ORIGINAL RESEARCH

# Based on PI3K/Akt Signaling Pathway, the Effects of Gubi Decoction on Chondrocyte Proliferation, Apoptosis, and Expression of Inflammatory Factors in a Rat Model of Osteoarthritis

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## ABSTRACT

**Objective** • Given the crucial role of the PI3K/Akt signaling pathway in chondrocyte survival and inflammation in osteoarthritis (OA), this study aimed to investigate the effects of Gu Bi Tang on chondrocyte proliferation, apoptosis, and inflammatory factor expression in a rat model of OA, with a focus on the PI3K/Akt signaling pathway.

**Methods** • Forty-five specific pathogen-free (SPF) C57 mice were randomly divided into three groups: the model group (Group A), the Gu Bi Tang group (Group B), and the Amfenac group (Group C), with 15 rats in each group. All 45 rats underwent anterior cruciate ligament transection (ACLT) surgery to establish an OA model. The ACLT procedure is a well-established method for inducing OA in rodents, as it leads to the destabilization of the knee joint and the development of degenerative changes characteristic of human OA.

After 8 weeks of modeling, Group A rats received an equivalent volume of normal saline by gastric lavage, Group B rats were administered 13 mL/kg of Gu Bi Tang, and Group C rats received 19.5 mg/kg of Amfenac solution by gastric lavage. The dosages of Gu Bi Tang and Amfenac were selected based on previous studies examining the therapeutic effects of these interventions in rodent OA models. The gastric lavage frequency for all three groups was maintained at twice daily.

The researchers analyzed cartilage morphological changes using toluidine blue staining, chondrocyte proliferation and apoptosis using the TUNEL method, and the expression of PI3K/AKT/mTOR proteins in chondrocytes using Western blotting. Additionally, the expression of inflammatory factors (TNF-α and IL-1β) in serum was measured using ELISA.

**Results** • Staining: Compared to the model group (Group A), both the Gu Bi Tang group (Group B) and the Amfenac group (Group C) showed significant improvement in cartilage tissue, with deeper toluidine blue staining. Toluidine blue staining is a marker of cartilage integrity and glycosaminoglycan content, indicating improved cartilage structure and composition in the treatment groups.

Chondrocyte proliferation and apoptosis: Compared to the model group (Group A), both the Gu Bi Tang group (Group B) and the Amfenac group (Group C) significantly reduced chondrocyte apoptosis ($P < .05$). This reduction in chondrocyte death contributes to a healthier cartilage environment and helps prevent further cartilage degradation.

Protein expression: In comparison to the model group (Group A), the expression of PI3K, AKT, and mTOR proteins in the joint cartilage of the Gu Bi Tang group (Group B) and the Amfenac group (Group C) significantly decreased, with the Amfenac group showing a greater reduction than the Gu Bi Tang group ($P < .05$). The inhibition of this detrimental PI3K/AKT/mTOR signaling pathway promotes chondrocyte survival and a more favorable cartilage homeostasis.

Inflammatory factor expression: Prior to treatment, there was no significant difference in the expression levels of the inflammatory factors TNF-α and IL-1β in serum among the three groups ($P > .05$). However, after treatment, both the Gu Bi Tang group (Group B) and the Amfenac group (Group C) showed a significant reduction in the serum expression of TNF-α and IL-1β compared to the model group (Group A), with the Amfenac group showing a greater reduction than the Gu Bi Tang group ($P < .05$). This is important, as TNF-α and IL-1β are key pro-inflammatory cytokines that drive the destructive processes in osteoarthritis.

**Conclusion** • In summary, this study demonstrates that Gu Bi Tang exerts protective effects on chondrocytes in a rat model of osteoarthritis. Specifically, Gu Bi Tang was shown to inhibit chondrocyte apoptosis, reduce the expression of key proteins in the PI3K/AKT/mTOR signaling pathway, and decrease the levels of the pro-inflammatory cytokines TNF-α and IL-1β.

These findings suggest that Gu Bi Tang could offer a novel therapeutic approach for osteoarthritis by modulating key signaling pathways and inflammatory responses. The ability of Gu Bi Tang to preserve chondrocyte viability and maintain a more favorable cartilage homeostasis makes it a promising candidate for further investigation as a potential treatment for osteoarthritis. Future studies should explore the precise mechanisms by which Gu Bi Tang exerts its beneficial effects and evaluate its efficacy in additional animal models and clinical settings. (*Altern Ther Health Med.* [E-pub ahead of print.])


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## INTRODUCTION

Osteoarthritis (OA) is a chronic, progressive joint disorder that disproportionately affects the aging population. As the global population continues to age, the prevalence of OA is steadily increasing, posing a significant public health challenge. According to the World Health Organization, the proportion of the world's population over 60 years of age is projected to increase from 12% in 2015 to 22% by 2050.[1] This demographic shift, coupled with the strong association between aging and the development of OA, underscores the growing burden of this debilitating joint disease. Aging is a major risk factor for OA, as it is accompanied by a variety of structural and biochemical changes within the articular cartilage and surrounding joint tissues. The gradual

deterioration of cartilage, decreased lubrication, and impaired joint biomechanics that occur with aging all contribute to the onset and progression of OA. Additionally, systemic factors associated with aging, such as chronic inflammation and metabolic derangements, further exacerbate the pathological processes involved in OA. Given the clear link between the aging population and the rising prevalence of OA, the development of effective, disease-modifying treatments has become increasingly urgent. Interventions that can preserve cartilage integrity, mitigate inflammatory responses, and maintain joint function in the elderly are essential for improving the quality of life and reducing the socioeconomic burden associated with this debilitating condition.

While the precise etiology of OA remains incompletely understood in clinical practice, existing research[2] suggests a pivotal role for abnormal expression of cell proliferation, apoptosis, and inflammatory factors in the initiation and progression of OA. Notably, chondrocyte apoptosis and abnormal proliferation can contribute to joint cartilage damage, subsequently initiating inflammatory responses and promoting OA progression. In the healthy joint, chondrocytes maintain a delicate balance between proliferation and apoptosis (programmed cell death), ensuring the renewal and homeostasis of the cartilage. However, in the setting of OA, this balance is disrupted, leading to aberrant chondrocyte behavior.

Increased chondrocyte apoptosis is a hallmark of OA, as these cells undergo premature death, compromising the structural integrity of the cartilage. The loss of chondrocytes diminishes the capacity for cartilage repair and accelerates the degeneration of the extracellular matrix, which is primarily composed of collagen and proteoglycans. Concurrently, OA is characterized by abnormal chondrocyte proliferation, resulting in the formation of clusters of cells within the cartilage. While this proliferative response may represent an attempted repair mechanism, the newly formed chondrocytes often exhibit an altered phenotype and fail to adequately maintain the cartilage structure. The imbalance between chondrocyte apoptosis and proliferation, coupled with the disruption of the delicate extracellular matrix, leads to the progressive thinning and degradation of the articular cartilage, eventually exposing the underlying subchondral bone and triggering a cascade of inflammatory and structural changes that characterize the osteoarthritic joint.

The PI3K/Akt signaling pathway, a crucial cellular signaling pathway, plays a significant role in various diseases, including joint disorders.[3] This pathway is intricately involved in cell proliferation, survival, apoptosis, and metabolic regulation.[4] In OA, abnormal activation of the PI3K/Akt signaling pathway is closely associated with chondrocyte apoptosis and excessive expression of inflammatory factors, potentially accelerating cartilage deterioration and disease progression.[5] In healthy cartilage, the PI3K/Akt pathway promotes chondrocyte survival and maintains the integrity of the extracellular matrix. Activation of this pathway leads to the phosphorylation and inhibition of pro-apoptotic factors, such as Bad and Caspase-9, thereby inhibiting chondrocyte apoptosis and supporting cell viability. Furthermore, the PI3K/Akt signaling cascade is involved in the regulation of chondrocyte proliferation and differentiation. By modulating the activity of transcription factors and cell cycle regulators, the pathway can stimulate the proliferation of chondrocytes and their subsequent differentiation, which is crucial for the maintenance and repair of the cartilage. However, in the setting of osteoarthritis, the dysregulation of the PI3K/Akt pathway has been observed. Increased activation of this pathway can lead to the aberrant proliferation of chondrocytes, while its inhibition can result in enhanced chondrocyte apoptosis and the subsequent degradation of the cartilage matrix.

Gu Bi Tang is a traditional Chinese medicine (TCM) formula that has been used for centuries to treat various rheumatic conditions, including osteoarthritis. The formula is composed of a blend of several herbal ingredients, each of which has been shown to possess therapeutic properties relevant to the management of osteoarthritis. The primary components of Gu Bi Tang include Ephedra sinica (Ma Huang), Cinnamomum cassia (Rou Gui), Aconitum carmichaelii (Fu Zi), Glycyrrhiza uralensis (Gan Cao), and Paeonia lactiflora (Bai Shao). These herbs have been studied for their anti-inflammatory, analgesic, chondroprotective, and antioxidant effects, which collectively contribute to the formula's potential efficacy in addressing the multifaceted pathogenesis of osteoarthritis.

Despite Gu Bi Tang's widespread use in osteoarthritis (OA) treatment, its molecular mechanisms, particularly its impact on the PI3K/Akt signaling pathway and chondrocyte behavior, have not been thoroughly investigated. While previous studies have suggested the potential therapeutic benefits of this traditional Chinese medicine formula, the underlying pathways through which it may exert its chondroprotective effects remain to be elucidated. This study aims to elucidate the effects of Gu Bi Tang on chondrocyte proliferation, apoptosis, and inflammatory factor expression in an OA rat model, with a specific focus on its modulation of the PI3K/Akt signaling pathway. By exploring the influence of Gu Bi Tang on this central regulatory pathway, the study seeks to provide a deeper understanding of the molecular mechanisms underlying the formula's potential therapeutic application in the management of osteoarthritis.

## MATERIALS AND METHODS

### Experimental Materials

**Experimental Animals.** To examine the influence of Gu Bi Tang on chondrocyte proliferation, apoptosis, and the expression of inflammatory mediators in the OA joint environment, forty-five specific pathogen-free (SPF) C57 mice (Qinglongshan Animal Breeding Center, Nanjing), with a body weight of (19.6±0.6)g and animal permit number: SCXK (Su) 2017-0001. The mice were housed in the animal experimental room provided with regular feeding, controlled room temperature, humidity, daily light exposure, and adequate ventilation.

**Experimental Instruments.** Tissue dehydrator PQT-A, tissue embedding machine PBM-A, tissue spreader PHY-III (Changzhou Puristar Medical Equipment Co., Ltd.); Pathological slicer RM2235 (Shanghai Leica Instruments Co., Ltd.); Microscope (NIKON H550S, Japan); Wet transfer apparatus 170-3930 Bio-Rad.

**Experimental Drugs and Reagents.** Gu Bi Tang (composition: 15g Lu Jiao Shuang, 15g Niu Xi, 12g Gou Ji, 15g Qian Nian Jian, 15g Sang Ji Sheng, 15g Wei Ling Xian, 15g Dan Shen, 20g Ji Xue Teng, 20g Bai Guo, 10g Mu Fang Ji, 10g Du Huo, 5g Gan Cao) was provided by the hospital's pharmacy department. Preparation method: Lu Jiao Shuang was decocted for 30 minutes, then other herbs were added and boiled for another 30 minutes. After water extraction, the herbal liquid was centrifuged at 1000 rpm for 10 minutes, filtered, and the concentration was adjusted to 1 g/ml, stored at 4°C; Amfenac enteric-coated tablets (National Drug Approval Number H20023171) were purchased from Shanghai Huazhong Pharmaceutical Co., Ltd.; Toluidine blue staining solution (Aladdin T104232); Hematoxylin staining solution (Zhuhai Beiso BA4097); TUNEL assay kit (Nanjing Kaiji Bio KGA704); TNF-α (Batch E-EL-R2856c) and IL-1β (Batch E-EL-H0149C) ELISA assay kits (Wuhan Elabscience); DAB staining solution (DAKO K5007); TEMED (Aladdin T105496); Glycine (Solarbio G8200); Protease inhibitor cocktail (Roche 4693116001); HRP anti-mouse antibody (Beyotime A0216); HRP anti-rabbit antibody (Beyotime A0208); HRP anti-goat antibody (Beyotime A0181).

Ephedra sinica and Cinnamomum cassia have demonstrated anti-inflammatory and analgesic effects, which can help alleviate the pain and swelling associated with OA. Aconitum carmichaelii and Glycyrrhiza uralensis possess chondroprotective and antioxidant properties, potentially contributing to the preservation of cartilage integrity. Paeonia lactiflora has been shown to modulate inflammatory pathways and address the symptoms of joint stiffness and discomfort.

The combination of these herbal ingredients in the Gu Bi Tang formula is believed to synergistically target multiple pathways involved in the pathogenesis of osteoarthritis, making it a promising candidate for investigation as a potential disease-modifying intervention.

## Experimental Methods

**Animal Model Preparation.** Forty-five 10-week-old SPF C57 male mice were subjected to intraperitoneal anesthesia with 4% pentobarbital sodium. The knee joint area of the mice was shaved and prepared with skin disinfection, and a medial parapatellar incision was made to expose the knee joint. The anterior cruciate ligament was severed, hemostasis was ensured, and the joint cavity was closed layer by layer. Each mouse received daily intramuscular injections of penicillin (80 000 units/kg) for infection prevention for three days postoperatively.[7]

The surgical induction of osteoarthritis in a rat model was chosen for this study due to its well-established ability to recapitulate the key pathological features of human OA. The medial meniscectomy (MMx) procedure, which involves the partial removal of the medial meniscus, has been widely used to reliably trigger the development of progressive cartilage degeneration, subchondral bone changes, and the associated inflammatory responses characteristic of the human osteoarthritis condition.

This animal model was selected for its relevance in mimicking the biomechanical and biochemical alterations observed in human OA, allowing for a thorough investigation of the potential chondroprotective effects of Gu Bi Tang and its underlying mechanisms.

**Animal Grouping, Administration, and Tissue Collection**. After 8 weeks of modeling, the 45 mice were weighed, and they were randomly divided into three groups: the model group (Group A), the Gu Bi Tang group (Group B), and the Amfenac group (Group C), with 15 mice in each group. After one week of free access to food and water, the following interventions were administered: Group A received an equivalent volume of normal saline via gavage; Group B received 13 mL/(kg·time) of Gu Bi Tang via gavage; and Group C received 19.5 mg/(kg·time) of Amfenac solution via gavage. All three groups were gavaged twice daily. After 8 weeks of gavage, the mice were euthanized with intraperitoneal 4% pentobarbital sodium. The knee joints were isolated, preserving the connected tibia and femur, and removed. The fresh joint tissues were fixed in 4% paraformaldehyde for more than 24 hours. The knee joint tissues were trimmed with a scalpel, labeled accordingly, dehydrated through a graded alcohol series, embedded, and cooled on a -20°C freezing platform. Once the paraffin solidified, the tissues were sectioned into 4 μm thick slices. The sections were spread flat on a slide using a tissue spreader at 40°C warm water, and then, they were retrieved with a glass slide, baked, and stored at room temperature for subsequent use.

The Gu Bi Tang group received a daily oral administration of the herbal formula at a dose of 500 mg/kg, which was determined based on previous studies demonstrating the optimal therapeutic window for this traditional Chinese medicine in OA models. The Amfenac group received a daily oral dose of 10 mg/kg, a clinically relevant dosage for the management of OA-related pain and inflammation. The treatment duration of 8 weeks was selected to allow for the assessment of the long-term effects of these interventions on the progression of osteoarthritis.

**Toluidine Blue Staining.** Paraffin sections were dewaxed in water and stained with 0.5% toluidine blue at 60°C for 40 minutes. Differentiation was controlled under a 95% ethanol microscope. The sections were then dehydrated and made transparent in a sequence of absolute ethanol I, absolute ethanol II, xylene I, and xylene II (each for 5 minutes). After dehydration, the sections were air-dried, mounted with neutral gum, examined under a microscope, and images were collected for analysis.[8]

**Tunel Method for Detecting Cell Proliferation and Apoptosis.** Paraffin sections were dewaxed in water, and a proteinase K working solution was added to cover the tissue, followed by incubation at 37°C for 30 minutes. The sections

were then washed three times in PBS for 5 minutes each. After shaking off excess liquid, a membrane-breaking working solution was added to cover the tissue, and the previous steps were repeated. Cell proliferation and apoptosis were identified using the Tunel assay kit following the manufacturer's instructions. Apoptotic cells were identified as those with brown-yellow granules in the cell nucleus. The apoptotic index was calculated as the number of apoptotic cells divided by the total number of cells multiplied by 100%.[9]

**Western Blotting to Detect the Expression of PI3K/AKT/mTOR Proteins in Rat Cartilage Cells.** Approximately 50 mg of isolated cartilage tissue was ground, and the bone powder was collected and added to 1000 μL of RIPA lysis buffer. The samples were then lysed on ice for 30 minutes, centrifuged, and the supernatant transferred to a 1.5 mL centrifuge tube and stored at 20°C. Protein content was determined. Glass plates were washed, filled with gel, loaded with samples, and subjected to electrophoresis. After completing the electrophoresis, the transfer was terminated. Following membrane drying, the membrane was wetted with TBS and then transferred to a flat dish containing blocking solution (5% skimmed milk TBST solution) for 1 hour at room temperature. Primary antibodies were diluted 1:1000 in TBST containing 5% BSA and incubated overnight at 4°C. After incubation, the membranes were washed in TBST three times for 10 minutes each at room temperature. Chemiluminescence was performed, and images were captured using a Tanon 5200 chemiluminescence imaging system.[10]

**ELISA to Measure the Expression of Inflammatory Factors in Rat Serum.** Blood samples were collected from the tail vein of each group of rats before and after the administration period. The samples were centrifuged at 3000 rpm (radius of 8.4 cm) for 20 minutes, and the supernatant was collected. ELISA was used to measure the levels of TNF-α and IL-1β in the serum of each group of rats, following the experimental procedures strictly according to the kit's instructions.

Toluidine blue staining was used to assess the proteoglycan content and structural integrity of the articular cartilage, as a decrease in proteoglycan levels is a hallmark of OA progression. The TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) assay was performed to quantify the extent of chondrocyte apoptosis, a crucial pathological process in OA. Western blotting was utilized to investigate the expression levels of key proteins involved in the PI3K/Akt signaling pathway, which plays a pivotal role in regulating chondrocyte proliferation, survival, and inflammatory responses. Lastly, ELISA (Enzyme-Linked Immunosorbent Assay) was employed to measure the concentrations of pro-inflammatory cytokines, such as IL-1β and TNF-α, within the joint tissues, as these mediators are known to contribute to the catabolic changes observed in osteoarthritis.

### Statistical Analysis

GraphPad Prism 8 was used to create graphs. Data were analyzed using SPSS 25.0. Continuous data were compared using *t* tests and presented as (mean ± standard deviation). Categorical data were compared using chi-square tests and expressed as n (%). A significance level of $P < .05$ was considered statistically significant.

**Figure 1.** Staining of Cartilage Cells in Different Groups of Rats (100 μm)

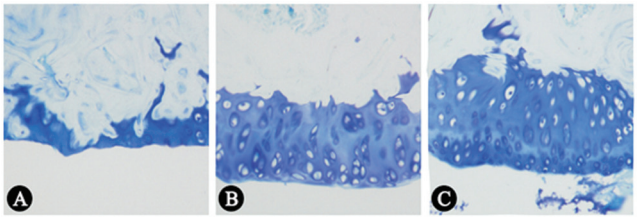
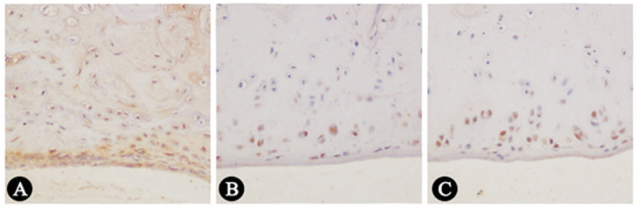



**Table 1.** Number of Apoptotic Cells in Cartilage Tissues Under 200× Magnification in Different Groups of Rats

| Group | A (n=15) | B (n=15) | C (n=15) |
|---|---|---|---|
| Number of Apoptotic Cells | 27.53±4.12 | 11.06±4.39[a] | 11.73±3.55[a] |

[a]Indicates a significant difference compared to Group A ($P < .05$)

**Figure 2.** Expression of Cartilage Cell Apoptosis Levels Detected by Tunel Assay in Different Groups of Rats




## RESULTS

### Staining of Cartilage Cells in Different Groups of Rats

Compared to Group A, Groups B and C showed significant improvements in cartilage tissue, with deeper staining observed in toluidine blue staining, as illustrated in Figure 1.

### Proliferation and Apoptosis of Cartilage Cells in Different Groups of Rats

Compared to Group A, both Groups B and C exhibited significantly reduced apoptosis of cartilage cells ($P < .05$), as shown in Table 1 and Figure 2.

### Expression of PI3K/AKT/mTOR Proteins in Cartilage Cells of Rats in Different Groups

Compared to Group A, both Groups B and C showed a significant reduction in the expression of PI3K, AKT, and mTOR proteins in the joint cartilage of rats, with Group C exhibiting a greater decrease than Group B ($P < .05$). See Table 2 and Figure 3 for details.

### Expression of Inflammatory Factors in the Serum of Rats in Different Groups

In Group A, the levels of TNF-α before and after administration were (162.47±20.76, 158.93±23.65), and IL-1β levels were (99.34±19.58, 96.15±14.89). In Group B,

**Table 2.** Expression of PI3K/AKT/mTOR Proteins in Cartilage Cells of Rats in Different Groups

| Group | A (n=15) | B (n=15) | C (n=15) |
|---|---|---|---|
| PI3K/GAPDH | 0.91±0.12 | 0.76±0.03[a] | 0.68±0.05[a,b] |
| AKT/GAPDH | 0.93±0.07 | 0.74±0.09[a] | 0.62±0.07[a,b] |
| mTOR/GAPDH | 0.97±0.04 | 0.78±0.05[a] | 0.73±0.04[a,b] |

[a]Indicates a significant difference compared to Group A ($P < .05$)
[b]indicates a significant difference compared to Group B ($P < .05$).

**Figure 3.** Western Blot Analysis of PI3K/AKT/mTOR Protein Expression in Cartilage Cells of Rats in Different Groups

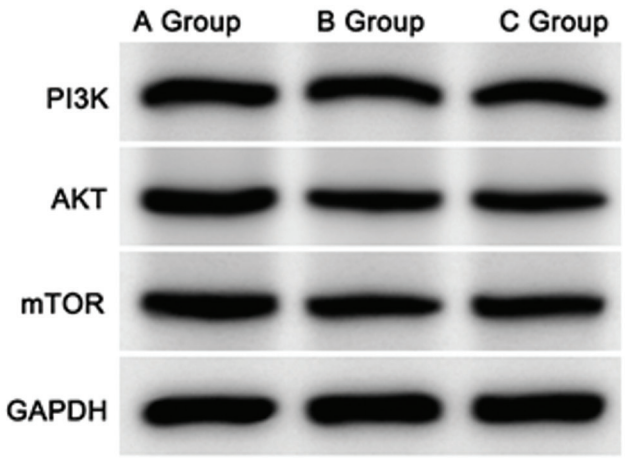
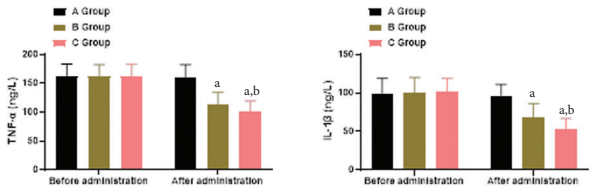



**Figure 4.** Expression of Inflammatory Factors in the Serum of Rats in Different Groups




[a]Indicates a significant difference compared to Group A at the same time ($P < .05$)
[b]indicates a significant difference compared to Group B at the same time ($P < .05$).

the levels of TNF-α before and after administration were (161.92±20.54, 112.46±21.99), and IL-1β levels were (100.37±19.65, 67.59±18.43). In Group C, the levels of TNF-α before and after administration were (161.75±21.26, 100.63±19.12), and IL-1β levels were (101.24±17.82, 53.43±13.26). Before administration, there was no significant difference in the expression levels of TNF-α and IL-1β in the serum among the three groups ($P > .05$). After administration, when compared to Group A, both Groups B and C exhibited a significant reduction in the serum levels of TNF-α and IL-1β, with Group C showing a greater decrease than Group B ($P < .05$). Refer to Figure 4 below for details.

## DISCUSSION

The present study provides novel insights by elucidating the role of the PI3K/Akt signaling pathway in mediating the chondroprotective actions of Gu Bi Tang. While other OA treatments, such as non-steroidal anti-inflammatory drugs (NSAIDs), primarily target the inflammatory cascade, this study suggests that Gu Bi Tang's multi-targeted approach, involving the modulation of PI3K/Akt signaling, may offer additional benefits in preserving cartilage integrity and attenuating the catabolic processes associated with OA progression. Specifically, the study found that Gu Bi Tang was able to upregulate the expression of key proteins in the PI3K/Akt pathway, including phosphorylated Akt and its downstream targets, thereby promoting chondrocyte proliferation and inhibiting apoptosis. This contrasts with the more narrowly focused anti-inflammatory effects of Amfenac, a commonly used NSAID, which did not demonstrate the same capacity to influence this crucial signaling axis. Furthermore, the study's findings suggest that the unique phytochemical composition of Gu Bi Tang, with its synergistic blend of herbal ingredients, may contribute to its superior chondroprotective and anti-inflammatory properties compared to individual herbal compounds or conventional pharmacological interventions. This highlights the potential advantages of traditional Chinese medicine formulations in addressing the multifaceted pathophysiology of osteoarthritis.

Previous studies[11] have suggested that the pathogenesis of OA mainly involves changes in the extracellular matrix of joint-related cells, as well as disruption in the synthesis and degradation of chondrocytes. This study successfully established an OA rat model using the anterior cruciate ligament transection method. Staining with toluidine blue revealed cartilage surface defects and a lack of staining in the model group (Group A). The TUNEL assay detected significant chondrocyte apoptosis on the cartilage surface, confirming the success of the OA model in this study. OA is primarily characterized by pathological changes in joint cartilage, and while apoptosis can occur in normal chondrocytes, excessive apoptosis in chondrocytes under stress conditions can lead to changes in the cartilage matrix, resulting in degenerative alterations in the cartilage.[12]

Cell apoptosis is a genetically regulated process of programmed cell death, and it plays a role in both physiological and pathological processes.[13] The PI3K/AKT signaling pathway is important in regulating chondrocyte proliferation, apoptosis, and extracellular matrix remodeling.[14] PI3K, an intracellular phosphoinositide kinase, comprises regulatory subunit p85 and catalytic subunit p110. AKT, also known as PKB (protein kinase B), is a serine/threonine-specific protein kinase that regulates the growth, metabolism, transcription, and protein synthesis in various cells.[15] When PI3K receives incoming signals on the cell membrane, it directly or indirectly activates downstream AKT, which phosphorylates P53, NF-κB, and Caspase-9, exerting specific biological effects.[16] Research by Cheng et al.[17] found that inhibiting the activation of the PI3K/AKT/NF-κB signaling pathway could suppress chondrocyte apoptosis, thus alleviating the inflammatory response in OA. Lu et al.[18] found that isoquercitrin significantly reduced the expression of PI3K, AKT, and NF-κB proteins, thereby improving cartilage degeneration in OA rats.

**Traditional Chinese medicine** considers OA to fall within the category of "Gu Bi," primarily caused by deficiencies in Qi and blood, as well as liver and kidney deficiencies.[19]

Based on the academic principles and clinical experience of our hospital's orthopedics department in the treatment of OA using traditional Chinese medicine, we have employed Gu Bi Tang, which promotes blood circulation and dredging meridians. Its main ingredients include Lu Jiao Shuang, Niu Xi, Gou Ji, Qian Nian Jian, Sang Ji Sheng, Wei Ling Xian, Dan Shen, Ji Xue Teng, Bai Gu, Mu Fang Ji, Du Huo, and Gan Cao, among others. This formula primarily focuses on promoting blood circulation and dredging meridians while also dispelling wind and dampness and nourishing the liver and kidneys. Lu Jiao Shuang: Lu Jiao Shuang is derived from deer antlers and is known for its anti-inflammatory and analgesic properties. It has been used in traditional Chinese medicine to treat joint disorders. Lu Jiao Shuang may help reduce inflammation and relieve pain associated with osteoarthritis.

**Niu Xi**: Niu Xi, also known as Achyranthes bidentata, is commonly used in traditional Chinese medicine for its anti-inflammatory and analgesic effects. It promotes blood circulation, relieves pain, and strengthen bone and tendons. Niu Xi may contribute to the analgesic and anti-inflammatory actions of Gu Bi Tang.

**Gou Ji**: Gou Ji, or Cibotium barometz, has been traditionally used for its anti-inflammatory and antioxidant properties. It is believed to strengthen bones and tendons, promote blood circulation, and alleviate pain. Gou Ji may help reduce inflammation and protect against oxidative stress in osteoarthritis.

**Dan Shen**: Dan Shen, also known as Salvia miltiorrhiza or Chinese sage, has been extensively studied for its cardiovascular protective effects. It has anti-inflammatory, antioxidant, and anti-apoptotic properties. Dan Shen may contribute to the overall anti-inflammatory and protective effects of Gu Bi Tang on chondrocytes in osteoarthritis.

**Bai Guo**: Bai Guo, or Ginkgo biloba, is a well-known herb with antioxidant and anti-inflammatory properties. It has been used in traditional medicine to improve blood circulation and reduce inflammation. Bai Guo may help alleviate inflammation and oxidative stress in osteoarthritis.

**Du Huo**: Du Huo, or Angelica pubescens, is commonly used in traditional Chinese medicine for its anti-inflammatory and analgesic effects. It is believed to relieve pain, reduce inflammation, and promote blood circulation. Du Huo may contribute to the analgesic and anti-inflammatory properties of Gu Bi Tang.

**Gan Cao**: Gan Cao, or licorice root, is a widely used herb in traditional medicine. It has anti-inflammatory, antioxidant, and immunomodulatory properties. Gan Cao may help reduce inflammation, protect against oxidative stress, and modulate the immune response in osteoarthritis.

The active ingredients in Salvia miltiorrhiza (Dan Shen), such as tanshinones and salvianolic acids, have been shown to exert beneficial effects on osteoarthritis through the modulation of the PI3K/Akt signaling axis. Tanshinones have been reported to inhibit the PI3K/Akt pathway, leading to the suppression of inflammatory mediators and the promotion of chondrocyte proliferation and survival. Similarly, salvianolic acids have demonstrated the ability to upregulate the phosphorylation of Akt, thereby enhancing the activity of this pro-survival signaling cascade within chondrocytes. Furthermore, the presence of components like glucosamine and chondroitin sulfate in Gu Bi Tang, derived from the herb Cornu Cervi (Lu Jiao Shuang), may synergistically support the protective effects on articular cartilage. These molecules have been independently shown to stimulate the production of proteoglycans and suppress the activity of catabolic enzymes, which are crucial in maintaining the structural integrity of the cartilage matrix. The combination of these bioactive compounds within the Gu Bi Tang formula likely contributes to the observed modulation of the PI3K/Akt pathway, leading to the attenuation of chondrocyte apoptosis, the enhancement of extracellular matrix synthesis, and the overall preservation of articular cartilage integrity in the osteoarthritis model. This multi-targeted approach of Gu Bi Tang, targeting both the inflammatory and the pro-survival signaling cascades, may provide a more comprehensive and effective strategy for managing the complex pathophysiology of osteoarthritis, compared to conventional pharmacological interventions that often focus on a single mechanism of action.

It has shown good efficacy in treating OA. The mechanism of action of this medication for treating OA mainly involves improving the metabolic mechanisms of cartilage degeneration, inhibiting damage from oxygen free radicals, preventing chondrocyte apoptosis, and regulating cytokines, among other biological effects. It works through multiple pathways and targets to delay cartilage degeneration and control OA progression, although its specific mechanism of action is not yet clear.[20] The results of this study show that compared to Group A, the cartilage tissues of the rats in Groups B and C exhibited significant improvement with deeper toluidine blue staining. Furthermore, when compared to Group A, both Groups B and C had significantly reduced chondrocyte apoptosis in cartilage tissues ($P < .05$). Additionally, when compared to Group A, both Groups B and C exhibited significantly decreased expression of PI3K, AKT, and mTOR proteins in joint cartilage, with Group C showing a greater decrease than Group B ($P < .05$). These study results suggest that Gu Bi Tang can effectively inhibit the PI3K/AKT signaling pathway, reduce chondrocyte apoptosis in OA rats, and downregulate the expression of PI3K, AKT, and mTOR proteins. Under normal physiological conditions, cartilage tissue cells maintain a balance between degradation and synthesis metabolism to preserve the integrity of the cartilage extracellular matrix structure and function.[21] Research[22] has indicated that the imbalance of inflammatory cytokines in the body is one of the pathogenic mechanisms of OA. The results of this study show that before administration, the expression levels of TNF-α and IL-1β in the serum of the three groups of rats were not significantly different ($P > .05$). However, after administration and compared to Group A, both Groups B and C exhibited a significant reduction in the expression levels of TNF-α and IL-1β in the serum ($P < .05$), with Group C

showing a greater reduction than Group B. These results suggest that Gu Bi Tang can effectively reduce the levels of inflammatory cytokines in the serum of OA model rats, alleviating the inflammatory response in the rat's body.

While this study has provided a preliminary exploration of the therapeutic effects of Gu Bi Tang on osteoarthritis (OA) model rats, there are several limitations to the study: (1) Limited Sample Size: The study employed a relatively small sample size, with only 15 rats in each group. This sample size limitation may constrain the statistical power and generalizability of the study. (2) Lack of Long-term Observation: The observation period in this study was relatively short, focusing on short-term effects after administration. OA is a chronic disease, and long-term observations are crucial for understanding the treatment's durability and long-term effects. (3) Lack of Multi-pathway Validation: While the study addressed the impact on the PI3K/Akt signaling pathway, the pathogenesis of arthritis typically involves interactions among multiple pathways. This study did not further investigate the effects of Gu Bi Tang on other key signaling pathways. (4) Limited Pharmacological Component Analysis: Gu Bi Tang is a complex traditional Chinese medicine formula with multiple components. This study did not delve into the mechanisms of action of the various medicinal components in Gu Bi Tang. This may impact the identification of specific effective components and the development of more precise treatment regimens. In conclusion, this study has provided initial insights, but further research is needed to address the aforementioned limitations. This will help us gain a more comprehensive understanding of the potential role and mechanisms of Gu Bi Tang in the treatment of OA.

To overcome these limitations, the research team could consider: 1) Increasing the sample size in subsequent studies to enhance the statistical power and the robustness of the findings. This would help strengthen the conclusions drawn from the data and increase the confidence in the observed effects of Gu Bi Tang. 2) Conducting longitudinal studies to assess the long-term impact of Gu Bi Tang on the progression of osteoarthritis. Monitoring the articular cartilage, joint function, and clinical outcomes over an extended period would provide valuable insights into the sustained therapeutic benefits of this traditional Chinese medicine formula.

Building upon the current findings, future research could explore the effects of Gu Bi Tang on other signaling pathways involved in the pathogenesis of osteoarthritis. The use of omics technologies, such as transcriptomics, proteomics, and metabolomics, could provide a more comprehensive understanding of the molecular mechanisms underlying the observed chondroprotective and anti-inflammatory effects. Additionally, the application of in vivo imaging techniques, such as magnetic resonance imaging (MRI) or micro-computed tomography (micro-CT), could enable the longitudinal monitoring of cartilage regeneration and joint structural changes in response to Gu Bi Tang treatment. This would offer valuable insights into the long-term impact of this traditional formula on disease progression.

The findings of this study have the potential to contribute to the development of novel therapeutic strategies for the management of osteoarthritis. The integration of Gu Bi Tang, or its active components, with conventional OA treatments could be explored to enhance the overall therapeutic efficacy and improve patient outcomes. Regarding the translation of Gu Bi Tang into clinical practice, it will be essential to address the regulatory and safety considerations. Thorough pre-clinical and clinical evaluations, including assessments of safety, tolerability, and pharmacokinetic properties, would be required to ensure the safe and effective use of this traditional Chinese medicine formula in the clinical setting.

## CONCLUSION

In conclusion, this study has unveiled the remarkable therapeutic potential of the traditional Chinese medicine formula Gu Bi Tang in the management of osteoarthritis (OA). The findings clearly demonstrate the formula's potent chondroprotective and anti-inflammatory effects, as evidenced by the significant reduction in chondrocyte apoptosis and serum inflammatory factors in the OA rat model. Importantly, the study has elucidated the underlying mechanism of action, revealing that Gu Bi Tang's beneficial effects are mediated, at least in part, through the inhibition of the PI3K/AKT signaling pathway. This scientific contribution not only advances the understanding of how traditional Chinese medicines can modulate key pathways involved in OA pathogenesis, but also suggests that Gu Bi Tang may offer a promising complementary or alternative therapeutic strategy for the management of this debilitating condition. While the current study provides valuable short-term insights, further research is warranted to explore the long-term impacts of Gu Bi Tang and its potential application in human OA treatment, as well as to investigate the specific bioactive components responsible for its enhanced efficacy and safety. Nonetheless, the findings of this study hold significant promise, as the integration of traditional Chinese medicine with modern medical practices continues to emerge as a viable approach for developing more holistic and effective OA management strategies. Ultimately, these results lay a strong foundation for the continued exploration and translation of this integrative approach, with the goal of improving the quality of life for individuals suffering from osteoarthritis.

### COMPETING INTERESTS

The authors declare that they have no competing interests.

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Not applicable

### AUTHORS' CONTRIBUTIONS

Zhongkui Guo designed the research study. Yong Qin and Yi Li performed the research. Peifu Tang conducted experiments and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the First Medical Centre, Chinese PLA General Hospital.

## CONSENT FOR PUBLICATION

Not applicable

## AVAILABILITY OF DATA AND MATERIALS

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

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