

# Vicenin 3 ameliorates ECM degradation by regulating the MAPK pathway in SW1353 chondrocytes

YUE-YUAN CHEN<sup>\*</sup>, XIAO-JIE YAN<sup>\*</sup>, XIAO-HUA JIANG,  
FENG-LAI LU, XUE-RONG YANG and DIAN-PENG LI

Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Institute of Botany,  
Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin, Guangxi 541006, P.R. China

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**Abstract.** Aberrant destruction of the articular extracellular matrix (ECM) has been considered to be one of the pathological features of osteoarthritis (OA) which results in chondrocyte changes and articular cartilage degeneration. The MAPK signaling pathway serves a key role by releasing cartilage-degrading enzymes from OA chondrocytes. However, the use of MAPK inhibitors for OA is hindered by their potential long-term toxicity. Vicenin 3 is one of the major components of the Jian-Gu injection which is effective in the clinical treatment of OA. However, its potential impact on OA remain poorly understood. Therefore, the present study aimed to assess the effects of vicenin 3 on interleukin (IL)-1 $\beta$ -treated SW1353 chondrocytes, which mimic the microenvironment of OA. These chondrocytes were pretreated with vicenin 3 (0, 5 and 20  $\mu$ M) for 1 h and subsequently stimulated with IL-1 $\beta$  (10 ng/ml) for 24 h. Nitric oxide (NO) production was measured using the Griess reaction, whereas the production of prostaglandin E2 (PGE2), matrix metalloproteinases (MMPs), A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTSs), collagen type II and aggrecan were measured using ELISA. The mRNA expression of MMPs and ADAMTSs were measured using reverse transcription-quantitative PCR. The protein expression levels

of MAPK were measured using western blotting. Vicenin 3 was found to significantly inhibit IL-1 $\beta$ -induced production of NO and PGE. Increments in the expression levels of MMP-1, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 induced by IL-1 $\beta$ , in addition to the IL-1 $\beta$ -induced degradation of collagen type II and aggrecan, were all reversed by vicenin 3 treatment. Furthermore, vicenin 3 suppressed IL-1 $\beta$ -stimulated MAPK activation, an effect that was similar to that exerted by SB203580, a well-known p38 MAPK inhibitor. In conclusion, vicenin 3 may confer therapeutic potential similar to that of the p38 MAPK inhibitor for the treatment of OA.

## Introduction

Osteoarthritis (OA) is characterized by the slow, progressive but irreversible erosion of the articular cartilage (1,2). It is also one of the most common musculoskeletal disorders and is a leading cause of disability, which results in the reduction in the quality of life in older adults. Among the various causes of OA, cartilage matrix degradation caused by inflammation is considered to be one of the main causes, which finally leads to the degradation of the extracellular matrix (ECM) (3,4). In particular, the proinflammatory cytokine interleukin (IL)-1 $\beta$  is considered to be a key inducer of OA acceleration by stimulating the release of cartilage degrading enzymes, including matrix metalloproteinases (MMPs), A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTSs) and other inflammatory mediators, such as nitric oxide (NO) and prostaglandin E2 (PGE2) (5,6). In addition, elevated levels of IL-1 $\beta$  have been previously detected in the synovial fluid and cartilage tissue of patients with OA (7). The MMP family of matrix-degrading enzymes serves an important role in OA cartilage degradation, of which MMP-13 is a key component in the catabolic processes of OA (8). It hydrolyzes protein structures in the ECM in the articular cartilage, including collagen type II and aggrecan (9). In addition, aggrecanases such as ADAMT-4 and -5 have been shown to induce degradation of the ECM by causing cleavage of proteoglycans and aggrecans within the matrix (10). These repeated cycles of inflammation and catabolism impair chondrocyte homeostasis and promote the irreversible degradation of the ECM in the cartilage, which occurs during OA (11). At present, conservative treatment options for OA mainly involve controlling pain and inflamma-

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*Correspondence to:* Professor Dian-Peng Li, Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, 85 Yanshan Street, Guilin, Guangxi 541006, P.R. China  
E-mail: phytoldp@hotmail.com

<sup>\*</sup>Contributed equally

**Abbreviations:** OA, osteoarthritis; ECM, extracellular matrix; NO, nitrite oxide; PGE2, prostaglandin E2; ADAMTS, A disintegrin-like and metalloproteinase with thrombospondin motifs; MMP, matrix metalloproteinase

**Key words:** vicenin 3, osteoarthritis, MMP-13, collagen type II and aggrecan, MAPK signaling pathway

tion using anti-inflammatory agents, including analgesics and nonsteroidal anti-inflammatory drugs, to attenuate articular cartilage injury at the early stages of OA (12,13). However, the majority of these treatment options are only short term that do not alleviate or prevent the progression of OA. In addition, they have been previously reported to cause adverse side effects, including the gastrointestinal hemorrhage (14) and renal impairment (15). By contrast, the other therapeutic strategy available, surgery, can increase risk (such as postoperative infection) and financial burden of the patient and family. Since there is currently no effective treatment strategies available to reverse OA progression, there is a demand to develop an alternative and efficient strategy to alleviate, delay or even reverse the process of OA.

*Premna fulva* Craib (Verbenaceae), also called 'Zhangu', is an ethnomedicine of the Zhuang people (a Chinese minority) that is used for the preparation of Jian-Gu injection (16). Clinically, Jian-Gu injection has been widely applied for the treatment of cervical spondylosis, lumbar sprain and OA (17-19). However, despite its use in the hospital, the mechanism of action has not been characterized in detail. Vicenin 3, which is also called (apigenin 6-C- $\beta$ -glucopyranosyl-8-C- $\beta$ -xylopyranoside (Fig. 1A), is a flavone di-C-glycoside purified from *Premna fulva* Craib that has been reported to be one of the major components in the Jian-Gu injection (20). Recently, flavonoid C-glycosides were reported to exert significant antioxidant, anti-tumor, anti-inflammatory and anti-diabetic activities (21,22). In general, C-glycosyl flavonoids exhibit more potent activity compared with that mediated by their corresponding O-glycosyl flavonoid and aglycone counterparts (23). Previous studies have shown that treatment with flavonoid C-glycosides are associated with a number of health benefits in the prevention and management of diseases such as cancer, diabetes, and cardiovascular disease (23), especially when used as a treatment for antigen-induced arthritis (24,25). However, the underlying mechanism of the effects of vicenin 3 on articular cartilage degeneration during OA remain poorly understood.

MAPK is a super family of intracellular serine-threonine protein kinases that serves as a major node of numerous signal transduction pathways (5). The MAPK pathway mainly includes three signal cascades: JNK, p38 MAPK and ERK (26,27). Previous studies have shown that the p38 signaling pathway serves a key role in the progression of several human diseases including cardiovascular disease, diabetes and cancer, especially in the development of OA (28,29). In addition, activation of the p38 MAPK signaling pathway may increase the expression of proinflammatory cytokines (IL-6, TNF- $\alpha$ ), chemokines (CCL3, CCL5), MMPs (MMP-3, MMP-13) and signaling enzymes (iNOS, COX-2) in human OA chondrocytes (30). Blocking the p38 MAPK pathway with a p38 inhibitor has been revealed to inhibit chondrocyte apoptosis and reduce the production of inflammatory cytokines to prevent the recruitment of inflammatory cells, which may alleviate bone and cartilage degradation (29). Therefore, in the present study, the effects of vicenin 3 on chondrocytes in an *in vitro* model of OA was investigated. In addition, the underlying mechanism of these effects, which particular focus on the MAPK signaling cascade, was also assessed.

## Materials and methods

**Reagents.** Vicenin 3 (purity  $\geq 98\%$ ) was isolated from *Premna fulva* Craib by recycling counter-current chromatography in Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization as previously described (20). FBS and DMEM were purchased from Gibco (Thermo Fisher Scientific, Inc.). Recombinant human IL-1 $\beta$  was purchased from R&D Systems, Inc. The Cell Counting Kit-8 (CCK-8) was purchased from Beyotime Institute of Biotechnology. The Griess reagent for NO estimation was obtained from Nanjing Jiancheng Bioengineering Institute. The ELISA kits for aggrecan (cat. no. E-EL-H0294c), collagen type II (cat. no. E-EL-H0777c), PGE2 (cat. no. E-EL-0034c), MMP-1 (cat. no. E-EL-H6073c) and MMP-13 (cat. no. E-EL-H0134c) were obtained from Elabscience Biotechnology Co., Ltd. whereas the MMP-3 (cat. no. CSB-E04677h) ELISA kit was purchased from Cusabio Biotech Co., Ltd. TRIzol reagent was purchased from Ambion; Thermo Fisher Scientific, Inc. HiScript QuantiTect Reverse Transcription kit was obtained from Vazyme Biotech Co., Ltd. Antibodies against JNK (#9252), phosphorylated (p-)-JNK (#9251), ERK (#9102), p-ERK (#9101), p38 (#8690), p-p38 (#9211) and GAPDH (#5174) were purchased from Cell Signaling Technology Inc. The p38 inhibitor SB203580 was obtained from MedChemExpress to block the activation of p38. Cultured chondrocytes were pretreated with vicenin 3 (20  $\mu$ M) or SB203580 (10  $\mu$ M) at 37°C for 1 h and then treated with 10 ng/ml IL-1 $\beta$  at 37°C for 24 h, western blot was used to detect the expression of p38, p-p38; PGE2, MMP-1, MMP-3, MMP-13, aggrecan and collagen type II were measured in the culture medium using an ELISA kit.

**Cell culture.** The human chondrocyte cell line SW1353 was acquired from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. They were cultured in DMEM containing 10% FBS and 2 mM glutamine, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin at 37°C in humidified atmosphere with 5% CO<sub>2</sub>.

**Cell viability assay.** The effects of vicenin 3 (6.25-100  $\mu$ M) on chondrocytes were determined using a CCK-8 kit according to the manufacturer's protocols. The SW1353 cells were seeded into 96-well plates (5,000 cells/well) at 37°C for 12 h and then treated with various concentrations of either vicenin 3 or IL-1 $\beta$  (10 ng/ml) alone at 37°C for 24 h. In an additional protocol, the cells were also pretreated with vicenin 3 (5 and 20  $\mu$ M) at 37°C for 1 h before IL-1 $\beta$  treatment (at 37°C for 24 h). Subsequently, 10  $\mu$ l CCK-8 was added to each well and incubated at 37°C for 4 h. Absorbance in each well was then measured at 450 nm using a microplate reader (Leica Microsystems GmbH). All experiments were performed in triplicate.

**NO measurement and ELISA.** The SW1353 cells (5,000 cells/well) were pretreated with different concentrations (5 and 20 M) of vicenin 3 at 37°C for 1 h, followed by treatment with IL-1 $\beta$  (10 ng/ml) at 37°C for 24 h, before the levels of NO accumulation in the culture medium were determined using the Griess reaction according to the manufacturer's protocols.

The protein levels of PGE2, MMP-1, MMP-3, MMP-13, aggrecan and collagen type II released from the chondrocyte

Table I. Primer sequences used for reverse transcription-quantitative PCR in the present study.

Gene	Primer	Sequence (5'-3')	PCR products
GAPDH	Forward	CAGCCTCAAGATCATCAGCA	106 bp
	Reverse	TGTGGTCATGAGTCCTTCCA	
MMP-1	Forward	CCAGGTATTGGAGGGGATG	273 bp
	Reverse	GTCACACGCTTTTGGGGTT	
MMP-13	Forward	CCCAACCCTAAACATCCAA	147 bp
	Reverse	AAACAGCTCCGCATCAACC	
MMP-3	Forward	TTCCTTGGATTGGAGGTGAC	248 bp
	Reverse	AGCCTGGAGAATGTGAGTGG	
ADAMTS-4	Forward	CAATCCTGTCAGCTTGGTGG	162 bp
	Reverse	GCTGTGTCAAAGTGGTCAGG	
ADAMTS-5	Forward	CTGCCACCACACTCAAGAAC	208 bp
	Reverse	TGGAGGCCATCGTCTTCAAT	

MMP, matrix metalloproteinase; ADAMTS, A disintegrin-like and metalloproteinase with thrombospondin motifs.

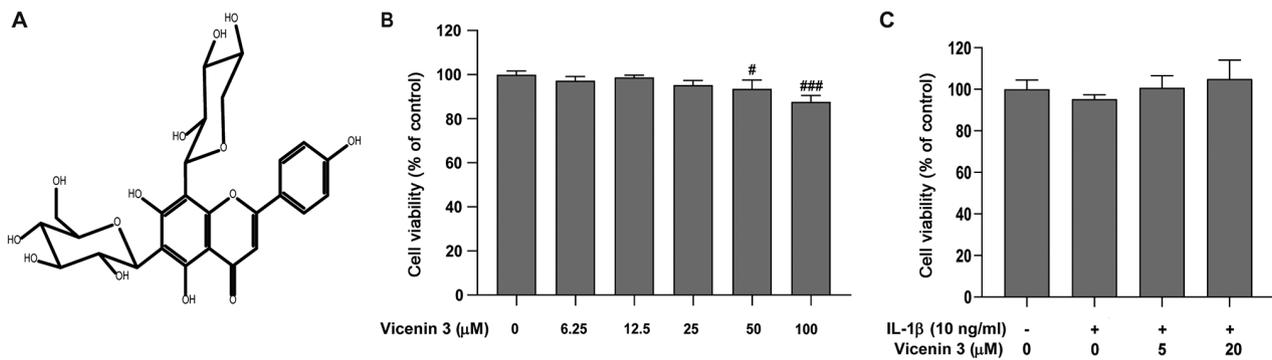


Figure 1. Effects of vicenin 3 and IL-1 $\beta$  on the cell viability of SW1353 human chondrocytes. (A) The structure of vicenin 3. Cell viability was analyzed using a Cell Counting Kit-8 assay after (B) vicenin 3 or (C) combined vicenin 3 and IL-1 $\beta$  treatment. #P<0.05 and ###P<0.001 vs. Control group. IL, interleukin.

cultured medium under the same conditions were evaluated using the ELISA kits according to the manufacturer's protocols. All assays were performed in triplicate.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was isolated from chondrocytes using TRIzol reagent (Ambion; Thermo Fisher Scientific, Inc., 15596-026) according to the manufacturer's protocol. The concentration was spectrophotometrically measured at 260 nm using NanoDrop 2000 (Thermo Fisher Scientific, Inc.). The A260/A280 ratio was calculated to test the quality and purity of the RNA samples. First-strand cDNA was synthesized from 3  $\mu$ g total RNA with the HiScript QuantiTect RT kit (Vazyme Biotech Co., Ltd, R101-01/02) at 25°C for 5 min, 50°C for 15 min, 85°C for 5 min and at 4°C for 10 min. qPCR was performed in the Bio-Rad CFX96 Touch Real-Time PCR Detection System (Bio Rad Laboratories, Inc., 1855196) under the following thermocycling conditions: 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. The level of target mRNA was normalized to the level of GAPDH and then compared with the control. mRNA expression was quantified using the 2<sup>- $\Delta\Delta$ Cq</sup> method (31). This assay was performed in triplicate. The primer sequences of the targeted genes are listed in Table I.

**Western blot analysis.** Western blotting was used to detect the protein expression and phosphorylation of JNK, ERK and p38. Total proteins were extracted from chondrocytes using RIPA (Beyotime Institute of Biotechnology) and PMSF (Shanghai Aladdin Biochemical Technology Co., Ltd) buffers. Lysates were sonicated (sonicated time: 40 sec, interval time 60 sec, power/frequency: 100 W/16 KHz) three times on finely-crushed dry ice for 5 min and centrifuged at 119 (x100 g) for 30 min at 4°C. Protein concentration was determined using the BCA protein assay kit. Protein samples (40  $\mu$ g/lane) was then separated by SDS-PAGE (10%) and transferred onto PVDF membranes. The membranes were blocked with 5% non-fat dry milk for 2 h at room temperature and subsequently washed three times for 5 min in TBS with 0.1% Tween-20 (TBST). The membranes were incubated sequentially with primary antibodies (1:1,000) against JNK, p-JNK, ERK, p-ERK, p38, p-p38 and GAPDH overnight at 4°C. After washing three times with TBST for 5 min, the membranes were incubated with HRP-conjugated secondary antibodies (BA1054, Wuhan Boster Biological Technology Co., Ltd.; 1:50,000) at room temperature for 2 h. Finally, proteins were visualized using the Immobilon Western Chemiluminescent HRP substrate (cat. no. WBKLS0500; EMD Millipore), and then the blots

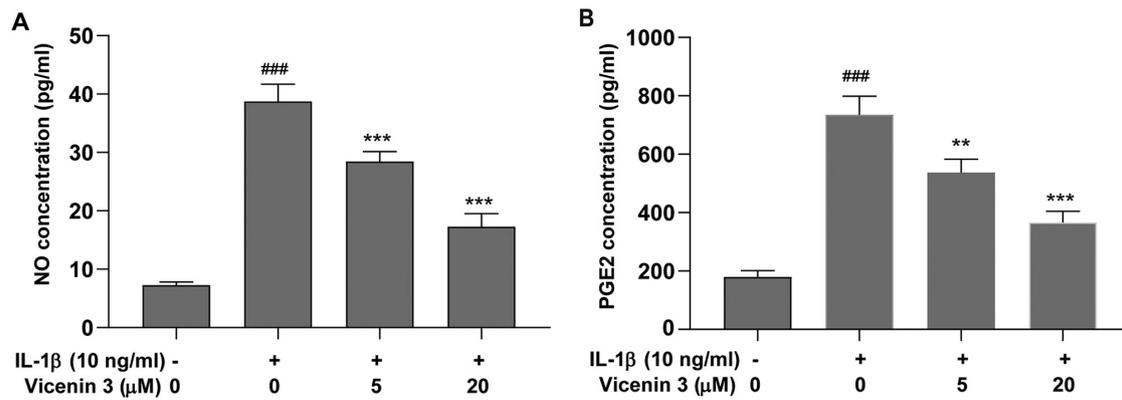


Figure 2. Effect of vicenin 3 on the IL-1 $\beta$ -induced NO and PGE2 production in SW1353 human chondrocytes. (A) NO levels in the culture medium were assessed using the Griess reaction. (B) PGE2 levels were determined using ELISA. ###P<0.001 vs. Control group. \*\*P<0.01 and \*\*\*P<0.001 vs. IL-1 $\beta$ . IL, interleukin; NO, nitric oxide; PGE2, prostaglandin E2.

were analyzed using the ChemiDoc XRS system (Bio Rad Laboratories, Inc.). The density of the protein bands was quantified using Image Lab 3.0 software (Bio-Rad Laboratories, Inc.). The relative protein levels of the target protein in each sample were determined before the levels of phosphorylated protein were normalized to those of their corresponding total protein.

**Statistical analysis.** The results are expressed as the mean  $\pm$  standard deviation. All assays were performed in triplicate. One-way analysis of variance followed by the Dunnett's test was used for multiple comparisons using the GraphPad Prism 8.0 software (Graphpad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

## Results

**Effects of vicenin 3 on SW1353 chondrocyte cytotoxicity.** The potential cytotoxicity of vicenin 3 on SW1353 chondrocytes was tested using CCK-8 assay. Vicenin 3 at the concentration range of 6.25–25  $\mu$ M did not exert cytotoxic effects on the cells. However, significant reductions in cell viability was observed at concentrations >50  $\mu$ M (Fig. 1B). In addition, 5 and 20  $\mu$ M vicenin 3 did not alter chondrocyte viability in combination with inflammatory stimuli (10 ng/ml IL-1 $\beta$ ; Fig. 1C). Therefore, 5 and 20  $\mu$ M vicenin 3 were chosen for subsequent experiments.

**Effect of vicenin 3 on NO and PGE2 production in chondrocytes.** The possible effects of vicenin 3 on IL-1 $\beta$ -induced NO and PGE2 production in SW1353 cells were next investigated. SW1353 cells were pretreated with vicenin 3 (5 and 20  $\mu$ M) for 1 h before subsequent IL-1 $\beta$  (10 ng/ml) stimulation for 24 h. NO concentration in the cell suspension was then determined using the Griess reaction whereas PGE2 levels were measured using ELISA. As shown in Fig. 2, the levels of NO and PGE2 in the supernatant were significantly increased after IL-1 $\beta$  treatment, which were significantly reversed by vicenin 3 in a dose-dependent manner.

**Effect of vicenin 3 on expression and secretion of MMP-1, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 in IL-1 $\beta$ -induced SW1353 chondrocytes.** ECM degradation by

matrix degrading enzymes, such as MMPs is a characteristic feature of OA (32). Therefore, the effect of vicenin 3 on the secretion levels of MMP-1, MMP-3 and MMP-13 was evaluated in IL-1 $\beta$ -induced SW1353 cells. As shown in Fig. 3A and B, IL-1 $\beta$  significantly upregulated the secretion and mRNA expression levels of MMP-1, MMP-3 and MMP-13, whilst treatment with the higher dose of vicenin 3 (20  $\mu$ M) resulted in the significant reversal of this IL-1 $\beta$ -induced increase in MMP-1, MMP-3 and MMP-13 mRNA expression and protein secretion. Although 5  $\mu$ M vicenin 3 did not significantly affect the secretion of MMP-3, it did significantly reverse the IL-1 $\beta$ -induced increase in MMP-1, MMP-3 and MMP-13 mRNA expression and MMP-1 and MMP-13 secretion. In addition, treatment with vicenin 3 significantly reversed the IL-1 $\beta$ -induced increase in ADAMTS-4 and ADAMTS-5 mRNA expression (Fig. 3C and D). These results suggest that vicenin 3 reduced the IL-1 $\beta$ -induced expression of matrix degrading enzymes in SW1353 chondrocytes.

**Effect of vicenin 3 on collagen type II and aggrecan secretion by IL-1 $\beta$ -induced SW1353 chondrocytes.** The effects of vicenin 3 on the secretion of collagen type II and aggrecan, major components of ECM in the articular cartilage (33), by IL-1 $\beta$ -stimulated SW1353 chondrocytes was next assessed using ELISA. As shown in Fig. 4, collagen type II and aggrecan levels were significantly decreased by IL-1 $\beta$ , whereas pre-treatment with vicenin 3 (5 and 20  $\mu$ M) significantly prevented this IL-1 $\beta$ -induced decrease in the secretion of collagen type II. In addition, vicenin 3 (20  $\mu$ M) significantly prevented the IL-1 $\beta$ -induced decrease in aggrecan expression. These results suggest that vicenin 3 has potent cartilage matrix-protective effects by suppressing IL-1 $\beta$ -induced degradation of collagen type II and aggrecan in SW1353 chondrocytes.

**Effect of vicenin 3 on MAPK activation in IL-1 $\beta$ -induced SW1353 chondrocytes.** Since IL-1 $\beta$ -induced MMP expression was previously found to be mediated by members of the MAPK kinase family, such as ERK, JNK, and p38 MAPK (34), the potential effects of vicenin 3 on the activity of these three MAPK kinases were next examined. In IL-1 $\beta$ -stimulated chondrocytes, the phosphorylation of ERK, JNK and p38 MAPK was found to be activated compared

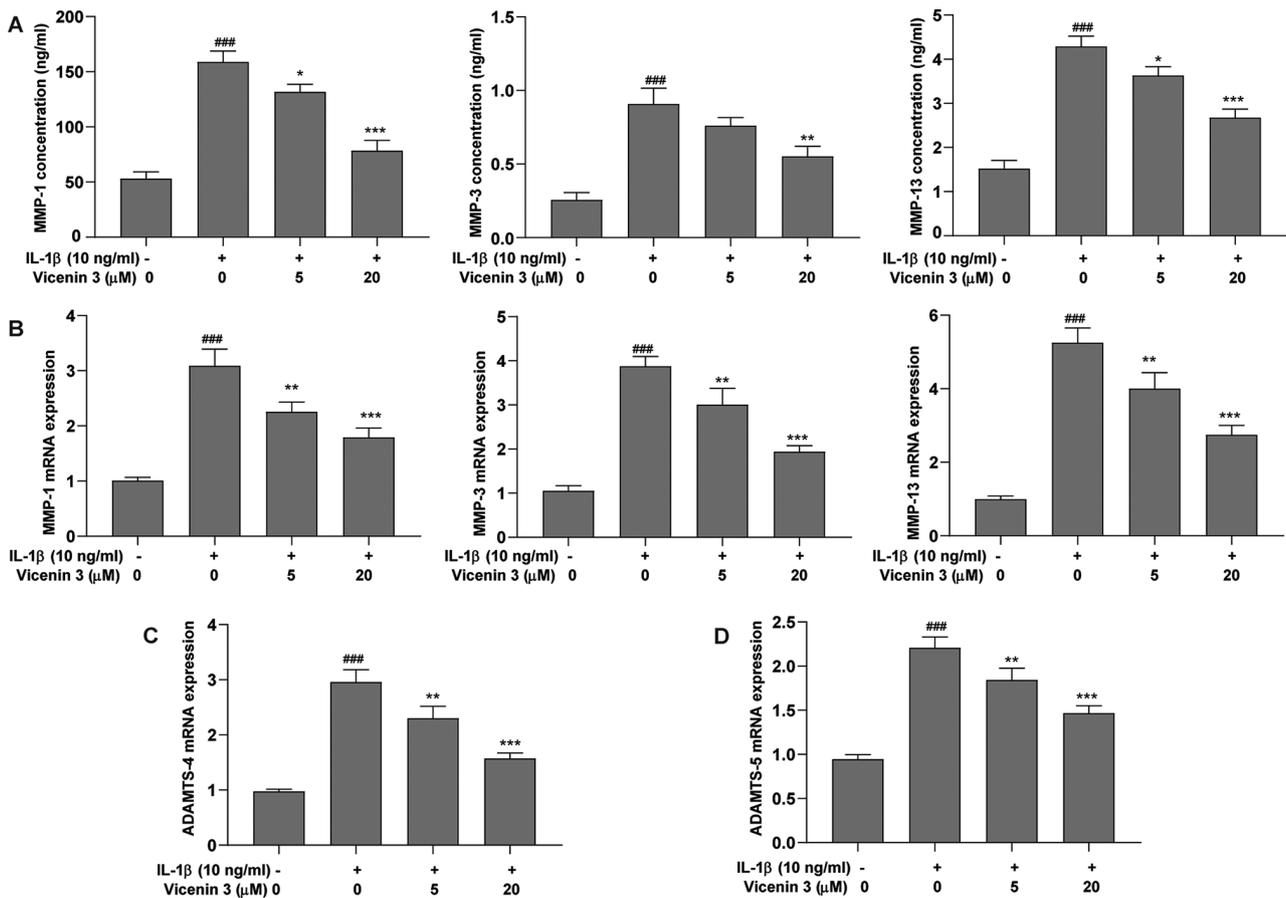


Figure 3. Effect of vicenin 3 on IL-1β-induced MMP-1, MMP-3, MMP-13, and ADAMTS-4 and ADAMTS-5 in SW1353 human chondrocytes. (A) The protein secretion levels of MMP-1, MMP-3 and MMP-13 were determined using ELISA. The mRNA expression levels of (B) MMP-1, MMP-3, MMP-13, (C) ADAMTS-4 and (D) ADAMTS-5 were assayed using reverse transcription-quantitative PCR. ###P<0.001 vs. Control group. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 vs. IL-1β. IL, interleukin; MMP, matrix metalloproteinase; ADAMTS, A disintegrin-like and metalloproteinase with thrombospondin motifs.

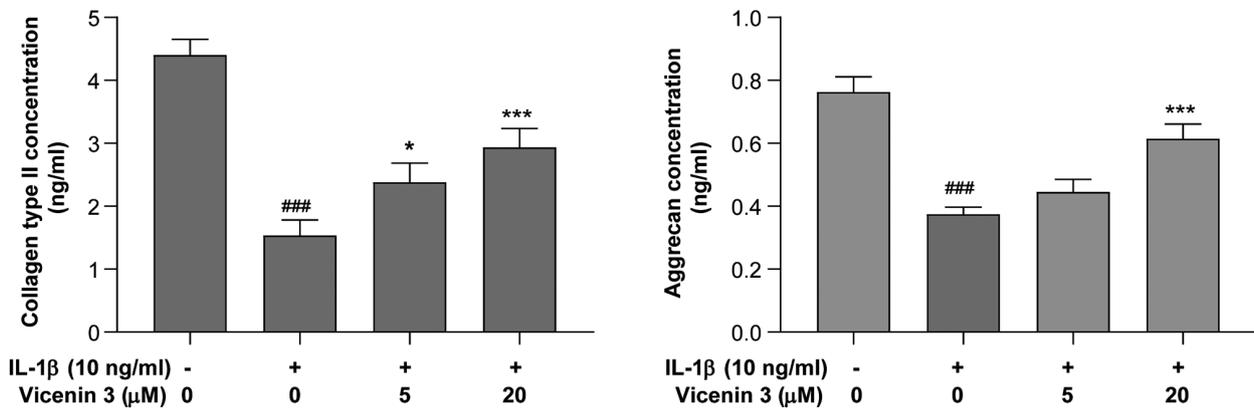


Figure 4. Effect of vicenin 3 on IL-1β-induced collagen type II and aggrecan expression in SW1353 human chondrocytes as measured using ELISA. ###P<0.001 vs. Control group. \*P<0.05 and \*\*\*P<0.001 vs. IL-1β. IL, interleukin.

with that in the control group but their corresponding total protein expression levels were not significantly affected (Fig. 5). However, pretreatment with vicenin 3 significantly reversed the IL-1β-induced phosphorylation of ERK, JNK and p38 MAPK (Fig. 5). These results suggest that the suppressive effects of vicenin 3 on the IL-1β-induced expression of cartilage degrading enzymes was mediated through MAPK signaling in SW1353 chondrocytes.

*Vicenin 3 ameliorates the degradation of ECM in IL-1β-treated SW1353 chondrocytes by blocking the p38 signaling pathway.* Western blot analysis demonstrated that vicenin 3 exerted a significant inhibitory influence on IL-1β-induced activation of ERK, JNK and p38 MAPK (Fig. 5). p38 MAPK has been reported to be associated with OA and has been considered to be a target for drug-mediated modulation in OA (35,36). Therefore, it was investigated whether vicenin 3 and the

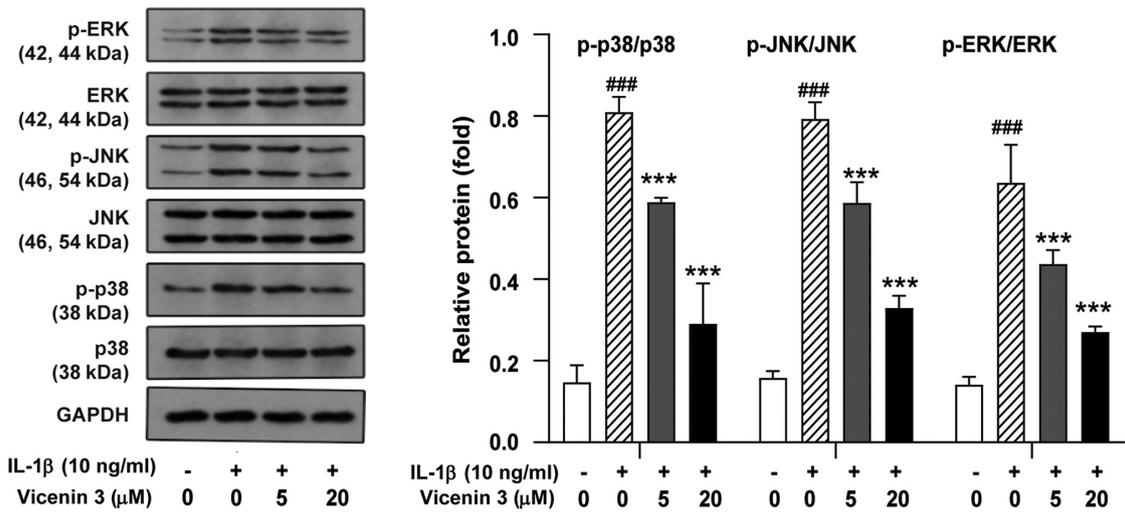


Figure 5. Effect of vicenin 3 on IL-1β-induced MAPK activation in SW1353 human chondrocytes. The protein levels of p-JNK, JNK, p-ERK, ERK, p-p38 and p38 were measured using western blotting and quantified. ###P<0.001 vs. Control group. \*\*\*P<0.001 vs. IL-1β. IL, interleukin.

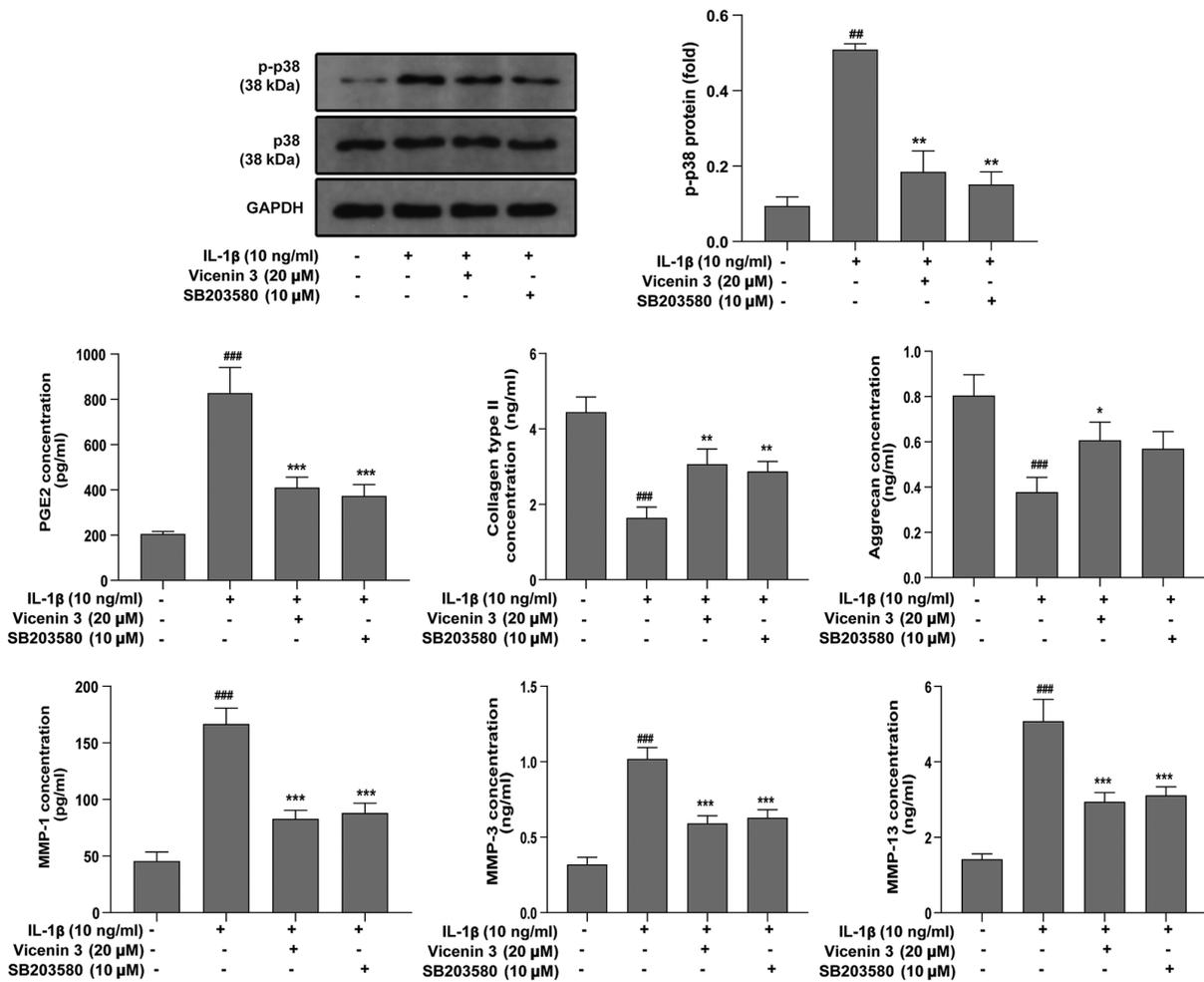


Figure 6. Effect of vicenin 3 and SB203580 on the degradation of ECM in IL-1β-treated SW1353 human chondrocytes and the p38 MAPK signaling pathway. The protein levels of p-p38 and total p38 were measured by western blot analysis. PGE2 production and protein secretion levels of MMP-1, MMP-3, MMP-13, aggrecan and collagen type II were measured in the culture medium using an ELISA kit. ##P<0.01 and ###P<0.001 compared with control group. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 vs. IL-1β. ECM, extracellular matrix; IL, interleukin; PGE2, prostaglandin E2; MMP, matrix metalloproteinase.

p38MAPK inhibitor (SB203580) had similar efficacy in IL-1β-stimulated SW1353 chondrocytes. As shown in Fig. 6, vicenin 3 was also found to exert similar effects compared with those mediated by SB203580, a p38 inhibitor, on blocking

p38 activation in IL-1 $\beta$ -stimulated SW1353 chondrocytes. Subsequently, in IL-1 $\beta$ -incubated SW1353 chondrocytes, which exhibited significantly higher protein secretion levels of PGE2, MMP-1, MMP-3 and MMP-13 compared with those in the control group, treatment with vicenin 3 or SB203580 significantly reduced the secretion of these enzymes (Fig. 6). By contrast, analysis of ELISA results revealed that the secretion levels of collagen type II by SW1353 chondrocytes was significantly decreased by IL-1 $\beta$ , which was significantly reversed by vicenin 3 or SB203580 treatment (Fig. 6). However, vicenin 3 exhibited a more potent inhibitory effect on the secretion of aggrecan compared with that by SB203580 (Fig. 6). Therefore, these results demonstrated that vicenin 3-related amelioration of ECM degradation by SW1353 chondrocytes was associated at least in part with the blocking of p38 MAPK signaling.

## Discussion

OA is a degenerative joint disease that is frequently observed among the elderly and exerts great burden on the society and economy due to the lack of therapeutic methods (37). The underlying pathological mechanism of OA remains to be fully elucidated. However, it is generally accepted that OA is caused at least in part by degradation of the cartilaginous matrix because of the inflammation-induced upregulation of catabolism in chondrocytes (4). A number of studies have highlighted the importance of chondrocyte function in the development of OA, since they are the only type of cells in the articular cartilage and are responsible for maintaining the anabolic and catabolic balance in the cartilage ECM (38,39). In the present study, a protective effect of vicenin 3 on chondrocyte was found, which was mediated through the inhibition of various pathological factors affecting OA, including NO-induced stress, degradation of articular ECM and the expression of pro-inflammatory cytokines and mediators. This effect was found to be due to the inhibition of MAPK signaling. Application of various p38 inhibitors, including synthetic pyridine imidazole small molecular compounds, for OA has conferred disappointing results owing to their long-term toxicities, such as gastrointestinal disorders, dizziness and rashes (40). Vicenin 3 is a natural flavone di-C-glycoside that can be purified from *Premna fulva* Craib (20). In the present study, it was found to suppress IL-1 $\beta$ -stimulated MAPK activation in a similar manner to that exerted by the p38 MAPK inhibitor SB203580. Taken together, these findings suggest that vicenin 3 may have therapeutic potential as a p38 MAPK inhibitor for the treatment of OA.

Inflammation is a potent catabolic inducer of the cartilage during OA pathogenesis, which apparently disrupts tissue maintenance and functionality as a result of chondrocyte apoptosis and hinders the regenerative ability of the joint (41). In particular, IL-1 $\beta$  has been found to trigger the initiation and acceleration of ECM degradation by chondrocytes, by stimulating the release of cartilage-degrading enzymes in the MMP and ADAMTS families and other catabolic factors, including NO and PGE2 (6). This consequently contributes to chondrocyte dysfunction (6). As a mediator of the inflammatory response, NO and PGE2 is involved in a number of inflammatory degenerative diseases, including OA and chronic neuronal diseases (42,43). In particular, the increase

of inflammatory mediators such as NO and PGE2 by IL-1 $\beta$  alleviate OA pathogenesis (44). Therefore, inhibition of these inflammatory mediators would be of therapeutic potential by assessing the effect of potential anti-inflammatory drugs on the joint degenerative process induced by inflammation. In the present study, the production of NO and PGE2 were increased by IL-1 $\beta$  treatment, but pre-treatment with vicenin 3 reverse this effect.

MMPs belong to a family of proteolytic enzymes and can facilitate OA development by mediating the irreversible degradation of collagen type II and proteoglycan in the cartilage (4). In particular, MMP-13 is considered to be a key player in the catabolic processes during OA because of its ability to degrade collagen type II and proteoglycan (9). In addition, ADAMTS-4 and -5 are considered to be the main enzymes responsible for the degradation of aggrecan and associated with OA (10). Therefore, the level of cartilage matrix components (collagen type II and proteoglycan) can be used as indicators for assessing the progression of cartilage destruction. In the present study, vicenin 3 inhibited the IL-1 $\beta$ -induced secretion and mRNA expression of MMP-1, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 in SW1353 chondrocytes. In addition, vicenin 3 reversed the IL-1 $\beta$ -induced reduction of collagen type II and aggrecan secretion. These results suggest that vicenin 3 has a protective effect on chondrocytes to inhibit the development of OA following IL-1 $\beta$ -related induction.

Numerous intracellular signaling systems have been reported to participate in regulating OA (45,46). Among these, MAPKs have been extensively studied, where they were reported to be involved in the development and progression of OA (47). In addition, activation of p38MAPK has been revealed to be associated with cartilage collagen degradation, chondrocyte apoptosis and the inflammation process in OA (35,48.). Therefore, p38 MAPK was proposed to be a therapeutic target for OA (36). In the present study, vicenin 3 was able to reduce the phosphorylation of MAPKs (ERK1/2, JNK and p38) *in vitro* induced by IL-1 $\beta$  stimulation. These results suggest that vicenin 3 may have the therapeutic potential for the treatment of OA by blocking MAPK signaling pathways.

In conclusion, IL-1 $\beta$ -induced production of inflammatory factors NO and PGE2 and cartilage-degrading enzymes MMP-1, MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 by SW1353 chondrocytes were found to be significantly reversed by vicenin 3 (especially at a concentration of 20  $\mu$ M) in the present study. Furthermore, vicenin 3 reversed the reduction in the production of ECM components collagen type II and aggrecan by SW1353 chondrocytes following IL-1 $\beta$  treatment. These findings suggest that vicenin 3, which is a main component of Jian-Gu injection, exerts protective effects against IL-1 $\beta$ -treated OA development.

Despite determining the discovery of the protective effects of vicenin 3 and the associated underlying mechanism of action using an *in vitro* OA model, the present study also has limitations. Although comparison to a previous study showed that SW1353 human chondrosarcoma is commensurate with the *in vitro* primary experimental human chondrocyte system (49), it would be more valuable to use chondrocytes isolated from patients with OA. In addition, further validation studies, such as the recommended optimal dose for use *in vivo*, are also required. Vicenin 3 is one of the five structurally related

flavone C-glycoside components reported to be present in the Jian-Gu injection (20). Therefore, further studies are required to clarify the protective effects of other flavone C-glycoside compounds on chondrocytes.

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### Availability of data and materials

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

### Authors' contributions

YYC and DPL designed this study. XJY and XHJ performed mechanistic and phenotypic experiments, respectively. XJY, XRY, and YYC analyzed the data and interpreted the results of the experiments. FLL analyzed the data and contributed to manuscript revision. DPL and FLL confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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