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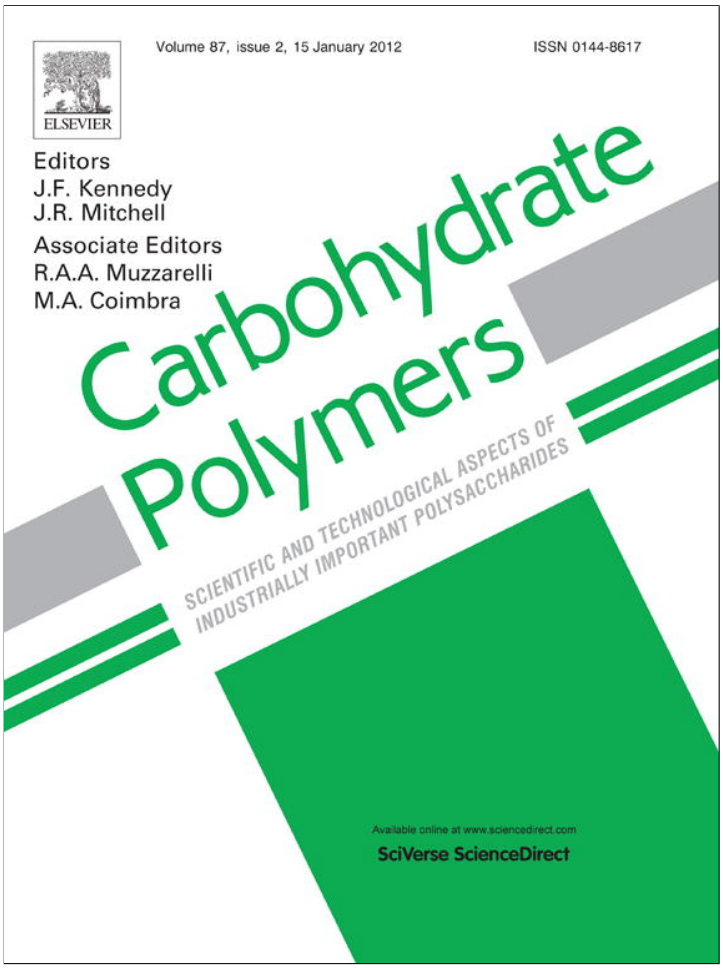

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# Preparation of xanthan gum injection and its protective effect on articular cartilage in the development of osteoarthritis

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ABSTRACT

This study was conducted to evaluate the protective effect of intra-articular (IA) injection of xanthan gum (XG) on articular cartilage in the papain-induced osteoarthritis (OA) model. The purified XG and injection were prepared and their qualities were evaluated. The rabbit knee OA model was induced through IA injection of papain and the protective effect on articular cartilage was evaluated through recording the width of knee joint, performing the morphological observation and histological evaluation of articular cartilage and synovium, measuring the sulphated glycosaminoglycans (GAGs) in cartilage. Our results showed that XG injection was of high transparency, low protein and endotoxin-free. IA injection of XG could protect the joint cartilage and this was probably an effective therapeutic method of OA.



## 1. Introduction

Osteoarthritis (OA), also known as degenerative joint disease, is one of the most common joint disorders. It is characterized by the degradation and loss of articular cartilage, osteophyte formation, subchondral bone sclerosis and inflammation of the synovial membrane (Yorimitsu et al., 2008). OA is a disease of high economical importance and the impact of OA has almost been compared to that of cardiovascular disease (Blagojevic, Jinks, Jeffery, & Jordan, 2010; Panicker, Borgia, Fhied, Mikecz, & Oegema, 2009).

Current treatment for OA is mainly aimed at maintaining the mobility in the joint, relieving clinical symptoms and retarding the degeneration of cartilage (Alcaraz, Megías, García-Arnandis, Clérigues, & Guillén, 2010). Intra-articular (IA) injection treatment has been one of the main methods for OA because of its effectiveness in increasing joint mobility and reducing joint pain (Uthman, Raynauld, & Haraoui, 2003). IA injection of hyaluronic acid (HA) is indicated as an effective treatment for OA due to its lubricating and cushioning properties (Abate, Pulcini, Di Iorio, & Schiavone, 2010; Amiel et al., 2003; Goldberg & Buckwalter, 2005; Gomis, Miralles, Schmidt, & Belmonte, 2007; Kumahashi et al., 2011). However, HA will be quickly degraded in vivo by hydrolytic or enzymatic reactions because of its instability (Barbucci et al., 2002; Zhong et al., 1994). Therefore, a compound which is similar to HA in the structure and function, but with a longer effect in the joint, will be needed.

Xanthan gum (XG) is a natural microbial extracellular heteropolysaccharide. It was discovered at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture. Typically, XG is made from fermentation of *Xanthomonas campestris* in well-agitated fermenter. The molecular weight of XG distribution ranges from $2 \times 10^6$ to $20 \times 10^6$ Da (García-Ochoa, Santos, Casas, & Gómez, 2000). XG contains repeating units of five monosaccharides formed by two D-glucose, two D-mannose and one D-glucuronic acid. The secondary structure of XG is a fivefold helical structure formed by the side chain wrapping back the main chain through hydrogen bonds. Side chains protect the main chain from the outside environment. The tertiary structure of XG is a network structure formed by helical complex (García-Ochoa et al., 2000).

XG is of many properties for its special structure such as high viscosity at low concentrations, temperature stability, pseudoplastic

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rheology, emulsion stabilization, synergistic effect with galactomannans and safety. XG is used in various medical and pharmaceutical applications such as controlled-release carriers, ophthalmic and antineoplastic (Koji & Hiroshi, 2009; Mundargi, Patil, & Aminabhavi, 2007; Shalviri, Liu, Abdekhodaie, & Wu, 2010; Takeuchi et al., 2009). XG is similar to HA in rheology and viscosity (Bewersdorff & Singh, 1988; García-Abuín, Gómez-Díaz, Navaza, Regueiro, & Vidal-Tato, 2011). XG solution is very stable in wide ranges of pH, ionic concentration and temperature (Dário, Hortêncio, Sierakowski, Queiroz Neto, & Petri, 2011; Nasr, Soudi, & Haghighi, 2007). The XG lyase is mainly produced by bacterium (Nankai, Hashimoto, Miki, Kawai, & Murata, 1999; Ruijssenaars, de Bont, & Hartmans, 1999), therefore, XG will not be easily degraded in vivo. IA injection of XG is probably an effective therapeutic method of OA due to XG injection is highly viscous. XG injection is also likely to have a long-lasting protective effect on articular cartilage to avoid numerous injections. In the present study, we evaluated macroscopically and microscopically the protective effect of IA injection of XG on articular cartilage in the rabbit model of papain-induced osteoarthritis.

## 2. Materials and methods

### 2.1. Purification and injection preparation of XG

XG batch fermentation (Linyi Freda Biochem Co., Ltd., Linyi, China) was carried out in bioreactor (Shanghai Ritai Medicine Equipment Project Co., Ltd., Shanghai, China). The working volume was 10,000 kg. The raw XG samples were obtained by precipitation using isopropanol (the ratio was one volume of XG fermented broth with two volumes isopropanol) and re-dissolved to 0.4% (w/v) containing 5% (w/v) KCl. The mixture solution was adsorbed firstly by 1% (w/v) diatomite, and then filtrated with the cake filtration followed by an addition of 20 ppm alkaline protease (Beijing Shuangxuan Microbe Culture Medium Products Factory Co., Ltd., Beijing, China) at 55 °C at a pH of 6.8 for 2 h. Finally, the mixture solution was adsorbed by 1% (w/v) active carbon, then filtrated with the cake filtration and a 0.45 μm membrane. XG samples were subjected precipitations twice using isopropanol for further purification. The purified XG white powder was dissolved in buffered physiological sodium chloride and sterilized at 121 °C for 15 min.

### 2.2. Determination of physical and chemical characteristics of purified XG

The physical and chemical properties of purified XG were characterized according to Chinese Pharmacopoeia 2010. The determination and standard of pH referred to European Pharmacopoeia 7.0. The amount of XG was determined according to United States Pharmacopeia 34. The amount of protein was determined by Bradford's protein assay (Bradford, 1976). The absorbances at 257 nm and 280 nm of 0.2% (w/v) XG solution were determined by Ultraviolet–visible Spectrophotometry (Shanghai Unico Instrument Co., Ltd., Shanghai, China). The molecular weight was analyzed using SEC-MALLS system consisting of an Agilent 1100 HPLC with RI detector and Wyatt DAWN EOS detector (Wyatt Technology Corp., USA). The flow rate was set to 0.6 ml/min. Samples were prepared of approximately 0.05 mg/ml and a refractive index increment (dn/dc) of 0.141 was used in the processing of the data.

### 2.3. Experimental animals

The animal experiment was carried out according to the internationally accredited guidelines with the approval of the Institutional Animal Care and Use Committee of Drug Safety Evalution Center of Shandong Institute of Pharmaceutical Industry (Jinan, China). Thirty adult male New Zealand white rabbits (2.8 ± 0.2 kg, 8 months old) were housed individually in steel cages of 45 cm × 30 cm × 60 cm dimensions and maintained under the same environmental conditions (temperature of 19–21 °C, relative humidity of 50–60%) for 2 weeks. The animals were fed with a standard rabbit diet (14% protein, 7% fat, 15% cellulose, 1–1.2% calcium and phosphorus) and filtered water.

### 2.4. Experimental OA model and treatment regimens

As been prescribed in the two articles (Inoue & Glimcher, 1982; Tanaka et al., 1992), papain (Sigma, St. Louis, MO, USA) and L-cysteine (Sigma, St. Louis, MO, USA) were dissolved in 0.9% (w/v) NaCl at a concentration of 2% (w/v) and 0.03 mol/L just before use and the solution was filtrated with a 0.22 μm membrane. IA injections of the solution into the right knees of all rabbits were performed three times on days 1, 3 and 5, respectively, under anesthesia (ketamine 25 mg/kg and midazolam 5 mg/kg intramuscularly). Then these animals were randomly divided into three groups of 10 each. 1% (w/v) XG injection 0.1 ml/kg was given intra-articularly into the right knees in weeks 2 and 4, while 0.9% (w/v) NaCl 0.1 ml/kg was injected in weeks 1, 3 and 5 (XG-treated group). 0.9% (w/v) NaCl 0.1 ml/kg (saline group) and 1% (w/v) HA 0.1 ml/kg (MW ≈ 1500–2500 kDa, Bausch & Lomb-Freda Pharmaceutical Co., Ltd., Jinan, China) (HA-treated group) were injected intra-articularly into the right knees once a week for 5 weeks. All animals were injected intra-articularly with 0.9% (w/v) NaCl 0.1 ml/kg into the left knees on days 1, 3 and 5 during the induction (papain) phase and once a week in the treatment phase. The treatment regimen of HA-treated group was determined according to previously described method (Kikuchi et al., 1997). The treatment regimen of XG-treated group was determined according to our pilot experimental results (data were not shown. Briefly, IA injections of XG once every 2 weeks for 5 weeks and once a week for 5 weeks have not shown a statistically significant difference). The animals were sacrificed in the sixth week by an intravenous overdose of anesthesia. Articular cartilage and synovium were harvested for further analysis. The body weight and knee joint width were detected throughout the experimental period.

### 2.5. Gross morphological observation and histological evaluation

For gross morphological observation, the femoral condyle and tibial plateau were collected and the cartilage lesions were evaluated using previously described method (Laverty, Girard, Williams, Hunziker, & Pritzker, 2010). The highest score, which is 4, indicates the most severe degeneration of cartilage. For histological evaluation, the medial femoral condyle and tibial plateau were fixed with 10% (w/v) buffered formalin for 48 h, decalcified in 10% (w/v) nitric acid for 5 days, dehydrated and embedded in paraffin. The cartilages of lateral femoral condyle and tibial plateau were collected for glycosaminoglycans (GAGs) measurement. Serial sections with 5 μm thickness collected from the same anatomical site on the femoral condyle and tibial plateau were prepared and stained with hematoxylin and eosin (H&E) for histological evaluation or with Safranin O for proteoglycans (PGs) staining. The histology was evaluated through double-blind observations using previously described method (Laverty et al., 2010). The highest score, which is 24, indicates the most severe degeneration of cartilage. The synovium was also processed and stained with H&E for histological evaluation. The severity of synovitis was graded using previously described method (Laverty et al., 2010). The highest score, which is 30, indicates the most severe degeneration of synovial tissue.

### 2.6. Measurement of sulphated glycosaminoglycans in knee joint cartilage

The sulphated GAGs were measured using 1,9-dimethylmethylene blue (DMB) colorimetric assay as previously described in the article (Williams, Rayan, Sumner, & Thonar, 2003). The DMB solution (pH 3.0) was prepared by dissolving 16 mg DMB (Sigma, St. Louis, MO, USA) in 1 L water containing 2.37 g NaC1, 3.04 g glycine and 95 ml 0.1 M HC1. The same dry weights of the collected cartilage samples were digested in 1.0 ml 20 mM sodium phosphate buffer (pH 6.8) containing 5 mM EDTA, 5 mM cysteine and 300 μg papain at 60 °C for 20 h. Each sample was diluted 20 times and then 100 μl of each sample was placed in a polystyrene tube, 2.5 ml DMB color reagent was added and $A_{525}$ was read immediately. The contents of sulphated GAGs were measured by reference to a calibration curve of chondroitin 4-sulphate sodium salt from bovine cartilage (Sigma, St. Louis, MO, USA).

### 2.7. Statistical analysis

Statistical analysis was performed using SPSS 13.0 statistical software package. All data were expressed as means ± standard deviation (SD). The data were initially evaluated for normal distribution. Statistical significances among groups were then tested using a one-way analysis of variance (ANOVA). Differences between groups were further confirmed by the Student's *t*-test and considered to be statistically significant if $P < 0.05$.

## 3. Results

### 3.1. Physical and chemical indicators of purified XG

The purified XG was white and free-flowing powder. The samples were soluble in water giving a highly viscous solution which was clear and transparent. There were almost no protein, endotoxin and other glycosaminoglycans in this solution. The absorbance at 257 nm was 0.009 ± 0.003 and that at 280 nm was 0.005 ± 0.002. The protein content was 0.042 ± 0.011% (w/w). The endotoxin level was less than 0.05 EU/mg. The amount of XG was 99.6% (w/w). The viscosity was 1597 ± 32 mPa s. The content of pyruvic acid was 4.8 ± 0.8% (w/v). The pH value was 7.2 ± 0.2. The molecular weight lay in the range of 5100 to 5400 kDa. All other parameters met the standards of Chinese Pharmacopoeia 2010.

### 3.2. Body weight and knee joint width

The body weight decreased in the first week after the injections of papain and then gradually increased in all of the two treatment groups and the saline group. The increased rate did not have significant differences among the two treatment groups and the saline group. The increased width of knee joint might reflect the severity of inflammation. The swelling of the right knee joint was the most severe in the second week after the initiation of papain injections and gradually lessened after that. These changes were not observed in the sham group. After treatment, the width of the right knee joints in the XG-treated group (20.40 ± 0.47, $n = 10$) was lower than that in the saline group (22.08 ± 0.36, $n = 10$) ($P < 0.001$), as seen in Fig. 1. However, no significant difference between the XG-treated group and the HA-treated group (20.58 ± 0.44, $n = 10$) was observed ($P = 0.396$).

### 3.3. Gross morphological observation

Macroscopically, articular cartilage surface was integrity, smooth and lustrous with a light white color in the sham group [Fig. 2(A) and (E)]. In the saline group, cartilage surface appeared

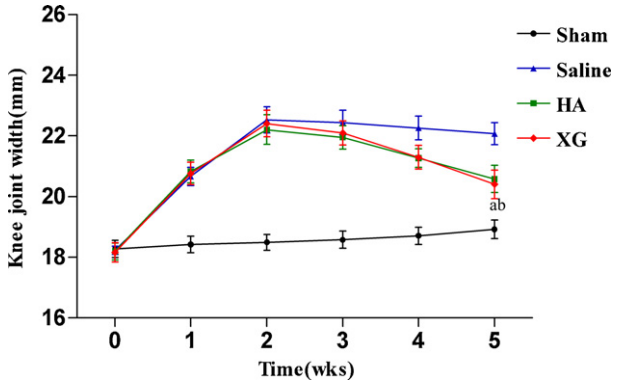


**Fig. 1.** Knee joint widths in all groups. Comparisons considered significant when $P < 0.05$. The width of the right knee joints in the XG-treated group was lower than that in the saline group, [a] $P < 0.001$ vs saline group. No significant difference between the XG-treated group and the HA-treated group, [b] $P = 0.396$ vs HA-treated group.

to be yellowish-white, uneven and was damaged [Fig. 2(B) and (F)]. In the two treatment groups, cartilage surfaces were lustrous with a white color, femoral condylar cartilages resembling the healthy cartilage were lesioned slightly [Fig. 2(C) and (D)]. The cartilages of tibial plateau displayed significant cracks and cartilage defects [Fig. 2(G) and (H)], but the severities were milder than that in the saline group. The scores of the XG-treated group were lower than that of the saline group ($P < 0.001$) (Table 1). However, no significant differences between the XG-treated group and the HA-treated group were observed (femoral condyle $P = 0.660$, tibial plateau $P = 0.556$).

### 3.4. Histological evaluation

#### 3.4.1. Articular cartilage

In the sham group, superficial layer of cartilage was smooth and integrity, chondrocytes were flattened and arranged in neat rows, the cartilage matrix was well stained with Safranin O. Tidemark was integrity [Fig. 3(A) and (E)]. In the saline group, moderate cartilage degradation was seen, including irregular superficial layer, fissure and complete degenerative and disarranged chondrocytes, moderate to severe loss of Safranin O staining intensity, which extended into the radial zone [Fig. 3(B) and (F)]. In the two treatment groups, the superficial layers of cartilages were smooth and mild loss of Safranin O staining intensity. Relative normality of chondrocytes and tidemark were observed [Fig. 3(C), (D), (G) and (H)]. The scores of the XG-treated group were lower than that of the saline group ($P < 0.001$) (Table 2). However, no statistical significance between the XG-treated group and the HA-treated group were observed (femoral condyle $P = 0.556$, tibial plateau $P = 0.484$).

#### 3.4.2. Synovium

Synovium surface was smooth and integrity without synovial lining cell hyperplasia and mononuclear infiltration in the sham group [Fig. 4(A)]. In the saline group, synovium showed moderate

**Table 1**
Gross morphological score of articular cartilage.

| Groups | Femoral condyle | Tibial plateau |
|---|---|---|
| Sham ($n = 30$) | 0.10 ± 0.32 | 0.20 ± 0.42 |
| Saline ($n = 10$) | 2.00 ± 0.67 | 2.30 ± 0.67 |
| HA ($n = 10$) | 0.7 ± 0.48 | 1.10 ± 0.32 |
| XG ($n = 10$) | 0.6 ± 0.52[a,b] | 1.20 ± 0.42[a,c] |

Values are the means ± SD. Comparisons considered significant when $P < 0.05$.
[a] $P < 0.001$ vs saline group.
[b] $P = 0.660$ vs HA-treated group.
[c] $P = 0.556$ vs HA-treated group.

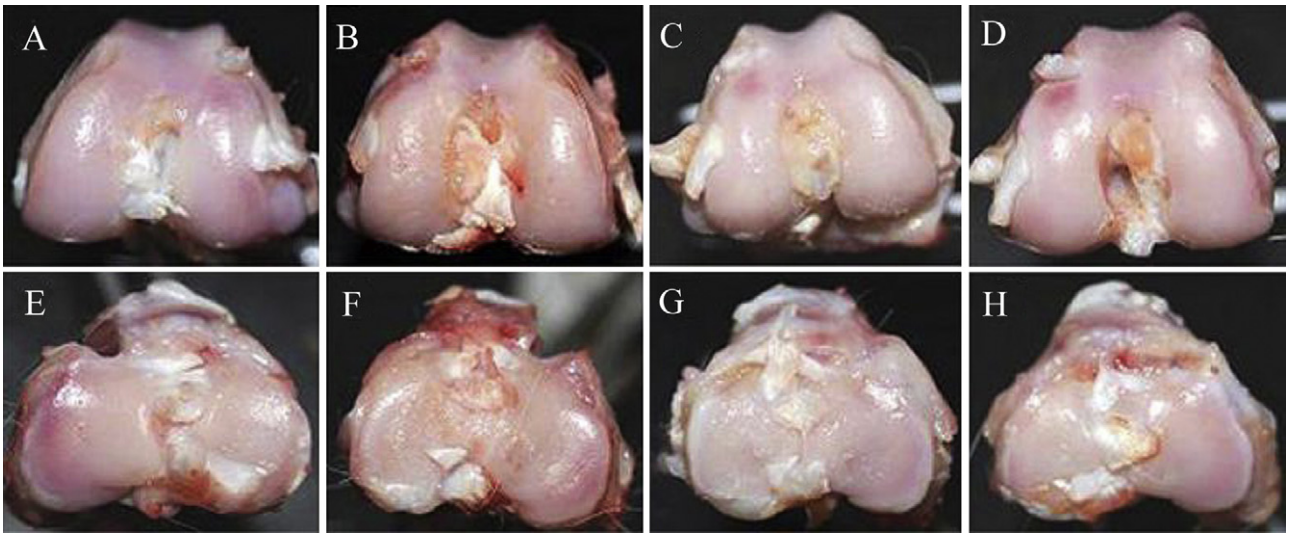


**Fig. 2.** Gross morphological observation of femoral condyle and tibial plateau. Femoral condyle: (A) sham group; (B) saline group; (C) HA-treated group; (D) XG-treated group. Tibial plateau: (E) sham group; (F) saline group; (G) HA-treated group; (H) XG-treated group. Normal cartilage surface was seen in (A). Cartilage surface was yellowish-white and damaged significantly in (B). Cartilage surfaces were lesioned slightly in (C) and (D). Normal cartilage surface was seen in (E). Cartilage surface was yellowish-white, uneven and damaged in (F). Cartilage surfaces were defective in (G) and (H), but the severities were milder than that in (F).

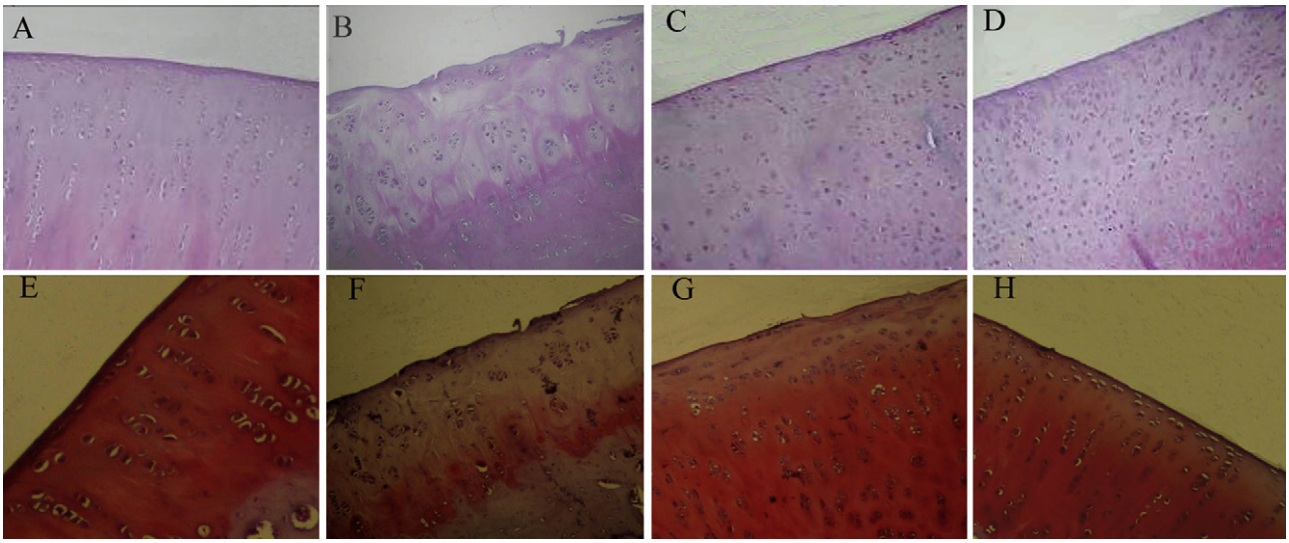


**Fig. 3.** Histological evaluation of tibial plateau (H&E, ×100 magnification): (A) sham group; (B) saline group; (C) HA-treated group; (D) XG-treated group (Safranin O, ×100 magnification): (E) sham group; (F) saline group; (G) HA-treated group; (H) XG-treated group. Normal cartilage structure and chondrocytes were seen in (A). Moderate cartilage degradation (irregular superficial layer and fissure) and disarranged chondrocytes (cluster formation) were seen in (B). Relative normality of cartilage and mild disarranged chondrocytes were seen in (C) and (D). Normal Safranin O staining intensity was seen in (E). Moderate loss of staining intensity was seen in (F). Mild loss of staining intensity was seen in (G) and (H).

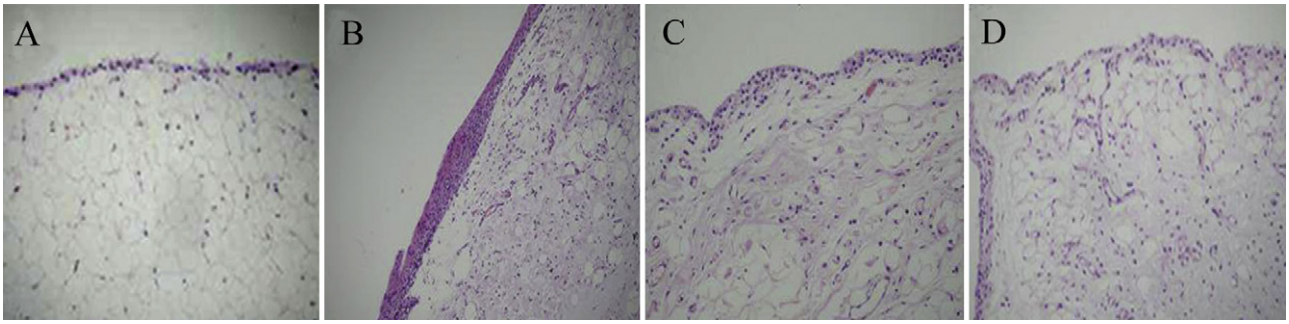


**Fig. 4.** Histological evaluation of synovium: (A) sham group; (B) saline group; (C) HA-treated group; (D) XG-treated group (H&E, ×100 magnification). Normal synovial structure and cells were seen in (A). Moderate cell hyperplasia, subsynovial tissue hypertrophy and moderate infiltration of mononuclear and polymorphonuclear cells were seen in (B). Mild cell hyperplasia, subsynovial tissue hypertrophy and infiltration of mononuclear and polymorphonuclear cells were seen in (C) and (D).

**Table 2**
Histological scores of articular cartilage and synovial membrane.

| Groups | Synovium | Femoral condyle | Tibial plateau |
|---|---|---|---|
| Sham ($n=30$) | 0.80 ± 0.63 | 0.20 ± 0.42 | 0.50 ± 0.71 |
| Saline ($n=10$) | 11.30 ± 1.57 | 8.10 ± 0.99 | 10.30 ± 0.95 |
| HA ($n=10$) | 5.10 ± 1.10 | 3.80 ± 0.63 | 5.30 ± 1.42 |
| XG ($n=10$) | 5.40 ± 1.07[a,b] | 3.60 ± 0.84[a,c] | 5.70 ± 1.06[a,d] |

Values are the means ± SD. Comparisons considered significant when $P<0.05$.
[a] $P<0.001$ vs saline group.
[b] $P=0.545$ vs HA-treated group.
[c] $P=0.556$ vs HA-treated group.
[d] $P=0.484$ vs HA-treated group.

cell hyperplasia, subsynovial tissue hypertrophy and moderate infiltration of mononuclear and polymorphonuclear cells. The vascularization of subsynovial tissue was moderate [Fig. 4(B)]. In the two treatment groups, synovium showed mild synovial lining cell hyperplasia and mononuclear cell infiltration [Fig. 4(C) and (D)]. The synovitis score was lower in the XG-treated group than that in the saline group ($P<0.001$) (Table 2). However, no significant difference between the XG-treated group and the HA-treated group were observed ($P=0.545$).

### 3.5. Amount of sulphated glycosaminoglycans in knee joint cartilage

Fig. 5 shows the results of the measurement of sulphated GAGs in the cartilage of tibial plateau. The contents of GAGs in the two treatment groups (XG 26.82 ± 2.22, $n=10$; HA 26.36 ± 1.89, $n=10$) and the saline group (16.49 ± 1.96, $n=10$) were lower than that in the sham group (32.49 ± 2.18, $n=30$) ($P<0.001$). But the content of GAGs in the XG-treated group was higher than that in the saline group ($P=0.004$). No significant difference between the XG-treated group and the HA-treated group were observed ($P=0.798$).

## 4. Discussion

The aim of this study is to evaluate the efficacy of IA injection of XG in the rabbit OA model. We induced the rabbit OA model through IA injection of the solution containing papain and L-cysteine into the right knees. The efficacy of XG treatment was evaluated by recording the width of rabbit right knee joints, performing the gross morphological observation and histological evaluation and measuring sulphated GAGs in knee joint cartilage.

XG for IA injection needs a high quality including high viscosity, low protein and endotoxin-free. However, XG fermented broth contains undissolved matter including bacterial cell residues and many other chemicals (Salah et al., 2010). Furthermore, XG fermented broth is very viscous and the purification process of XG needs to not only obtain a high quality product but also meet large-scale production. Therefore, the purification of XG is difficult to do. Studies have indicated that enzymatic treatment is an effective approach to solubilize the undissolved matter (Kobzeff et al., 2002). In this study, we combined the following methods of cake filtration, enzymatic treatment, active carbon absorption and repeated isopropanol precipitations to obtain good quality of XG. This XG injection was of high transparency, low protein and endotoxin-free. Furthermore, the purification process was easy to meet large-scale production. This XG injection was highly viscous as a result of stable fermentation process and high molecular weight. This XG injection would be of good lubricating and cushioning effects when injected intra-articularly due to XG is similar to HA in rheology and viscosity.

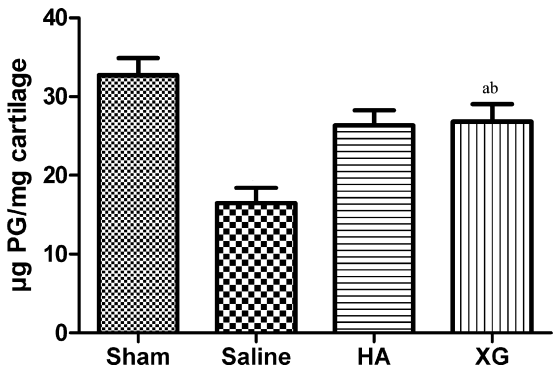


**Fig. 5.** Contents of sulphated GAGs in the cartilage of tibial plateau. Comparisons considered significant when $P<0.05$. The content of GAGs in the XG-treated group was higher than that in the saline group, [a]$P=0.004$ vs saline group. No significant difference between the XG-treated group and the HA-treated group were observed, [b]$P=0.798$ vs HA-treated group.

Experimentally induced OA in animals is valuable in the evaluation of pathogenesis and therapeutic methods of OA (Aigner et al., 2010). The histological structure of articular cartilage of rabbit is similar to that of human and the biochemical parameters in rabbit OA model is consistent with those in human OA. Furthermore, the rabbit knee joint is wide and easy to dose. Therefore, the rabbit OA model used to study the pathogenesis of cartilage degeneration and to evaluate potential therapeutic methods of OA is more appropriate. The rabbit OA models can be induced using various techniques, including IA injection of agents (e.g. enzymes, cytokines, chemicals, etc.), immobilization and surgical induction (Little & Smith, 2008). Among these techniques, IA injection of papain causes joint change similar to those in human OA with erosion of the articular cartilage. Papain as a proteolytic enzyme can deplete the proteoglycan content and remove the cartilage cell. Consequently, the method will appear to be useful for studying the earlier changes of degeneration and repair of articular cartilage in the development of OA and evaluating the therapeutic methods (Inoue & Glimcher, 1982). In this study, we intra-articularly injected the solution containing 2% (w/v) papain and 0.03 mol/L L-cysteine into the right knees of rabbits, which initiated a sequence of degenerative changes, mimicking OA closely. The experiment was of short period and good repeatability. Furthermore, the rabbits survived the experimental period and showed no signs of a generalized toxic reaction.

Maintaining the mobility in the joint, relieving clinical symptoms and retarding cartilage degeneration are current treatment for early OA (Auw Yang, Saris, Dhert, & Verbout, 2004). In this study, we selected these dosing conditions after considering those used in the clinical therapy of osteoarthritis of the knee and the dose used in an experimental model of osteoarthritis in rabbits (Kikuchi et al., 1997). The results showed that the severity of cartilage destruction in tibial plateau was higher than that in femoral condyle, which might be due to the habitual posture of rabbit or the intra-articularly injection point and direction. IA injection of XG once every 2 weeks for 5 weeks decreased the severity of swelling of the knee joint, reduced the damage of cartilage surfaces, inhibited the cells changes, structural changes and loss of Safranin O staining intensity of femoral condyle and tibial plateau and also inhibited cells hyperplasia and infiltration of mononuclear cells in the synovium. Therefore, IA injection of XG may inhibit the continuous lesions on the cartilage and delay the progression of OA. Furthermore, no significant differences between the XG-treated group (IA injection of XG once every 2 weeks for 5 weeks) and the HA-treated group (IA injection of HA once a week for 5 weeks) were observed.

OA is an articular cartilage injury caused by an imbalance in the degradation and synthesis of chondrocytes and extracellular matrix that occurs during the development of the disease (Goldring & Goldring, 2007). The extracellular matrix is composed mainly of a high concentration of PGs entangled in a dense network of collagen fibers and a large amount of water. Articular cartilage is of high flexibility because PGs can maintain the water of articular cartilage and provide important biomechanical functions, such as load bearing,

load distribution and shock absorption. GAGs accounted for 90% (w/w) of PGs molecular weight or more and is the decisive functional group of PGs (Martel-Pelletier, Boileau, Pelletier, & Roughley, 2008). GAGs are more suitable for the transformation parameters than PGs. In this study, we found that the contents of sulphated GAGs were higher in the XG-treated group in comparison to that in the saline group. The results suggest that IA injection of XG should be of marked inhibitory effect on cartilage degeneration. Furthermore, no significant difference between the XG-treated group (IA injection of XG once every 2 weeks for 5 weeks) and the HA-treated group (IA injection of HA once a week for 5 weeks) was observed.

## 5. Conclusions

Our results showed that IA injection of XG could protect the joint cartilage and reduced the papain-induced OA progression. This was probably an effective therapeutic method of OA. An important finding of our present study was that XG could be injected fewer times than HA to get the same treatment results under current treatment regimen, because IA injection of XG once every 2 weeks for 5 weeks and IA injection of HA once a week for 5 weeks had similar effectiveness. Moreover, the injection of XG was heat-stable which might be sterilized at 121 °C for 15 min. This finding is a valuable contribution to the development of a new therapeutic method for OA. However, further study is needed to understand the detailed action mechanism, long-term protective effects and the pharmacokinetics of XG in OA treatment in the future. At present, we are studying the clearance following a single IA injection of XG in rabbits and biocompatibility according to ISO 10993.

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