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ORIGINAL RESEARCH

Exploring the Therapeutic Potential of FSGTC for Osteoarthritis: A Comprehensive Study Combining Nested Case Analysis, Network Pharmacology, and Experimental Validation

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Objective: This research aims to clarify the clinical efficacy and potential mechanisms of Fengshi Gutong capsule (FSGTC) in improving inflammatory response and hypercoagulability in osteoarthritis (OA) patients, and to evaluate the safety of FSGTC.

Methods: A nested case-control study and association rule analysis were used to evaluate the effects of FSGTC on inflammation, coagulation, and liver and kidney function in OA patients. Screening key pathways for FSGTC treatment of OA through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Subsequently, Hematoxylin-eosin staining (HE), Safranine O-Fast Green staining (S&O), and Immunohistochemistry (IHC) were used to evaluate the effects of FSGTC on cartilage injury, inflammatory cell infiltration, and protein expression in OA rats induced by monosodium iodoacetate (MIA). ELISA detects the expression of pro-inflammatory and procoagulant factors. Organ index and HE staining of organs to evaluate the safety of FSGTC treatment. Subsequently, further validate the above results in IL-1β - stimulated chondrocytes.

Results: The clinical data analysis showed that FSGTC can significantly improve inflammation and coagulation indicators in OA patients. The KEGG pathway enrichment analysis results showed that PI3K/AKT is a key signaling pathway for FSGTC intervention in OA. Animal experiments have shown that FSGTC can alleviate cartilage damage and reduce inflammatory cell infiltration in OA rats, while having no effect on organs such as liver, heart, spleen, and kidney. The cell experiment results further confirmed that FSGTC increases chondrocyte viability and reduces the expression levels of COX2, PGE2 and PAI-1 by inhibiting the activation of the PI3K/AKT signaling pathway.

Conclusion: FSGTC can alleviate inflammation and hypercoagulability in OA, and this therapeutic effect is attributed to its inhibition of PI3K/AKT pathway activation, thereby reducing the release of pro-inflammatory and procoagulant factors in OA patients, and the above drugs do not affect the liver and kidney function of patients.

Keywords: osteoarthritis, fengshi gutong capsule, inflammatory response, hypercoagulable state, PI3K/AKT pathway

Introduction

Osteoarthritis(OA) is a joint pain and functional impairment caused by characteristic lesions of joint tissue, clinically manifested as joint pain, stiffness, and limited mobility.[1,2] Its pathogenesis involves multiple aspects such as chondrocyte aging, inflammatory response, Extracellular Matrix(ECM) degradation, and bone remodeling.[3–6] According to the latest data from the Global Burden of Disease (GBD), OA currently affects 595 million people worldwide and is the 7th leading cause of disability globally.[7] It seriously affects the quality of life of patients and imposes a heavy burden on national health and the economy.[8,9]

Hypercoagulable state, also known as hypercoagulability or pre thrombotic state, refers to a state in which blood clotting factors are excessively active, leading to abnormal coagulation of the blood.[10] This state is caused by various

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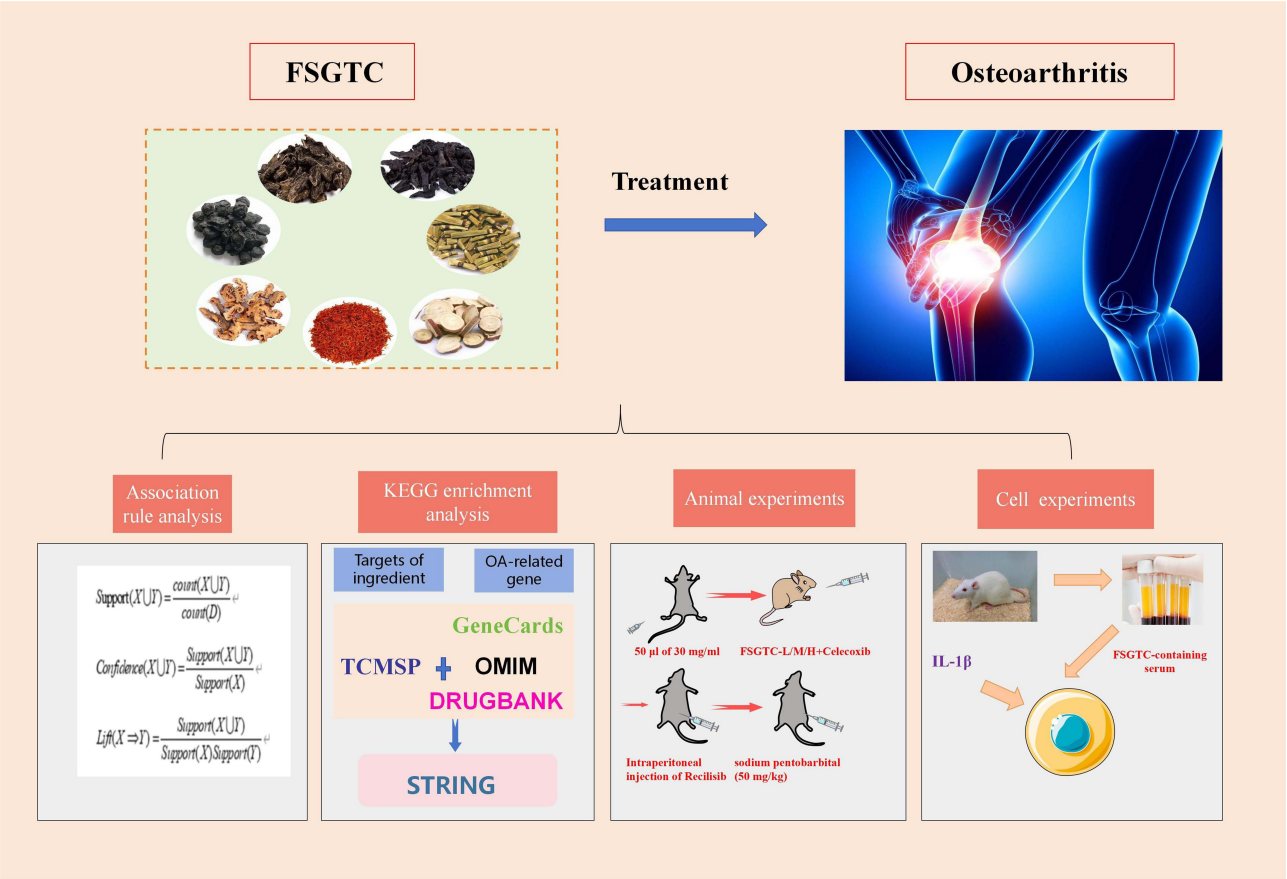
Graphical Abstract



factors such as genetics, diseases, and lifestyle habits, which may lead to serious complications such as deep vein thrombosis (DVT), pulmonary embolism, stroke, and heart disease in patients, and have a profound impact on human health.[11,12] Laboratory testing is an important means of diagnosing hypercoagulable state, and commonly used detection indicators include activated partial thromboplastin time (APTT), fibrinogen (FBG), platelet (PLT), prothrombin time (PT), and thrombin time (TT). Abnormal elevation of these indicators indicates that the blood is in a hypercoagulable state or has a tendency to form blood clots.[13] According to reports, pro-inflammatory factors promote the degradation of extracellular matrix in chondrocytes, causing joint tissue damage, while also causing damage to vascular endothelial cells, directly or indirectly activating the coagulation fibrinolysis system, and interfering with anticoagulation, leading to microcirculation disorders, systemic blood circulation abnormalities, and ultimately causing hypercoagulability in OA patients.[14–16] A data mining study based on 717 OA patients confirmed that OA patients have hypercoagulability, and inflammation is a risk factor for hypercoagulability in OA patients.[17] Similarly, another cohort study based on 3747 OA patients also confirmed the above conclusion. In addition, the study found that the incidence of hypercoagulability in OA patients was about 25.54%, and the interaction between inflammation and hypercoagulability increased the risk of readmission in OA patients.[18] Therefore, taking appropriate measures to suppress the inflammatory response of OA, maintain the balance of the fibrinolytic system, improve the inflammatory response and hypercoagulable state are the latest strategies to reduce the readmission risk of OA patients.

As a heterogeneous and multifactorial disease, the currently available treatment options for OA have not produced fully satisfactory results.[19] The recommended surgical or medication regimen, such as nonsteroidal anti-inflammatory

drugs (NSAIDs), may not fully meet the expectations of patients and may even cause serious side effects.[20,21] In this context, exploring effective and feasible treatment methods is still imperative. In recent years, with the gradual deepening of traditional Chinese medicine research, the role of traditional Chinese medicine(TCM) compound preparations in the treatment of OA has been widely recognized.[22,23] TCM theory refers to OA as "Bi", specifically, it refers to the invasion of wind, cold, and dampness pathogens into the human body's muscles, veins, and joints, causing blockages in meridians and qi and blood, leading to the onset of the disease. Therefore, it emphasizes the use of drugs with the effect of "unblocking meridians and relieving pain" for treatment.

FSGTC originated from the Aconitum Decoction formula in Zhang Zhongjing's "Synopsis of the Golden Chamber" during the Han Dynasty. It is a traditional herbal formula approved by the China Food and Drug Administration (No. Z34020025) with the functions of warming the meridians, dispelling cold, and relieving pain. FSGTC is composed of seven herbs including Aconitum carmichaelii Debeaux (https://mpns.science.kew.org/), Aconitum kusnezoffii Rchb (https://mpns.science.kew.org/), Carthamus tinctorius L.(https://mpns.science.kew.org/), Chaenomeles speciosa (Sweet) Nakai(https://mpns.science.kew.org/), Prunus mume (Siebold) Siebold & Zucc.(https://mpns.science.kew.org/), Ephedra sinica Stapf (https://mpns.science.kew.org/), and Glycyrrhiza uralensis Fisch. ex DC.(https://mpns.science.kew.org/) in the ratio of 1:1:1:1:1:1:1. Our previous studies used high-performance liquid chromatography (HPLC) technology to analyze the fingerprint spectra of 12 batches of FSGTCs, and the results showed that the 12 batches of FSGTCs had high similarity (0.973~0.998). We have established a quality control method that combines qualitative and quantitative methods with higher testing efficiency, lower detection costs, and better specificity by combining TLC technology, thin-layer bioautography technology, and HPLC technology, in order to achieve strict quality control.[24–26] In addition, we have revealed the pharmacological mechanism of FSGTC by identifying the key active ingredients involved in its entry into the bloodstream. Please refer to the supplementary materials for specific details (Supplementary, Supplementary Figure 1, and Supplementary Figure 2). Meta analysis of clinical observational studies shows that FSGTC has advantages in reducing inflammatory response, improving clinical symptoms, and enhancing patients' quality of life.[27,28]

Although previous studies have shown the strong therapeutic effect of traditional Chinese medicine FSGTC on OA patients. However, the therapeutic effect and related mechanisms of FSGTC on hypercoagulable state in OA patients are still unclear. Therefore, this study conducted a nested case-control study and association rule analysis to clarify whether the addition of FSGTC to conventional treatment can improve hypercoagulability in OA patients, and further clarify the efficacy and safety of FSGTC drugs through animal and cell experiments, exploring its mechanism of improving OA inflammation and coagulation factor disorders.

Materials and Methods

Clinical Information of OA Patients

All data used in the nested case-control study were from the Department of Rheumatology at the First Affiliated Hospital of Anhui University of Chinese Medicine. We used the diagnostic criteria for primary symptomatic OA defined by the American College of Rheumatology and randomly selected samples from hospitalized OA patients based on gender, age, and completeness of laboratory indicators.[29] Finally, data from 36 OA patients who received FSGTC treatment and had complete laboratory indicators were collected. The collected clinical data includes basic patient information (gender, age) and clinical laboratory indicators. Clinical laboratory indicators include high-sensitivity C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), APTT, PLT, FBG, TT, PT, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), and urea nitrogen (BUN).This study study complies with the Declaration of Helsinki. This study protected the privacy of the patients during the process of information collection and did not interfere with the treatment plan. The Ethics Committee of the First Affiliated Hospital of Anhui University of Chinese Medicine waived the need for informed consent.

Confirmation of Case Group and Control Group

The main outcome is the changes in laboratory indicators of hospitalized patients. Subsequently, each case was randomly matched to a control group that did not use FSGTC based on age, gender, and comorbidities. The observation dates

assigned to each control group correspond to the observation dates of matched patients, ensuring that the observation probabilities of all included subjects during the study period are the same.

Association Rule Analysis

Association rules reflect the correlation between things, and their most famous algorithm is the Apriori algorithm.[30] As one of the data mining methods, the effectiveness verification of association rules is mainly measured through support and confidence. Support expresses the probability of the association between the two occurring in the population.[31] The formula is as follows:

$$\text{support}(X \rightarrow Y) = \frac{X \cup Y}{N\sigma}$$

Among them, X and Y are project variables; N is the overall number of projects; (X ∪ Y) is the probability of X and Y occurring simultaneously; σ is the support value of N.

The confidence level represents the probability of the occurrence of another feature attribute Y when one feature attribute X that constitutes the association rule occurs, reflecting the strength of the association between the two. The calculation formula is as follows:

$$\text{confidence}(X \rightarrow Y) = \frac{X \cup Y}{\sigma\ (X)}$$

Where X and Y are project variables; (X ∪ Y) is the probability of X and Y occurring simultaneously; σ (X) is the probability of X occurring.

The lift reflects the mutual relationship between the two. A degree of improvement of 1 indicates no correlation between the two; If the degree of improvement is less than 1, it indicates a negative correlation between the two, and the involved feature attributes are mutually exclusive; A degree of improvement greater than 1 indicates a positive correlation between the two, and the involved feature attributes are symbiotic. The formula is as follows:

$$\text{lift}(X \rightarrow Y) = \text{confidence}\frac{(X \rightarrow Y)}{\sigma\ (Y)}$$

This study assigned values based on whether FSGTC was used and whether the patient's laboratory indicators improved after treatment (with "yes" set to 1 and "no" set to 0). The minimum support level was set to 30%, the minimum confidence level was set to 50%, and the maximum number of leading terms was set to 1. IBM SPSS Modeler 18.0 software was used for association rule analysis.

Target Acquisition and KEGG Pathway Enrichment Analysis

FSGTC was composed of seven herbs, namely Aconitum carmichaelii Debeaux, Aconitum kusnezoffii Rchb, Carthamus tinctorius L., Chaenomeles speciosa (Sweet) Nakai, Prunus mume (Siebold) Siebold & Zucc., Ephedra sinica Stapf, and Glycyrrhiza uralensis Fisch. ex DC.These herbs were then input into the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) to determine their active ingredients. The active compounds were screened according to oral bioavailability (OB) values ≥ 30% and drug-likeness (DL) values ≥ 0.18. Download the 2D structure of the chemical components of FSGTC drugs from the Public Chemistry Database (PubChem, https://pubchem.ncbi.nlm.nih.gov/). Then, import the structure into the Swiss Target Prediction database, set the attribute to "Homo sapiens", remove targets with scores less than 0, merge and deduplicate the remaining targets to obtain the component targets of FSGTC drugs.

"Osteoarthritis" was used as a keyword to search four databases (the DrugBank database, the Genecards, the Therapeutic Target Database (TTD), and the Online Mendelian Inheritance in Man (OMIM)) in order to acquire the OA-related targets.Then, the drug-related targets were intersected with the disease targets, followed by the plotting of a Venn diagram of the intersected targets with Online drawing tools (https://www.bioinformatics.com.cn/). Subsequently, the intersecting targets will be imported into the Metascape database (https://metascape.org) for KEGG pathway enrichment analyses.

KEGG pathways were ranked by count. Obtain the P value enriched by KEGG. Then carry out multiple test correction to get the FDR value[FDR=POWER (log10 (p), log10 (q))]. With FDR≤0.05 as the threshold, the KEGG term meeting this condition is defined as the KEGG term significantly enriched in the differentially expressed genes. Then, the top 20% enriched KEGG pathways were selected for further analyses.

Drugs and Reagents

Fetal bovine serum (FBS) (Cat.No.G4207-500ML) were obtained from Servicebio (Wuhan, China). Cell counting kit-8 (CCK-8)(Cat.No.GK100001) were obtained from GLPBIO (Shanghai, China). Human chondrocyte culture medium (Cat. No.CM-H107) were obtained from Procell Life Science&Technology Co., Ltd (Wuhan, China). PI3k/AKT inhibitor (LY294002, Cat.No.HY-10108) and agonist (Recilisib, Cat.No.HY-101625/CS-6712) were obtained from MedChemexpress (Shanghai, China).IL-1β (Cat.No.CG93) were obtained from Novoprotein Scientific Inc. (Suzhou, China).PGE2 ELISA kit (Cat.No.RX105367H), COX2 ELISA kit (Cat.No.RX106931H) and PAI-1 ELISA kit (Cat.No. RX105072H) were obtained from Ruixin Biotechnology Co., Ltd (Quanzhou, China). MIA (Cat.No.M9337) were obtained from Abmole (Beijing, China).BCA Protein Concentration Determination Kit (Cat.No.P0010S) were obtained from Beyotime Biotechnology (Shanghai, China).β-actin (Cat.No.TA-09), Goat anti mouse IgG(Cat.No.ZB-2305) and Goat anti rabbit IgG(Cat.No.B-2301) were obtained from Zs-BIO(Beijing, China).P-AKT(Cat.No.AF0016), AKT(Cat. No.AF6261) and P-PI3K (Cat.No.AF3241) were obtained from Affinity Biosciences(Beijing, China).PI3K (Cat. No.60225-1-Ig) were obtained from Proteintech Group, Inc(Wuhan, China).Hematoxylin staining solution (Cat. No. 10092410), alcohol soluble eosin staining solution (Cat. No. 10132415), and safranin solid green staining solution (Cat. No. 10122416) were obtained by ebiogo (Anhui, China).ALT(Cat.No.C009-2-1), AST(Cat.No.C010-2-1) Cr(Cat. No.C011-2-1), and BUN(Cat.No.C013-2-1) were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

Rat PGE2 ELISA kit (Cat.No.JYM0446Ra), Rat COX2 ELISA kit (Cat.No.JYM0885Ra) and Rat PAI-1 ELISA kit (Cat.No.JYM0450Ra) were obtained from Wuhan Gene Beauty Technology Co., Ltd(Wuhan, China).

Animals

Sixty-eight clean-grade male Sprague-Dawley (SD) rats (aged 6–8 weeks, weighing 200 ±20 g; Pizhou Oriental Breeding Co., Ltd., Jiangsu, China) were fed in the standard clean laboratory animal room of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine under a quiet indoor environment with a temperature of 18–22°C and a relative humidity of 50–75%. Ventilation and exhaustion were maintained at 10–20 times per hour. All animal studies in this research were performed in accordance with the principles of animal ethics and the Regulations for the Administration of Experimental Animals established by the People's Republic of China. These experiments were reviewed and approved by the Laboratory Animal Ethics Committee of Anhui University of Traditional Chinese Medicine (AHUCM-rats-2022068).

OA Rat Model Establishment and Rat Grouping

Twenty rats were randomly categorized into normal serum and drug-containing serum groups (10 rats/group). FSGTC-containing serum was prepared according to our previously published article.[24] Forty-eight rats were randomly allocated into control (n = 6) and OA (n = 42) groups. Rats in the OA group were given 50 μL of 30 mg/mL MIA intra-articularly for 7 days to establish the OA model and then randomly arranged into model, low-dose FSGTC (FSGTC-L), medium-dose FSGTC (FSGTC-M), high-dose FSGTC (FSGTC-H), Celecoxib, Recilisib and Recilisib+FSGTC groups (n = 6 rats/group). After the establishment of the model, the Recilisib and Recilisib+FSGTC groups were intraperitoneally injected with Recilisib (1 mg Recilisib dissolved in 50mL physiological saline was indocalculated intraperitoneally).According to the principle of equal dosage per unit body surface area among various experimental animals, drug conversion was carried out with the following formula: clinical equivalent dose = X mg/kg × 70 kg × 0.018/200 g = 6.3 X mg/kg (X: clinical dose; 70 kg: adult mass; 200 g: rat mass).[32] Low, medium, and high dose groups were set up at 2, 4, and 8 times of the clinical equivalent dose, with 1 mL/100 g as the standard gavage. Finally, rats in the FSGTC-L/M/H groups were given FSGTC at 7.56/15.12/30.24 g/kg/day by gavage. Rats in the Celecoxib group received Celecoxib at 10.08 g/kg/day

(8 times of the clinical equivalent dose) by gavage and rats in the control and model group were gavaged with 0.9% NaCl at 1 mL/100 g/day. During the experiment, no rats died. Gavage was continued for 14 consecutive days. Rats were euthanized with pentobarbital sodium (50 mg/kg, 20200216; Xiensi Biochemical Technology Co., Ltd., Tianjin, China), followed by the collection of blood from the abdominal aorta of rats. Next, serum was obtained subsequent to 15-min centrifugation of blood at 3000 r/min. The knee joints of rats were separated, fixed in 4% paraformaldehyde, demineralized, and embedded with paraffin as described previously.[33] Then, the samples were sectioned consecutively at 4 μm for pathological examination.

Cell Model Construction and Grouping

Resuscitate and culture human chondrocytes in culture medium, and freeze them after passage for future use. The experimental groups are as follows: CHs, CHs+IL-1β, IL-1β+FSGTC, IL-1β+LY294002(PI3k/AKT inhibitor), IL-1β +FSGTC+Recilisib(PI3k/AKT agonist). The CHs+IL-1β group was treated with 10ng/mL IL-1β+ normal rat serum(10%) for 24 hours, while the IL-1 β+FSGTC group was pre treated with 15% drug containing serum for 24 hours.[34] A culture medium containing 10ng/mL IL-1β+15% drug containing serum was prepared for medium exchange and further cultured for 24 hours. The IL-1β+LY294002 group was treated with 25 μmol/L LY294002 for 1 hour, and a culture medium containing 10ng/mLIL-1β+10% normal serum was prepared for medium exchange. The culture was continued for 24 hours. The IL-1β+FSGTC+Recilisib group was treated with 15% medicated serum for 24 hours, and then a culture medium containing 10ng/mL IL-1β+15% medicated serum+50 μmol/L Recilisib was prepared for medium exchange. The culture was continued for another 24 hours. After cultivation, collect cells from each group for subsequent experiments.

Biochemical and ELISA Testing

The corresponding biochemical detection kit was used to detect the levels of ALT, AST, Cr, and BUN in the blood of rat abdominal aorta.The corresponding ELISA kit was used to detect the levels of PGE2, COX2, and PAI-1 in cell supernatant and rat synovial fluid.

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 was used to detect the cell viability of chondrocytes in each group to evaluate the protective effect of FSGTC on chondrocytes. The specific steps can be found in our previously published papers.[35]

Western Blot (WB)

RIPA lysis buffer (Biosharp) was used to lyse CHs and extract proteins. The antibodies employed in this study were as follows: goat anti-rabbit IgG (1:10,000) and goat anti-mouse IgG (1:10,000). β-Tubulin (1:1000) and PI3K(1:5000) antibodies are derived from mouse.P-AKT(1:500), AKT(1:1000) and P-PI3K(1:500) antibodies are derived from rabbit. Finally, the protein was measured with a hypersensitive electrochemiluminescence kit. The OD value of the target protein was calculated with β-actin as the internal control. Image J software was used for analyzing protein bands.

Hematoxylin-Eosin Staining (HE)

The cartilage sections were baked in a drying oven at 66°C for 30 min and treated with xylene three times (5 min/times) and gradient ethanol (100%, 95%, and 80%; 3 min/concentration). The sections were slowly rinsed with running water to remove ethanol, cleared, immersed in hematoxylin for 2−5 min, and rinsed thoroughly with running water, followed by 1% hydrochloric acid alcohol differentiation and complete running water rinsing. The sections were returned to blue with saturated lithium carbonate solutions, rinsed with running water, dehydrated with 95% ethanol for 2 min, and shaken up and down several times. The sections were immersed in eosin solutions (alcohol-soluble), treated with 95% ethanol two times (5−10 s/time), dehydrated with 100% ethanol two times (1 min/time), and cleared with phenol, xylene I, and xylene II (2 min each), followed by neutral gum sealing and microscopic observation.

Safranine O-Fast Green Staining (S&O)

Cartilage sections were routinely dewaxed and hydrated, with the hydrophobic circle drawn. The sections were added dropwise with Weigert staining solutions for 5 min, rinsed with distilled water, differentiated with acidic differentiation solutions, and rinsed with distilled water before fast green staining for 5−10 min and distilled water washing. The sections were quickly washed with weak acid solutions to remove residual fast green staining solutions, stained with safranin O staining solutions for 5−10 min, and washed with distilled water, followed by conventional dehydration, xylene clearing, neutral gum sealing, and microscopic observation.

Immunohistochemistry (IHC)

After being trypsinized, the cartilage sections were washed with xylene and ethanol, cultured at room temperature in 3% H2O2 for 20 min, and washed with distilled water three times. After the hydrophobic circle was drawn, the sections were washed with phosphate-buffered saline (PBS) with Tween 20 (PBST) three times. After excess liquids were removed, the sections were incubated with primary antibodies at 37°C for 60 min, rinsed with PBST three times, and incubated with secondary antibodies in a 37°C incubator for 30 min, followed by three PBST washes. The sections were developed with 3,3'-diaminobenzidine. Color development was controlled under the microscope and terminated if positive. Next, the sections were rinsed with distilled water, counter-stained with hematoxylin for 2−5 min, and washed thoroughly with water, followed by 1% hydrochloric acid alcohol differentiation and water rinsing. The sections were returned to blue with lithium carbonate solutions for 30s and washed with water. Following conventional dehydration and xylene clearing, the sections were sealed with neutral gum and observed under the microscope.

Statistical Methods

Clinical data were processed with SPSS v.23.0 (IBM, Armonk, NY, USA), and experimental data were analyzed with GraphPad Prism (version 9.0.0) software. Measurement data with normal distribution were represented by mean ± standard deviation, with the *t*-test for inter-group comparisons. Measurement data with skewed distribution were presented as median (interquartile range), that is, M (P25, P75). Differences were considered statistically significant at $P < 0.01$.

Results

FSGTC Significantly Improves Inflammation and Coagulation Indicators in OA Patients

The final study cohort included 72 patients. During the entire study period, a total of 36 pairs of OA patients with and without FSGTC were included in the study. The average age of both groups of patients is 66 years old. According to the random matching process, there was no difference between the two groups in terms of initial demographic data and comorbidities. The detailed information of baseline related variables is shown in Table 1. Subsequently, we analyzed

Table 1 Population Characteristics

Variables	Number (N,%)	Cases (N=36, %)	Controls (N=36, %)	Standardized Difference
Age(Years)				
≤70	48(66.67)	23(63.89)	25(69.44)	0.132
>70	24(33.33)	13(36.11)	11(30.56)	0.023
Gender				
Male	12(16.67)	6(16.67)	6(16.67)	1.000
Female	60(83.33)	30(83.33)	30(83.33)	
Treatment time (days)	12.17 (7.78,16.15)	12.20 (7.35,16.15)	12.13 (7.79, 16.15)	0.153
Complications (N/%)				
Hypertension (N/%)	22(30.56)	10(27.78)	12(33.33)	0.609
Hyperlipidemia (N/%)	22(30.56)	13(36.11)	9(25.00)	0.306
Diabetes (N/%)	15(20.83)	8(22.22)	7(19.44)	0.772

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Table 2 Effect of FSGTCon Clinical Laboratory Indexes of OA Patients

Index	Cases			Controls		
	Before Treatment	After Treatment	P-value	Before Treatment	After Treatment	P-value
ESR(mm/h)	22.94(16.25,30.75)	11.11(8.00,15.00)	<0.01	22.71(11.00,30.75)	21.00(10.29.25)	0.03
HCRP (mg/L)	12.23(8.2,16.24)	5.18(4.29,5.39)	<0.01	13.47(8.33,11.91)	9.16(8.37,11.93)	0.93
PLT(×10^9/L)	267.03(179.00,331.50)	205.56(134.25,198.00)	<0.01	269.22(176.75,309.5)	224.22(134,232.50)	0.06
FBG(g/L)	3.71(2.88,4.53)	3.00(2.37,3.38)	<0.01	3.50(2.99,4.16)	3.09(2.44,3.32)	0.03
TT(sec)	18.49(16.88,19.45)	18.53(16.88,19.56)	0.18	19.7(17.6,21.95)	19.63(17.53,21.95)	0.32
PT(sec)	11.10(10.13,11.70)	11.07(10.13,11.63)	0.65	11.63(10.45,11.9)	11.65(10.45,11.9)	0.18
APTT(sec)	29.54(24.48,32.78)	29.58(24.48,32.78)	0.66	28.47(24.33,31.95)	28.51(24.33,31.95)	0.66
ALT (U/L)	18.92(12.00,22.50)	17.06(12.00,20.00)	0.10	17.81(10.25,19.75)	18.22(11.25,24.00)	0.68
AST(U/L)	19.22(16.00,21.00)	17.81(14.25,20.00)	0.02	19.5(16.00,21.00)	20.42(16.25,22.75)	0.45
CREA(umol/L)	56.22(45.70,58.15)	55.18(46.25,56.08)	0.10	64.78(50.20.65.90)	72.57(50.2,70.18)	0.11
BUN(mmol/L)	5.73(4.11,6.76)	5.35(3.94,6.05)	0.15	5.99(4.62,6.59)	5.91(4.36,6.63)	0.65

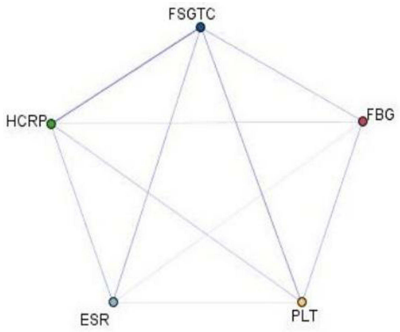
laboratory indicators of two groups of OA patients before and after treatment. The results showed that compared with before treatment, the use of FSGTC improved inflammation indicators (HCRP, ESR) and coagulation indicators (PLT, FBG) in OA patients (P<0.01), and there were no abnormalities in liver function indicators (ALT, AST) and kidney function indicators (CREA, BUN) before and after treatment. This indicates that FSGTC can reduce inflammatory response, improve hypercoagulability in OA patients, and have no liver or kidney function damage. (Table 2). Subsequently, we further confirmed through association rule analysis that the use of FSGTC is strongly associated with improvements in indicators such as HCRP, ESR, PLT, and FBG in patients. The results are shown in Figure 1.

PI3K/AKT is a Key Signaling Pathway for FSGTC Treatment of OA

The data mining results show the therapeutic effect of FSGTC on inflammation and coagulation indicators in OA patients. Subsequently, interdisciplinary network pharmacology analysis methods were introduced to predict the key signaling pathways of FSGTC in treating OA (Figure 2A). We obtained 750 FSGTC active ingredient targets and 961 OA disease targets through database retrieval, and ultimately obtained 170 intersecting genes (Figure 2B). We imported 170 intersecting genes into the Metascape database for KEGG enrichment analysis, and obtained the top five signaling pathways Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, Diabetic cardiomyopathy and Efferocytosis (Figure 2C). Based on the characteristics of OA disease, we ultimately determined that PI3K/AKT is the key signaling pathway for FSGTC treatment of OA.

FSGTC Significantly Reduces Cartilage Damage, Inflammatory Cell Infiltration, and PI3K/AKT Expression in Rats

Photographic analysis of rat knee joints showed that treatment with FSGTC significantly reduced joint swelling in rats (Figure 3A). Compared with the blank group, the HE staining results of the model group showed that the knee joints of



Items(LHS➪RHS)	Support(%)	Confidence(%)	Lift	*P* value
{FSGTC}➪{ESR↓}	50.00	100.0	1.76	<0.01
{FSGTC}➪{HCRP↓}	45.83	91.67	1.50	<0.01
{FSGTC}➪{FBG↓}	34.72	69.44	1.32	<0.01
{FSGTC}➪{PLT↓}	30.56	61.11	1.42	<0.01

Figure 1 Analysis of the association rules between FSGTC and inflammation and coagulation indicators in OA patients.

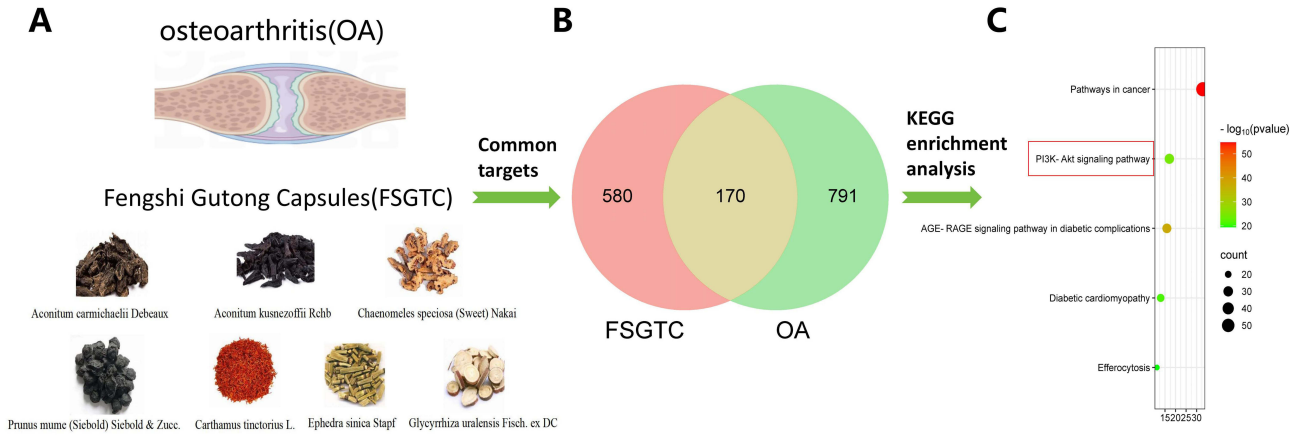


Figure 2 KEGG enrichment analysis of FSGTC treatment for OA. (**A** and **B**) Key targets of FSGTC intervention in OA; Figure (**C**) KEGG pathway enrichment analysis of key targets.

rats exhibited fibrous tissue proliferation and inflammatory cell infiltration (Figure 3B). The S&O staining of the model group showed shallow staining with yellow red pigment O, significant joint swelling, abnormal cartilage structure, and disordered arrangement (Figure 3C). The immunohistochemical staining results of the model group showed a significant increase in the positive expression of PI3K and AKT (Figure 3D and E). On the contrary, histopathological staining results of the FSGTC-L/M/H group and Celecoxib group showed fibrous tissue proliferation and decreased inflammatory cells in the knee joint of rats, normal cartilage structure, and significantly decreased positive expression of PI3K and AKT (Figure 3B-E). In addition, ELISA detection results showed that the levels of pro-inflammatory and procoagulant factors COX2, PGE2, and PAI-1 were significantly increased in the model group rats, while the expression levels of these cytokines were significantly decreased in the FSGTC-L/M/H group and Celecoxib group (Figure 3F-H). Specifically, the levels of procoagulant factor PAI-1 in the FSGTC-L/M/H group were significantly lower than those in the Celecoxib group (Figure 3H). These findings further demonstrate that FSGTC can alleviate cartilage damage in OA rats, reduce inflammatory cell infiltration, inhibit PI3K/AKT expression, and thus suppress inflammation and disruption of the coagulation fibrinolysis system in OA rats.

Subsequently, we tested the safety indicators of rats. The abdominal aortic blood test results of OA rats showed that compared with the blank group, the ALT indicators of the model group rats increased (Figure 4A), while the AST, Cr, and BUN indicators showed no significant changes (Figure 4B-D). There were no statistically significant differences in ALT, AST, Cr, and BUN between the FSGTC-L/M/H group and Celecoxib group compared to the blank group (Figure 4A-D). The results of weight statistics and organ coefficient (liver, spleen, kidney, heart) analysis showed that compared with the blank group, there was no statistically significant difference in weight and organ coefficient among the groups (Figure 4E-I). The HE staining results showed that there were no significant pathological changes in organs such as heart, liver, spleen, and kidney in each group of rats. The above results indicate that FSGTC intervention has significant therapeutic effects and is safe in OA rats (Figure 4J).

FSGTC Improves Cartilage Injury and Inflammatory Cell Infiltration in Rats by Inhibiting the Activation of PI3K/AKT Signaling Pathway

The previous research results confirmed the therapeutic effect of FSGTC and the expression changes of PI3K/AKT in OA rats. To confirm whether FSGTC improves cartilage damage and inflammatory cell infiltration in rats by inhibiting the activation of the PI3K/AKT signaling pathway, we further validated it by intraperitoneal injection of the PI3K/AKT pathway activator Recilisib in rats. The histopathological staining results showed that compared with the blank group, the Recilisib group had fibrous tissue proliferation, inflammatory cell infiltration, abnormal cartilage structure, disordered arrangement, and significantly increased positive expression of PI3K and AKT in the knee joint of rats (Figure 5A-D), while the Recilisib+FSGTC group had the opposite results. The ELISA detection results showed that the levels of pro-

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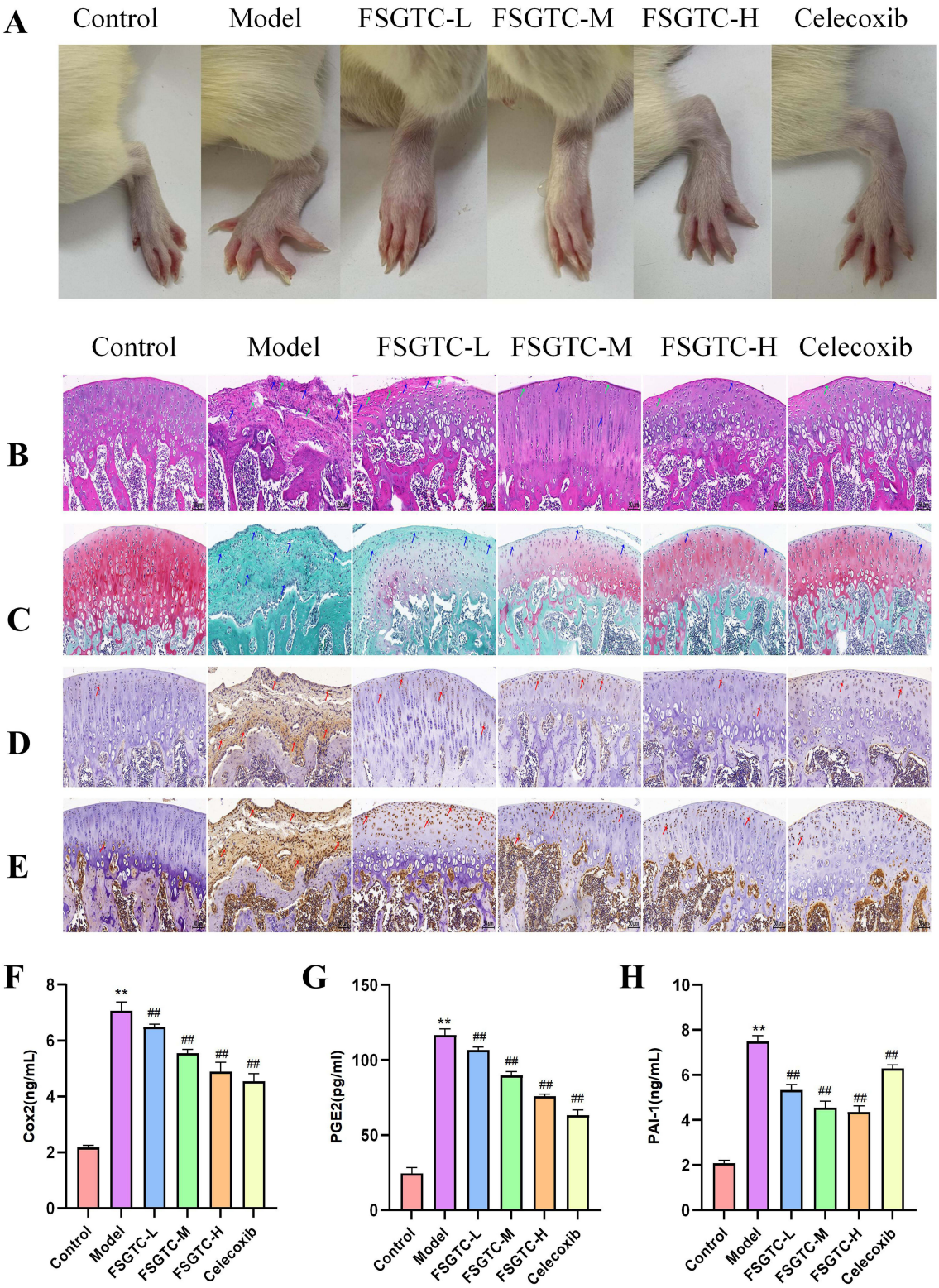

Figure 3 The effect of FSGTC on cartilage injury in rats. (**A**) Representative images of rat paw. (**B**) HE staining detection of cartilage fibrous tissue proliferation and inflammatory cell infiltration. Green arrow: fibrous tissue proliferation; Blue arrow: infiltration of inflammatory cells. (**C**) S&O staining was performed to observe to detect cartilage damage. Blue arrow: Indicates cartilage degeneration. (**D** and **E**) IHC was used to detect the expression of PI3K and AKT. Red arrow: Indicates positive expression. (**F–H**) ELISA was used to detect the expression levels of COX2, PGE2, and PAI in rats.**P < 0.01 vs the Control, ##P < 0.01 vs the Model.

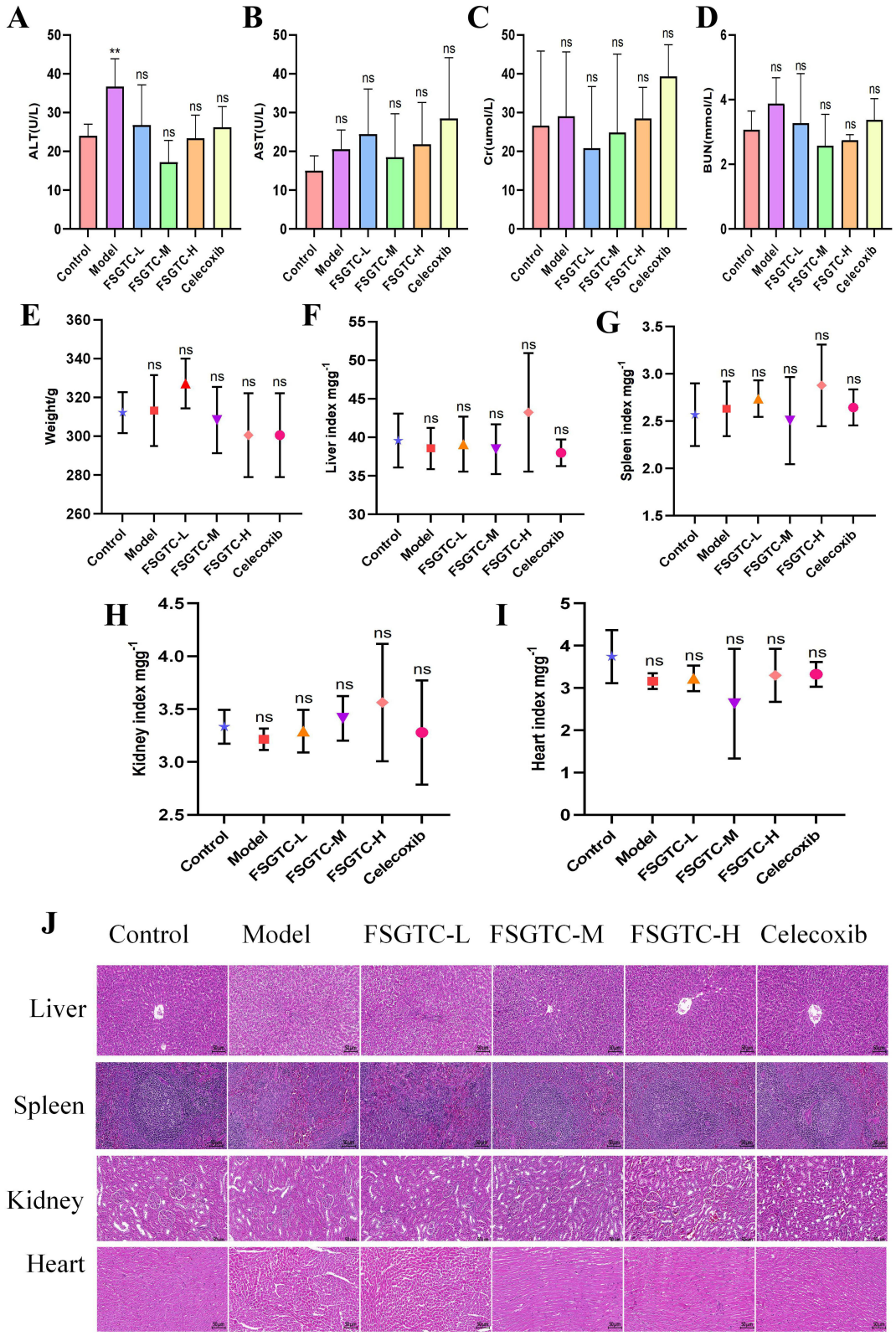

Figure 4 Safety validation of FSGTC treatment in OA rats. (**A**–**D**) Levels of ALT, AST, Cr, and BUN in the abdominal aortic blood of OA rats. (**E**)Weight statistics of rats. (**F**–**I**) Rat organ coefficients (heart, liver, spleen, and kidneys). (**J**) HE staining detection of liver, spleen, kidneys and heart.**P < 0.01 vs the Control, ^{ns}P > 0.01 vs the Control.

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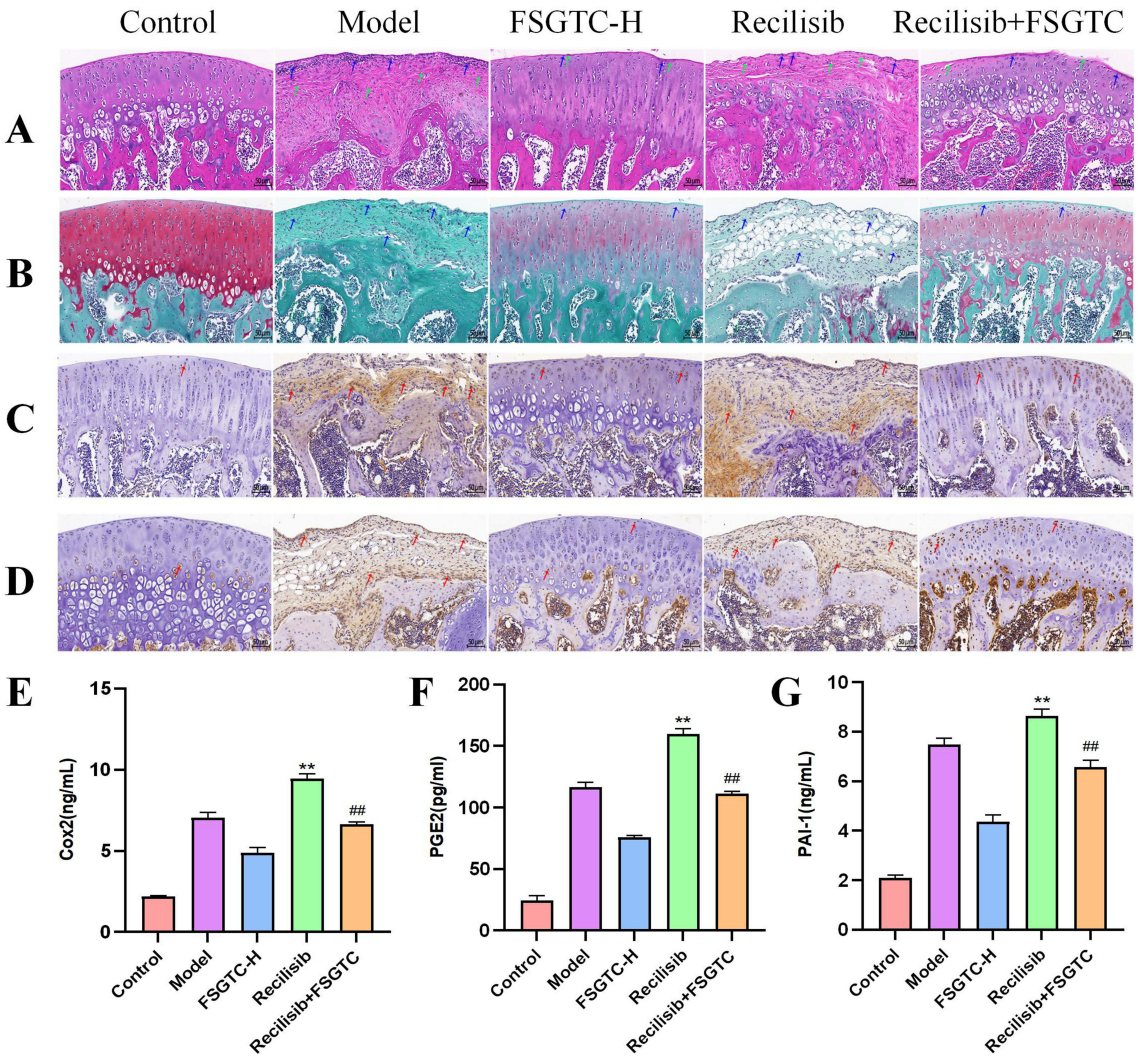

Figure 5 The impact of PI3K/AKT signaling pathway on OA and the intervention effect of FSGTC. (**A**) HE staining detection of cartilage fibrous tissue proliferation and inflammatory cell infiltration. Green arrow: fibrous tissue proliferation; Blue arrow: infiltration of inflammatory cells. (**B**) S&O staining was performed to detect cartilage damage. Blue arrow: Indicates cartilage degeneration. (**C** and **D**) IHC was used to detect the expression of PI3K and AKT. Red arrow: Indicates positive expression. (**E**–**G**) ELISA is used to detect the expression levels of COX2, PGE2, and PAI in rats.** $P < 0.01$ vs the Control, $^{\#\#}P < 0.01$ vs the Recilisib.

inflammatory and procoagulant factors COX2, PGE2, and PAI-1 were significantly increased in the Recilisib group, while the expression levels of these cytokines were significantly decreased in the Recilisib+FSGTC group (Figure 5E-G). This confirms that FSGTC can improve cartilage injury and inflammatory cell infiltration in rats by inhibiting the activation of the PI3K/AKT signaling pathway, thereby inhibiting inflammation and disruption of the coagulation and fibrinolysis system in OA rats.

The Effect of Different Concentrations of FSGTC Containing Serum on Chondrocyte Viability and the Construction of OA-CHs Model

In cell experiments, we first evaluated the safety of FSGTC and screened the optimal concentration of action by intervening with serum containing different concentrations of FSGTC on the viability of chondrocytes. The results showed that CHs exhibited high activity at different concentrations (5%, 10%, 20%, 30%, 40%) and at different times (12h, 24h, 48h). And under the intervention of 20% FSGTC containing serum for 24 hours, the activity of CHs was the highest, so we used this concentration and time for subsequent experiments (Figure 6A).

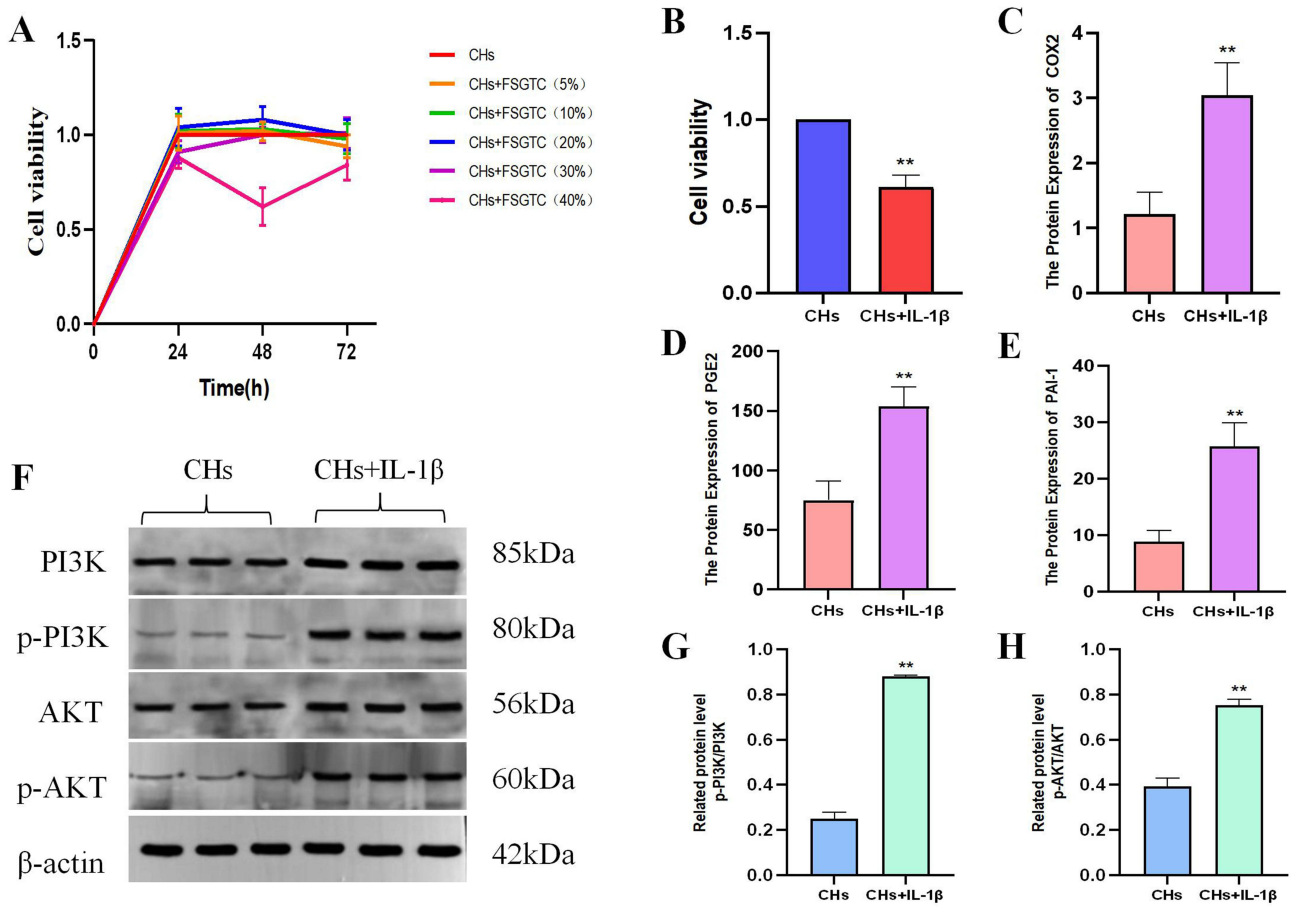

Figure 6 The effect of different concentrations of FSGTC containing serum on chondrocyte viability and the construction of OA-CHs model. (**A** and **B**) CCK8 was used to determine the cell viability of CHs; (**C**–**E**) ELISA is used to evaluate the levels of COX2, PGE2, and PAI-1; (**F**–**H**) WB was used to determine the expression of PI3K, p-PI3K, AKT and p-AKT protein. **P < 0.01 vs the IL-1β.

Subsequently, we stimulated CHs with IL-1 β (10 μg/L) for 24 hours to construct an OA CHs cell model. The results showed that the cell viability of the OA CHs group significantly decreased ($p<0.01$), while the expression levels of inflammatory and coagulation factors COX2, PGE2, and PAI-1 significantly increased ($p<0.01$)(Figure 6B-E). The levels of p-PI3K/PI3K and p-AKT/AKT also significantly increased ($p<0.01$), indicating that we successfully constructed a cell model simulating OA patients (Figure 6F-H).

FSGTC and PI3K/AKT Signaling Pathways are Involved in Regulating OA Chs Inflammation and Coagulation Factor Disorders

As a PI3K inhibitor, LY294002 can block PI3K activity and inhibit the regulation of AKT by the PI3K signaling pathway. Therefore, we further clarified the regulatory role of the PI3K/AKT signaling pathway in OA Chs inflammation and coagulation factor disorders by adding LY294002. The results showed that compared with the IL-1 β group, the addition of FSGTC containing serum and LY294002 increased the viability of CHs cells, significantly decreased the expression levels of COX2, PGE2, and PAI-1 ($p<0.01$), and significantly decreased the protein levels of p-PI3K/PI3K and p-AKT/AKT ($p<0.01$) (Figure 7). This indicates that FSGTC and the PI3K/AKT signaling pathway are jointly involved in regulating OA Chs inflammation and coagulation factor disorders.

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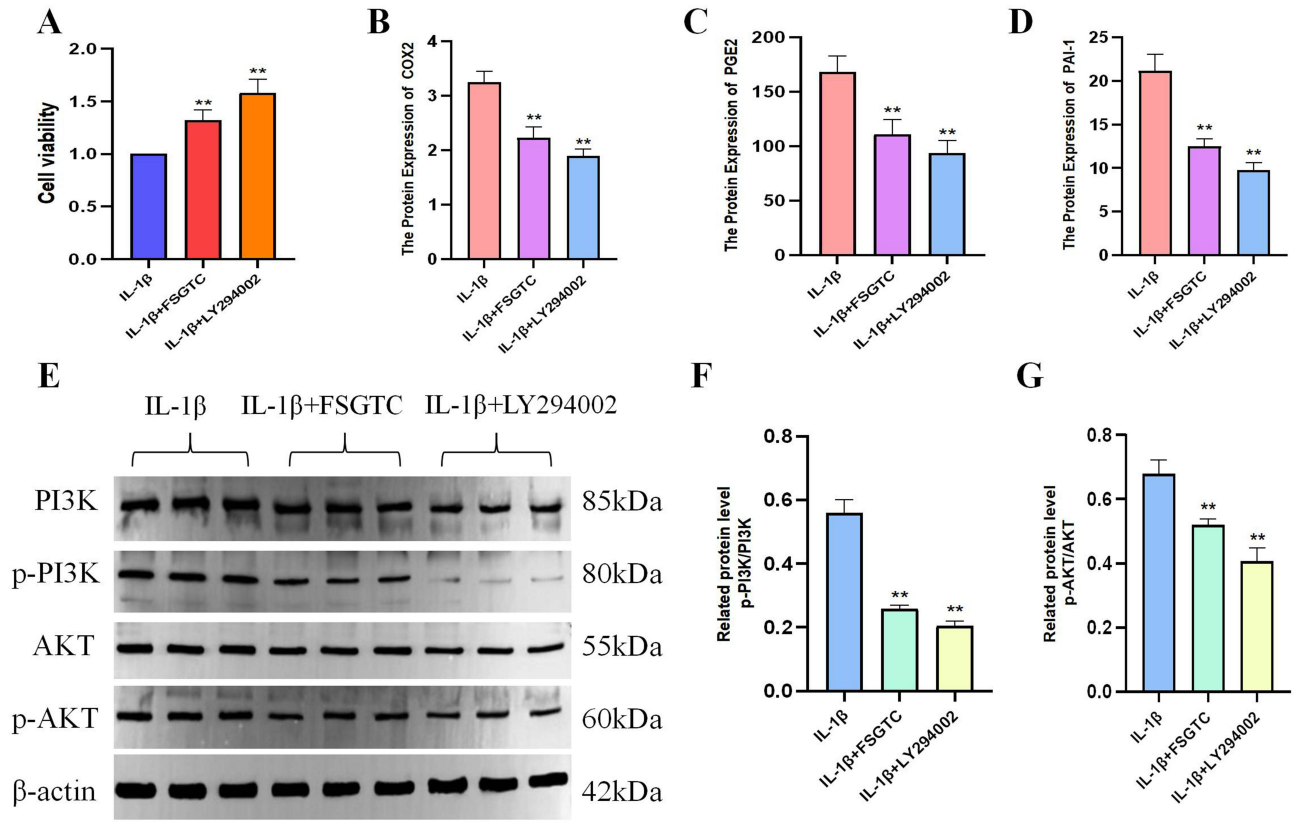

Figure 7 Regulatory effects of FSGTC and PI3K/AKT pathway inhibitors on OA-CHs inflammation and coagulation factors. (**A**) CCK8 was used to determine the cell viability of CHs; (**B**–**D**) ELISA is used to evaluate the levels of COX2, PGE2, and PAI-1; (**E**–**G**) WB was used to determine the expression of PI3K, p-PI3K, AKT and p-AKT protein.**P < 0.01 vs the IL-1β.

FSGTC Improves Chondrocyte Inflammation and Coagulation Factor Disorder by Inhibiting the Activation of PI3K/AKT Signaling Pathway

Through the above experiments, we found that activation of the PI3K/AKT signaling pathway in OA CHs leads to chondrocyte inflammation and coagulation factor disorder, while FSGTC and PI3K inhibitor LY294002 can improve the above situation. Subsequently, we further clarified through response experiments whether FSGTC inhibits the activation of the PI3K/AKT signaling pathway, improves chondrocyte inflammation, and coagulation factor disorders. The results showed that adding the PI3K/AKT signaling pathway to activate Recilisib on the basis of FSGTC intervention reversed the protective effect of FSGTC on CHs activity and the improvement effect on chondrocyte inflammation and coagulation factor disorders (Figure 8). Indicating that FSGTC improves chondrocyte inflammation and coagulation factor disorders by inhibiting the activation of the PI3K/AKT signaling pathway.

Discussion

In this study, we demonstrated that FSGTC can effectively improve inflammation and hypercoagulability in OA patients without liver or kidney function damage, based on clinical patient laboratory indicators, combined with cell experiments and animal experiments. In addition, this study also found that FSGTC inhibits the activation of the PI3K/AKT pathway, thereby suppressing inflammation and abnormal expression of coagulation factors (COX2, PGE2, and PAI-1), restoring the activity of the coagulation fibrinolysis system, and improving OA cartilage damage.

In TCM theory, "promoting blood circulation", "unblocking collaterals", and "relieving pain" are key steps in treating osteoarthritis. FSGTC originated from the Aconitum Decoction formula in Zhang Zhongjing's "Synopsis of the Golden Chamber" during the Han Dynasty. It is composed of seven Chinese herbs and has the effects of warming the meridians, dispelling cold, and relieving pain. The principle of "Jun Chen Zuo Shi" in TCM formulas is an important principle of

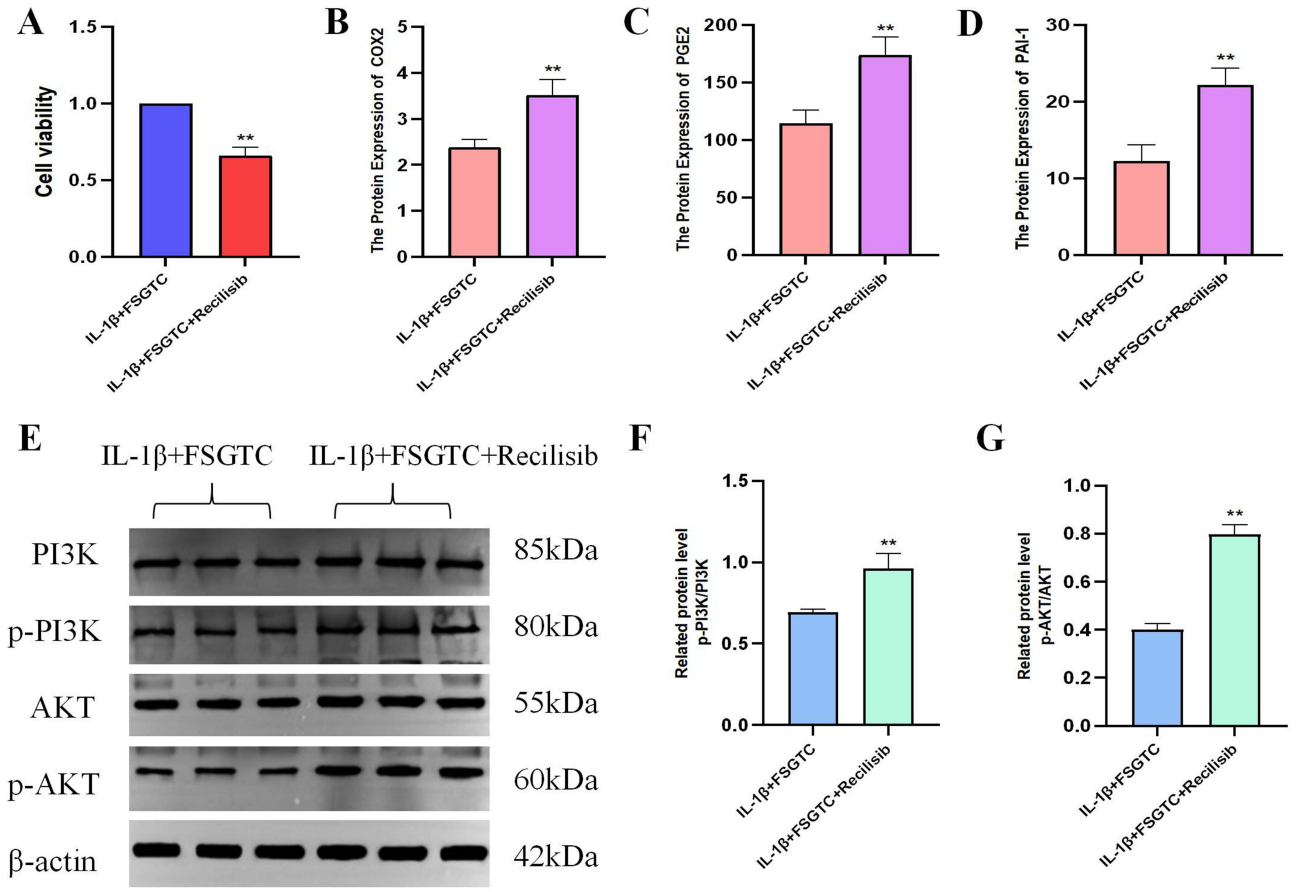

Figure 8 The effect of PI3K/AKT signaling pathway on OA and the intervention effect of FSGTC. (**A**) CCK8 was used to determine the cell viability of CHs; (**B**–**D**) ELISA is used to evaluate the levels of COX2, PGE2, and PAI-1; (**E**–**G**) WB was used to determine the expression of PI3K, p-PI3K, AKT and p-AKT protein. **P < 0.01 vs the IL-1β +FSGTC.

compatibility and a characteristic of TCM diagnosis and treatment. Reasonable combination can enable drugs to support each other, thereby exerting synergistic effects and achieving the best therapeutic effect. In this formula, Aconitum carmichaelii Debeaux and Aconitum kusnezofii Rchbe are used as the main drugs to exert the effects of dispelling wind, dampness, warming meridians, and relieving pain.Ephedra sinica Stapf, a Chen (Minister) drug, is used for dispelling the wind and cold. Carthamus tinctorius L., Chaenomeles speciosa (Sweet) Nakai, and Prunus mume (Siebold) Siebold & Zucc. are used as Zuo (assistant) drugs, Enhance the therapeutic effect of both imperial and imperial medicines in promoting blood circulation, unblocking collaterals, and relieving pain.Specifically, in TCM theory, Carthamus tinctorius L. has the function of promoting blood circulation, unblocking collaterals, and improving blood hypercoagulability.[36] Glycyrrhiza uralensis Fisch. ex DC, a Shi (guide) drug, is used to coordinate drug interactions.Modern pharmacological research has shown that all the above-mentioned drugs have anti-inflammatory and analgesic effects.[37–44] Specifically, the Ephedra sinica Stapf in the formula also has pharmacological effects of promoting blood circulation and anticoagulation.[45,46] In this study, by observing laboratory indicators before and after treatment in OA patients, it was found that compared to patients who did not use FSGTC, OA patients who used FSGTC showed significant improvement in inflammation indicators (HCRP, ESR) and coagulation indicators (PLT, FBG), and no liver or kidney function damage. In addition, HE staining and immunohistochemistry results of rat articular cartilage showed that FSGTC treatment reduced cartilage damage and inflammatory cell infiltration in OA rats, while maintaining cartilage structure. The cell experiment results showed that FSGTC treatment can significantly improve the vitality of chondrocytes.

The PI3K/Akt pathway is an important signaling pathway for FSGTC treatment of OA, and is associated with the expression of COX2, PGE2, and PAI-1. According to reports, the PI3K/AKT pathway is widely involved in pathological processes such as OA cartilage degeneration, synovitis, and cartilage degeneration.[47,48] Previous studies have shown that inflammatory factors can

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activate receptor tyrosine kinase (RTK) and G protein coupled receptor (GPCR), thereby initiating PI3K/AKT signaling, promoting AKT phosphorylation, and activating various downstream substrates such as COX-2.[49,50] During the progression of osteoarthritis, chondrocytes and synovial cells produce inflammatory mediators (such as IL-1β) to accelerate cartilage degradation while activating the PI3K/AKT signaling pathway, which in turn accelerates the secretion of inflammatory mediators.[48,51] COX-2 is a well-known inflammatory mediator that primarily converts arachidonic acid into prostaglandins, including PGE2.[52] In OA, PGE2 is mainly produced by synovial cells, chondrocytes, and macrophages/monocytes, while COX-2 levels are upregulated in activated joint cells.[53] In addition, the PI3K/Akt pathway also plays an important role in regulating vascular wall fibrinolysis balance.According to reports, activation of the PI3K/Akt pathway can regulate the transcriptional and post transcriptional levels of PAI-1.[54,55] PAI-1 is an inhibitor of tissue type and urokinase type plasminogen activators, and is a negative regulator of the fibrinolytic system.[56,57] Research has shown that PAI-1 increases the risk of thrombosis by inhibiting the activity of plasminogen activator and reducing the degradation of fibrin.[58] Interestingly, both hypoxia induced PAI-1 expression and nerve growth factor induced PAI-1 expression can be inhibited by PI3K inhibitors.[59,60] In the mouse endotoxemia model, treatment with PI3K inhibitors increases TNF-α induced PAI-1 expression and exacerbates cytokine induced hypercoagulability.[61] In this study, we found that the PI3K/AKT pathway was activated in OA CHs, and the expression levels of COX2, PGE2, and PAI-1 increased. However, after the addition of PI3K/AKT pathway inhibitors and FSGTC, the expression levels of COX2, PGE2, and PAI-1 significantly decreased, demonstrating the important role of the PI3K/AKT pathway in OA. In the response experiment, we were pleasantly surprised to find that FSGTC can exert anti-inflammatory and anticoagulant effects by inhibiting the activation of the PI3K/AKT pathway.

Drug safety has always been a focus of public concern and a concern for clinical doctors when using drugs. In terms of drug safety, we first established the HPLC fingerprint of Fengshi Gutong Capsules and evaluated their inter batch consistency, strictly controlling the quality of FSGTC.[26,62] At the same time, thin-layer chromatography was used to identify ephedra, processed Aconitum carmichaelii, and processed Aconitum carmichaelii in FSGTC, improve the quality standards of the compound, and enhance its quality control level.[25] In addition, we explore the quality transfer law of the production process based on quantitative measurement and fingerprint spectrum, aiming to improve the overall quality standards of FSGTC and provide a basis for clinical application.[63] In the past, after evaluating the toxic effects of FSGTC through animal experiments, we found that FSGTC only had mild toxic reactions at 60 times the clinical dosage (mild cloudy swelling in rat hearts observed under microscopy), and had no toxicity at 35 times the clinical dosage, indicating that the clinical application of FSGTC is safe.[64] In this study, we fed rats with clinical doses of 2, 4, and 8 times (7.56/15.12/30.24 g/kg) for 14 days and found that the general condition of rats in each dose group was good. There were no significant differences in body weight, organ coefficient, and liver and kidney function indicators between the groups and the control group. HE staining of organs such as the heart, liver, spleen, and kidneys showed no significant pathological changes.

Several important advantages of this study are worth commenting on. Firstly, this study has a rich research foundation. In the early stage, we conducted a comprehensive evaluation of the quality, safety, and clinical efficacy of FSGTC formula through HPLC, clinical observations, and meta-analysis. Secondly, this study closely combines clinical patient data, focuses on changes in patient laboratory indicators, and explores the possible mechanism of FSGTC formula in treating hypercoagulable state in OA patients, providing feasible traditional Chinese medicine diagnosis and treatment plans for OA hypercoagulable state patients. At the same time, the evaluation of drug safety runs through every part of this study to eliminate patients' concerns about drug safety.

Nevertheless, certain limitations should be acknowledged. Firstly, given the difficulty of collecting clinical patient data across regions, this study only collected data from patients using FSGTC at the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. In the future, we will expand the scope of data collection and further evaluate the benefits of FSGTC for patients with OA hypercoagulability. Secondly, in this study, we demonstrated the regulatory role of the PI3K/AKT signaling pathway in FSGTC intervention of OA by adding agonists and inhibitors, but did not delve into its upstream regulatory genes. In the future, we will integrate multidisciplinary research methods and explore the deep mechanisms of FSGTC treatment for OA from the perspective of gene modification through sequencing, bioinformatics analysis, and other methods. Finally, the main approach of compound intervention is to achieve precise treatment of complex diseases through the synergistic effects of multiple components, multiple pathways of action, and targeting multiple biological targets. In the future, we will combine MS, molecular docking, molecular dynamics simulation, and cell thermal displacement research methods to integrate multiple active ingredients and targets, providing a more comprehensive mechanism analysis for FSGTC intervention in the treatment of OA.

Conclusions

Based on clinical data of patients, we found that FSGTC can effectively improve inflammation and coagulation indicators in OA patients without liver or kidney function damage. Subsequently, network pharmacology, animal experiments, and cell experiments confirmed the results of clinical data mining, and we found that FSGTC may improve the inflammatory response and hypercoagulability of OA patients by inhibiting the activation of the PI3k/AKT pathway, alleviating cartilage damage and reducing inflammatory cell infiltration in OA rats and the expression of OA pro-inflammatory cytokines (COX2, PGE2) and procoagulant factor 1 (PAI-1).

Abbreviations

FSGTC, Fengshi Gutong capsule; OA, osteoarthritis; PAI-1, Plasma plasminogen activator inhibitor-1; COX2, cyclooxygenase-2; PGE2, Prostaglandin E2; hs-CRP, high-sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CREA, creatinine; BUN, urea nitrogen; CHs, chondrocytes; IL-1β, interleukin-1β; HPLC, High Performance Liquid Chromatography; KEGG, Kyoto Encyclopedia of Genes and Genomes; HE, Hematoxylin-eosin staining; S&O, Safranine O-Fast Green staining; IHC, Immunohistochemistry; MIA, monosodium iodoacetate; APTT, activated partial thromboplastin time; FBG, fibrinogen; PLT, platelet; PT, prothrombin time; TT, thrombin time; TCM, traditional Chinese medicine.

Data Sharing Statement

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding authors.

Ethics Approval and Consent to Participate

This study was reviewed and ratified by the Laboratory Animal Ethics Committee of Anhui University of Traditional Chinese Medicine (AHUCM-rats-2022068).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Yue L, Berman J. What is osteoarthritis? *Jama-J Am Med Assoc*. 2022;327(13):1300. doi:10.1001/jama.2022.1980
2. Kloppenburg M, Namane M, Cicuttini F. Osteoarthritis. *Lancet*. 2025;405(10472):71–85. doi:10.1016/S0140-6736(24)02322-5
3. Attwaters M. Targeting senescence in OA. *Nat Rev Rheumatol*. 2022;18(6):305. doi:10.1038/s41584-022-00788-2
4. Zhu X, Chan YT, Yung PSH, et al. Subchondral bone remodeling: a therapeutic target for osteoarthritis. *Front Cell Dev Biol*. 2021;8:607764. doi:10.3389/fcell.2020.607764
5. Kloppenburg M. Inflammation is a relevant treatment target in osteoarthritis. *Lancet*. 2023;402(10414):1725–1726. doi:10.1016/S0140-6736(23)01726-9
6. Peng Z, Sun H, Bunpetch V, et al. The regulation of cartilage extracellular matrix homeostasis in joint cartilage degeneration and regeneration. *Biomaterials*. 2020;268:268120555. doi:10.1016/j.biomaterials.2020.120555
7. Steinmetz JD, Culbreth GT, Haile LM. Global, regional, and national burden of osteoarthritis, 1990-2020 and projections to 2050: a systematic analysis for the global burden of disease study 2021. *Lancet Rheumatol*. 2023;5(9):e508–e522. doi:10.1016/S2665-9913(23)00163-7

8. Chaidou A, Veliou K, Gianniki M, et al. AB1006 Impact of osteoarthritis on the quality of life in patients with knee pain. *Ann Rheum Dis*. 2022;81 (Suppl 1):1627.2–1627. doi:10.1136/annrheumdis-2022-eular.5379
9. Weng Q, Chen Q, Jiang T, et al. Global burden of early-onset osteoarthritis, 1990-2019: results from the global burden of disease study 2019. *Ann Rheum Dis*. 2024;83(7):915–925. doi:10.1136/ard-2023-225324
10. Lipinski S, Bremer L, Lammers T, et al. Coagulation and inflammation. Molecular insights and diagnostic implications. *Hamostaseologie*. 2010;31 (2):94–102,104. doi:10.5482/ha-1134
11. Zhang W, Yu T, Wang Y, et al. Clinical significance of the combined test of seven blood coagulation indexes, lipids and platelet agglutination on hypercoagulable state of blood in parturient women. *Pak J Med Sci*. 2024;40(4):741–746. doi:10.12669/pjms.40.4.7530
12. Middeldorp S. Indicators of hypercoagulability and recurrent venous thromboembolism in the elderly: rethinking age and thrombophilia. *Res Pract Thromb Haemost*. 2023;7(1):100056. doi:10.1016/j.rpth.2023.100056
13. Yingjie Z, Huijun W, Rongwei H, et al. Clinical application of the four coagulation factors. *J Lab Med Clin Med*. 2013;10(04):450–452.
14. Ren XM, Wang M, Shen XY, et al. Clinical observation on acupoint irradiation with combined laser or red light on patients with knee osteoarthritis of yang deficiency and cold coagulation type. *Zhongguo Zhen Jiu*. 2010;30(12):977–981. PMID: 21290832.
15. Iba T, Gando S, Murata A, et al. Predicting the severity of systemic inflammatory response syndrome (SIRS)-associated coagulopathy with hemostatic molecular markers and vascular endothelial injury markers. *J Trauma*. 2007;63(5):1093–1098. doi:10.1097/01.ta.0000251420.41427.d3
16. Kisand K, Tamm AE, Lintrop M, et al. New insights into the natural course of knee osteoarthritis: early regulation of cytokines and growth factors, with emphasis on sex-dependent angiogenesis and tissue remodeling. A pilot study. *Osteoarthr Cartilage*. 2018;26(8):1045–1054. doi:10.1016/j.joca.2018.05.009
17. Mingyu H, Liu J, Xin L, et al. Analysis of changes in coagulation indicators and their correlation with immune, inflammatory, and lipid metabolism indicators in 717 patients with osteoarthritis. *J Gansu Univ Trad Chin Med*. 2021;38(03):68–73. doi:10.16841/j.issn1003-8450.2021.03.15
18. Mingyu H, Liu J, Wang G, et al. Chinese medicine treatment reduces the risk of readmission in patients with high coagulation status of osteoarthritis: a retrospective cohort study based on OA population. *J Shanxi Univ Trad Chin Med*. 2024;25(09):963–970. doi:10.19763/j.cnki.2096-7403.2024.09.04
19. Lawford BJ, Bennell KL, Haber T, et al. Osteoarthritis year in review 2024: rehabilitation and outcomes. *Osteoarthr Cartilage*. 2024;32 (11):1405–1412. doi:10.1016/j.joca.2024.08.001
20. Zhang Z, Hu Z, Zhao D, et al. Arthroscopic surgery is not superior to conservative treatment in knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trails. *BMC Musculoskelet Disord*. 2024;25(1):712. doi:10.1186/s12891-024-07813-3
21. Gong D, Wu X, Wu M, et al. Knowledge, attitude, and practice toward nonsteroidal anti-inflammatory drugs among osteoarthritis patients: a cross-sectional study. *Sci Rep*. 2024;14(1):24953. doi:10.1038/s41598-024-76171-w
22. Chen X, Liu J, Wang G, et al. Validation of Jianpi Qingre Tongluo recipe in reducing inflammation and dyslipidemia in osteoarthritis via Lnc RNA HOTAIR/APN/PI3K/AKT. *Int J Gen Med*. 2024;17:3293–3318. doi:10.2147/IJGM.S466148
23. Liu J, Wang Y, Sun Y, et al. Efficacy and safety of Xinfeng capsule in the treatment of osteoarthritis: a multicenter, randomized, double-blinded, controlled trial. *J Tradit Chin Med*. 2020;40(2):284–295. PMID: 32242394.
24. Sun Y, Liu J, Wang J, et al. Network pharmacology integrated with experimental validation revealed the mechanism of Fengshi Gutong Capsule in the treatment of osteoarthritis. *J Ethnopharmacol*. 2023;319(Pt 1):117261. doi:10.1016/j.jep.2023.117261
25. Chen L, Liu S, Lin Q, et al. Research on qualitative and quantitative quality control methods of Fengshi Gutong capsules. *Shanghai J Trad Chin Med*. 2022;56(01):81–88. doi:10.16305/j.1007-1334.2022.2104116
26. Chen L, Chen X, Wang L, et al. Evaluation of consistency between batches of Fengshi Gutong capsule by HPLC fingerprint combined with pattern recognition analysis. *Trad Chinese Patent Med Simple Prep*. 2021;43(12):3446–3449.
27. Mingyu H, Liu J, Sun Y, et al. Meta analysis of clinical efficacy of rheumatoid bone pain medications in the treatment of rheumatic diseases. *Chin J ClinHealth Care*. 2022;25(06):837–841.
28. Wang L, Wang L. Clinical experience of Fengshi Gutong capsule combined with glucosamine hydrochloride in treating knee osteoarthritis. *JETCM*. 2009;18(1):132–133.
29. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and therapeutic criteria committee of the American rheumatism association. *Arthritis Rheumatol US*. 1986;29(8):1039–1049. doi:10.1002/art.1780290816
30. Sun D. Research on web data mining technology based on apriori algorithm. *Inform Technol Info*. 2022;07:91–94.
31. Fang Y, Liu J, Xin L, et al. Identifying Compound effect of drugs on rheumatoid arthritis treatment based on the association rule and a random walking-based model. *Biomed Res Int*. 2020;2020:4031015. doi:10.1155/2020/4031015
32. Xu SY, Bian RL, Chen X. *Pharmacological Experimental Methodology*. 3rd ed. Beijing: People's Health Publishing House; 2002.
33. Sun Y, Liu J, Xin L, et al. Xinfeng capsule inhibits inflammation and oxidative stress in rheumatoid arthritis by up-regulating LINC00638 and activating Nrf2/HO-1 pathway. *J Ethnopharmacol*. 2022;301:115839. doi:10.1016/j.jep.2022.115839
34. Niu S, Sun Z, Wang C. The effect and mechanism of Gynostemma pentaphyllum saponin A regulating lncRNATTTY15/miR-335-5p pathway on chondrocyte damage in osteoarthritis. *Hebei Med J*. 2022;44(7):981-985.
35. He M, Liu J, Sun Y, et al. FSGT capsule inhibits IL-1β-induced inflammation in chondrocytes and ameliorates osteoarthritis by upregulating LncRNA PACER and downregulating COX2/PGE2. *Immun Inflamm Dis*. 2024;12(6):e1334. doi:10.1002/iid3.1334
36. Fang X, Xia L, Liu X. Clinical observation of safflower yellow in the treatment of elderly patients with diabetes nephropathy complicated with acute myocardial infarction. *China Pharm*. 2017;28(29):4140–4143.
37. Liangni C, Xuemei C, Yong C, et al. Research progress on pharmacological action, toxicity and quality control methods of Radix Aconiti Chuanwu. *Trad Chinese Patent Med Simple Prep*. 2021;43(03):722–729.
38. Xiao D, Wang J, Zhao D, et al. Experimental study on the analgesic and anti-inflammatory effects of compound Chuancao Wu mixture. *Western Trad Chin Med*. 2024;37(09):16–20.
39. Wu X, Yuan M, Xiong X, et al. Research progress on chemical composition and pharmacological effects of Caowu. *Drug Eval*. 2021;18 (24):1534–1536. doi:10.19939/j.cnki.1672-2809.2021.24.16
40. Zhuo X, Chen J, Tian M, et al. Research progress on chemical composition and pharmacological effects of ephedra. *Trad Chin Med Info*. 2021;38 (02):80–83. doi:10.19656/j.cnki.1002-2406.210216

41. Sen D, Rathee S, Pandey V, et al. Exploring saffron's therapeutic potential: insights on phytochemistry, bioactivity, and clinical implications. *Curr Pharm Design*. 2024;31. doi:10.2174/0113816128337941240928181943
42. Dai Q, She Y, Ye Q, et al. Research progress on chemical composition and pharmacological effects of traditional Chinese medicine papaya and papaya seeds. *Asia Pacific Trad Med*. 2021;17(08):219–223.
43. Yang Y, Wang R, Qian C, et al. Research progress in chemical constituents and pharmacological effects of Fructus Mume and prediction of quality markers. *Trad Chin Patent Med Simple Prep*. 2023;45(05):1583–1588.
44. Deng T, Peng C, Peng D, et al. Research progress on chemical components and pharmacological effects of licorice and exploration of quality markers. *Chin J Trad Chin Med*. 2021;46(11):2660–2676. doi:10.19540/j.cnki.cjcmm.20210304.201
45. Zhang D, Rao X, Xi Y, et al. Comparative study on the chemical composition of decoction of Ephedra sinica and its "QuJie" decoction. *New Chin Med Clin Pharmacol*. 2019;30(9):1112–1117.
46. Cao R, Bai L, Yanan X, et al. Study on the active ingredients and modern pharmacological effects of various herbs in Dedu Honghua-7 Wei San. *Pharm Res*. 2019;38(1):38–41.
47. Lin W, Kang H, Niu Y, et al. Cartilage degeneration is associated with activation of the PI3K/AKT signaling pathway in a growing rat experimental model of developmental trochlear dysplasia. *J Adv Res*. 2021;35:109–116. doi:10.1016/j.jare.2021.04.006
48. Wang L, Xu H, Li X, et al. Cucurbitacin E reduces IL-1β-induced inflammation and cartilage degeneration by inhibiting the PI3K/Akt pathway in osteoarthritic chondrocytes. *J Transl Med*. 2023;21(1):880. doi:10.1186/s12967-023-04771-7
49. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet*. 2006;7:606–619. doi:10.1038/nrg1879
50. De Santis MC, Gulluni F, Campa CC, et al. Targeting PI3K signaling in cancer: challenges and advances. *BBA-Rev Cancer*. 2019;1871(2):361–366. doi:10.1016/j.bbcan.2019.03.003
51. Liu S, Cao C, Zhang Y, et al. PI3K/Akt inhibitor partly decreases TNF-α-induced activation of fibroblast-like synoviocytes in osteoarthritis. *J Orthop Surg Res*. 2019;14(1):425. doi:10.1186/s13018-019-1394-4
52. Yagami T, Koma H, Yamamoto Y. Pathophysiological roles of cyclooxygenases and prostaglandins in the central nervous system. *Mol Neurobiol*. 2016;53(7):4754–4771. doi:10.1007/s12035-015-9355-3
53. Li Z, Ma D, Peng L, et al. Compatibility of Achyranthes bidentata components in reducing inflammatory response through Arachidonic acid pathway for treatment of Osteoarthritis. *Bioengineered*. 2022;13(1):1746–1757. doi:10.1080/21655979.2021.2020394
54. Blum S, Issbrüker K, Willuweit A, et al. An inhibitory role of the phosphatidylinositol 3-kinase-signaling pathway in vascular endothelial growth factor-induced tissue factor expression. *J Biol Chem*. 2001;276(36):33428–33434. doi:10.1074/jbc.M105474200
55. Mukai Y, Wang CY, Rikitake Y, et al. Phosphatidylinositol 3-kinase/protein kinase Akt negatively regulates plasminogen activator inhibitor type 1 expression in vascular endothelial cells. *Am J Physiol-Heart C*. 2006;292(4):H1937–42. doi:10.1152/ajpheart.00868.2006
56. Huber K, Christ G, Wojta J, Gulba D. Plasminogen activator inhibitor type-1 in cardiovascular disease. Status report 2001. *Thromb Res*. 2001;103 (Suppl 1):S7–S19. doi:10.1016/s0049-3848(01)00293-6
57. Nordt TK, Peter K, Ruef J, et al. Plasminogen activator inhibitor type-1 (PAI-1) and its role in cardiovascular disease. *Thromb Haemostasis*. 1999;82 Suppl 1:14–18. PMID: 10695479.
58. Kohler HP, Grant PJ. Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med*. 2000;342:1792–1801. doi:10.1056/NEJM200006153422406
59. Kietzmann T, Jungermann K, Gorlach A. Regulation of the hypoxiadependent plasminogen activator inhibitor 1 expression by MAP kinases. *Thromb Haemostasis*. 2003;89(4):666–673. doi:10.1055/s-0037-1613573
60. Takahashi H, Uno S, Watanabe Y, Arakawa K, Nakagawa S. Expression of nerve growth factor-induced type 1 plasminogen activator inhibitor (PAI-1) mRNA is inhibited by genistein and wortmannin. *NeuroReport*. 2000;11(5):1111–1115. doi:10.1097/00001756-200004070-00040
61. Schabbauer G, Tencati M, Pedersen B, Pawlinski R, Mackman N. PI3K-Akt pathway suppresses coagulation and inflammation in endotoxemic mice. *Arterioscler Thromb Vasc Biol*. 2004;24:1963–1969. doi:10.1161/01.ATV.0000143096.15099.ce
62. Mingrui L, Liang Y, Zhang J, et al. Determination of ephedrine hydrochloride, benzoyl aconitine, and oleanolic acid in Fengshi Gutong capsules by reversed phase high performance liquid chromatography. *Eval Anal Drug Use ChinHospitals*. 2020;20(10):1212–1215. doi:10.14009/j.issn.1672-2124.2020.10.016
63. Chen L. Research on improving the quality standards of fengshi gutong capsules. *Guangxi Univ Trad Chin Med*. 2021. doi:10.27879/d.cnki.ggxzy.2021.000499
64. Xiang Q, Peng D, Liu Q. Study on the efficacy and toxicity of Fengshi Gutong capsule. *Trad Chin Patent Med Simple Prep*. 1996;03:32–34.

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