Int Surg 2021;**105:**774–783
DOI: 10.9738/INTSURG-D-20-00016.1

Effects of Catalpa Alcohol From *Rehmannia glutinosa* on Calcium-Binding Protein, Interleukin-1β, and Galectin-3 in Synovial Tissues of Rats With Knee Osteoarthritis

Qiang He[1,2], Yixin Liu[1,2], Hong Yin[2], Weiqing Qian[2], Nianwei Yao[2], Zhen Wang[1,2], Lei Xu[1,2], Dapeng Zhang[1,2], Kun Yan[1,2], Fenglei Dai[2]

[1]*Department of Orthopedic Surgery, Nanjing University of Traditional Chinese Medicine, Nanjing, China*

[2]*Department of Orthopedic Surgery, Nanjing TCM Hospital, Affiliated to Nanjing University of Traditional Chinese Medicine, Nanjing, China*

Objectives: We aimed to evaluate the effects of catalpa alcohol from *Rehmannia glutinosa* on the expressions of calcium-binding protein (S100A12), interleukin-1β (IL-1β), and galectin-3 in the synovium of rats with early knee osteoarthritis (KOA).

Methods: Fifty-two adult male Wistar rats aged 3 to 8 weeks were divided into normal control (n = 16), model (n = 12), low-dose (n = 12), and high-dose groups (n = 12). On the 10th day after modeling, 6 rats in normal control group and 6 in the other 3 groups were randomly selected. X-ray and 3-dimensional computed tomography (3D CT) images of the left knee joint were taken under live anesthesia. The joint cavity of sacrificed rats was opened to observe cartilage surface. After 28 consecutive days of administration, the synovial tissue of left knee joint was collected.

Results: The S100A12, IL-1β, and galectin-3 levels in synovial tissue were detected by immunohistochemistry and ELISA. There were articular cartilage defects in left knees. Radiologic examination showed significant joint space narrowing and hyperplasia, and 3D CT joint space value decreased ($P < 0.05$). The Mankins and OARSI scores of synovial histopathology were significantly different ($P < 0.05$). The S100A12, IL-1β, and galectin-3 levels in synovial tissue of the model group significantly exceeded those of the normal control group ($P < 0.01$). Compared with the model group, such levels of low-dose ($P < 0.05$) and high-dose groups ($P < 0.01$) were significantly lower.

Corresponding author: Fenglei Dai, Department of Orthopedic Surgery, Nanjing TCM Hospital, Affiliated to Nanjing University of Traditional Chinese Medicine, 157 Da Ming Road, Nanjing 210000, China.
E-mail: karlgeorg-kippert@web.de

Conclusions: The S100A12, IL-1β and galectin-3 levels in synovium tissue decreased with rising concentration of catalpa alcohol from *R. glutinosa*. Therefore, this drug is potentially suitable for inhibiting an inflammatory response to delay the progression of KOA.

Key words: Rehmannia glutinosa – Catalpa alcohol – Immunohistochemistry – Enzyme-linked immunosorbent assay – Calcium-binding protein – Interleukin-1β – Galectin-3

Knee osteoarthritis (KOA), as one of the most common forms of arthritis worldwide, is typified by progressive degeneration of articular cartilage, synovial hyperplasia, and bone remodeling.[1,2] In recent years, researchers have endeavored to clarify the pathogenesis of KOA.[3] Synovitis has been associated with the severity of KOA.[2] Interleukin-1β (IL-1β) is an inflammatory cytokine that is widely expressed during KOA as a key mediator of cartilage degradation.[4] Proinflammatory factor galectin-3 is a member of the chimeric galactoside-binding protein family,[5] which shows proinflammatory effects mostly by enhancing the activation of macrophages, mast cells, natural killer cells, and T and B lymphocytes.[6] Calcium-binding protein (S100A12), a member of the S100 family, is a low-molecular-weight protein that plays a proinflammatory role by activating mast cells and participating in the metastasis of neutrophils to inflammatory sites.[7,8] Besides, IL-1β can inhibit chondrocyte proliferation and promote the release of inflammatory cytokines,[1] galectin-3 is important for IL-1β production,[9] and S100A12 can cause progressive cartilage damage by degrading the extracellular matrix (ECM). All 3 factors play crucial roles in the pathogenesis of KOA.

As an iridoid glycoside extracted from *Rehmannia glutinosa*, catalpa alcohol has a variety of pharmacologic effects,[10] including antiapoptotic and anti-inflammatory properties.[11] Until now, raw *R. glutinosa* has been widely used to treat inflammatory diseases,[12] but systematic basic studies remain limited. In this study, the rat model of KOA was constructed by 4% papain combined with 0.03 mol/L cysteine solution, and the effects of different doses of catalpa alcohol from *R. glutinosa* on S100A12, IL-1β, and galectin-3 expressions in the synovial tissues of rats with KOA were assessed, aiming to explore the underlying mechanism.

Materials and Methods

Animals

Fifty-two healthy adult male Wistar rats weighing 0.29 ± 0.1 kg were provided by Qinglongshan Animal Breeding Farm (License No. SCXK[Jiangsu]2017-0001; Nanjing, China). All animals were kept in a pathogen-free environment and fed ad libitum. The procedures for care and use of animals were approved by the Ethics Committee of our hospital, and all applicable institutional and governmental regulations concerning the ethical use of animals were followed.

Apparatus and reagents

ELISA kits for S100A12 (Batch E20181201A, Item Ck-e30711r), galectin-3 (Batch E20181201A, Item Ck-e94644r), and IL-1β (Batch E20181201A, Item ck-e30419r) were used.

Immunohistochemistry was performed using antibodies against galectin-3 (Item 14979-i-ap, Batch 10006432; Proteintech), IL-1β (Item 66737-i-ig, Batch 10006432; Proteintech), and S100A12 (Item BS7539, Batch XS20181105000; Bioworld).

Other reagents and apparatus included catalpa alcohol from *R. glutinosa* (Xi'an Anacreontic Technology Biological Co, Ltd), 4% papain (Shanghai Hengyuan Biotechnology Co, Ltd), hematoxylin (Batch 160401100; Wuxi Jiangyuan Industrial Technology and Trade Corporation), horseradish peroxidase (Item BS10950, Batch CI33171; Bioworld), horseradish peroxidase color development kit (Batch 033016160503; Beyotime Biotechnology Co., Ltd. [Nantong Branch]), an Axioplan 2 imaging optical microscope (Zeiss), and ELx800 microplate reader (Boteng Instrument).

Modeling and success criteria

According to previous literature,[13] 52 adult male Wistar rats aged 3 to 8 weeks were selected and divided into a normal control group (n = 16), a model group (n = 12), a low-dose group (n = 12), and a high-dose group (n = 12). The latter 3 groups were injected with 4% papain and 0.03 mol/L cysteine (0.2 mL) after conventional shaving and disinfection of the left knee joint on days 1, 4, and 7 after the experiment began. The treatment method and injection site of the normal control group were

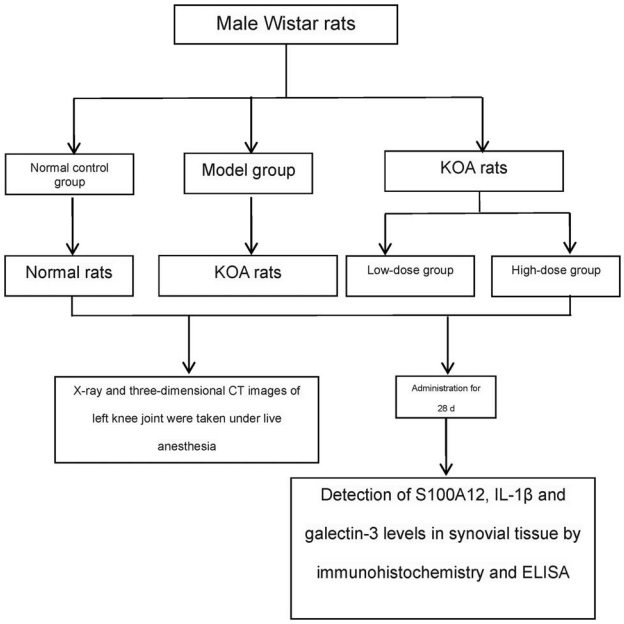

Fig. 1 Flow chart of experimental procedure.

identical, and the same amount of normal saline was injected into the joint cavity with a syringe. Six rats were randomly selected from the normal control group 10 days after modeling, and 6 rats were selected from the 3 groups (2 in each group; Fig. 2). The rats were anesthetized by intraperitoneal injection of 7% chloral hydrate according to the body mass (5 mL/kg). After X-ray and 3-dimensional computed tomography (3D CT) examinations of the left knee joint, the rats were killed. The articular cavity of the left knee was opened to observe the cartilage surface, and histopathologic examination was conducted to verify the success of modeling.

Administration method

The recommended daily dose of adults was converted into the daily dose of experimental rats according to the formula of dose estimation in pharmacology research of traditional Chinese medicine: $dB = dA \times KB/KA$.[14] The high- and low-dose groups were administered 100 and 10 mg/kg catalpa alcohol, respectively, and each rat was given 0.2 mL/kg of the drug through gavage. Normal control and model groups were given the same amount of normal saline by gavage. Intragastric administration was carried out once daily (8:00 AM) for 28 consecutive days.

Detection of indices

After 28 days of continuous gavage, synovial tissue was collected from the left knee joint.[15] S100A12, IL-1β, and galectin-3 in the synovial membrane were detected by immunohistochemistry and ELISA strictly in accordance with the kits' instructions.

Immunohistochemistry

After drug intervention, the rats were killed under excessive anesthesia by injection with 7% chloral hydrate into the hearts. After being killed, the rats were immersed in 10% strong disinfectant cold solution for 10 minutes, placed on an ultra-clean bench, fixed in the inverted position, and routinely sterilized in the surgical field. After the right knee joint cavity was opened on the medial side of the right hind limb, smooth and bright yellowish synovial tissue was found extending upward from the lower margin of the patella. The synovial tissue was completely stripped off and then cut off with a

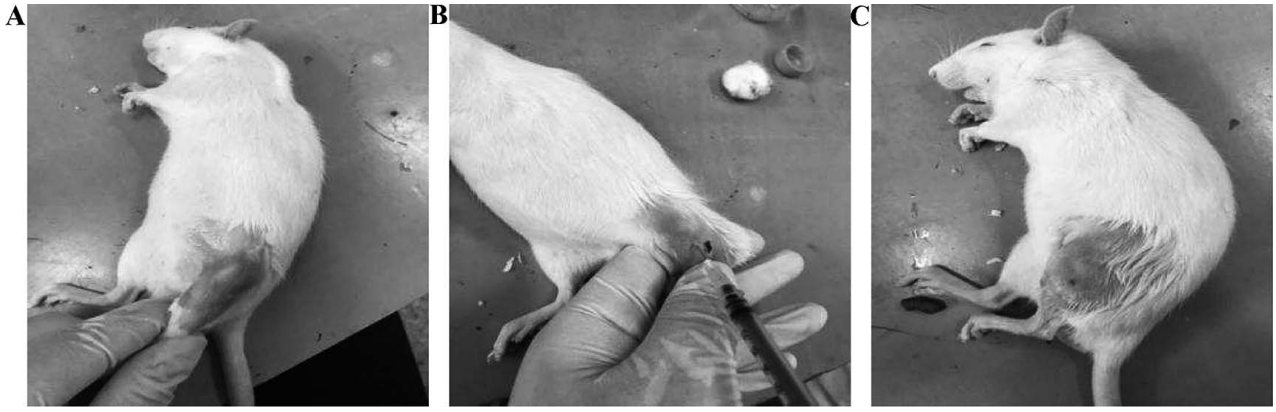

Fig. 2 Drug injection processes to the left knee articular cavity. (A) Shaving and disinfection before injection. (B) Intra-articular drug injection into the left knee. (C) After drug injection.

surgical blade. Soft tissues around the articular surface of the medial femoral condyle of the right hindlimb were removed, and a 0.3-cm × 0.3-cm block of articular cartilage was chiseled according to the elasticity. A small amount of subchondral bone was added to the cartilage on the surface of each joint. The removed synovial tissue was washed repeatedly with phosphate-buffered saline (PBS) and marked clearly in a disposable plastic embedding frame; the plastic embedding frame was dehydrated in gradient concentrations of ethanol solutions for 2 hours; the tissue was transparentized for 2 hours with 50% ethanol-xylene and xylene successively; after immersion and embedding in paraffin, the tissue block was sectioned into 6-μm-thick sections; and the glass slide holder was put in a 60°C oven, baked for about 5 hours, and stored in a 4°C refrigerator.

The sections were routinely deparaffinized with xylene and gradient concentrations of solutions. Endogenous peroxidase in the sections was inactivated by 3% H_2O_2. The slide holder was placed in 0.01 mol/L citrate buffer (pH 6.0) at 95°C and incubated with 5% normal goat serum prepared by PBS at 37°C for 10 minutes, and then excess liquid was discarded. According to the instructions, primary and secondary antibodies were diluted with 5% bovine serum albumin (BSA), and 150 μL primary antibody was added. The sections were incubated at room temperature for 1 hour, washed 3 times with PBS (5 minutes each time), incubated with 150 μL secondary antibody at room temperature for 1 hour, and washed with PBS 3 times (5 minutes each time). The samples were stained brownish yellow under the microscope after 150 μL DAB solution was added. When the degree of staining was appropriate, the sections were rinsed immediately with PBS for 10 minutes. After routine hematoxylin staining, the sections were dehydrated with gradient concentrations of ethanol solutions, transparentized with xylene, mounted with neutral resin, and photographed under the light microscope (×400). The staining results of immunohistochemistry were analyzed by Image J software (National Institutes of Health, Bethesda, Maryland). Brown staining was determined positive, and IOD/area (average optical density) was evaluated.

ELISA

On the first day after drug administration, the synovial tissue was collected using the same method. Soft tissues around the articular surface of the medial femoral condyle of the right hindlimb were removed, and a 0.3-cm × 0.3-cm section of articular cartilage was chiseled according to the elasticity. A small amount of subchondral bone was added to the cartilage on the surface of each joint. The synovial tissue was separated, and 0.9% precooled normal saline was added according to the weight (g)/volume (mL) ratio of 1:9 and mechanically homogenized into a 10% homogenate. After centrifugation at 3000 rpm for 10 minutes, the supernatant was collected and stored in a –80°C refrigerator.

ELISA was performed strictly following the kits' instructions. (1) S100A12, IL-1β, and galectin-3 kits were prepared within 24 hours before use. The required slats were taken out of the aluminum foil bag after 20 minutes of equilibrium at room temperature, and the remaining slats were sealed with a self-sealing bag and stored at 4°C. (2) Standard wells were added at 50 μL standard at different concentrations, the sample well was added at 10 μL sample and 40 μL diluent, and blank wells were not added. (3) Except for the blank well, 100 μL horseradish peroxidase–labeled detection antibody was added to the standard and sample wells, and the reaction well was sealed with sealing plate membrane and incubated at 37°C in a water bath or an incubator for 60 minutes. (4) After the liquid was discarded and the plate was pat-dried by absorbent paper, each well was filled with washing buffer and left still for 1 minute, and the plate was pat-dried by absorbent paper after the washing buffer was shaken off, which were repeated 5 times. (5) Substrates A and B were added to each well, followed by incubation at 37°C in the dark for 15 minutes. (6) Fifty microliters of stopping buffer was added to each well, and OD was measured at 450 nm with the microplate reader within 15 minutes. In the SPSS worksheet, a linear regression curve of standard was plotted by using the standard concentration as the abscissa and the corresponding OD as the ordinate, and sample concentrations were calculated according to the curve equation.

Statistical analysis

All data were statistically analyzed by SPSS 25.0 software and expressed as mean ± SD. One-way analysis of variance (ANOVA) was used for pairwise comparison between groups. $P < 0.05$ indicated a statistically significant difference.

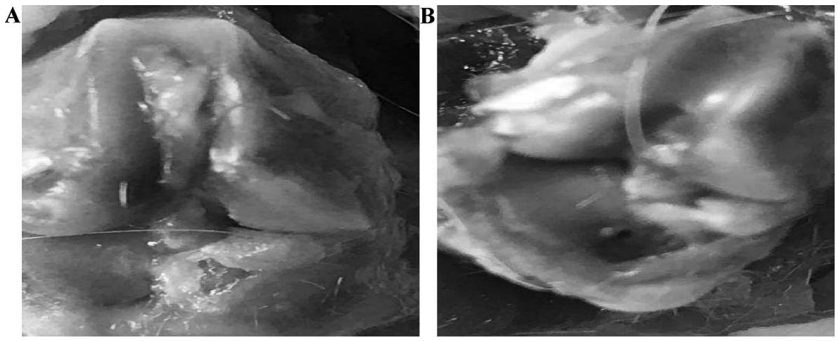

Fig. 3 Gross observation of articular cartilage incision of left knee. (A) Normal control group. (B) Model group.

Results

General state of rats after articular cavity injection

After 3 injections, the model group had decreased appetite and activity, obvious claudication, significant swelling of the knee joint at the injection site, reluctance to land on the hind limbs, and evident weight loss. In contrast, appetite, activity, or body weight barely changed in the normal control group, and limb movement disorders or lameness occasionally occurred. Slight swelling of the knee joint at the injection site disappeared 1 to 2 days after each injection.

Gross observation of articular cartilage of the left knee

In the normal control group, the articular cartilage was grayish white with bright color, and the cartilage surface was smooth. There were no cracks or damages on the articular surface, subchondral bone exposure, or hyperplasia (Fig. 3A). The articular cartilage of the model group was pale yellowish white with dim color, and the cartilage surface was rough and exfoliated, with subchondral bone exposure and hyperplasia (Fig. 3B). Compared with the model group, the cartilage surfaces of low- and high-dose groups became smooth, and the articular cartilage color became brighter.

X-ray examination results of left knee joint

On the 10th day after modeling, photographs were taken according to the methods mentioned above. Orthotopic X-ray revealed that the normal control group had normal knee joint space and smooth bone edge, without stenosis or osteophytes (Fig. 4A). In the model group, the knee joint space narrowed, and osteophytes formed at the bone margin (Fig. 4B). With the left leg extended, X-ray imaging showed that the joint space of the normal control group was significantly larger than that of the model group (Fig. 4C). Compared with the model group, the joint spaces of low- and high-dose groups markedly increased.

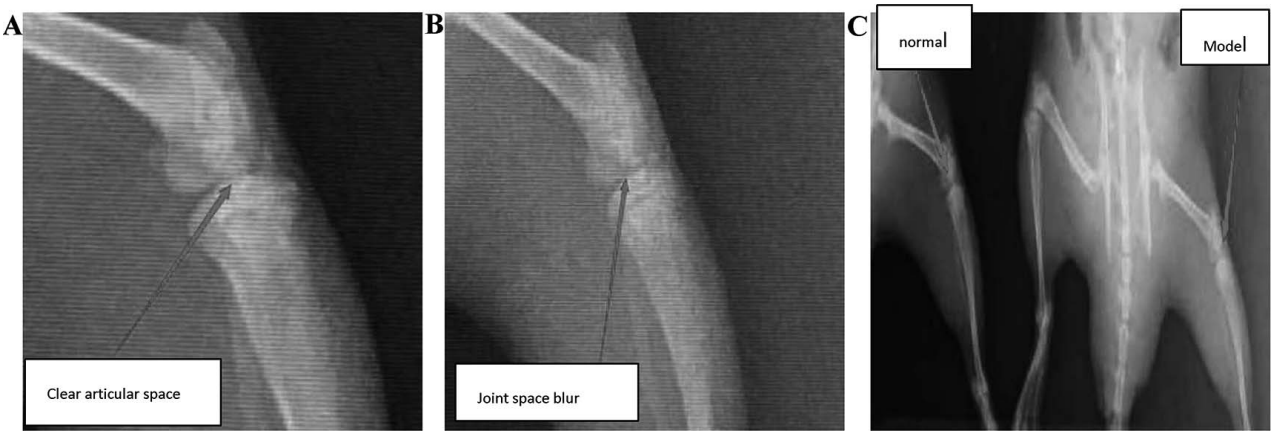

Fig. 4 X-ray images of extended left knee joint. (A) Normal control group. (B) Model group. (C) Difference between normal control and model groups.

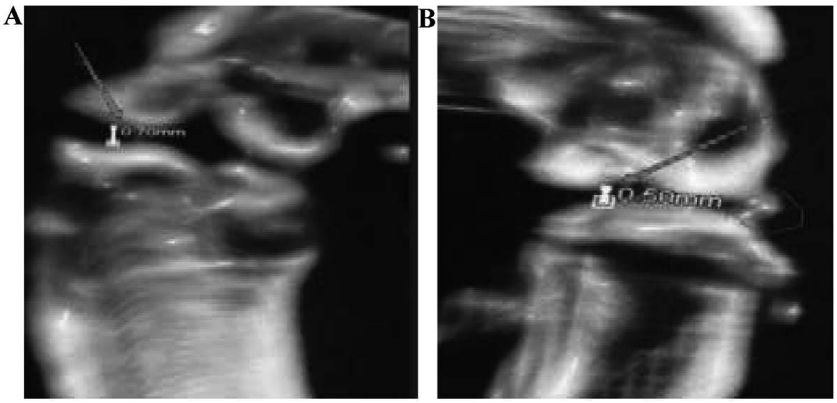

Fig. 5 3D CT images of left knee joint. (A) Normal control group. (B) Model group.

3D CT results of left knee joint

The 3D CT images of the knee joints of the normal control group showed clear and normal joint space and a smooth bone edge, without stenosis (Fig. 5A). For the model group, the joint space narrowed significantly, and osteophytes formed clearly. The bone edges and articular cartilage surfaces were rough (Fig. 5B). Besides, the joint space values of normal control and model groups were significantly different ($P < 0.05$). Compared with the model group, the joints of low- and high-dose groups were significantly enlarged ($P < 0.05$; Table 1).

Histopathologic observation results

The Mankin and OARSI scores of the model group were significantly higher than those of the other 3 groups ($P < 0.05$), and the scores of low- and high-dose groups significantly exceeded those of the model group ($P < 0.05$; Table 2).

Levels of S100A12, IL-1β, and galectin-3 in the synovium of the left knee joint detected by immunohistochemistry

The expressions of S100A12, IL-1β, and galectin-3 in the synovium of left knee joint were detected by immunohistochemistry (Table 3). The levels of S100A12, IL-1β, and galectin-3 in the synovial membrane of the model group significantly exceeded those of the normal control group ($P < 0.01$). Compared with the model group, such levels significantly decreased in administration groups, especially in the high-dose group ($P < 0.01$; Figs. 6–8).

Table 1 Joint space values of normal control and model groups

Group	n	Joint space (mm)
Normal control group	6	0.68 ± 0.016
Model group	6	0.58 ± 0.034
Low-dose group	6	0.64 ± 0.011
High-dose group	6	0.67 ± 0.012

Compared with normal control group, $P < 0.05$.

Levels of S100A12, IL-1β, and galectin-3 in the synovium of the left knee joint detected by ELISA

The expressions of S100A12, IL-1β, and galectin-3 in the synovial membrane of left knee joint were detected by ELISA (Table 2). The levels of S100A12, IL-1β, and galectin-3 in the synovial membrane of the model group surpassed those of the normal control group ($P < 0.01$). Such levels of the low-dose group ($P < 0.05$) and high-dose group ($P < 0.01$) were significantly lower than those of the model group (Table 4; Fig. 9).

Discussion

KOA is a whole joint disease characterized by progressive degeneration of articular cartilage, synovial hyperplasia, and bone remodeling in various joint tissues. The risk factors for KOA include aging, acute or chronic mechanical stress, joint trauma, and metabolic disease, which impair the homeostasis between ECM degradation and

Table 2 Mankin and OARSI scores of cartilage injury (mean ± SD n = 4)

Group	Mankin score	OARSI score
Normal control group	1.53 ± 0.71	0.36 ± 0.42
Model group	8.23 ± 2.12[a]	2.15 ± 0.78[a]
Low-dose group	4.45 ± 0.72[b]	1.10 ± 0.21[b]
High-dose group	2.34 ± 0.22[b]	0.45 ± 0.18[b]

Compared with normal control group, [a]$P < 0.05$; compared with model group, [b]$P < 0.05$.

Table 3 IL-1β, galectin-3, and S100A12 levels in synovial tissues detected by immunohistochemistry (average OD)

Group	IL-1β	Galectin-3	S100A12
Normal control group	8.50 ± 0.84	8.75 ± 6.50	7.62 ± 1.23
Model group	33.93 ± 1.63[a]	52.94 ± 2.24[a]	30.6 ± 1.34[a]
Low-dose group	28.84 ± 0.56[b]	26.62 ± 1.70[b]	26.99 ± 2.89
High-dose group	6.95 ± 0.78[b]	25.26 ± 1.33[b]	24.29 ± 1.65[b]

Comparison between model and normal control groups, and comparison between administration and model groups: [a]$P < 0.05$; **$P < 0.01$.

repair. Synovitis precedes the destruction of articular cartilage, as a predictor for the progression of KOA.[16] In the case of KOA, chondrocytes and other tissues are activated, and homeostasis is altered by exposure to abnormal environmental damage. The release of proinflammatory factors from the synovium begins catabolic activation, leading to the net degradation of ECM. KOA is now recognized as a complex syndrome that affects multiple tissues within the synovial joint and involves many sophisticated homeostatic pathways.[17] S100A12, IL-1β, and galectin-3 are important factors participating in the degradation and destruction of cartilage matrix in the progression of KOA.[18]

As an inflammatory cytokine, IL-1β dominantly mediates cartilage degradation by inhibiting chondrocyte proliferation and promoting the release of inflammatory cytokines, which is considerably expressed on KOA.[4] Meanwhile, the proinflammatory cytokine environment can drive the increase of IL-1β level.[19] Long *et al*[20] found that the IL-1β level of the arthritis group was significantly higher than that of the normal group, and a higher level suggested that the disease was more severe.

Galectin-3 is associated with the upstream regulation of NF-κB signal transduction in chondrocytes to promote human KOA.[21] Additionally, it is a broad-spectrum upstream effector on KOA. At a concentration as low as 1 to 10 μg/mL, galectin-3 functions as a proinflammatory cytokine and an inducer of matrix metalloproteinase expression.[22] Salamanna *et al*[23] induced osteoarthritis in 30 male Sprague-Dawley rats aged 12 weeks by destabilizing the medial meniscus for 4 weeks and found that the galectin-3 level in synovial tissue significantly increased. Furthermore, galectin-3 can amplify IL-1β–mediated inflammatory response in cells.[24] Lacobini *et al*[25] reported that the expression of IL-1β affected galectin-3, as well as accelerated the loss of trabecular bone reduction of bone strength and progression of KOA.

S100A12 is a low-molecular-weight protein in the S100 family.[7] It is essentially involved in immune defense and inflammatory response; it also regulates cell growth and differentiation while inhibiting

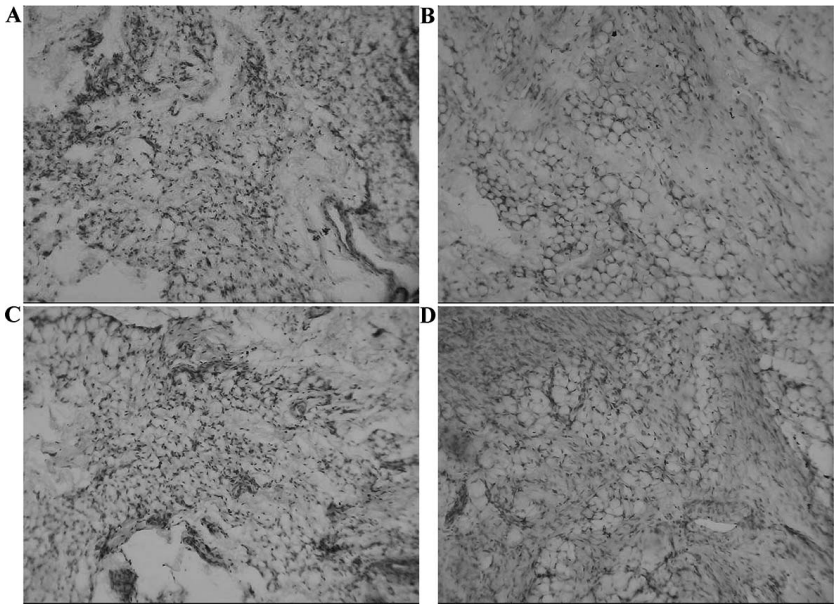

Fig. 6 S100A12 in synovial tissue observed by microscopy. (A) Normal control group. (B) Model group. (C) Low-dose group. (D) High-dose group.

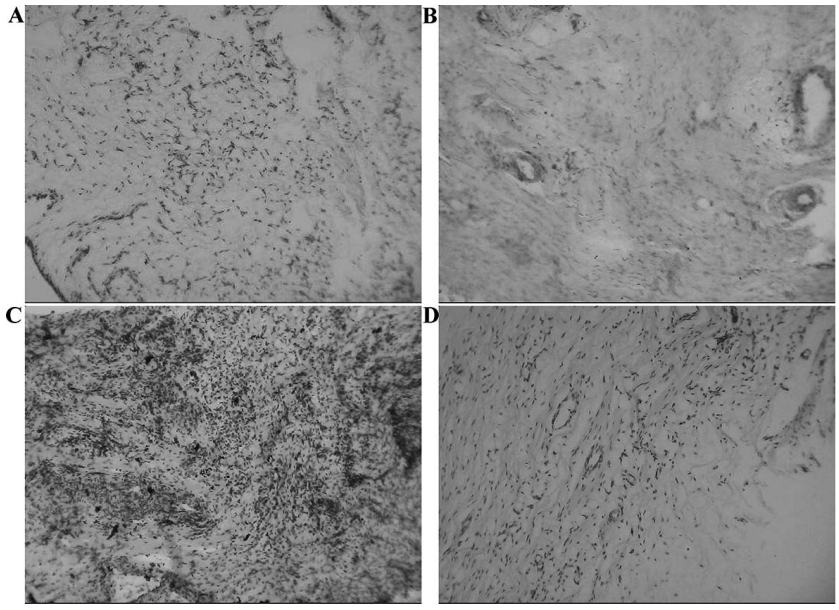

Fig. 7 IL-1β in synovial tissue observed by microscopy. (A) Normal control group. (B) Model group. (C) Low-dose group. (D) High-dose group.

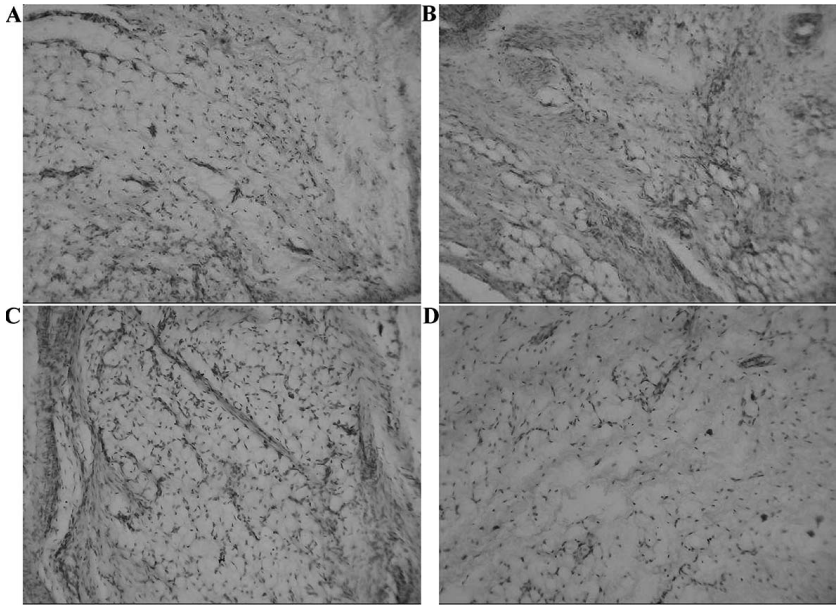

Fig. 8 Galectin-3 in synovial tissue observed by microscopy. (A) Normal control group. (B) Model group. (C) Low-dose group. (D) High-dose group.

Table 4 Expression levels of IL-1β, galectin-3, and S100A12 in synovial tissues detected by ELISA (mean ± SD, ng/mL)

Group	n	IL-1β	Galectin-3	S100A12
Normal control group	10	896.42 ± 190.92	1.69 ± 0.33	7.61 ± 1.54
Model group	10	3170.975 ± 168.18	6.97 ± 0.35	32.78 ± 2.25
Low-dose group	10	2359.97 ± 213.83	3.77 ± 0.46	28.13 ± 1.26
High-dose group	10	1328.03 ± 143.57	2.46 ± 0.47	10.32 ± 2.22

Comparison between model and normal control groups, $P < 0.01$; comparison between low-dose and model groups, $P < 0.05$; comparison between high-dose and model groups, $P < 0.01$.

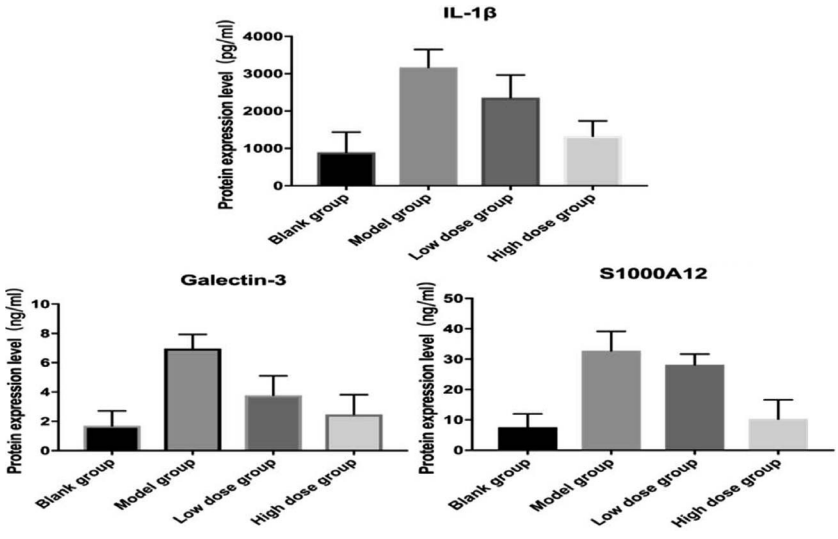

Fig. 9 IL-1β, galectin-3, and S100A12 levels in the synovium measured by ELISA.

apoptosis. Our group has previously reported that S100A12 secreted in the blood and extra-articular tissues of KOA patients promoted synovitis after entering the blood circulation and reaching periarticular tissues. Moreover, the severity of KOA was positively correlated with the expression level of S100A12.[26] The patients with hip osteoarthritis have similar clinical results.[27]

R. glutinosa was first recorded in "Shennong Bencaojing" and ranked the top of 3 herbal drugs. It tastes sweet, bitter, and cold, can nourish Yin, can tonify the kidney, and facilitates the secretion of saliva. The rhizoma of *R. glutinosa* has been widely used to treat inflammatory diseases. Catalpa alcohol is an iridoid glycoside extracted from *R. glutinosa*, which was stipulated by the Chinese Pharmacopoeia (2015 Edition) as an index for the quality control of *R. glutinosa*.[28] In this study, immunohistochemistry and ELISA were conducted to evaluate the effects of catalpa alcohol from *R. glutinosa* on the expression levels of IL-1β, galectin-3, and S100A12 in the synovial tissues of rats with early KOA. Such levels significantly increased in the synovial tissues of KOA rats, which were decreased by low- and high-dose catalpa alcohol ($P < 0.05$ and $P < 0.01$, respectively), suggesting that this drug managed to control early KOA. Notably, the controlling effect of high-dose catalpa alcohol was superior to that of low-dose drug. In summary, catalpa alcohol from *R. glutinosa* can be used to slow down the progression of KOA by reducing the contents of inflammatory factors in synovial tissue and relieving inflammatory response. This study still has limitations. The numbers of rats in low- and high-dose groups are small, so the results may be biased. Further in-depth studies using more rats are ongoing in our group to verify the results.

Acknowledgments

The authors declare no conflicts of interest. This study was financially supported by General Program of Medical Technology Development in Nanjing, Jiangsu Province (Grant YKK17124). Qiang He and Yixin Liu contributed equally to this study.

References

1. Bastick AN, Belo JN, Runhaar J, Bierma-Zeinstra SM. What are the prognostic factors for imaging progress of knee osteoarthritis. *Clin Orthop Relat Res* 2015;**473**(9):2969–2989
2. Kato T, Miyaki S, Ishitobi H, Nakamura Y, Nakasa T, Lotz MK *et al*. Exosomes from IL-1β stimulated synovial fibroblasts induce osteoarthritic changes in articular chondrocytes. *Arthritis Res Ther* 2014;**16**(4):R163
3. Andriacchi TP, Favre J, Erhart-Hledik JC, Chu CR. A systems view of risk factors for knee osteoarthritis reveals insights into the pathogenesis of the disease. *Ann Biomed Eng* 2015;**43**(2): 376–387
4. Chien SY, Huang CY, Tsai CH, Wang SW, Lin YM, Tang CH. Interleukin-1β induces fibroblast growth factor 2 expression and subsequently promotes endothelial progenitor cell angiogenesis in chondrocytes. *Clin Sci (Lond)* 2016;**130**(9):667–681
5. Reesink HL, Nixon AJ, Su J, Liu S, Sutton RM, Mann S *et al*. Galectins-1 and-3 increase in equine post-traumatic osteoarthritis. *Front Vet Sci* 2018;**5**:288

6. Sciacchitano S, Lavra L, Morgante A, Ulivieri A, Magi F, De Francesco GP *et al*. Galectin-3: one molecule for an alphabet of diseases, from A to Z. *Int J Mol Sci* 2018;**19**(2)
7. Nishida M, Saegusa J, Tanaka S, Morinobu A. S100A12 facilitates osteoclast differentiation from human monocytes. *PLoS One* 2018;**13**(9):e0204140
8. Huang X, Zeng Y, Xing X, Zeng J, Gao Y, Cai Z *et al*. Quantitative proteomics analysis of early recurrence/metastasis of huge hepatocellular carcinoma following radical resection. *Proteome Sci* 2014;**12**:22
9. Simovic Markovic B, Nikolic A, Gazdic M, Bojic S, Vucicevic L, Kosic M *et al*. Galectin-3 plays an important pro-inflammatory role in the induction phase of acute colitis by promoting activation of NLRP3 inflammasome and production of IL-1β in macrophages. *J Crohns Colitis* 2016;**10**(5):593–606
10. Wang L, Xue GB. Catalpol suppresses osteosarcoma cell proliferation through blocking epithelial-mesenchymal transition (EMT) and inducing apoptosis. *Biochem Biophys Res Commun* 2018;**495**(1):27–34
11. Zhu P, Wu Y, Yang A, Fu X, Mao M, Liu Z. Catalpol suppressed proliferation, growth and invasion of CT26 colon cancer by inhibiting inflammation and tumor angiogenesis. *Biomed Pharmacother* 2017;**95**:68–76
12. Jhun JY, Na HS, Shin JW, Jung KA, Seo HB, Ryu JY *et al*. Notoginseng radix and rehmanniae radix preparata extract combination (YH23537) reduces pain and cartilage degeneration in rats with monosodium iodoacetate-induced osteoarthritis. *J Med Food* 2018;**21**(8):745–754
13. Wang L, Wang Z, Liu Q, Su J, Wang T, Li T. Effect of whole body vibration on HIF-2α expression in SD rats with early knee osteoarthritis. *J Bone Miner Metab* 2020
14. Chen Q. *Methodology of Pharmacology Research of Traditional Chinese Medicine*. Beijing, China: People's Medical Publishing House; 1993
15. Vaamonde-García C, Loureiro J, Valcárcel-Ares MN, Riveiro-Naveira RR, Ramil-Gómez O, Hermida-Carballo L *et al*. The mitochondrial inhibitor oligomycin induces an inflammatory response in the rat knee joint. *BMC Musculoskelet Disord* 2017; **18**(1):254
16. Zeng C, Li YS, Lei GH. Synovitis in knee osteoarthritis: a precursor or a concomitant feature. *Ann Rheum Dis* 2015; **74**(10):e58
17. Schiphof D, van den Driest JJ, Runhaar J. Osteoarthritis year in review 2017: rehabilitation and outcomes. *Osteoarthritis Cartilage* 2018;**26**(3):326–340
18. Blom AB, van der Kraan PM, van den Berg WB. Cytokine targeting in osteoarthritis. *Curr Drug Targets* 2007;**8**(2):283–292
19. Kessel C, Lippitz K, Weinhage T, Hinze C, Wittkowski H, Holzinger D *et al*. Proinflammatory cytokine environments can drive interleukin-17 overexpression by γ/δ T cells in systemic juvenile idiopathic arthritis. *Arthritis Rheumatol* 2017;**69**(7): 1480–1494
20. Long CY, Li FZ, Liang JH, Zhong LY, Liang Y, Jiang GJ *et al*. [Effect of Qingdu Shenjin Decoction on serum TNF-α and IL-1 β levels in rats with collagen induced arthritis]. *Acta Chin Med* 2017;**32**(1):93–95
21. Weinmann D, Schlangen K, André S, Schmidt S, Walzer SM, Kubista B *et al*. Galectin-3 induces a pro-degradative/ inflammatory gene signature in human chondrocytes, teaming up with galectin-1 in osteoarthritis pathogenesis. *Sci Rep*. 2016; **6**:39112
22. Hu Y, Yéléhé-Okouma M, Ea HK, Jouzeau JY, Reboul P. Galectin-3: a key player in arthritis. *Joint Bone Spine* 2017;**84**(1): 15–20
23. Salamanna F, Giavaresi G, Parrilli A, Martini L, Nicoli Aldini N, Abatangelo G *et al*. Effects of intra-articular hyaluronic acid associated to Chitlac (arty-duo®) in a rat knee osteoarthritis model. *J Orthop Res* 2019;**37**(4):867–876
24. Uchino Y, Woodward AM, Mauris J, Peterson K, Verma P, Nilsson UJ *et al*. Galectin-3 is an amplifier of the interleukin-1β-mediated inflammatory response in corneal keratinocytes. *Immunology* 2018;**154**(3):490–499
25. Iacobini C, Blasetti Fantauzzi C, Bedini R, Pecci R, Bartolazzi A, Amadio B *et al*. Galectin-3 is essential for proper bone cell differentiation and activity, bone remodeling and biomechanical competence in mice. *Metabolism* 2018;**83**:149–158
26. Wang LC, Zhang HY, Shao L, Chen L, Liu ZH, He X *et al*. S100A12 levels in synovial fluid may reflect clinical severity in patients with primary knee osteoarthritis. *Biomarkers* 2013; **18**(3):216–220
27. Lafeber FP, van Spil WE. Osteoarthritis year 2013 in review: biomarkers; reflecting before moving forward, one step at a time. *Osteoarthritis Cartilage* 2013;**21**(10):1452–1464
28. NP Committee. China Pharmacopoeia (2015 edition).