

Network pharmacology to investigate the potential mechanism of Qianghuo against osteoarthritis and experimental validation

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Research Article

Keywords: Osteoarthritis, Notopterygium incisum, Network Pharmacology, Molecular Docking, traditional Chinese medicine

Posted Date: April 17th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9338081/v1>

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Additional Declarations: No competing interests reported.

Abstract

Notopterygium incisum (Qianghuo, QH) is commonly used for Osteoarthritis (OA), yet its bioactive components and molecular targets remain unclear. This study systematically investigated the pharmacological basis and mechanism of QH against OA. Active compounds and targets were screened using TCMSP and GeneCards databases. Protein-protein interaction (PPI), GO, and KEGG enrichment analyses were performed to predict mechanisms. Molecular docking verified binding affinities. In a papain-induced OA rat model, histopathological changes were evaluated using HE and Safranin O-Fast Green staining, while inflammatory cytokine expression and key protein levels were assessed via qRT-PCR and Western blot. Network analysis identified 15 active compounds and 55 targets, with AKT1, MAPK1, and CASP3 as core hubs. Molecular docking demonstrated strong binding energy (< -7 kcal/mol) between active ingredients and these targets. *In vivo* experiments showed that QH significantly alleviated cartilage erosion, improved histopathological scores, and downregulated mRNA expression of IL-1 β , IL-6, and TNF- α . Furthermore, QH significantly inhibited AKT1 and MAPK1 phosphorylation and reduced Cleaved-Caspase-3 levels. In conclusion, QH exerts anti-OA effects by mitigating cartilage degeneration and suppressing inflammation. The mechanism involves inhibiting excessive AKT1/MAPK1 activation, thereby suppressing downstream Caspase-3 cleavage and blocking chondrocyte apoptosis, providing a scientific basis for QH in OA treatment.

1 INTRODUCTION

Osteoarthritis (OA) is a prevalent and incapacitating condition on a global scale, with nearly all joint tissue abnormalities being linked to its onset to some extent¹. It is estimated that nearly 500 million individuals worldwide are afflicted with OA, with Knee Osteoarthritis (KOA) accounting for the majority of these cases. Recent epidemiological studies reveal that KOA affects about 15% of individuals aged 30 years and older, marking it as a significant public health challenge². Knee osteoarthritis is a heterogeneous disease characterized by various clinical manifestations³. Central to its pathogenesis is the gradual degradation of articular cartilage. This structural deterioration precipitates debilitating symptoms such as persistent pain, stiffness and deformity—which significantly impair the patient's quality of life⁴. Due to the limited regenerative capacity of articular cartilage, failure to provide timely and effective intervention can ultimately result in disability⁵. At the advanced stage of the disease, prosthetic joint replacement often becomes the only viable option, imposing a heavy burden on both individuals and healthcare systems⁶.

Osteoarthritis has long been regarded as a disease caused by mechanical wear, but modern research reveals that the pathological mechanism of OA is a multi-dimensional interactive process of biomechanics, inflammation and metabolic factors^{7,8}. Current clinical guidelines prioritize pharmacological interventions, particularly nonsteroidal anti-inflammatory drugs (NSAIDs)⁹. However, while these medications can alleviate pain, they fail to halt cartilage degeneration and disease progression; prolonged use may also lead to gastrointestinal and cardiovascular adverse reactions¹⁰. Traditional Chinese medicine (TCM) has long served as an alternative therapy for OA. Given its demonstrated efficacy, both TCM and its bioactive small-molecule components are increasingly attracting research attention¹¹. *Notopterygium incisum*, known in Chinese as "Qianghuo" (QH), is a traditional Chinese medicinal herb that is listed as the top grade in the "Shennong Materia Medica Classic"¹². While QH is widely used in TCM formulas and exhibits potent anti-inflammatory and anti-apoptotic properties, its specific bioactive ingredients and molecular targets as a standalone agent remain largely undefined¹³. Therefore, there is a critical

need to bridge the research gap by elucidating the precise interactions between QH components and OA-related targets ⁴.

Due to the lack of contemporary research techniques in related fields like molecular biology, pharmacology, and bioinformatics, traditional Chinese medicine has been disregarded and labeled as empirical medicine over the past few decades ¹⁴. Since most TCM active ingredients are small molecules that exert effects through multiple biological targets, a more comprehensive approach is required. Network pharmacology serves as an effective tool for dissecting 'gene-target-pathway' interactions ¹⁵. In this study, we applied this approach to establish a 'drug-components-targets-pathways-disease' network to investigate the potential molecular basis of QH in the treatment of Osteoarthritis (OA). Furthermore, recognizing that Knee Osteoarthritis (KOA) represents the most prevalent and debilitating form of OA, we selected the papain-induced KOA rat model as a representative system to experimentally validate the predicted targets via molecular docking and in vivo studies. This work uncovers the potential molecular mechanisms underlying the therapeutic effects of QH on osteoarthritis.

2 METHODS

2.1 Pharmacokinetics and Component Screening of QH

The active ingredients of QH were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database ¹⁶. Oral bioavailability (OB) and drug-likeness (DL) were selected as key pharmacokinetic parameters. According to TCMSP guidelines and recent studies ^{17,18}, the threshold was set to OB $\geq$ 30% and DL $\geq$ 0.18. Compounds meeting these criteria were identified as candidate bioactive ingredients. Subsequently, the potential targets of these ingredients were predicted via the PharmMapper server. Targets with a Norm Fit score $>$ 0.7 were selected for high-confidence prediction. Finally, the UniProt database was employed to standardize the target names into official gene symbols, limiting the species to "Homo sapiens".

2.2 Acquisition of six QH compounds specific for OA targets

The database was searched for OA-related targets using the phrase "osteoarthritis". The databases included The Online Mendelian Inheritance in Man (OMIM) (<http://omim.org/>), the DrugBank (<https://www.drugbank.ca/>), the human gene database GeneCards (<http://www.genecards.org/>), The Therapeutic Target Database (TTD) (<https://db.idrblab.net/ttd/>) and The DisGeNET database (<https://www.disgenet.org/>). The link between the targets for the OA disease and the targets for the QH component was compared using a Venn diagram.

2.3 Construction of networks and pathway analysis

The potential targets were examined for Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment using the Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>) v6.8. The target proteins involved in the biological processes (BPs), molecular functions (MFs), and cellular components (CCs) were also outlined. The hub genes were highlighted in the KEGG mapper (<http://www.kegg.jp.org>) to highlight the pathway's specific molecular process ¹⁹. Using Cytoscape software (version 3.8.2), a Drug-Compound-Target-Pathway-Disease (D-C-T-P-D) Network was created to fully comprehend the molecular processes of the anti-OA action of QH ²⁰. The STRING database was then accessed to conduct a protein-protein interaction analysis on the associated targets of QH anti-OA. Based on software analysis using Cytoscape 3.7.0, the network topology was displayed. The hub targets of the QH anti-OA were then gathered using the MCODE (molecular complex detection) and Cytohubba plug-ins to create the regulatory

network. CytoNCA calculates degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC), and then uses threshold values to further filter for hub genes.

2.4 Molecular Docking of Hub Genes and QH

We chose hub genes of important enrichment pathways as molecular receptors and QH for molecular docking study in order to assess the accuracy of QH and OA target interactions and explore appropriate binding modalities. Protein molecules' raw files were downloaded from www.rcsb.org, and the raw file of QH (in MOL2 format) was obtained from the PubChem database. The files were eventually converted to PDBQT format for molecular docking in PyMOL software (<https://pymol.org/2/>; version 2.4.1) after the appropriate AutoDock Tool processing.

2.5 Experimental verification of network analysis results

2.5.1. Materials

TCM decoction pieces of QH (SCXK:20210469) were purchased from Anhui Fuming Traditional Chinese Medicine Slices Co., Ltd. (Anhui, China), SD rats (6 weeks old, weighing 150-200g), in grade Specific Pathogen Free (SPF) (SCXK: 2020-0001), were purchased from Liaoning Changsheng Biotechnology Co., Ltd. Papain (G8430) and cysteine (C0012) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China); Modified Safranin O and Fast Green Stain Kit (G1371) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). TRIzol (15596018) was purchased from Ambion Co., Ltd. (Texas, USA). Universal SYBR qPCR Master Mix (BL697A) purchased from Biosharp Co., Ltd. (Hefei, China). AKT1 (#4691T), MAPK1 (#4695T), and Caspase-3(#9662S) All provided by Cell Signaling Technology (Boston, USA). GAPDH (60004-1-Ig) provided by Proteintech Group, Inc. (Chicago, USA).

2.5.2 Preparation of QH extraction

QH (100 g) was crushed and extracted in 10-fold (v/w) distilled water. The mixture was refluxed twice for 30 min each time. The residues were removed by filtration, and the filtrates from both extractions were combined. The solution was then concentrated to a final volume of 100 mL to obtain a standard aqueous extract (equivalent to 1 g raw herb/mL). Finally, the extract was stored at 4°C for further use.

2.5.3 Establishment of OA rat model and Drug Administration

According to the random number table, 30 SD rats were divided into three groups, each consisting of 10 rats: the negative control (NC) group, the OA group, and the QH group. On days 1, 3, and 7 in the OA group and QH group, respectively, the right knee joint of the rats received a single injection of 0.2 mL of 4% papain and its activator, 0.03 M cysteine. A similar quantity of 0.9% sodium chloride solution was injected into the knee joint of the NC group at the same time. After the last injection of papain solution, the medication was administered by gavage. The administration group calculated the intragastric dose of the drug solution based on the human to animal dose conversion formula: the dose of rat (mg/kg) = $W \times \text{human (mg/kg)}$, $W = 6.2^{21,22}$. According to the drug compatibility dose in "Qianghuo Shengshi decoction", the medicinal dose of adult QH is 9 g/day, which is calculated as 150mg/kg for a 60 kg person, and the clinical equivalent dose of rats is $150 \times 6.2 = 930\text{mg/kg}$. where the rats in the QH group were divided into a low-dose group (0.93g/kg) and a high-dose group (1.86g/kg). The NC group and the OA group were given an equal amount of 0.9% sodium chloride solution by gavage for a total of 8 weeks. On week 9, after the rats were sacrificed, the cartilage was harvested from the right knee joint.

All animal experiments were performed in accordance with the relevant guidelines and regulations. This study is reported in accordance with ARRIVE guidelines.

2.5.4 Histological Analysis

At 8 weeks, all animals were sacrificed, and samples of articular cartilage were harvested. Articular cartilage samples from each group were fixed for 48 hours in 4% paraformaldehyde before being decalcified for a month in 10% Ethylene Diamine Tetraacetic Acid (EDTA) (pH 7.4). The cartilage tissues were then cut into 4.0-mm sections, embedded in paraffin, and dehydrated in a graded ethanol series. The degeneration of articular cartilage was then assessed using two staining methods. The cartilage samples were first stained with hematoxylin for five minutes, followed by an eosin stain for forty seconds, and then they were observed under a microscope. This procedure is known as hematoxylin and eosin (HE) staining. The samples were stained with 0.02% fast green for 30 minutes, 1% acetic acid for 10 seconds, and 1.5% Safranin O for 3 minutes for the Safranin O/fast green staining procedure. The cartilage samples were dehydrated and mounted with neutral balsam before being examined under a microscope. Using Safranin O/fast green staining, two experienced independent researchers who were blinded to the study assessed the articular cartilage degeneration of each knee joint. They also assessed and quantified the cartilage degeneration of osteoarthritis using the Mankin and the Osteoarthritis Research Society International (OARSI) histological scoring system. The higher the degree of injury, the higher the score ^{23,24}.

2.5.5 Quantitative Real-Time PCR

Following the manufacturer's instructions, total RNA was extracted from the cartilage of the right knee joint using TRIzol reagent, and the PrimeScript RT reagent kit (Takara) was used to reverse-transcribe the RNA into cDNA. Real-time PCR was then carried out utilizing Takara's SYBR® GreenER SuperMix. The GAPDH gene expression levels were utilized as the endogenous control, and the relative fold changes in gene expression were calculated using the 2CT technique. Table 1 provides a summary of the primer sequences.

Table 1
The primer sequences used for the RT-qPCR analysis

Gene	Forward primer	Reverse primer
IL-1β	5'- GAGCTGAAAGCTCTCCACCT – 3'	5'- TTCCATCTTCTTCTTTGGGT – 3'
IL-6	5'- ATTGTATGAACAGCGATGATGCAC – 3'	5'- CCAGGTAGAAACGGA ACTCCAGA – 3'
TNF-α	5'- TCAGTTCCATGGCCCAGAC – 3'	5'- GTTGTCTTTGAGATCCATGCCATT – 3'
GAPDH	5'- TCTCTGCTCCTCCCTGTTCT – 3'	5'- CTTGCCGTGGGTAGAGTCAT – 3'

2.5.6 Western Blot

Take out the liquid nitrogen-frozen cartilage tissue, mix it in a RIPA lysate for five minutes, and then extract the whole protein. The concentration of total proteins was verified by bicinchoninic acid (BCA) assays. Using 8%–12% SDS–PAGE, proteins were isolated and then put into polyvinylidene fluoride membranes. Add primary antibodies (Caspase 3, 1:1000; AKT1, 1:1000; GAPDH, 1:5000) at 4°C overnight after sealing with skimmed milk powder at room temperature for two hours. The membranes were TBST-washed and treated with the appropriate secondary antibodies for 90 minutes at room temperature.

3 RESULTS

3.1 Active ingredients and target information

According to the screening requirements of $OB \geq 30\%$ and $DL \geq 0.18$, 15 components extracted from QH were identified from the TCMSP database (Table 2). For further study, the targets of each molecule discovered in QH were retrieved from the PharmMapper database by choosing with norm fit > 0.7 . We identified 1,195 targets related to the candidate compounds. After removing duplicates, 255 potential targets were selected. Through the use of the GeneCards, DrugBank, TTD, DisGeNET, and OMIM databases, 1377 OA-related targets were found. A total of 55 OA-related targets were identified by intersecting the potential targets with the disease targets (Fig. 1)

Table 2
The ADME characteristics of QH active compounds according to the TCMSP database.

NO.	Molecule ID	Molecule name	OB(%)	DL	Molecular weight
1	MOL001941	Ammidin	34.55	0.22	270.3
2	MOL011962	6'-Feruloylnodakenin	32.02	0.67	584.62
3	MOL011963	8-geranoxo-5-methoxypsoralen	40.97	0.5	368.46
4	MOL011968	coumarin, glycoside	33.07	0.78	534.61
5	MOL011969	Demethylfuropinnarin	41.31	0.21	270.3
6	MOL011971	diversoside_qt	67.57	0.31	332.43
7	MOL011975	notoptol	62.97	0.48	354.43
8	MOL001951	Bergaptin	41.73	0.42	338.43
9	MOL001956	Cnidilin	32.69	0.28	300.33
10	MOL000359	sitosterol	36.91	0.75	414.79
11	MOL004792	nodakenin	57.12	0.69	408.44
12	MOL000358	beta-sitosterol	36.91	0.75	414.79
13	MOL001942	isoimperatorin	45.46	0.23	270.3
14	MOL002644	Phellopterin	40.19	0.28	300.33
15	MOL002881	Diosmetin	31.14	0.27	300.28

3.2 GO and KEGG Enrichment Analysis

Further analysis of the cellular localization, biological impact, pathway, and function of the 55 potential targets of QH anti-OA was aided by GO and KEGG pathway enrichment analysis. Based on the number of genes enriched in the function, the top 10 GO analyses were chosen in the GO analysis. As seen in Fig. 2A, biological processes connected to OA included signal transduction, proteolysis, positive regulation of gene expression, positive regulation of cell proliferation, and positive regulation of transcription. Higher enrichment was detected in the cytosol, cytoplasm, extracellular region, plasma membrane, and extracellular space in terms of cellular components. Protein binding, identical protein binding, ATP binding, zinc ion binding, and protein serine/threonine kinase activity were the key in molecular activities. The number of genes that were enriched in the first 10 pathways was also chosen. The main enriched pathways included MAPK signaling, PI3K-Akt signaling, osteoclast

differentiation, FoxO signaling, apoptosis, and IL-17 signaling (Figs. 2B and 2C). Due to the greatest number of potential targets ($n = 14$), the P13K and MAPK signaling pathways are substantially linked with osteoarthritis. MAPK has a higher degree of enrichment than P13K. The QH anti-OA targets in the MAPK pathway were highlighted in red using the KEGG mapper (Fig. 2D).

3.3 The D-C-T-P-D network

Figure 3 illustrates the intricate interaction between QH and osteoarthritis by displaying the D-C-T-P-D network of QH for the treatment of osteoarthritis. It has 82 total nodes (15 compound nodes, 55 target nodes, 10 core routes, one QH node, and one OA node), 848 edges, and displays the intricate interaction between QH and osteoarthritis. The top five compounds in the compound section are bergaptin, diversoside_qt, coumarin-glycoside, 8-geranoxymethoxypsoralen, and notoptol, with degrees of 38, 38, 36, 35, and 34, respectively. Similarly, the top degree of the MAPK pathway was 10, which was the most important signaling pathway.

3.4 The networks and pathway analysis

STRING was used to import 55 potential targets for PPI network analysis. The network was visualized and examined using the Cytoscape software by determining centrality and other metrics. These specifications led to the arrangement of all the targets into circles. A significant role in the network was signified by the high centrality value. The hub targets were then chosen by the plugins MCODE and CytoHubba. In this study, the top 15 target points in the PPI network were chosen as hub genes using the topology-related MCC algorithm. ALB, AKT1, MAPK1, CASP3, ANXA5, ESR1, MAPK14, IGF1R, AR, MAPK8, PPARG, GSK3B, PARP1, CDK2, and ESR2 were the 15 hub genes. Analyzing the aforementioned hub genes further using CytoNCA based on degree centrality (DC), betweenness centrality (BC), and closeness centrality (CC). In the end, four main targets—ALB, AKT1, CASP3, and MAPK1—were attained using the threshold values of $DC \geq 14$, $BC \geq 4.341$, and $CC \geq 1$ (Fig. 4). The MAPK signaling pathway also includes AKT1, CASP-3, and MAPK1, which again suggested that these three genes would be the key targets in QH for the treatment of OA. Similarly, the number of hub genes in the OA-related targets of the top 5 drugs was determined, as shown in Fig. 5. Bergaptin and notoptol's OA-related targets included 14 hub genes, indicating that they may be the most significant active ingredient in the treatment of osteoarthritis.

3.5 Molecular Docking

In our research, we docked three key targets with five active QH monomers. The results showed that majority of the results were -7 kcal/mol and that bergaptin and notoptol have much lower binding energies to important proteins than other compounds of QH (Table 3). By investigating the particular binding sites and the spatial distance in the UCFS chimera, the docking results of QH and three targets were refined. For instance, Bergaptin formed hydrogen bonds with GLN-79 amino acid residues in AKT1 (PDB ID: 4EJN), at a distance of 2.2 Å. Bergaptin formed hydrogen bonds with amino acid residues GLN-161 (2.2 Å), ARG-64 (2.2 Å), and ARG-207 (2.4 Å) in CASP3 (PDB ID: 3KJF). Bergaptin formed hydrogen bonds with the amino acid residues SER-208 and THR-206 in MAPK1 (PDB ID: 8AOA), with the distances being 2.9 Å and 3.2 Å, respectively (Fig. 6).

Table 3
The detailed molecular docking messages of five QH compounds with AKT1, MAPK1, and CASP3

No	Gene	PDB-ID	Binding Energy /(kcal·mol ⁻¹)				
			Bergaptin	notoptol	8-geranoxy-5-methoxypsoralen	diversoside_qt	coumarin,glycoside
1	AKT1	4EJN	-10	-9.9	-9.6	-8.8	-9.6
2	CASP3	3KJF	-7.7	-7.6	-6.6	-7.0	-7.3
3	MAPK1	8AOA	-7.3	-7.3	-7.2	-7.0	-7.2

3.6 Experimental verification of network analysis results

3.6.1 The anti-OA effects of QH

In the OA group, the rats displayed symptoms such as weight loss, uneven hair, decreased appetite, and abnormal gait. Compared with the OA group, the symptoms in the QH group were significantly improved. The NC group's articular cartilage was smooth and uniform, devoid of redness or swelling, with an intact cartilage surface, neat edges, and no osteophyte growth, according to general observation of articular cartilage; In the OA group, the articular cartilage was rough and uneven, with more redness and edema. In the QH group, the articular cartilage appeared slightly rough with rough edges; however, redness, swelling, and cartilage destruction were reduced compared to the OA group, with the most significant improvement observed in the 1.86 g/kg dose group. As shown by HE and safranin solid green staining, the cartilage surface of the NC group rats was smooth, with normal cell morphology, uniform arrangement, no clusters or clustering, and the staining was generally uniform. The cartilage in the OA group displayed surface deterioration, erosion, structural disarray, and decreased staining. The cartilage surface in the QH group was a little rougher, had little damage, and had a more consistent cell arrangement (Fig. 7A). The OARSI and Mankin's score in the OA group were higher than that in the NC, after continuous administration of QH for 21 days, the score significantly decreased, with the 1.86g/kg dose group being the most significant ($P < 0.05$) (Fig. 7B and C).

Real-time PCR results showed that the OA group displayed a significant increase in the mRNA levels of proinflammatory cytokines (IL-1 β , IL-6, TNF- α) in rat cartilage tissue. However, QH treatment effectively regulated the mRNA levels of these, with the 1.86g/kg dose group being the most significant. The above results showed that QH had good anti-OA effects in vivo (Fig. 8A-C).

3.6.2 Anti-OA effects of QH in vivo and the underlying mechanisms

Based on network pharmacology and molecular docking results, in order to further verify the mechanism of the anti-OA effect of QH, we investigated the activity of the p-AKT1, p-MAPK1 and C-CASP3 using Western blot analysis. In the OA group, the proteins expression of p-AKT1, p-MAPK1 and C-CASP3 in articular cartilage tissue was significantly increased. After continuous administration of QH for 21 days, the proteins expression of p-AKT1, p-MAPK1 and C-CASP3 was significantly reduced, with the 1.86g/kg dose group being the most significant.

4. DISCUSSION

Osteoarthritis (OA), a prevalent degenerative disease that affects over 240 million people, is the leading cause of disability²⁵. At present, pharmacological interventions (NSAIDs) are often accompanied by adverse reactions, so it is urgent to develop safer treatment strategies. Because of its unique advantages in treating OA, TCM is used as an alternative therapy by many professionals. QH is a key ingredient in anti-OA formulae, but its molecular efficacy as a single drug and the specific signal pathway mechanism involved have not been fully explored. It is worth noting that the progress of OA is closely related to inflammatory reaction and chondrocyte apoptosis, in which the abnormal activation of MAPK and PI3K/Akt signaling pathways accelerates cartilage degradation^{26,27}. Previous studies have indicated that QH extracts possess potent anti-inflammatory properties. Therefore, this study used network pharmacology combined with molecular docking and experimental verification to explore the bioactive components and molecular mechanism of QH in the treatment of OA.

Network pharmacology is consistent with TCM theory's holistic view. By constructing "drug-target-disease" interaction networks, it provides a systematic framework for predicting bioactive components in herbal medicines and disease-related pathways. We collected the active components of 15 species of *Notopterygium* from the TCMSP database. Among them, Bergapten, Notoptol, and Coumarin-glycoside were identified as key active components based on their high degree values in the network. This result is highly consistent with previous pharmacological studies. For example, studies indicate that Bergapten suppresses the expression of pro-inflammatory cytokines such as IL-6 and TNF- α , thereby mitigating extracellular matrix degradation and effectively preserving the chondrocyte phenotype²⁸. Similarly, coumarin derivatives have been demonstrated to inhibit the production of inflammatory cytokines while enhancing the proliferative capacity of chondrocytes²⁹.

Further analysis of the protein-protein interaction (PPI) network suggests that AKT1, MAPK1, and CASP3 may serve as key hub targets for QH in the treatment of OA. To validate the interactions between the identified bioactive compounds and these key proteins, we performed molecular docking simulations. The results indicate that key active ingredients, particularly Bergapten and Notoptol, possess high binding affinities, with binding energies consistently lower than -7 kcal/mol. Notably, the binding energy of QH components with AKT1 was significantly lower than that of other targets, suggesting AKT1 might be a primary molecular target. These findings suggest that QH may exert its comprehensive therapeutic effects by simultaneously promoting cell survival and ameliorating the inflammatory microenvironment.

To validate the predicted results, we conducted animal experiments. The results demonstrated that *Notopterygium* alleviated articular cartilage damage, as confirmed by improvements in OARSI and Mankin scores. Subsequently, we investigated the underlying reasons at the molecular level. The progression of osteoarthritis is closely associated with inflammatory responses and chondrocyte apoptosis³⁰. Our qPCR results showed that QH suppressed the mRNA expression of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α), key mediators that directly trigger the matrix metalloproteinase (MMP) cascade, resulting in irreversible structural damage in articular cartilage^{31,32}. For instance, research has demonstrated that the aqueous extract of QH could significantly reduce the activity of matrix metalloproteinases (MMP-2 and MMP-9), thereby effectively preventing the enzymatic degradation of the extracellular matrix³³.

To further investigate the intracellular signaling pathways controlling these changes, we performed Western blot analysis. Results showed that in the OA group, protein levels of p-AKT1, p-MAPK1, and C-CASP3 were significantly elevated, indicating abnormal pathway activation under inflammatory stress. Notably, treatment with QH reduced the expression levels of p-AKT1, p-MAPK1, and C-CASP3. These findings align with the pathological roles of these

kinases in OA. As a serine/threonine protein kinase, AKT1 regulates cell growth and survival by phosphorylating downstream signaling targets ³⁴. However, within the inflammatory microenvironment of osteoarthritis, driven by pro-inflammatory cytokines such as IL-1 β , AKT1 remains in a state of persistent overactivation. This leads to chondrocyte hypertrophy and catabolic metabolism of the extracellular matrix ³⁵. Similarly, activation of the MAPK1 (ERK2) signaling pathway is closely associated with the release of pro-inflammatory cytokines, ultimately leading to chondrocyte apoptosis ³⁶. QH may exert its chondroprotective effects by inhibiting the excessive activation of these inflammation-driving kinases, thereby suppressing the cleavage of downstream Caspase-3, consistent with our research findings.

This study still has several limitations. For instance, Network pharmacology analysis depends on existing scientific databases, which are constantly being updated. Therefore, some active components or potential targets of QH may not have been fully captured in our current analysis. Furthermore, while we validated the protective effects of QH in animals, we did not perform cell experiments to confirm the direct influence of specific ingredients on the identified proteins. Future research should focus on testing these individual components in cell models to better understand how they work together.

Conclusion

In summary, this study demonstrates that Qianghuo (QH) exerts a significant therapeutic effect on Osteoarthritis (OA) by mitigating cartilage degeneration and suppressing inflammatory responses. Through an integrated approach combining network pharmacology, molecular docking, and in vivo experimental validation, we discovered that Bergapten and Notoptol are likely the key bioactive ingredients. Our findings indicate that the anti-OA mechanism of QH is primarily mediated by the inhibition of the MAPK and PI3K-Akt signaling pathways, specifically through the downregulation of p-AKT1, p-MAPK1, and C-CASP3. While the direct interaction between these active ingredients and targets warrants further verification in cellular models, this work establishes a solid theoretical and experimental foundation for developing QH-derived agents as potential OA therapeutics.

Declarations

Acknowledgements

Not applicable.

Funding

This research study was sponsored by the National Natural Science Foundation of China (No. 82474538) and Tianjin Jinmen Medical Talent Program (TJSJMYXYC-D2-028)

Availability of data and materials

The repository/repositories presented in this study can be found in the article. The data of animal experiments in this study are available from the corresponding author upon request.

Authors' contributions

AL designed the study. GC and WW analyzed the data, conducted animal OA modeling, and drafted the manuscript. BS and EX collected the data, generated all figures, and prepared the supplementary information. GC

and WW contributed equally to this work. All authors read and approved the submitted version.

Ethics approval and consent to participate

The animal study was reviewed and approved by the Committee of Ethics on Animal Experiments at the Anhui Wanbei Coal-Electricity Group General Hospital [No. WBYY2023034].

Clinical Trial Registration

Clinical trial number: not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

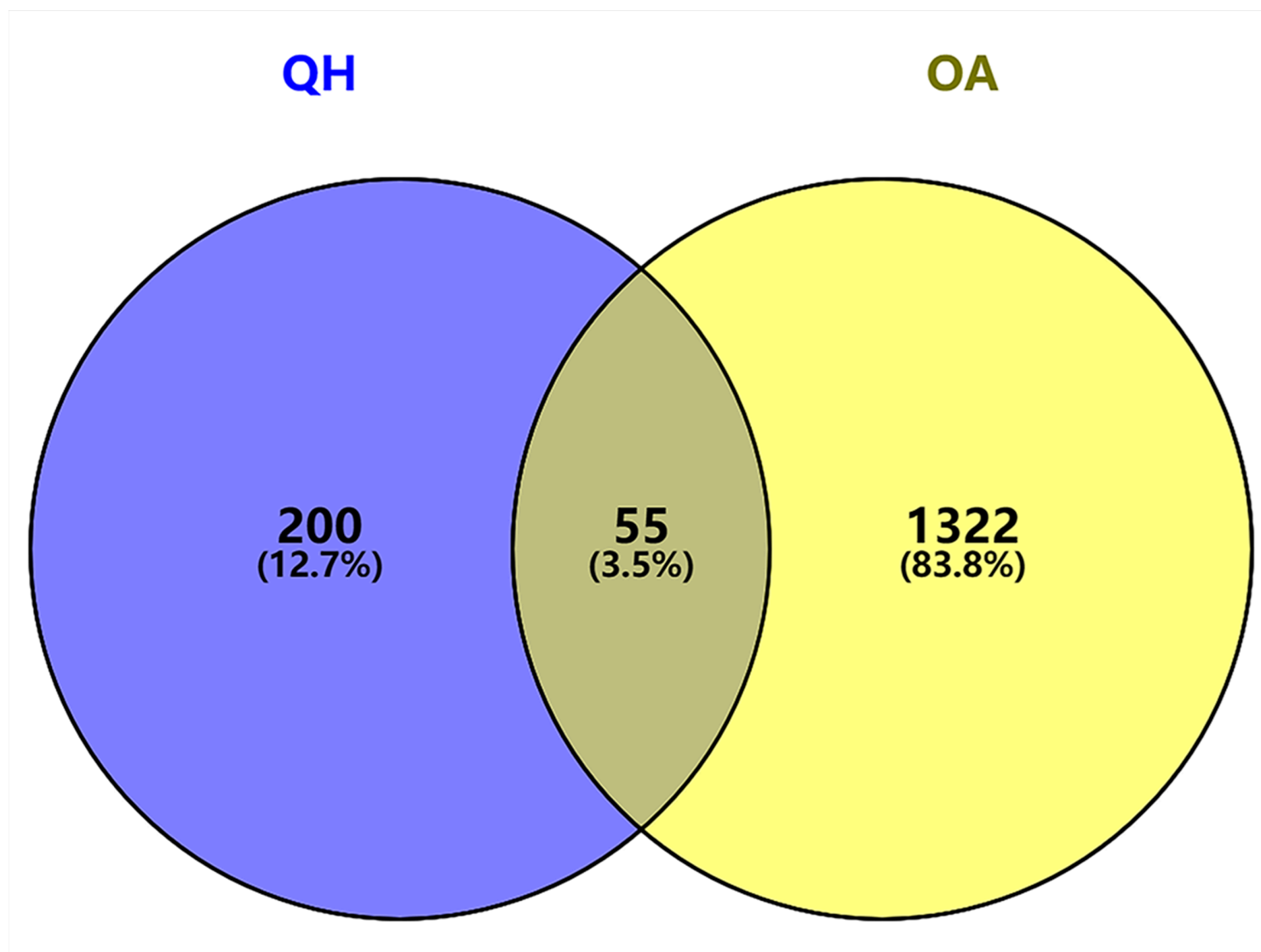


Figure 1

Intersection of QH and OA targets.

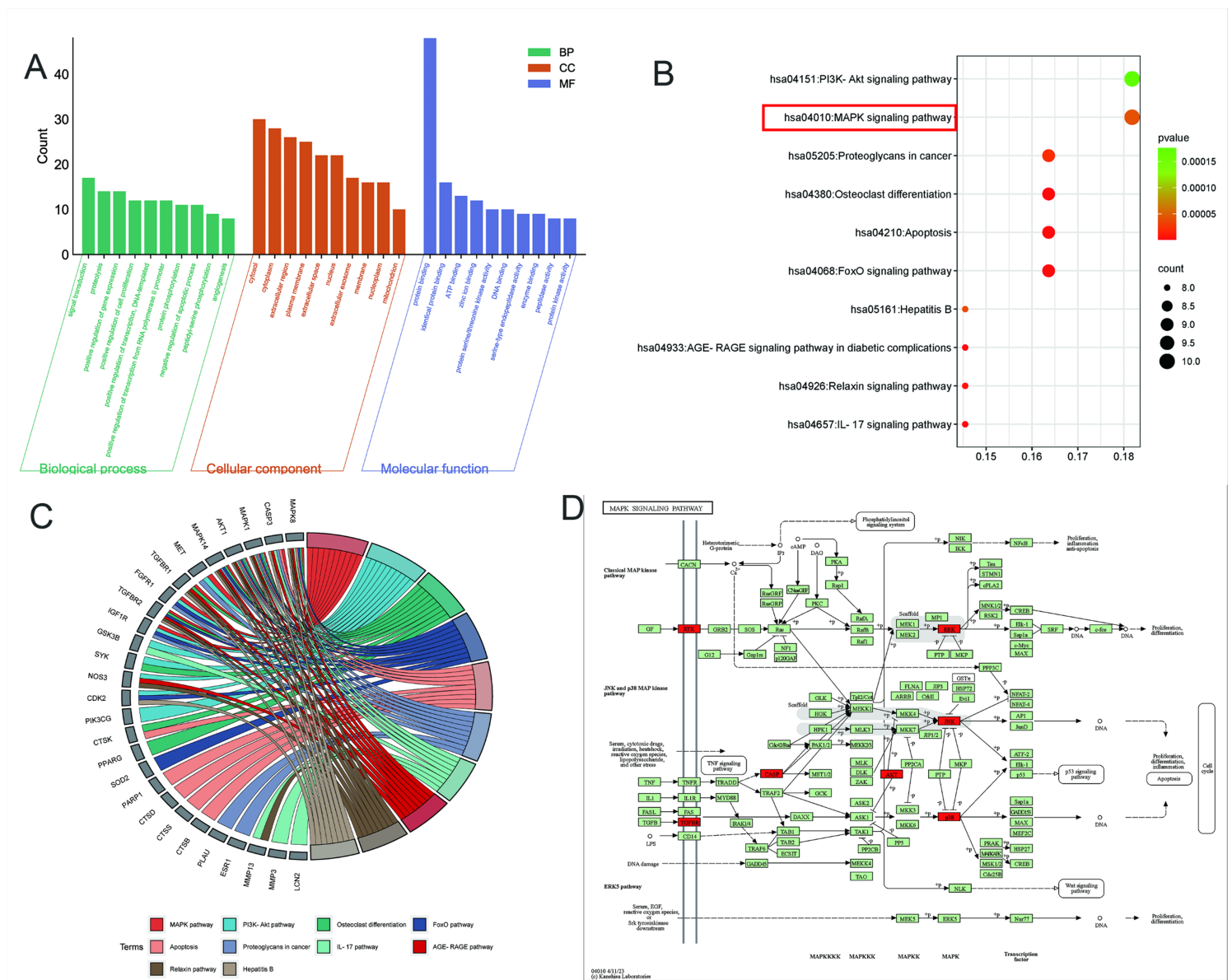


Figure 2

GO and KEGG enrichment analysis of potential targets for QH against OA. (A) Results of GO enrichment analysis of intersection targets. (B) Results of KEGG enrichment analysis of cross-targets. (C) The chord diagram showing significantly enriched top 10 signaling pathways in KEGG. (D) Mechanism diagram of the MAPK signaling pathway.

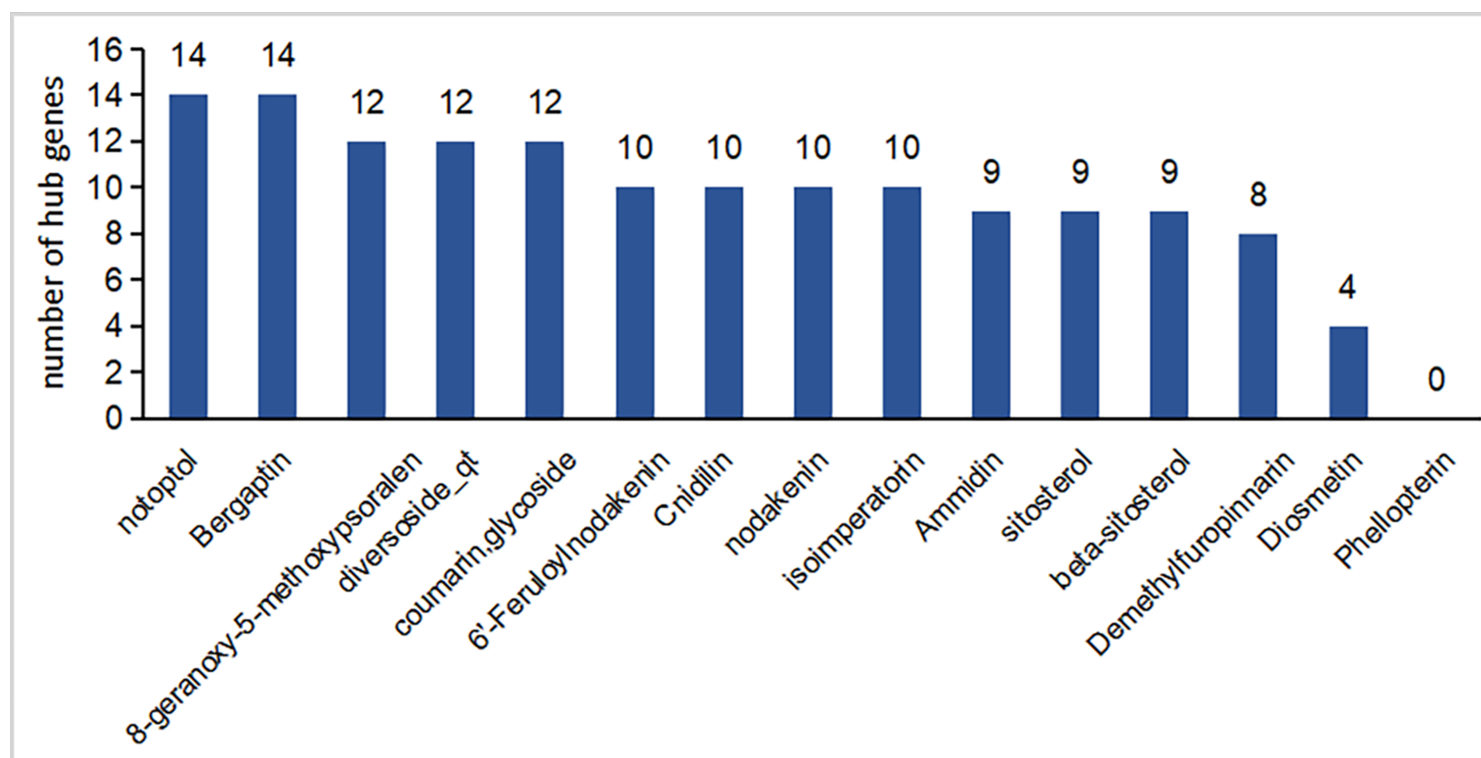


Figure 5

Ranking of QH active ingredients by hub gene count.

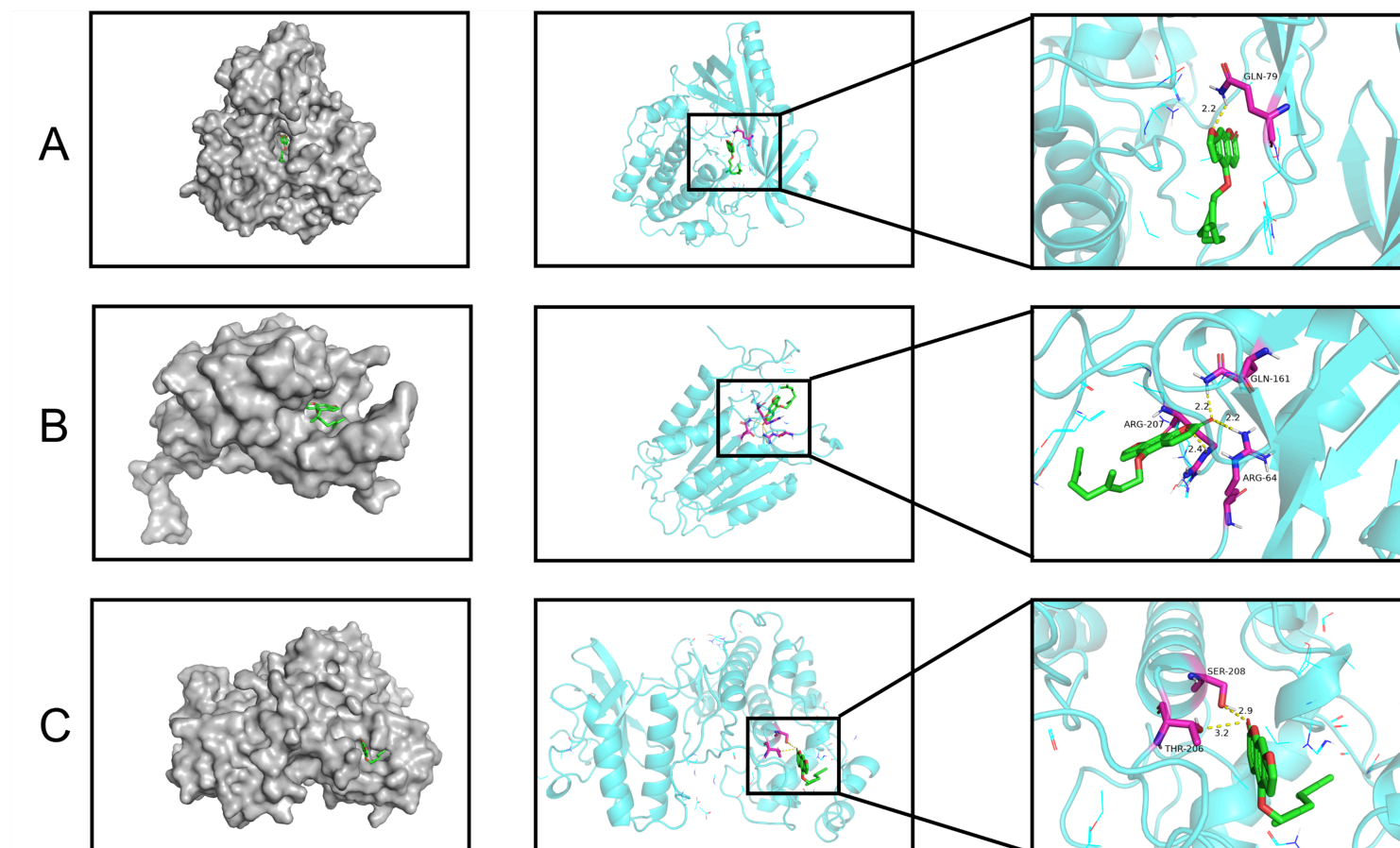


Figure 6

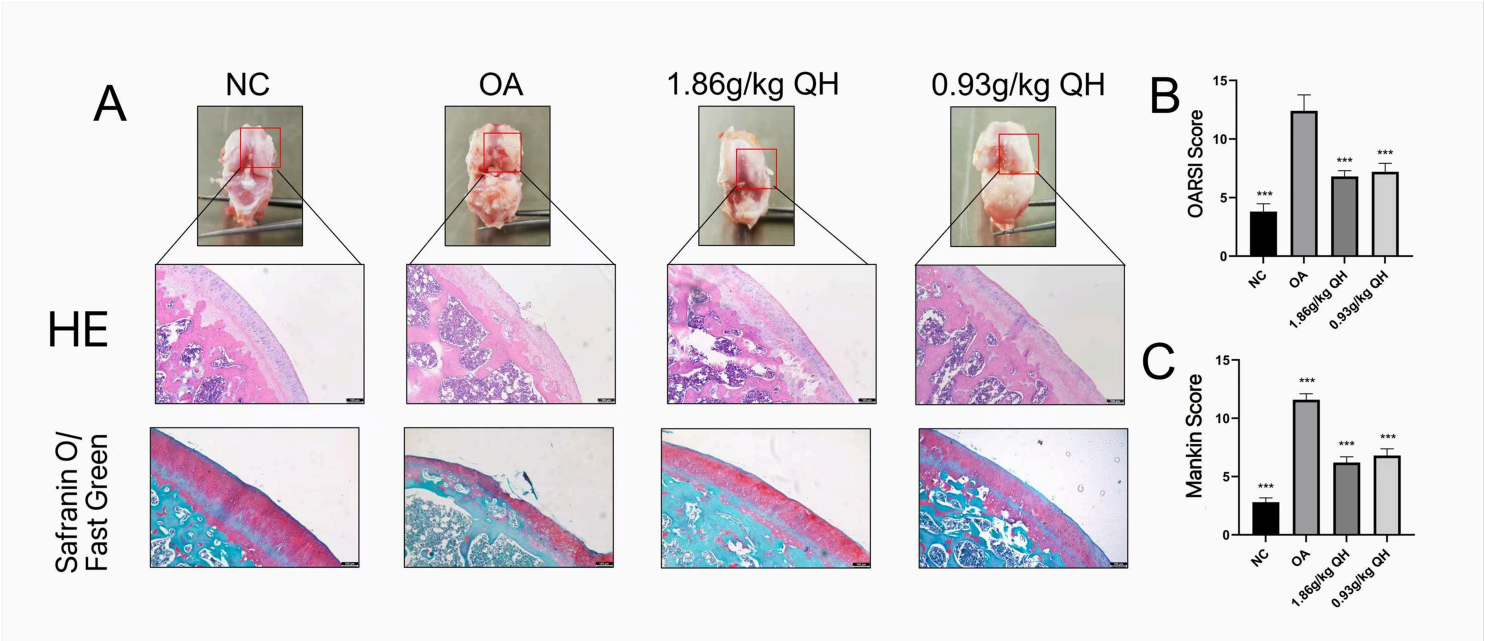


Figure 7

Histological evaluation of articular cartilage in rats. (A) Representative images of articular cartilage stained with HE and Safranin O/Fast Green in different groups. (B) OARSI scores of different groups. (C) Mankin scores of different groups.

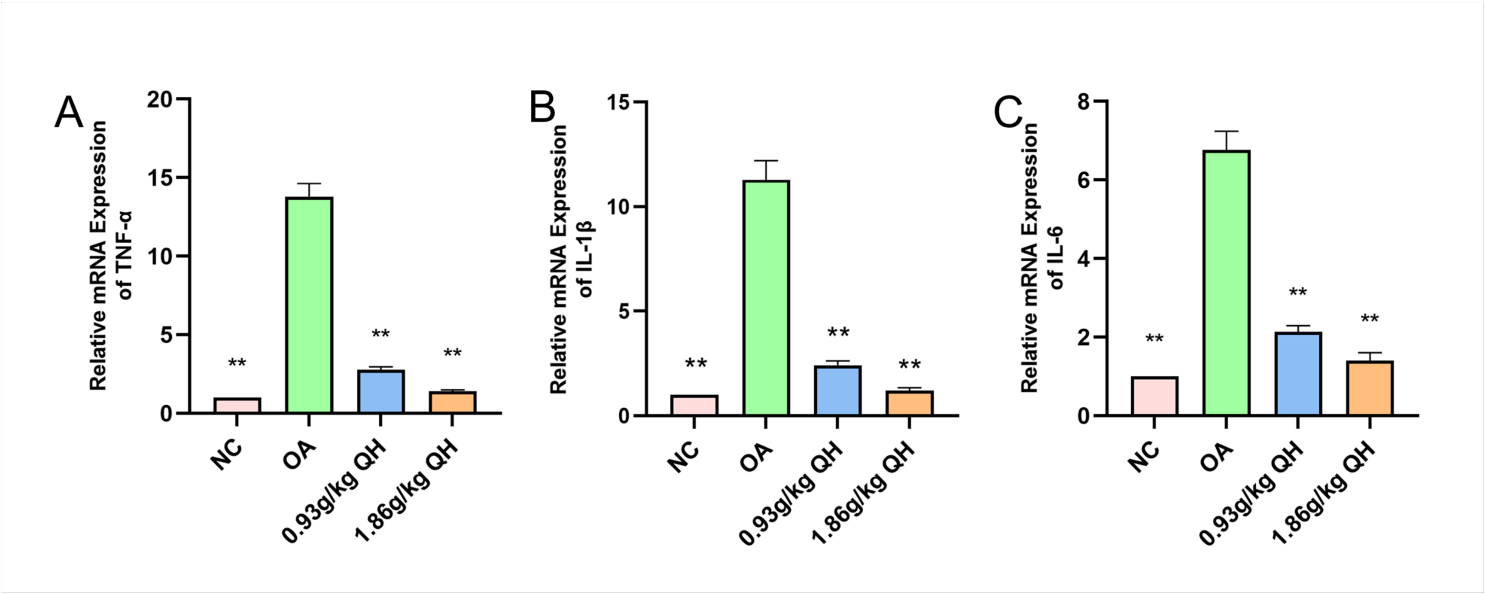


Figure 8

Effect of QH on the mRNA expression of pro-inflammatory cytokines in rat articular cartilage. (A) Relative mRNA expression of TNF-α in cartilage of rats in different groups. (B) Relative mRNA expression of IL-1β in cartilage of rats in different groups. (C) Relative mRNA expression of IL-6 in cartilage of rats in different groups.

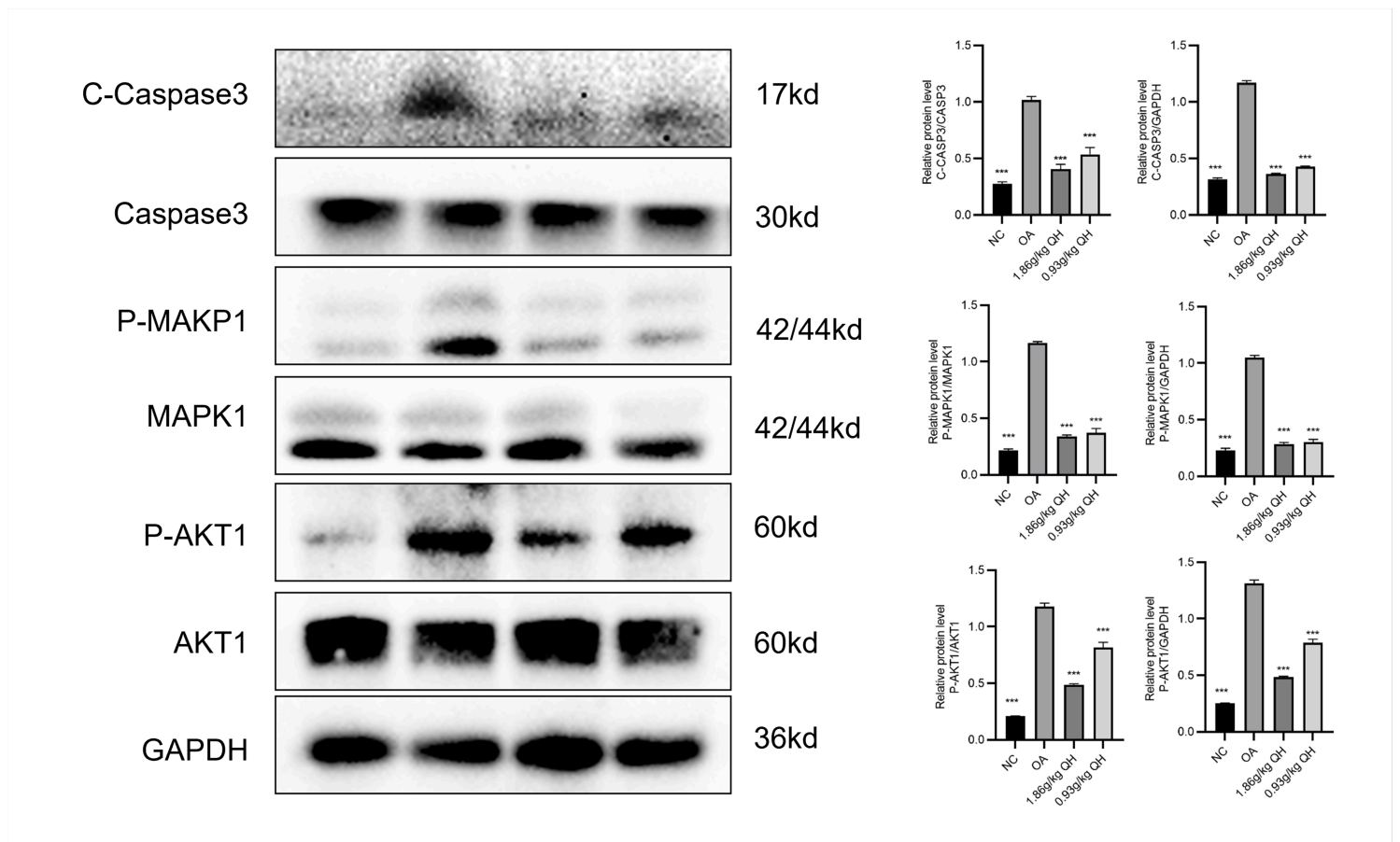


Figure 9

Western blot analysis of key pathway proteins (AKT1, MAPK1, and Caspase-3) in rat articular cartilage.

Supplementary Files

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- [Supplementarymaterial.pdf](#)