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Research article

Epimedin C alleviated osteoarthritis development by regulating chondrocyte Nrf2-mediated NLRP3 inflammasome axis

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A R T I C L E I N F O

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A B S T R A C T

Osteoarthritis (OA) is a prevalent musculoskeletal disorder globally. This study explored the therapeutic potential of Epimedin C (Epi C) in OA and its mechanisms. We isolated primary chondrocytes from mice and induced inflammatory damage using interleukin-1β (IL-1β) to evaluate Epi C's capacity to preserve cell viability and inhibit apoptosis, employing cell counting kit (CCK8) assays, EdU staining, and flow cytometry. Additionally, its anti-inflammatory effects were quantified using enzyme-linked immunosorbent assay (ELISA), Western blot, and real-time fluorescence quantitative polymerase chain reaction (RT-qPCR), alongside assessments of extracellular matrix (ECM) degradation. *In vivo*, OA was induced in mice through destabilization of the medial meniscus (DMM), followed by Epi C administration. Cartilage integrity was evaluated via micro-computed tomography (CT) and histology. Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway involvement was investigated through siRNA knockdown and oxidative stress markers, while NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome expression was measured to establish Epi C's modulatory effect. Our study revealed that Epi C protected against IL-1β-induced chondrocyte damage by enhancing cell viability, reducing apoptosis, and dampening inflammatory responses. The *in vivo* studies demonstrated Epi C's role in preserving cartilage structure, activating nuclear factor erythroid 2-related factor 2 (Nrf2), and inhibiting NLRP3 expression in DMM-induced OA mice. Conclusively, our findings provide substantial evidence of Epi C's therapeutic efficacy in OA, primarily through its modulation of the Nrf2-mediated NLRP3 inflammasome pathway, offering novel insights into its management role in OA.

1. Introduction

Osteoarthritis (OA) is a prevalent chronic degenerative joint disease primarily affecting the elderly population [1]. It is

Abbreviations: OA, Osteoarthritis; Epi C, Epimedin C; IL-1β, interleukin-1β; ECM, extracellular matrix; DMM, destabilization of medial meniscus; Nrf2, Nuclear factor erythroid 2-related factor 2; NLRP3, NOD-like receptor thermal protein domain associated protein 3; ROS, reactive oxygen species; H&E, hematoxylin and eosin; OARSI, Osteoarthritis Research Society International; HC, hyaline cartilage; CC, calcified cartilage; CT, Micro-computed tomography; BSA, bovine serum albumin; TNF-α, tumor necrosis factor-α; NO, nitric oxide; PGE2, prostaglandin E2; MDA, malonaldehyde; PBS, phosphate buffer saline; RT-PCR, Real-time polymerase chain reaction; DHE, dihydroethidium.

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characterized by the gradual erosion and breakdown of joint cartilage, resulting in pain, swelling, and functional impairment. Contributing factors include aging, overuse, genetics, joint injuries, and obesity [2]. OA significantly impacts the quality of life for affected individuals and is a leading cause of joint-related disabilities worldwide. Current treatments focus on pain relief, inflammation reduction, and preserving joint function, encompassing medication, physical therapy, and surgical interventions. Despite the range of available treatment options, effectively managing OA over the long term remains a significant challenge in both clinical practice and scientific research [3].

Research indicates a strong correlation between osteoarthritis and factors like inflammatory responses and cellular apoptosis. These elements disrupt the balance between the synthesis and catabolism metabolism of chondrocyte extracellular matrix (ECM), ultimately leading to its degradation [4]. This degradation process initiates and exacerbates the development of osteoarthritis. Emerging evidence highlights the crucial role of pro-inflammatory cytokines in the degradation of the articular cartilage matrix [5]. Both external stimuli and internal injury signals can trigger the production of reactive oxygen species (ROS), which in turn activate the NLRP3 inflammasome [6]. Studies on fibroblast-like synovial cells, animal OA models, and knee OA patients have demonstrated the significant role of NLRP3 in this condition. These findings underscore the potential of anti-inflammatory therapies targeting the NLRP3 inflammasome as a promising strategy for treating OA.

Nuclear factor erythroid 2-related factor 2 (Nrf2) serves as a crucial transcription factor within the body's anti-oxidative stress defense system. When exposed to oxidative stress conditions, Nrf2 initiates endogenous defense mechanisms and preserves mitochondrial integrity [7]. This process is instrumental in upholding cellular oxidative homeostasis and attenuating the detrimental impact of oxidative stress on cellular structures. Recent studies have demonstrated that Nrf2 inhibits NLRP3-mediated inflammation in OA [8]. Thus, regulating this pathway is a promising therapeutic chance for the amelioration of osteoarthritis.

In recent years, significant progress has been made in the research on traditional Chinese medicine for the treatment of osteoarthritis [9]. Numerous herbal extracts and medicinal plants have been discovered to exhibit anti-inflammatory, analgesic, and joint function-enhancing properties. For instance, components like loganin [10], nodakenin [11], and curcuminoids [12] have been scientifically proven to possess the potential for alleviating OA symptoms. Epimedium is recognized for its varied pharmacological applications, encompassing anti-tumor, anti-diabetic, hepatoprotective, and anti-osteoporosis functions [13]. Epimedin C (Epi C) is a natural flavonoid compound commonly extracted from plants belonging to the Epimedium genus [14]. Recent research has unveiled its diverse biological properties, including antioxidative [15], anti-inflammatory [16], anticancer effects [17]. Furthermore, investigations have delved into the potential applications of Epi C in the treatment of bone and joint diseases [18]. Nevertheless, the protective effect of Epi C on OA and its underlying mechanisms remain unclear.

This study aims to identify and explore the potential therapeutic effects of Epi C on OA. The study evaluated the chondroprotective properties and therapeutic efficacy of Epi C through *in vitro* induction of mouse chondrocytes with IL-1β and an OA model induced by DMM surgery. Additionally, the study investigated the regulatory role of the Nrf2/NLRP3 signaling pathway in mediating these effects. Our research provides valuable insights into OA treatment and lays a significant foundation for the development of effective therapeutic strategies.

2. Method

2.1. Material and reagents

Epimedin C (Epi C, purity >98 %, CAS: 110642-44-9) was purchased from MedChemExpress (NJ, USA). Antibodies are as follows: Aggrecan (1:1000, Abcam, ab313636), Collagen II (1:1000, Abcam, ab307674), MMP13 (1:1000, Cell signaling technology, #69926), ADAMTS5 (1:1000, Cell signaling technology, #12897), iNOS (1:1000, Cell signaling technology, #13120), COX-2 (1:1000, Cell signaling technology, #12282), Nrf2 (1:1000, Cell signaling technology, #12721), SOD-2 (1:1000, Cell signaling technology, #13141), NQO1 (1:1000, ABclonal, A23486), HO-1 (1:1000, Cell signaling technology, #70081), NLRP3 (1:1000, Cell signaling technology, #15101), ASC (1:1000, Abcam, ab283684), Pro-Caspase-1 (1:1000, Cell signaling technology, #24232), Caspase-1 P10 (1:1000, abcam, ab179515), IL-1β (1:1000, Cell signaling technology, #12242), GAPDH (1:1000, Cell signaling technology, #2118) and Rabbit anti-goat secondary antibody (1:1000, ABclonal, AS014). Hochest (#4082) was acquired from Cell signaling technology. RIPA lysate (P0013B) was purchased from Beyotime Biotechnology. JC-1 dyeing reagent was obtained from ThermoFisher Scientific.

2.2. Construction and treatment of the mice OA model

Male C57BL/6J mice aged 12 weeks were randomly divided into four groups: control, model, and Epi C (20, 40 mg/kg). Post-traumatic osteoarthritis (OA) was induced using destabilization of medial meniscus (DMM) surgery. In brief, anesthesia was induced in the mice through intraperitoneal injection of 2 % pentobarbital. The medial menisco-tibial ligaments were delicately incised using a microsurgical bistoury during the surgery. In the sham-operated group, knee joint incisions were made without removing any tissue. All animal experiments were conducted in strict compliance with relevant regulations and were approved by the Ethics Committee of Nanjing University of Chinese Medicine (Ethics No. ACU230307). After 8 weeks, the animals were euthanized, and knee joint samples were collected for subsequent analysis.

2.3. Histopathology

The knee joint samples underwent a fixation process in 4 % paraformaldehyde for 48 h, followed by decalcification in 10 % thylene

diamine tetraacetic acid (EDTA) over a period of 4 weeks. The knee joints were then cut into slices measuring 6 μm in thickness. Then, the samples were subjected to staining procedures, including hematoxylin and eosin (H&E), as well as Safranin O (S.O.)/Fast Green staining. Experimental images were captured using an upright microscope (Olympus Optical Ltd, Tokyo, Japan). Cartilage degeneration levels were assessed using the Osteoarthritis Research Society International (OARSI) scoring system and the hyaline cartilage (HC) versus calcified cartilage (CC) ratio.

2.4. Micro-computed tomography (CT)

After the knee joints of mice were fixed in 4 % paraformaldehyde, micro-CT imaging was performed on the knee joint samples using the Skyscan-1176 scanning system (USA). Subsequently, the acquired scan data were reconstructed using CT-Vol software.

2.5. Immunofluorescent staining

Slides were incubated with anti-Nrf2 and anti-NLRP3 antibodies at 4 °C overnight. After a 1 h incubation with secondary antibodies, cells were stained with DAPI for nuclear visualization. Images were observed and captured under a confocal laser scanning microscope (Leica, Heidelberg, Germany).

Cells were fixed with 4 % paraformaldehyde for 30 min, permeabilized with 1 % Triton X-100 (Sigma-Aldrich), and blocked with bovine serum albumin (BSA) for 1 h. Subsequently, cells were incubated overnight with anti-Collagen II and anti-Nrf2 primary antibodies. After washing with PBS, cells were incubated with secondary antibodies for 2 h and stained with Hoechst for nuclear visualization. The samples were observed under a confocal laser scanning microscope (Leica, Heidelberg, Germany).

2.6. Isolation and culture of chondrocytes

Articular cartilage samples of mice were harvested and subjected to enzymatic digestion for the isolation of chondrocytes. The isolated cells were then cultured *in vitro* according to the protocol established by Bar Oz et al. [19]. Briefly, sterile cartilage tissues were cut into small pieces measuring 0.5–1.0 mm. These cartilage fragments were then subjected to a 30-min treatment with 0.1 % trypsin. Following this, a digestion process was carried out using 0.2 % recombinant collagenase II in a 5 % CO_2 environment at 37 °C for 6 h. The resulting digest was centrifuged to isolate the chondrocytes. After rinsing with PBS, the isolated chondrocytes were suspended in DMEM/F-12 supplemented with 10 % FBS and 100 U/mL of penicillin-streptomycin. The resuspended chondrocytes were subsequently cultured in an incubator at 37 °C with 5 % CO_2. To replicate the *in vitro* OA environment, the culture medium was switched to DMEM-F12 supplemented with 10 % FBS, 1 % penicillin-streptomycin, and IL-1β at a concentration of 10 ng/mL. Chondrocytes at passages 1 to 2 were used for the subsequent experiment.

2.7. Cell viability assay

Cells were seeded into a 96-well plate and allowed to adhere. Cells were then stimulated with 10 ng/mL IL-1β (Beyotime Biotechnology) for 24 h. After treatment with Epi C (10, 20 μM) for 24 h, the culture medium was aspirated carefully, and a 10 % CCK-8 (Dojindo Molecular Technologies, Inc.) reagent was added to each well. Following an incubation at 37 °C for 1 h, the absorbance was measured at a wavelength of 450 nm using a microplate reader.

2.8. Flow cytometry

Apoptotic cells were detected using the Cell Apoptosis Detection Kit (Beyotime Biotechnology). Briefly, cells were collected and incubated with 10 μL Annexin V and 5 μL PI. After adjusting cell density with staining buffer, samples were detected using flow cytometry (Thermo Fisher Scientific, USA). Data analysis was performed using FlowJo software.

2.9. EdU staining

Cell proliferation was assessed using a cell proliferation assay kit (Beyotime Biotechnology). The reaction mixture was prepared according to the instructions and added to the cells under examination, followed by incubation at room temperature in the dark for 30 min. Cells were washed three times with washing solution, each time for 5 min. Nuclei were stained with Hoechst dye. Observation and recording were conducted under a fluorescence microscope (Olympus Optical Ltd, Tokyo, Japan).

2.10. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of tumor necrosis factor-α (TNF-α), IL-6, nitric oxide (NO), prostaglandin E2 (PGE2) and malonaldehyde (MDA) were quantified using ELISA, following the manufacturer's instructions (Elabscience Biotechnology Co. Ltd). In brief, samples were added to the ELISA plate, followed by the addition of detection antibodies. After incubation and subsequent washing steps, enzyme was introduced, followed by additional incubation and washing. Substrate was then added for color development, and the absorbance was measured using an microplate reader.

2.11. Western blot assay (WB)

The cells were rinsed with ice-cold phosphate buffer saline (PBS) and lysed with lysis buffer supplemented 1 % PMSF. The total protein concentration was examined using BCA assay (Beyotime Biotechnology). Equal protein was separated by SDS-PAGE, transferred to PVDF membranes, blocked in 5 % milk for 1 h and subsequent incubated at 4 °C overnight with a primary antibody. The membrane was incubated for 1 h with secondary antibody at room temperature. Protein bands were visualized with enhanced chemiluminescence and were analyzed using the Image Lab software.

2.12. Real-time polymerase chain reaction (RT-PCR)

RNA was extracted from tissue samples using Trizol reagent, and the RNA concentration was measured using a NanoDrop 2000 UV–Vis spectrophotometer. Following the instructions provided with the *Evo M-MLV* reverse transcription kit, RNA was converted into cDNA. Specific primers were designed, and the reaction mixture was prepared using the SYBR® Green Pro Taq HS premix qPCR kit. Real-time PCR was conducted using a qPCR machine. The relative cDNA levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers are as follows:

Gene	Sequence
Aggrecan	Forward: 5′-CTCACCCCAAGAATCAAGTGG-3′ Reverse: 5′-GATCTCCAAGGTAGCATCGC-3′
Collagen II	Forward: 5′-CTTAGTGCAGGAAACTTCGCG-3′ Reverse: 5′-ACCAGGATTGCCTTGAAATCC-3′
ADAMTS5	Forward: 5′-GCAAAGTGGGCTACCTTGTC-3′ Reverse: 5′-GTTTCTACAGAGGCACCGTG-3′
MMP13	Forward: 5′-GATGGACCTTCTGGTCTTCT-3′

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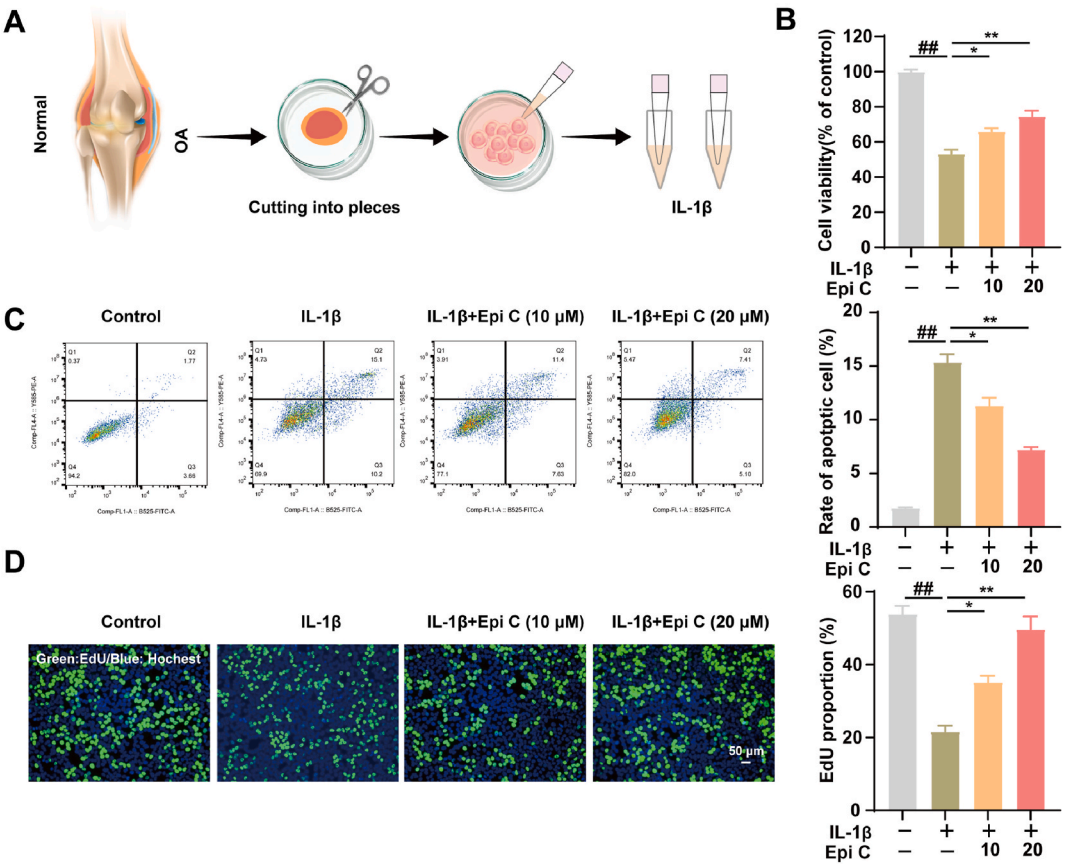

Fig. 1. Effect of Epi C on IL-1β-induced chondrocytes. (A) Diagram of chondrocyte extraction process. (B) The effect of Epi C on cell viability was evaluated by CCK8. (C) The effect of EpiC on chondrocyte apoptosis was detected by flow cytometry. (D) Representative images stained by EdU after EpiC administration. Data represented the mean ± SEM from three independent experiments. n = 3 for each group. $^{\#\#}p < 0.01$ vs. Control group; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. IL-1β group.

(*continued*)

Gene	Sequence
TNF-α	Reverse: 5′-GCTCATGGGCAGCAACAATA-3′ Forward: 5′-ACTCCAGGCGGTGCCTATG-3′
INOS	Reverse: 5′-GTGAGGGTCTGGGCCATAGAA-3′ Forward: 5′-CTCCTGCCTCATGCCATTG-3′
IL-6	Reverse: 5′-AGCTCATCCAGAGTGAGCTG-3′ Forward: 5′-TCCAGTTGCCTTCTTGGGAC-3′
COX-2	Reverse: 5′-CTGTTGGGAGTGGTATCCTC-3′ Forward: 5′-GATAACCGAGTCGTTCTGCC-3′
GAPDH	Reverse: 5′-AATCCTGGTCGGTTTGATGC-3′ Forward: 5′-TGTTTCCTCGTCCCGTAGAC-3′ Reverse: 5′-GTTGAGGTCAATGAAGGGGTC-3′

2.13. Dihydroethidium (DHE) staining

Cells were incubated with 2 μM dihydroethidium (Sigma–Aldrich) in the dark at room temperature for 30 min. After washing with

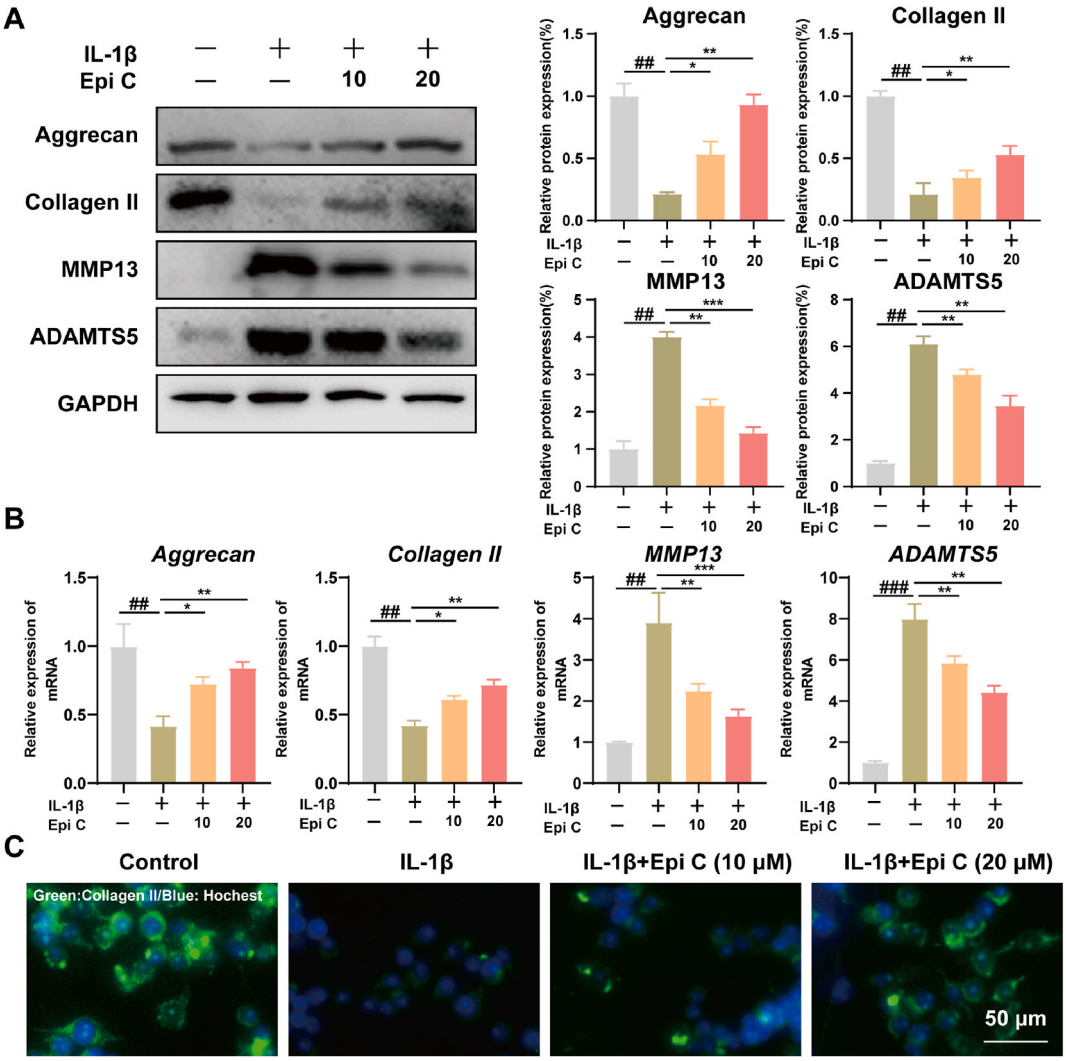

Fig. 2. Effect of Epi C on ECM degradation in IL-1β-induced chondrocytes. (A) Western blot assays were used to determine the protein levels of Aggrecan, Collagen II, MMP13 and ADAMTS5 (n = 3 for each group). (B) The mRNA expressions of Aggrecan, Collagen II, MMP13 and ADAMTS5 were detected by RT-qPCR (n = 3 for each group). (C) Representative images of Collagen II immunofluorescence staining. Data represented the mean ± SEM from three independent experiments. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. IL-1β group.

PBS, the cells were observed and recorded under a fluorescence microscope (Olympus Optical Ltd, Tokyo, Japan).

2.14. Nrf2 siRNA transcription

Nrf2 siRNA and NC-siRNA were synthesized by Thermo Fisher Scientific. Cells (2×10^5) were seeded in 6-well plate and allowed to incubate overnight. The cells were transfected with Nrf2-siRNA and NC-siRNA using Lipofectamine 3000 following the instructions provided by Invitrogen. Subsequently, after additional treatment, cellular samples were collected for biochemical analyses.

2.15. Statistical analysis

The data were expressed as mean ± standard deviations (SD). Statistical analysis was performed using Prism 9.0. To compare multiple groups, a one-way analysis of variance (ANOVA) was applied, and statistical significance was considered for results with $p < 0.05$.

3. Results

3.1. Effect of Epi C on proliferation and apoptosis in IL-1β-induced primary chondrocytes

To initially investigate the therapeutic effect of Epi C on OA, mouse chondrocytes were isolated (Fig. 1A). Cell viability assay was assessed using the CCK-8 assay, and the results revealed that IL-1β significantly suppressed cell viability, while Epi C (10, 20 μM) substantially ameliorated IL-1β-induced reduction in cell viability (Fig. 1B). Furthermore, chondrocyte apoptosis was observed in IL-1β group, whereas was markedly reduced after treatment with Epi C (10, 20 μM) (Fig. 1C). In parallel, EdU staining results also revealed that IL-1β significantly inhibited cell proliferation, and this inhibitory effect was significantly alleviated after treatment with Epi C (10, 20 μM) (Fig. 1D).

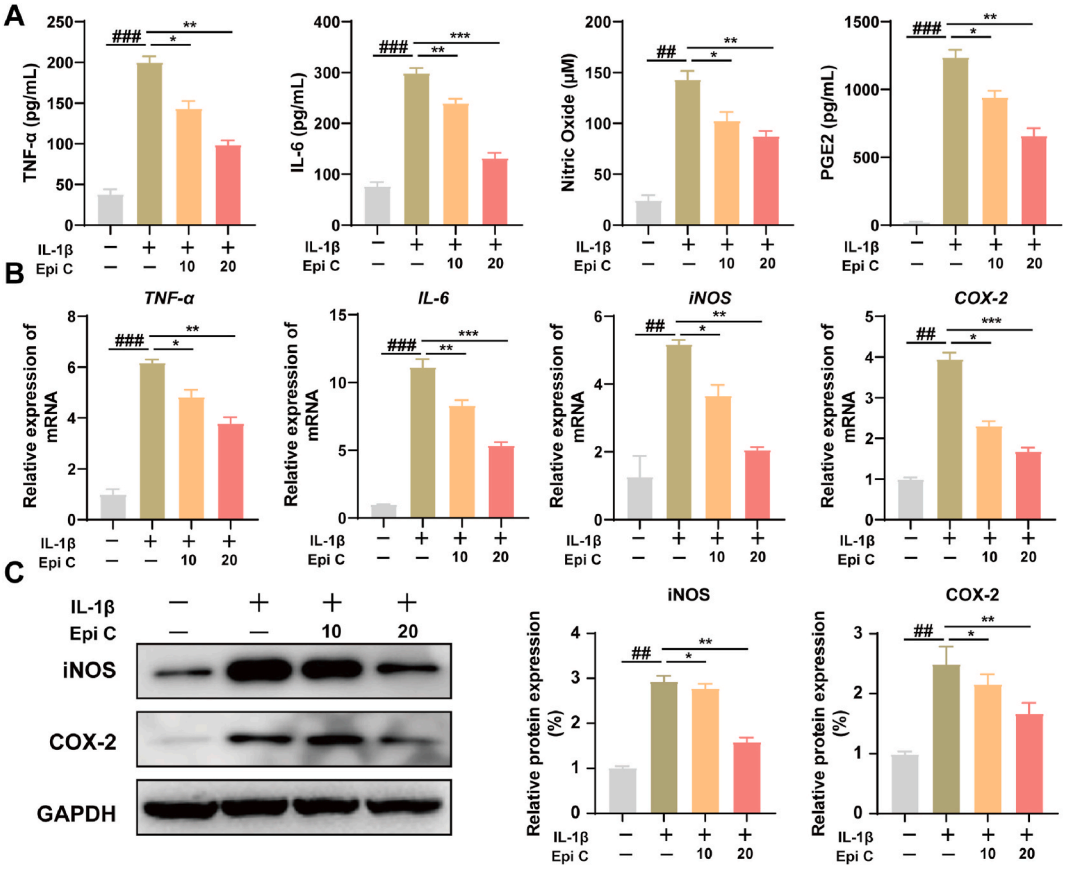

Fig. 3. Effect of Epi C on inflammatory mediators in IL-1β-induced chondrocytes. (A) The contents of TNF-α, IL-6, NO and PGE2 were detected by ELISA (n = 3 for each group). (B) The mRNA expressions of *TNF-α*, *IL-6*, *iNO* and *COX-2* were detected by RT-qPCR (n = 3 for each group). (C) The protein levels of iNO and COX-2 were detected by Western blot (n = 3 for each group). Data represented the mean ± SEM from three independent experiments. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. IL-1β group.

3.2. Effect of Epi C on extracellular matrix (ECM) degradation in IL-1β-induced chondrocytes

Beyond chondrocytes, the extracellular matrix (ECM) constitutes another critical component of cartilage tissue, playing a pivotal role in its structure and function. Therefore, Western blot was used to detect the transcriptomic expression of genes related to matrix synthesis (Aggrecan and Collagen II) and degradation (MMP13 and ADAMTS5). These results revealed that Epi C effectively suppressed the IL-1β-induced decrease of Aggrecan and Collagen II, actively participating in ECM anabolism. Furthermore, Epi C inhibited the IL-1β-mediated upregulation of ECM degradation-related proteins MMP13 and ADAMTS5 (Fig. 2A). RT-qPCR experiments were conducted to confirm these findings at the gene expression level, aligning with the results obtained from Western blot analysis (Fig. 2B). Additionally, immunofluorescence staining demonstrated that IL-1β led to a decline in Collagen II protein expression in chondrocytes, while treatment with Epi C resulted in a notable increase in Collagen II protein levels (Fig. 2C).

3.3. Effect of Epi C on inflammatory mediators in IL-1β-induced chondrocytes

Subsequently, our investigation focused on examining the influence of STE on the production of key inflammatory markers (TNF-α, IL-6, NO and PGE2), in chondrocytes activated by IL-1β. To accomplish this, we utilized ELISA kits, RT-qPCR and Western blotting, providing a comprehensive analysis of these mediators. The results revealed that IL-1β significantly increased the levels of tumor necrosis factor-α (TNF-α), IL-6, nitric oxide (NO), and Prostaglandin E2 (PGE2). However, upon treatment with Epi C, these levels were markedly reduced (Fig. 3A). RT-qPCR analysis confirmed the upregulation of TNF-α, IL-6, iNOS, and COX-2 mRNA expression in response to IL-1β. Notably, Epi C treatment led to a significant decrease in their expression levels (Fig. 3B). Additionally, Western blot experiments demonstrated that Epi C effectively attenuated the IL-1β-induced elevation in iNOS and COX-2 protein levels (Fig. 3C).

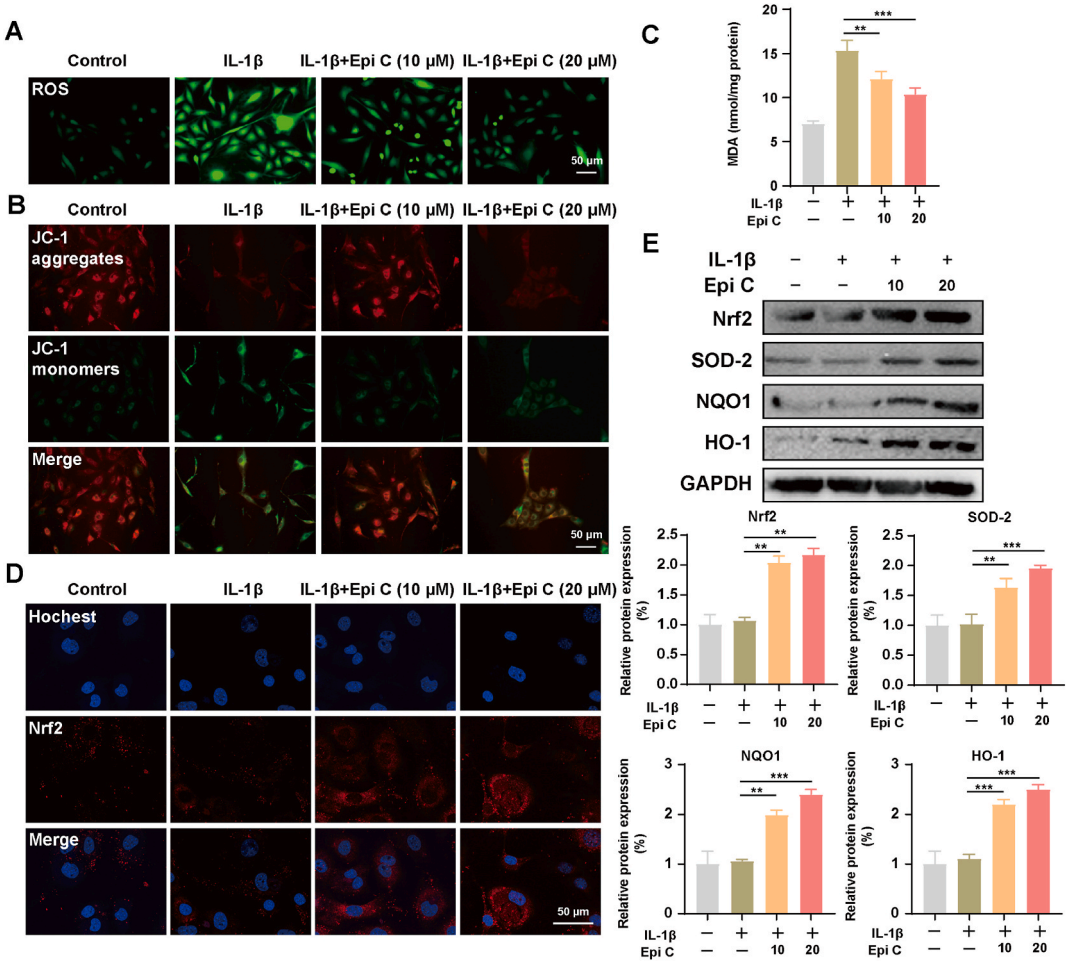

Fig. 4. Effect of Epi C on NRF2 in IL-1β-induced chondrocytes. (A) Representative images of ROS staining. (B) Representative images of JC-1 aggregates (red)/JC-1 monomers (green) staining. (C) The contents of MDA were detected by ELISA (n = 3 for each group). (D) Representative images of Nrf2 staining. (E) The protein levels of Nrf2, SOD-2, NQO1 and HO-1 were detected by Western blot (n = 3 for each group). Data represented the mean ± SEM from three independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. IL-1β group.

3.4. Effect of Epi C on NRF2-dependent antioxidant in IL-1β-induced chondrocytes

Based on previous research findings, the activation of the Nrf2 pathway has been reported to inhibit the progression of osteoarthritis [20]. Nrf2, a pivotal antioxidant transcription factor, regulates the expression of antioxidant enzymes and detoxifying enzymes, aiding cells in coping with oxidative stress [21]. In our experiments, we quantitatively measured the ROS levels in chondrocytes. The results showed a significant elevation in ROS following IL-1β treatment, indicative of enhanced oxidative stress. Contrastingly, administration of Epi C led to a notable reduction in ROS production, suggesting its potential in mitigating oxidative damage in chondrocytes. (Fig. 4A). Furthermore, our results demonstrated that treatment with IL-1β significantly induced mitochondrial depolarization in the cells. In contrast, treatment with Epi C markedly mitigated these changes. (Fig. 4B). Meanwhile, malonydialdehyde (MDA), an oxidative damage marker, was also significantly decreased by Epi C in the chondrocytes (Fig. 4C). Moreover, the antioxidant pathway Nrf2 signaling pathway was detected. As decipted in Fig. 4D, Western blot analysis showed that Epi C increased the expression of Nrf2, superoxide dismutase 2 (SOD-2), NAD(P)H: quinone oxidoreductase 1 (NQO1), and heme oxygenase-1 (HO-1) in chondrocytes treated with IL-1β. In addition, the immunofluorescence test showed that Epi C facilitated Nrf2 nuclear translocation (Fig. 4E). In conclusion, our findings suggested that the anti-oxidative properties of Epi C are attributable to two primary mechanisms: direct scavenging of reactive oxygen species (ROS) and restoration of the Nrf2-mediated cellular antioxidant defense system.

3.5. The cytoprotective effect of Epi C depends on Nrf2

To determine the role of Nrf2 signaling in the antioxidative effects of Epi C, we employed an *in vitro* approach where Nrf2 expression was selectively knocked down in chondrocytes using Nrf2-specific siRNA. Following the inhibition of Nrf2, the effectiveness of Epi C in reducing IL-1β-induced apoptosis in chondrocytes was significantly diminished, with no notable difference compared to Nrf2 knockdown alone, as illustrated in Fig. 5A and Fig. S1. This trend was also evident in the context of ROS production, where the ability of Epi C to attenuate ROS generation was similarly compromised post-Nrf2 knockdown (Fig. 5B). Additionally, the Nrf2 suppression adversely affected the proliferative influence of Epi C on chondrocytes, as depicted in Fig. 5C. Collectively, these findings highlight that the chondroprotective effects of Epi C against IL-1β-induced damage in chondrocytes are critically dependent on the presence of functional Nrf2 signaling.

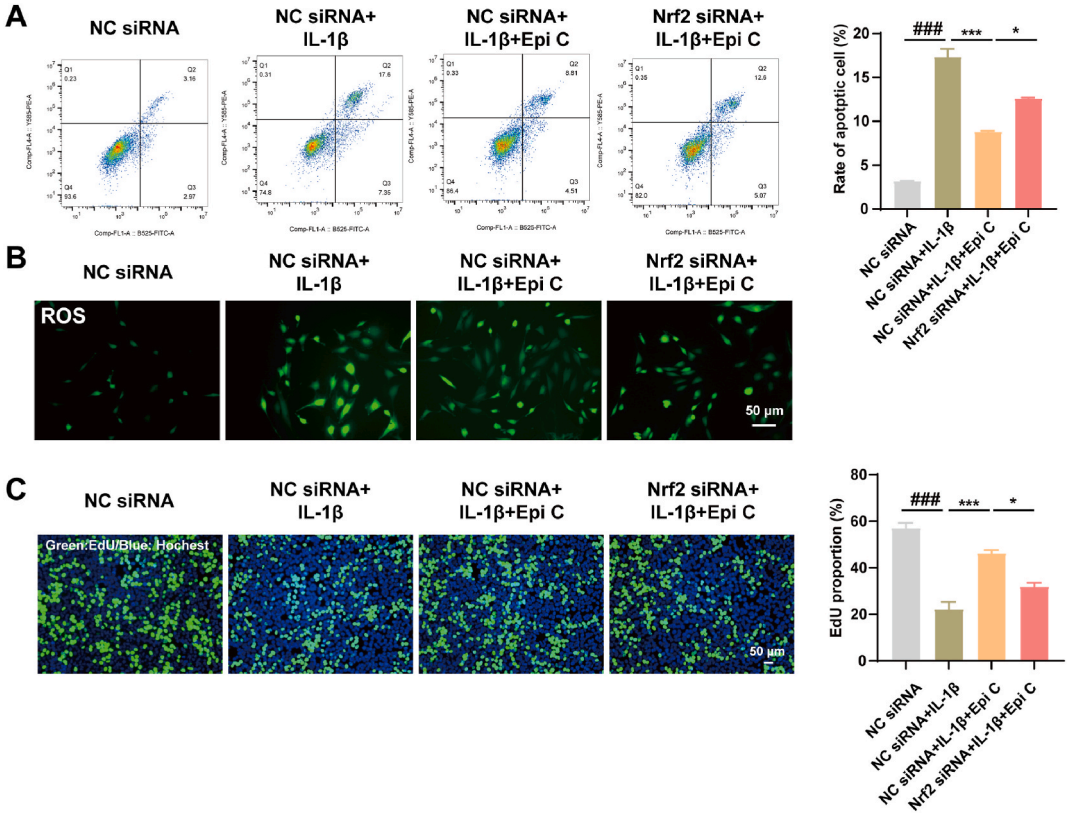

Fig. 5. The cytoprotective effect of Epi C depends on Nrf2. (A) Apoptosis was detected by flow cytometry (n = 3 for each group). (B) Representative images of ROS staining. (C) Representative images of EdU staining (n = 3 for each group). Data represented the mean ± SEM from three independent experiments. $^{###}p < 0.001$ vs. Control group; $^{*}p < 0.05$, $^{***}p < 0.001$ vs. IL-1β group.

3.6. Inhibition of NLRP3 inflammasome by Epi C in IL-1β-induced chondrocytes is associated with the Nrf2 pathway

The NLRP3 inflammasome plays a key role in the pathological responses of chondrocytes to IL-1β treatment, driving inflammation and tissue damage. In this context, the activation of Nrf2 emerges as a crucial regulatory mechanism, as it effectively inhibits both the formation and activation of the NLRP3 inflammasome, thereby modulating the inflammatory responses in chondrocytes [22]. Based on

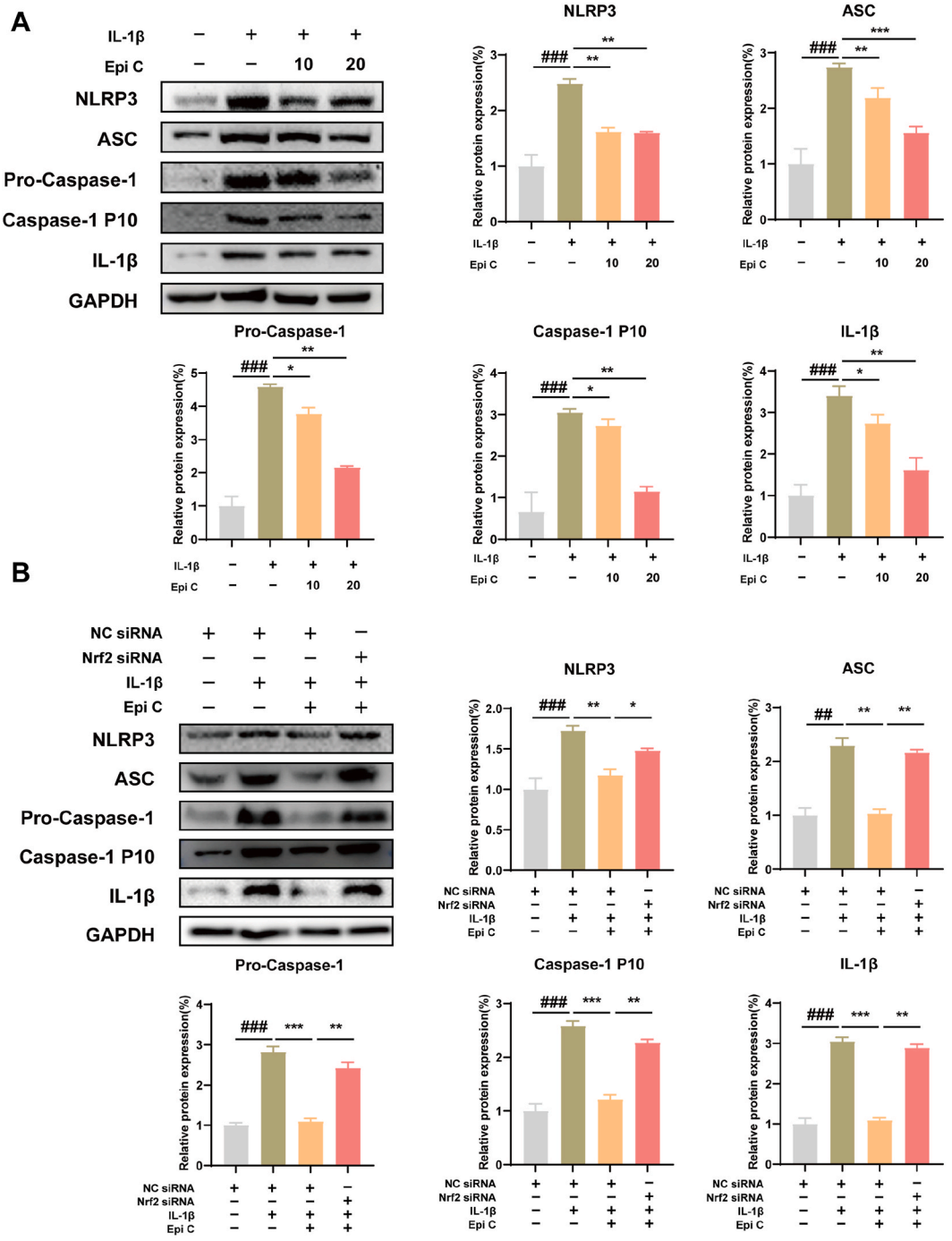

Fig. 6. Epi C inhibited NLRP3 in IL-1β-induced chondrocytes. (A, B) The protein levels of NLRP3, ASC, Pro-caspase-1 and Caspase-1 P10 were detected by Western blot (n = 3 for each group). Data represented the mean ± SEM from three independent experiments. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Control group; $^{*}p < 0.05$, $^{**}p < 0.01$,$^{***}p < 0.001$ vs. IL-1β group.

these theoretical foundations, we detected the expression of NLRP3-related protein by Western blot. Fig. 6A demonstrated that treatment with IL-1β significantly upregulated the expression of NLRP3, ASC, Pro-Caspase-1, Caspase-1 P10, and IL-1β. In contrast, Epi C treatment notably reduced the expression levels of these proteins, indicating its inhibitory effect on the NLRP3 inflammasome pathway. However, when Nrf2 was knocked down using siRNA, this inhibitory effect of Epi C was markedly diminished, as shown in Fig. 6B. Collectively, these findings suggested that the suppressive action of Epi C on the NLRP3 inflammasome is mediated through the upregulation of Nrf2 nuclear transcription."

3.7. Epi C alleviated DMM-induced cartilage degradation via Nrf2/NLRP3 aixs

To further explore whether Epi C could attenuate OA progression *in vivo*, DMM was used for mouse OA modelling. Utilizing micro-CT scanning, as outlined by McErlain et al. (2008), we monitored the subchondral bone alterations in the knee joints of OA models. The three-dimensional reconstruction of the coronal plane images of the knee joint disclosed notable damage to the articular surfaces of both the femur and tibia in the DMM model, as depicted in Fig. 7A. Statistically, bone volume (BV) and bone volume/total tissue volume (BV/TV) decreased following Epi C treatment (Fig. 7A). Remarkably, treatment with Epi C, a significant repair and improvement in the condition of these articular surfaces were observed. The progression of osteoarthritis (OA) was assessed histologically using HE and S.O. staining techniques. As illustrated in Fig. 7B, Epi C restored the reduced ratio of HC to CC caused by DMM (Fig. 7B). 8 weeks following DMM surgery, the DMM group exhibited more pronounced cartilage destruction, as evidenced by their cartilage condition. In contrast, the group treated with DMM + Epi C displayed a notably smoother and more intact cartilage surface, accompanied by a reduced OARSI score, indicating lesser severity of cartilage damage (Fig. 7C).

Immunohistochemical analysis was performed to examine the expression levels of Nrf2 and NLRP3. The findings, presented in Fig. 7D, showed a significant increase in the proportion of Nrf2-positive chondrocytes in the DMM + Epi C groups. Additionally, the number of NLRP3-positive cells was markedly lower in the DMM groups compared to the sham groups. Notably, Epi C treatment effectively reversed this decrease in NLRP3 expression. The data collectively revealed that the positive effects of Epi C treatment might be crucial to the Nrf2/NLRP3 axis.

4. Discussion

Osteoarthritis (OA), the most prevalent chronic joint disorder, is characterized by the gradual hardening of joints and the deterioration of cartilage. Currently, there is a lack of standardized and universally effective treatments for OA, with available options primarily focused on managing symptoms and improving joint function [3]. Non-pharmacological interventions, such as physical therapy and weight management, are often recommended alongside pain-relieving medications and, in severe cases, joint replacement surgery [23]. In recent years, traditional Chinese medicine has made significant strides in the treatment of osteoarthritis (OA). Compounds like danshensu [24], dihydroartemisinin [25], and scutellarin [26] have shown promising results. Studies have highlighted the remarkable impact of Epi C in enhancing the synthesis of collagen and non-collagen matrix proteins in human osteoarthritic chondrocytes [16]. Moreover, Epi C exhibits potent anti-inflammatory effects, potentially achieved through the inhibition of phosphorylation in the NF-κB signaling pathway [27]. These findings strongly imply the potential therapeutic role of Epi C in OA. However, its effectiveness in OA mice models induced by destabilization of the medial meniscus (DMM) surgery, remains to be thoroughly explored.

Pro-inflammatory cytokines, particularly IL-1β, are crucial in initiating osteoarthritis (OA), as evidenced in several studies [28]. IL-1β acts as a potent inflammatory mediator, triggering responses within the body and is found in elevated levels in the joints of OA patients. This cytokine is a key pro-inflammatory agent, significantly contributing to OA progression. Given its prominent role in the disease, the IL-1β-induced chondrocyte inflammatory model has become an established *in vitro* approach for osteoarthritis research, providing valuable insights into the inflammatory mechanisms driving OA progression [24]. In this model, chondrocytes tend to overproduce inflammatory mediators, including proteases, cytokines, and reactive oxygen species, which lead to cell apoptosis, collagen degradation, and inflammation within the cartilage tissue. Initially, our observations indicated a decrease in chondrocyte viability, increased apoptosis, and reduced cell proliferation following IL-1β treatment, confirming the successful establishment of the *in vitro* model. Importantly, intervention with Epi C was found to effectively restore cell proliferation and inhibit apoptosis, underscoring its therapeutic potential.

Aggrecan and Collagen II are essential components of the cartilage matrix, playing a pivotal role in maintaining the stability and flexibility of cartilage tissue [29]. They are crucial for facilitating smooth joint movement and providing support to the skeletal structure. MMP13, a matrix metalloproteinase, degrades type II collagen and other matrix molecules in joint cartilage. Elevated MMP13 activity during osteoarthritis leads to the degradation and damage of cartilage. ADAMTS5 functions as a primary aggrecanase, breaking down the cartilage matrix in joints [30]. Coincidence with previous reports, IL-1β reduces the expression of Aggrecan and Collagen II, elevated the expression of MMP13 and ADAMTS5. However, Epi C intervention effectively restored the abnormal protein expression induced by IL-1β. TNF-α and IL-6 play critical roles in inflammatory responses, triggering inflammation and inducing cell apoptosis [31]. The synthesis of prostaglandins, driven by the activity of iNOS and COX2, respectively, is a key contributing factor to inflammation in cartilage [32]. In our research, we observed an increase in inflammatory factors mediated by IL-1β in chondrocytes; however, after Epi C intervention, the expression of these inflammatory factors significantly decreased.

As individuals age, a decline in chondrocyte proliferation is observed, accompanied by mitochondrial senescence and dysfunction. This cellular aging process leads to elevated production of ROS and an increase in the levels of fatty acids, glucose, and inflammatory mediators, ultimately resulting in progressive cartilage damage. ROS inflict oxidative damage on proteins, lipids, and DNA, which

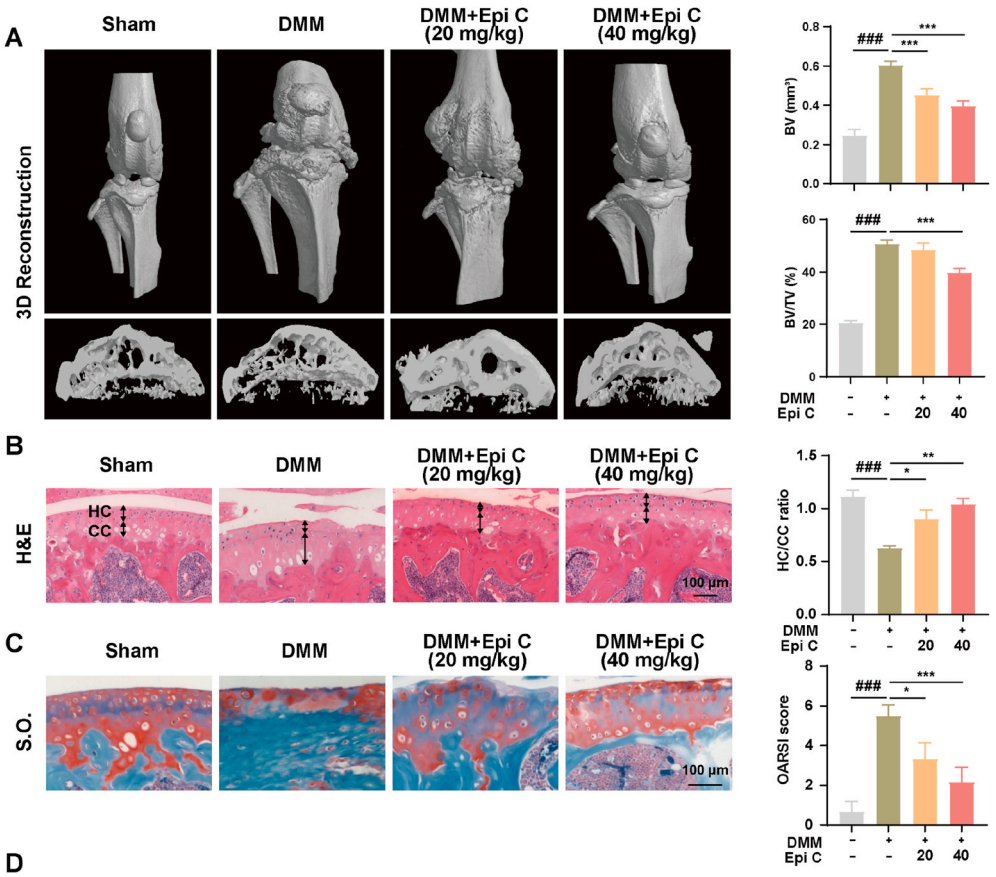

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Fig. 7. Epi C alleviated OA development. (A) A three-dimensional reconstruction illustrating the development of osteophyte, along with quantitative data on bone volume (BV) and bone volume/total tissue volume (BV/TV) for each group. (B) Representative images showcasing H&E-stained articular cartilage (n = 6 for each group). Measurements were taken of the HC to CC ratio. (C) Representative images displaying articular cartilage following staining with safranin O and fast green. OARSI grading was utilized for assessment (n = 6 for each group). (D) Representative images of Nrf2 and NLRP3 staining. Data represented the mean ± SEM from three independent experiments. $^{###}p < 0.001$ vs. Sham group; $^{*}p < 0.05$, $^{**}p < 0.01$,$^{***}p < 0.001$ vs. DMM group.

accelerates chondrocyte apoptosis and exacerbates cartilage degradation [33]. Furthermore, excessive ROS generation leads to a decline in mitochondrial membrane potential, thereby impairing mitochondrial functionality. This impairment disrupts critical cellular processes, including energy production and the regulation of apoptosis, further influencing the pathophysiology of cartilage deterioration in osteoarthritis [34]. Malondialdehyde (MDA) serves as a marker of cellular lipid peroxidation and is commonly used to assess oxidative damage [35]. Nrf2, a transcription factor involved in the antioxidant stress response, plays a pivotal role in regulating the expression of intracellular antioxidants such as SOD-2, NQO1, and HO-1, enabling cells to counteract oxidative damage [36]. In the context of OA, Nrf2 activation is believed to have a protective effect against oxidative stress and inflammatory responses [37]. Our research findings demonstrated that IL-1β stimulation in chondrocytes leads to the induction of oxidative stress, characterized by an increased intracellular accumulation of ROS and MDA. This oxidative burden is further associated with a notable reduction in mitochondrial membrane potential. Notably, treatment with Epi C was found to effectively counteract these adverse effects, suggesting its potential as a protective agent against oxidative damage in chondrocytes. Additionally, our data, supported by immunofluorescence and Western blot analyses, confirmed that Epi C activates Nrf2. This activation resulted in upregulation of antioxidative enzymes such as SOD-2, NQO1, and HO-1, and facilitated the translocation of Nrf2 into the nucleus, highlighting the role of Epi C in enhancing cellular antioxidant defense mechanisms. To substantiate our results, we employed siRNA to specifically knock down Nrf2 expression in chondrocytes. Consistent with our hypothesis, this genetic manipulation led to in a marked attenuation of Epi C's protective effects on chondrocytes, underscoring the pivotal role of Nrf2 in mediating these protective mechanisms.

Emerging evidence robustly suggests that Nrf2 activation plays a critical role in attenuating the formation and activation of NLRP3 inflammasomes, leading to a notable reduction in the secretion of inflammatory cytokines [38]. NLRP3, an integral member of the NOD-like receptor (NLR) family, acts as a pivotal intracellular receptor protein in this context. During inflammasome assembly, the adaptor protein ASC connects NLRP3 and Caspase-1, facilitating the conversion of Pro-Caspase-1 into Caspase-1 P10, which promotes the formation of fully functional inflammasomes [39]. The assembly results in the production of inflammatory mediators such as IL-1β and IL-18. Our research demonstrates that Epi C significantly impedes IL-1β-induced NLRP3 activation. Crucially, silencing Nrf2 via siRNA markedly diminished the inhibitory influence of Epi C on NLRP3, highlighting the essential role of Nrf2 in mediating the suppressive effects of Epi C on NLRP3 inflammasome activity.

The DMM surgical procedure effectively induces knee joint instability in mice, simulating the characteristics of human OA lesions. This model is distinguished by its high controllability and reproducibility, accurately reflecting the progressive nature of joint inflammation [39]. A key aspect of OA pathology involves the damage to hyaline cartilage and subsequent formation of calcified cartilage, which are principal contributors to the pain and functional impairment experienced by patients. In our constructed DMM mouse model, administration of Epi C was observed to mitigate joint damage significantly. This was evidenced by an increased ratio of hyaline to calcified cartilage, thus effectively attenuating the severity of arthritis symptoms. Furthermore, Epi C demonstrated a potent effect in activating Nrf2 pathways in DMM mice, while concurrently inhibiting the expression of NLRP3.

In summary, our study confirmed the efficacy of Epi C in treating osteoarthritis, potentially achieved through modulation of the Nrf2/NLRP3 signaling pathway. Despite the promising findings, several limitations should be acknowledged. First, the research primarily utilized *in vitro* and animal models, which may not fully replicate human osteoarthritis. Further clinical studies are needed to validate the efficacy of Epi C in patients. Additionally, the long-term effects and potential side effects of Epi C treatment require further investigation. While we focused on the Nrf2/NLRP3 signaling pathway, other mechanisms may also play a role in Epi C's protective effects, necessitating further exploration.

5. Conclusions

In conclusion, our research highlighted the promising therapeutic efficacy of Epi C in the management of OA. The findings suggested that the beneficial effects of Epi C are largely due to its ability to modulate the NLRP3 inflammasome pathway, which is achieved through the activation of the Nrf2 signaling pathway. This mechanistic insight positions Epi C as a potential key agent in osteoarthritis treatment strategies.

CRediT authorship contribution statement

Changchang Liu: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation. **Guangyu Duan:** Writing – original draft, Visualization, Software, Formal analysis, Data curation, Conceptualization. **Shengjie Xu:** Validation, Resources, Project administration, Methodology. **Teng Li:** Validation, Project administration, Methodology, Data curation, Conceptualization. **Xin Sun:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Ethics statement

The animal experiments were approved by the Ethics Committee of Nanjing University of Chinese Medicine [Ethics No. ACU230307].

Data availability

All data generated or analyzed during this study are included in this published article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40458.

References

[1] B. Abramoff, F.E. Caldera, Osteoarthritis: pathology, diagnosis, and treatment options, Med. Clin. N. Am. 104 (2) (2020) 293–311.
[2] J. Martel-Pelletier, A.J. Barr, F.M. Cicuttini, P.G. Conaghan, C. Cooper, M.B. Goldring, S.R. Goldring, G. Jones, A.J. Teichtahl, J.P. Pelletier, Osteoarthritis, Nat. Rev. Dis. Prim. 2 (2016) 16072.
[3] S.T. Skou, E.M. Roos, Physical therapy for patients with knee and hip osteoarthritis: supervised, active treatment is current best practice, Clin. Exp. Rheumatol. 37 (5) (2019) 112–117. Suppl 120.
[4] B. Xia, Di Chen, J. Zhang, S. Hu, H. Jin, P. Tong, Osteoarthritis pathogenesis: a review of molecular mechanisms, Calcif. Tissue Int. 95 (6) (2014) 495–505.
[5] J.N. Katz, K.R. Arant, R.F. Loeser, Diagnosis and treatment of hip and knee osteoarthritis: a review, JAMA, J. Am. Med. Assoc. 325 (6) (2021) 568–578.
[6] H. Sun, Z. Sun, X. Xu, Z. Lv, J. Li, R. Wu, Y. Fei, G. Tan, Z. Liu, Y. Liu, D. Shi, Blocking TRPV4 Ameliorates Osteoarthritis by inhibiting M1 macrophage polarization via the ROS/NLRP3 signaling pathway, antioxidants-basel 11 (12) (2022).
[7] E.H. Kobayashi, T. Suzuki, R. Funayama, T. Nagashima, M. Hayashi, H. Sekine, N. Tanaka, T. Moriguchi, H. Motohashi, K. Nakayama, et al., Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription, Nat. Commun. 7 (2016) 11624.
[8] Z. Chen, H. Zhong, J. Wei, S. Lin, Z. Zong, F. Gong, X. Huang, J. Sun, P. Li, H. Lin, B, et al., Inhibition of Nrf2/HO-1 signaling leads to increased activation of the NLRP3 inflammasome in osteoarthritis, Arthritis Res. Ther. 21 (1) (2019) 300.
[9] M. Yang, L. Jiang, Q. Wang, H. Chen, G. Xu, Traditional Chinese medicine for knee osteoarthritis: an overview of systematic review, PLoS One 12 (12) (2017) e189884.
[10] J. Hu, J. Zhou, J. Wu, Q. Chen, W. Du, F. Fu, H. Yu, S. Yao, H. Jin, P. Tong, et al., Loganin ameliorates cartilage degeneration and osteoarthritis development in an osteoarthritis mouse model through inhibition of NF-kappaB activity and pyroptosis in chondrocytes, J. Ethnopharmacol. 247 (2020) 112261.
[11] N. Yi, Y. Mi, X. Xu, N. Li, B. Chen, K. Yan, K. Tan, B. Zhang, L. Wang, G. Kuang, et al., Nodakenin attenuates cartilage degradation and inflammatory responses in a mice model of knee osteoarthritis by regulating mitochondrial Drp1/ROS/NLRP3 axis, Int. Immunopharmacol. 113 (Pt A) (2022) 109349.
[12] J. Feng, Z. Li, L. Tian, P. Mu, Y. Hu, F. Xiong, X. Ma, Efficacy and safety of curcuminoids alone in alleviating pain and dysfunction for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials, BMC Complement. Med. 22 (1) (2022) 276.
[13] L. Ke, X. Duan, J. Cui, X. Song, W. Ma, W. Zhang, Y. Liu, Y. Fan, Research progress on the extraction technology and activity study of Epimedium polysaccharides, Carbohyd. Polym. 306 (2023) 120602.
[14] C. Wang, S. Tian, Q. Tang, Z. Zhou, X. Peng, X. Cai, Y. Xu, Systematic quality evaluation of epimedium wushanense T. S. Ying based on two quality control standards: total flavonoid glycosides and epimedin C, Chem. Biodivers. 20 (3) (2023) e202200579.
[15] X.H. Yang, L. Li, Y.B. Xue, X.X. Zhou, J.H. Tang, Flavonoids from Epimedium pubescens: extraction and mechanism, antioxidant capacity and effects on CAT and GSH-Px of Drosophila melanogaster, PeerJ 8 (2020) e8361.
[16] R. Ziadlou, A. Barbero, I. Martin, X. Wang, L. Qin, M. Alini, S. Grad, Anti-inflammatory and chondroprotective effects of vanillic acid and epimedin C in human osteoarthritic chondrocytes, Biomolecules 10 (6) (2020).
[17] Y.M. Liu, X.Q. Li, X.R. Zhang, Y.Y. Chen, Y.P. Liu, H.Q. Zhang, Y. Chen, Uncovering the key pharmacodynamic material basis and possible molecular mechanism of extract of Epimedium against liver cancer through a comprehensive investigation, J. Ethnopharmacol. 317 (2023) 116765.
[18] Y. Xu, S. Chen, L. Huang, W. Han, Y. Shao, M. Chen, Y. Zhang, R. He, B. Xie, Epimedin C alleviates glucocorticoid-Induced suppression of osteogenic differentiation by modulating PI3K/AKT/RUNX2 signaling pathway, Front. Pharmacol. 13 (2022) 894832.
[19] A. Mobasheri, C. Matta, R. Zakany, G. Musumeci, Chondrosenescence: definition, hallmarks and potential role in the pathogenesis of osteoarthritis, Maturitas 80 (3) (2015) 237–244.
[20] L. Wang, C. He, Nrf2-mediated anti-inflammatory polarization of macrophages as therapeutic targets for osteoarthritis, Front. Immunol. 13 (2022) 967193.
[21] A. Mata, S. Cadenas, The Antioxidant transcription factor Nrf2 in cardiac ischemia-reperfusion injury, Int. J. Mol. Sci. 22 (21) (2021).
[22] J. Zhou, Q. Wang, Daphnoretin relieves IL-1beta-mediated chondrocytes apoptosis via repressing endoplasmic reticulum stress and NLRP3 inflammasome, J. Orthop. Surg. Res. 17 (1) (2022) 487.
[23] R.M. Ferreira, R.T. Torres, J.A. Duarte, R.S. Goncalves, Non-pharmacological and non-surgical interventions for knee osteoarthritis: a systematic review and meta-analysis, Acta Reumatol. Port. 44 (3) (2019) 173–217.
[24] Z. Xu, T. Ke, Y. Zhang, L. Guo, F. Chen, W. He, Danshensu inhibits the IL-1beta-induced inflammatory response in chondrocytes and osteoarthritis possibly via suppressing NF-kappaB signaling pathway, Mol. Med. 27 (1) (2021) 80.

[25] D. Ding, J. Yan, G. Feng, Y. Zhou, L. Ma, Q. Jin, Dihydroartemisinin attenuates osteoclast formation and bone resorption via inhibiting the NF-kappaB, MAPK and NFATc1 signaling pathways and alleviates osteoarthritis, Int. J. Mol. Med. 49 (1) (2022).
[26] S.H. Ju, L.R. Tan, P.W. Liu, Y.L. Tan, Y.T. Zhang, X.H. Li, M.J. Wang, B.X. He, Scutellarin regulates osteoarthritis in vitro by inhibiting the PI3K/AKT/mTOR signaling pathway, Mol. Med. Rep. 23 (1) (2021).
[27] M. Huang, Y. Wei, J. Dong, Epimedin C modulates the balance between Th9 cells and Treg cells through negative regulation of noncanonical NF-kappaB pathway and MAPKs activation to inhibit airway inflammation in the ovalbumin-induced murine asthma model, Pulm. Pharmacol. Ther. 65 (2020) 102005.
[28] B.W. Wang, Y. Jiang, Z.L. Yao, P.S. Chen, B. Yu, S.N. Wang, Aucubin protects chondrocytes against IL-1beta-induced apoptosis in vitro and inhibits osteoarthritis in mice model, Drug Des. Devel. Ther. 13 (2019) 3529–3538.
[29] Y. Luo, D. Sinkeviciute, Y. He, M. Karsdal, Y. Henrotin, A. Mobasheri, P. Onnerfjord, A. Bay-Jensen, The minor collagens in articular cartilage, Protein Cell 8 (8) (2017) 560–572.
[30] J. Li, M. Jiang, Z. Yu, C. Xiong, J. Pan, Z. Cai, N. Xu, X. Zhou, Y. Huang, Z. Yang, Artemisinin relieves osteoarthritis by activating mitochondrial autophagy through reducing TNFSF11 expression and inhibiting PI3K/AKT/mTOR signaling in cartilage, Cell. Mol. Biol. Lett. 27 (1) (2022) 62.
[31] J. Zhang, H. Chi, H. Xiao, X. Tian, Y. Wang, X. Yun, Y. Xu, Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) single nucleotide polymorphisms (SNPs), inflammation and metabolism in gestational diabetes mellitus in inner Mongolia, Med. Sci. Monitor. 23 (2017) 4149–4157.
[32] A. Pupe, R. Moison, P. De Haes, G.B. van Henegouwen, L. Rhodes, H. Degreef, M. Garmyn, Eicosapentaenoic acid, a n-3 polyunsaturated fatty acid differentially modulates TNF-alpha, IL-1alpha, IL-6 and PGE2 expression in UVB-irradiated human keratinocytes, J. Invest. Dermatol. 118 (4) (2002) 692–698.
[33] M. Arra, G. Swarnkar, K. Ke, J.E. Otero, J. Ying, X. Duan, T. Maruyama, M.F. Rai, R.J. O'Keefe, G. Mbalaviele, J. Shen, Y. Abu-Amer, LDHA-mediated ROS generation in chondrocytes is a potential therapeutic target for osteoarthritis, Nat. Commun. 11 (1) (2020) 3427.
[34] D. Nolfi-Donegan, A. Braganza, S. Shiva, Mitochondrial electron transport chain: oxidative phosphorylation, oxidant production, and methods of measurement, Redox Biol. 37 (2020) 101674.
[35] D. Tsikas, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges, Anal. Biochem. 524 (2017) 13–30.
[36] X. Wang, J. Liu, X. Zhang, S. Zhao, K. Zou, J. Xie, X. Wang, C. Liu, J. Wang, Y. Wang, Seabuckthorn berry polysaccharide extracts protect against acetaminophen induced hepatotoxicity in mice via activating the Nrf-2/HO-1-SOD-2 signaling pathway, Phytomedicine 38 (2018) 90–97.
[37] J.W. Li, R.L. Wang, J. Xu, K.Y. Sun, H.M. Jiang, Z.Y. Sun, Z.Y. Lv, X.Q. Xu, R. Wu, H. Guo, et al., Methylene blue prevents osteoarthritis progression and relieves pain in rats via upregulation of Nrf2/PRDX1, Acta Pharmacol. Sin. 43 (2) (2022) 417–428.
[38] B.I. Arioz, B. Tastan, E. Tarakcioglu, K.U. Tufekci, M. Olcum, N. Ersoy, A. Bagriyanik, K. Genc, S. Genc, Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway, Front. Immunol. 10 (2019) 1511.
[39] J. Fu, H. Wu, Structural mechanisms of NLRP3 inflammasome assembly and activation, Annu. Rev. Immunol. 41 (2023) 301–316.