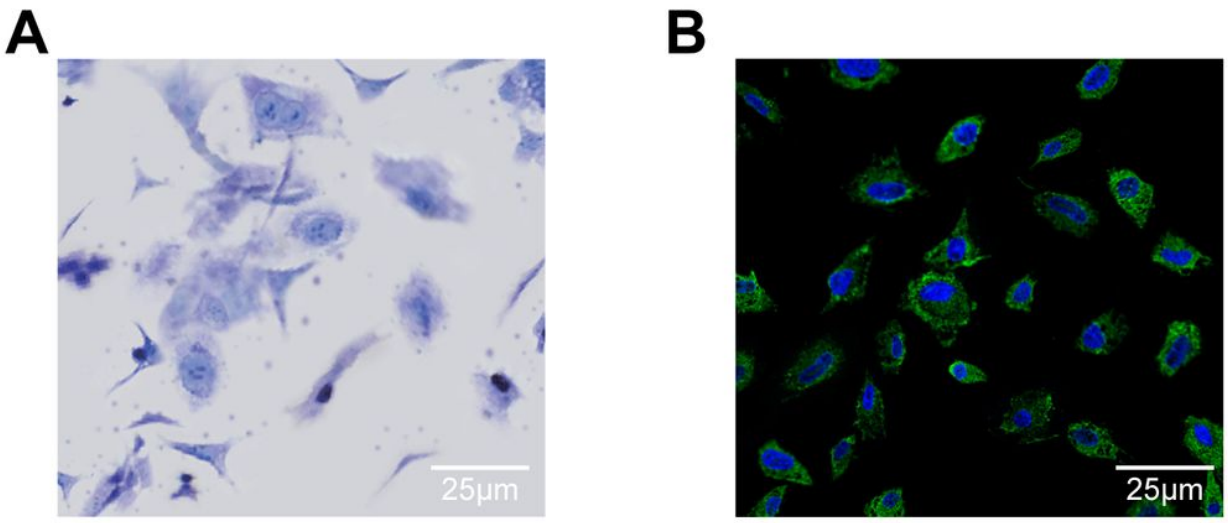
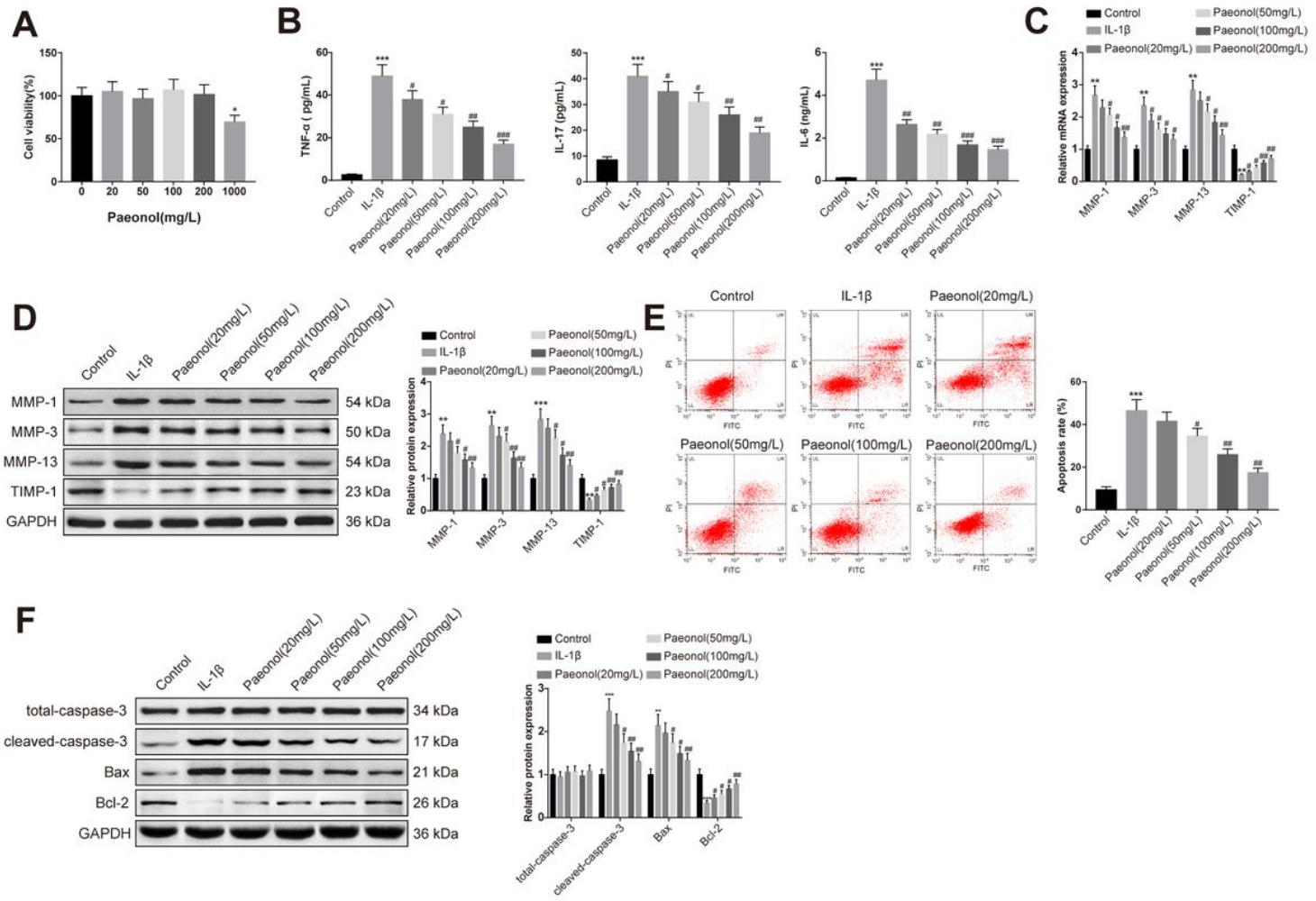


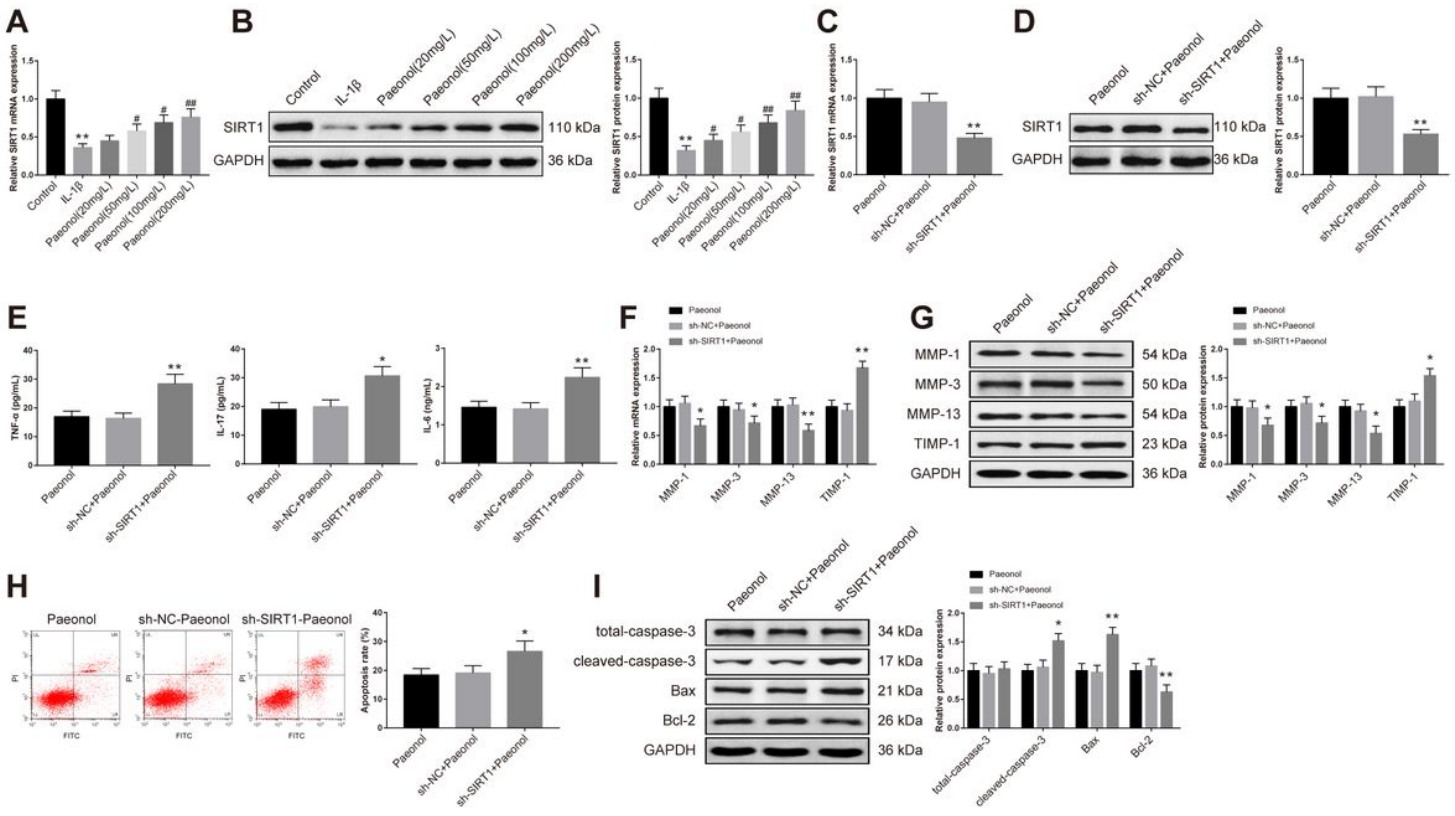

**Figure 1**

Extracted cells were identified by toluidine blue staining and immunofluorescence staining of type II collagen. Note: Primary chondrocytes were stained using toluidine blue staining, in which the aggrecan was in blue violet (A); type II collagen in chondrocyte was subjected to immunofluorescence staining, and green fluorescence represented type II collagen (B).



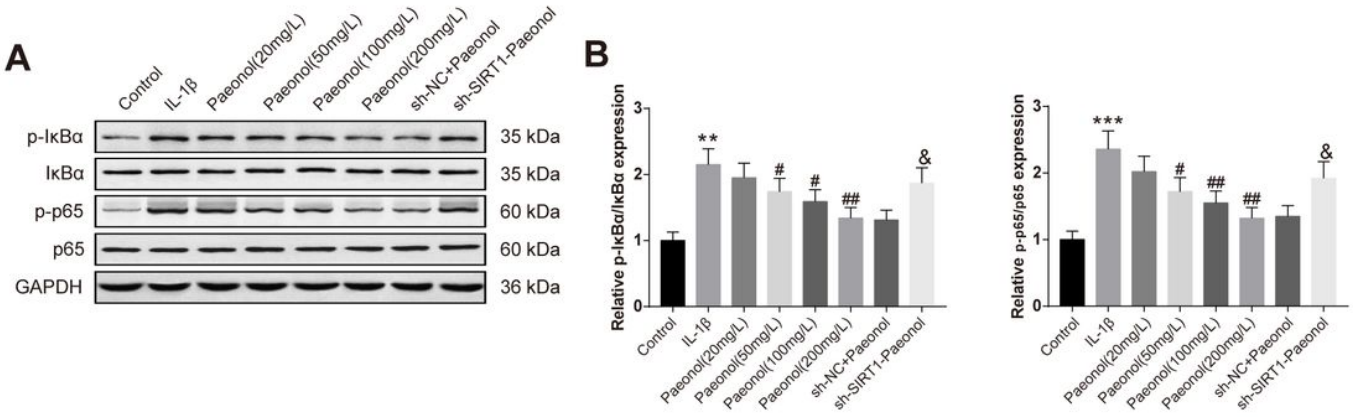

## Figure 2

Paeonol ameliorated OA-induced chondrocytes inflammation and protected cartilage. Note: Toxicity of paeonol (0, 20, 50, 100, 200, 1000 mg/L) on chondrocytes was measured using MTT assay (A); After the chondrocytes were treated with paeonol (0, 20, 50, 100, 200 mg/L) and 10 ng/ml of IL-1β, ELISA was applied to assess the expressions of TNF-α, IL-17 and IL-6 (B); qRT-PCR (C) and Western blot (D) quantified the expressions of MMP-1, MMP-3, MMP-13 and TIMP-1 in chondrocytes; apoptotic rate as measured by flow cytometry (E); cleaved-caspase-3, Bax, and Bcl-2 expressions as quantified by Western blot analysis (F). *P < 0.05, * *P < 0.01, ***P < 0.001, compared to Control group, #P < 0.05, ##P < 0.01, ###P < 0.001, compared to IL-1β group. OA, osteoarthritis.




## Figure 3

Paeonol exerted protective role through upregulating SIRT1. After the chondrocytes were treated with paeonol (0, 20, 50, 100, 200 mg/L) and IL-1β (10 ng/ml), the expression of SIRT1 as measured by qRT-PCR (A) and Western blot (B); detection on SITR1 expression by qRT-PCR (C) and Western blot (D) after the chondrocytes were transfected with sh-SIRT1 or sh-NC and then treated with paeonol (200 mg/L) and IL-1β (10 ng/ml); the expressions of TNF-α, IL-17 and IL-6 in chondrocytes as measured by ELISA (E); qRT-PCR (F) and Western blot (G) quantified the expressions of MMP-1, MMP-3, MMP-13 and TIMP-1 in chondrocytes; flow cytometry determined the apoptotic rate (H); the levels of cleaved-caspase-3, Bax and Bcl-2 were detected using Western blot (I). *P < 0.05, * *P < 0.01, compared to Control group or sh-NC + Paeonol group, #P < 0.05, ##P < 0.01, compared to IL-1β group.




## Figure 4

Inhibition of paeonol on NF-κB signaling pathway. Note: After the transfection of sh-SIRT1 or sh-NC or/and the treatment of paeonol (0, 20, 50, 100, 200 mg/L) and IL-1β (10 ng/ml), the expressions of p-IκBa and p-p65 were determined by qRT-PCR (A) and Western blot (B). **P < 0.01, ***P < 0.001, compared to Control group or sh-NC-Paeonol group, #P < 0.05, ##P < 0.01, compared to IL-1β group, &P < 0.05, compared to sh-NC-Paeonol group.