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The effect and mechanism of *QufengZhitong capsule* for the treatment of osteoarthritis in a rat model

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ABSTRACT

Objective: The purpose of this study was to evaluate the therapeutic effects and mechanism of *Qufeng Zhitong (QFZT)capsule* for the treatment of osteoarthritis (OA) in a rat model.
Methods: 8-10-week-old male Sprague–Dawley rats were randomly divided into the sham group (vehicle-treated), OA group (vehicle-treated), high-dose, middle-dose, low-dose of *QFZT capsule*-treated groups. OA was induced by transecting the medial collateral ligament and the medial meniscus in the right limb. The Sprague–Dawley rats were treated daily for 12 weeks with different concentrations of *QFZT capsule*: low (QFZT-L, 128 mg/kg), medium (QFZT-M, 256.5 mg/kg), and high (QFZT-H, 513 mg/kg) by gavage administration for a period of 4 and 12 weeks respectively. Vehicle-treated rats served as controls and administered 0.5% Carboxymethyl Cellulose Sodium (CMC-Na) by gavage on the same schedule. Weekly measurement of dynamic weight-bearing capacity, grip strength, joint swelling was were performed to monitor the progression of disease for 3 weeks. After euthanasia, the knee joints were articular cartilage changes. Pro-inflammatory gene expression in synovial joints was examined to assess the bone and cartilage changes. Gene expression of pro-inflammatory cytokines in synovial joints was measured to determine the therapeutic effect of QFZT.
Results: 2 weeks after the treatment, the grip strength and weight-bearing capacity were significantly increased in the QFZT-M and QFZT-H groups, compared with the OA group. The joint widths were decreased significantly in the QFZT-L and QFZT- H groups, compared with the OA group as well. The mRNA level in the articular cartilage of knee joint of IL-1β in the QFZT-L group and IL-6 in the QFZT-H group was significantly suppressed at week 4, compared with the OA group. The radiology score was significantly decreased in the QFZT-H group compared with the OA group 12 weeks after treatment. Furthermore, the rats on QFZT treatment decreased the progression of OA, which was characterised by decreased cartilage degradation. However, the bone changes were no different in OA group and QFZT groups.
Conclusion: In a rat model of OA, *QFZT capsule* shows the tendency to reduce the destruction of cartilage, joint swelling and bone erosion which provides new evidence for the therapeutic potential of *QFZT capsule* in the treatment of OA in clinics.
The translational potential of this article: The QFZT capsule can improve the symptoms of the OA in rodent animal rats by attenuating pain and retarding cartilage damage. This study indicated that the QFZT capsule has the potential clinical application of in OA therapy.

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1. Introduction

Osteoarthritis (OA) is the most common form of joint disease and a leading cause of disability according to the World Health Organization (WHO), and knee OA is the most common form of arthritis affecting about 3.8% of the global population with effective disease-modifying drugs available to date [1,2]. The main symptoms and signs of OA are pain, prolonged morning stiffness and muscle weakness. Although the efficacy of current pharmaceutical therapies for OA treatment in terms of pain relief has been well documented, the side-effects such as gastrointestinal bleeding events have also been reported [3]. Thus, the development of new therapeutic approaches against OA for prolonged use is required.

Chinese herbal medicines have attracted attention recently. Several natural products have been tested for their potential for treating OA patients [4], such as *Boswellia serrata* [5], *Curcumin* [6], and *Danshen* (*Salvia miltiorrhiza*) [7]. The Chinese herbal formula, *Qufengzhitong (QFZT) capsule* consists of seven herbs including *Geranium wilfordii*, *Viscum album*, *Radix dipsaci*, *Clematis chinensis*, *R. angelicae pubescentis*, *R. aconiti kusnezoffii preparata*, and *Carthamus tinctorius*. It has been reported that *QFZT capsule* has been used in the clinic for the treatment of rheumatoid arthritis (RA) decreasing synovium hyperplasia and cartilage erosion and expression of anti-inflammatory cytokines [8]. Based on the traditional Chinese medicine (TCM)theory, OA, which was called "Bi" syndrome was caused by "wind and moisture", the *QFZT capsule* was reported, "dispelling wind and relieving pain". Therefore, *QFZT capsule* may have the therapeutic effect for OA management.

In our previous study, *QFZT capsule and its active ingredients* gallic acid, syringin and osthole increased mechanical properties of cortical bone in ovariectomised rats [9]. These three main ingredients have been reported anti-inflammation effects. About 30 years ago, gallic acid was found to possess anti-inflammatory activity towards zymosan-induced acute food pad swelling in mice [10]. In recent years, the gallic acid has been reported to inhibit NO, PGE2, and IL-6 in LPS-induced RAW 267.4 macrophages [11]. Syringin alleviated inflammation in colitis [12] and also significantly inhibited iNOS-induced NO and COX-2-induced PGE2 production in LPS-treated RAW 264.7 cells [13]. Osthole treatment also can ameliorate the inflammatory via suppress the level of the pro-inflammatory cytokines IL and blocked the activation of nuclear factor kappa B (NF-κB) [14,15]. These evidences were indicated that QFZT capsules might have potential application for treating inflammatory disorders, such as OA. In this study, we aimed to determine whether QFZT capsules modulates OA-induced pathological changes in the knee joint using a rat model.

2. Materials and methods

2.1. Animals

One hundred twenty male Sprague–Dawley rats (8–10-week-of-age) were obtained from the Guangdong Medicine Experimental Animal Center (Guangdong, China). The animals were housed under special pathogen-free conditions at the animal facility. All the experiments were carried out according to the National Institutes of Health Guide for Care

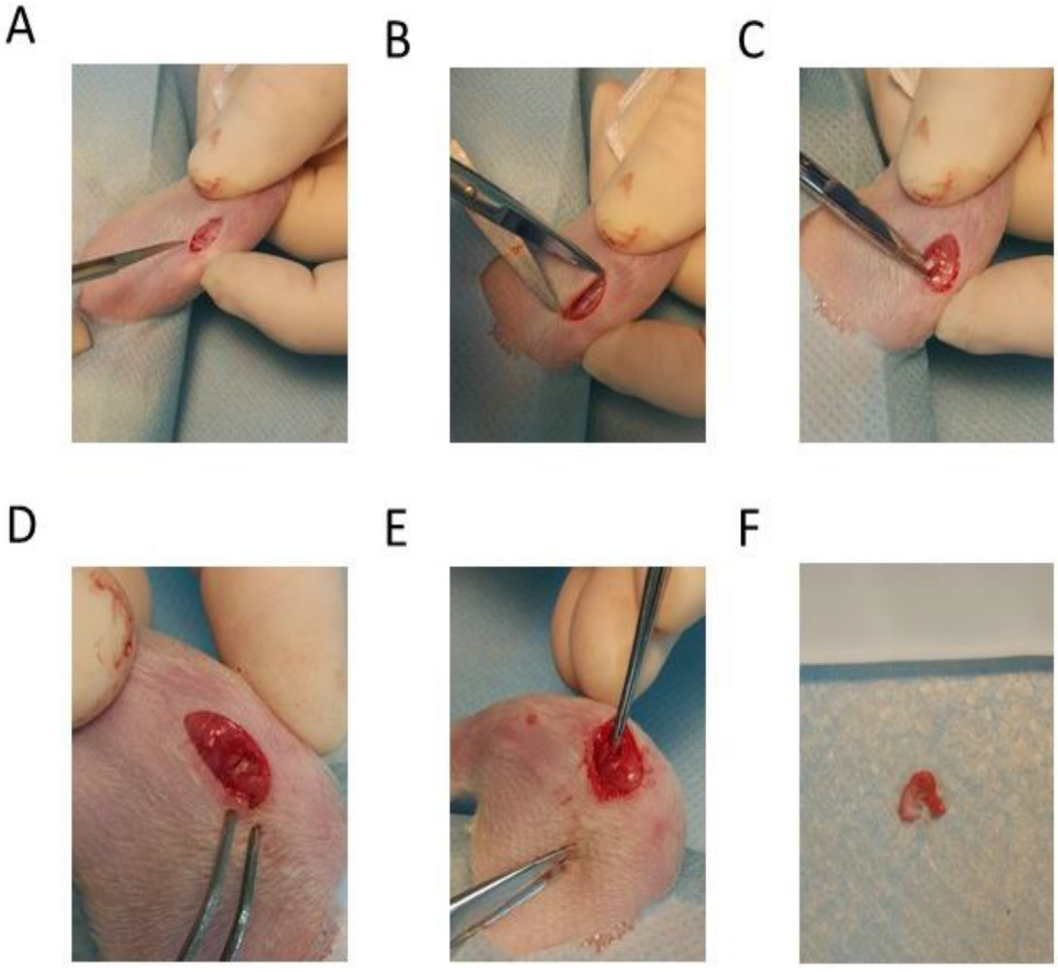


Figure 1. Surgical procedure of OA model. A. Incise the skin; B. Expose the lateral collateral ligament; C. Cut off the lateral collateral ligament; D. Expose the meniscus; E & F. Stripping the meniscus.

and Use of Laboratory Animals and approved by the Animal Ethics Committee of the Chinese Academy of Sciences Shenzhen Institute of Advanced Technology (SIAT-IRB-160223-YGS-ZHANGP-A0167).

2.2. OA model preparation

The rats were anesthetised intraperitoneally withisoflurane (Sigma–Aldrich, St. Louis, MO, USA). OA was induced by transecting the medial collateral ligament and medial meniscus. The sham animals were subjected to the similar surgical procedure, with the exception being the ligament or meniscus was not excised or manipulated by any means. A schematic diagram showing the experimental procedure was shown in Fig. 1 [16–18].

2.3. QFZT capsule treatment

The clinical dose of *QFZT capsule* is 60 mg/kg/day. Dose conversion from human to animal was translated based on body surface area according to previous study [19]. Rat equivalent dose (mg/kg/day) = 60 mg/kg/dayx6.17xKm ratioxcorrection factor (Medium dose). The rats in the sham and surgery (OA) groups received a vehicle (0.5% Carboxymethyl Cellulose Sodium, CMC-Na (Sigma–Aldrich, St. Louis, MO, USA) low (QFZT-L), medium (QFZT-M), and high (QFZT-H) dosage of QFZT capsule (Shaanxi Buchang Pharmaceutical Co., Ltd., Shaanxi, China) dissolved in 0.5% CMC-Na at 128, 256.5, and 513 mg/kg/day, respectively. All the treatment started from the day of surgery by gavage administration once a day for 4 or 12 weeks.

2.4. Tissue preparation

The animals were euthanised at 4 and 12 weeks after QFZT treatment. The right hind limbs were harvested and fixed in 10% formalin overnight. Tissue samples were used for *ex vivo* imaging and histological analyses of cartilage and bone. The articular cartilages were harvested and stored at −80 °C for mRNA extraction.

2.5. Evaluation of dynamic weight-bearing (DWB)

DWB measurement was performed by the incapacitance meter (Model 600, IITC Life Science, Woodland Hills, California, USA) once a week after surgery for 3 weeks [20]. Each hind paw was placed on a transducer pad then recorded over 4 s and the weight distribution in newton.

2.6. Grip strength

Grip strength was measured once a week after treatment for 3weeks. Rats were held with their forelimbs and left hind limbs immobilised. Then the rats were induced to grasp rigid bars attached to a digital force gauge (Aikoh Engineering Corporation, Osaka, Japan) with their right hind limb. Each rat was gently pulled backward, and the tension reading of the digital force gauge was defined as the grip strength [21]. The test was performed three consecutive times, and the mean value of the three tests was recorded as the grip strength.

2.7. Knee joint width measurement

The diameter of knee joints was measured with a three-button digital calliper once a week from 1 week before surgery to 3 weeks after treatment. The results are presented as the percentage change from baseline diameter.

2.8. Cytokines expression in mRNA level

Cartilages of the right knee were obtained and stored in liquid nitrogen. Total RNA of the cartilage cells was isolated using a commercial total RNA mini-prep kit (Axygen, USA) according to the manufacturer's instructions. Each sample was reverse transcribed using a cDNA synthesis kit (TaKaRa, Japan) according to the manufacturer's protocol. Real-time PCR (RT-PCR) analysis was performed using SYBR Green PCR Premix Ex Taq II reagents (TaKaRa, Japan) on a Light Cycler 480 II real-time system (Roche, USA). PCR primer sequences are listed in Table 1. The housekeeping gene GAPDH was used for normalisation.

2.9. Radiology assessment

Knee joint radiographs were captured using In-Vivo DXS PRO x-ray (DXS4000 Pro, Bruker Corporation, Germany). All sample images were processed at 3 × magnification and were positioned horizontally with the centre of the beam at the knee joint. The clinical evaluation standard was used to quantify joint-space and osseous changes [22,23].

All right knee joints were scanned by the SKYSCAN 1176 micro-CT (Bruker, Belgium). Micro-CT data were used to reconstruct a scout 3D image of the knee joints and analyse exactly the same area of interest that we selected in the medial aspect of the tibial plateau. The settings recommended by the manufacturer with exposure of 60 kV and 400 μA was used.The reconstructed data of the tibial plateau medial region were used to create a 3D model. The medial tibial plateau above the growth plate was selected, and then the grayscale setting was adjusted between 80 and 255. Image-Pro Plus (Version 6.0, Media Cybernetics) was used to analyse the picture. The ratio of the worn area of the medial plateau to the whole area of the medial tibial plateau was determined.

2.10. Histology assessment

For histology, the samples were fixed overnight in 10% formalin, and then dehydrated and embedded in paraffin (AR1071, BOSTER). Hematoxylin and eosin (H&E) and toluidine blue (T-blue) staining was performed. The histological images were used to semi-quantitatively determine cartilage abrasion. The distance between the top points of both ends of the tibial plateau was chosen as the total projected cartilage area, and the damaged tibial plateau surface was chosen to be the cartilage abrasion area. We generated this histological scoring (Table 2) by ref cartilage damage image [24], meanwhile taken in accordance with the actual situation. We observed that joint surface wear was increased even in normal rats because the weight of animals reached more than 700 g at the end of the experiment, but without influence on sport ability animals. Then we defined that no more than 30% depth abrasion was the normal condition (Grade 0). When the cartilage is damaged, the area and depth of the wear will be further increased (Grade 1–5).

Table 1
Forward and reverse primer sequences for RT-PCR.

Gene	Forward Primer	Reverse Primer
TNF-α	GGCTGCCCCGACTACGT	AGGGCAAGGGCTCTTGATG
IL-1β	AAAGAAGAAGATGGAAAAGCGGTT	GGGAACTGTGCAGACTCAAACTC
IL-6	ATGGATGCTTCCAAACTGGAT	TGAATGACTCTGGCTTTGTCT
GAPDH	GAACATCATCCCTGCATCCA	GCCAGTGAGCTTCCCGTTCA

Table 2
Cartilage damage score.

Parameter	Grade	Description
Cartilage abrasion	0	No more than 30% depth abrasion
	1	Minimal abrasion, 5–10% of the total projected cartilage area affected by cartilage abrasion
	2	Mild abrasion, 11–25% damage
	3	Moderate abrasion, 26–50% damage
	4	Marked abrasion, 51–75% damage
	5	Severe abrasion, greater than 75% damage

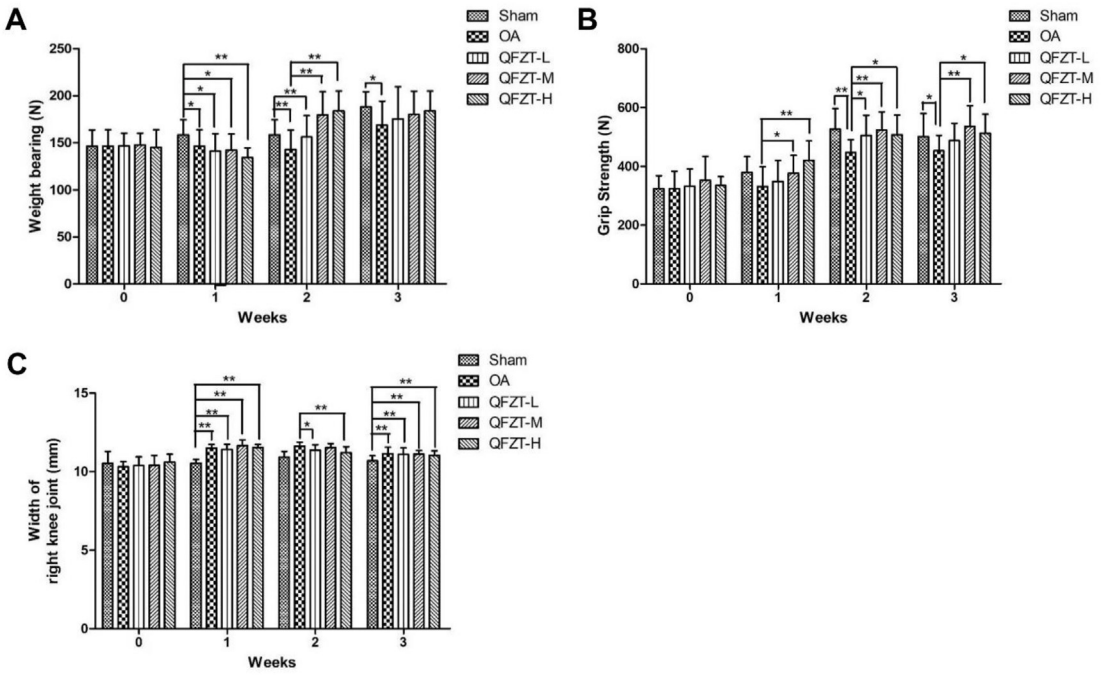


Figure 2. QFZT treatment alleviates pain in OA rats. A. Weight-bearing capacity of the right hind limb. (note: $^{*}P < 0.05$; $^{**}P < 0.01$, n = 12); B. Grip strength of the right hind limbs. (note: $^{*}P < 0.05$; $^{**}P < 0.01$, n = 12); C. Degree of knee joint swelling in this research. (note: $^{*}P < 0.05$; $^{**}P < 0.01$, n = 12).

2.11. Statistical analysis

Data were given as mean ± S.D. All the data were analysed with one-way ANOVA followed by Dennett's t-test to understand whether the differences between the groups are statistically different by SPSS software (Version 19, IBM SPSS Statistics). The *P*-values less than 0.05 and 0.01were considered statistically significant.

3. Results

3.1. Functional recovery

3.1.1. Dynamic weight-bearing

The weight-bearing capacity of the right hind limb in all OA and QFZT treatment groups decreased significantly after one week treatment, compared to the sham group. The weight-bearing capacity of the right

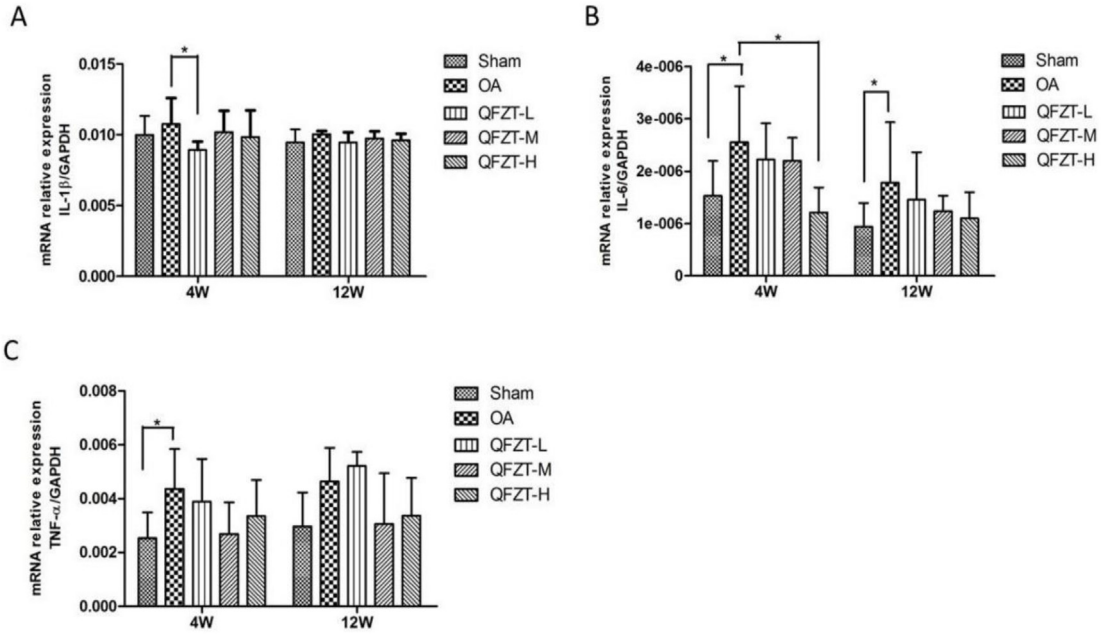


Figure 3. mRNA levels of inflammatory cytokines. A. mRNA expression of IL-1β; B. mRNA expression of TNF-α; C. mRNA expression of IL-6. (Note: $^{*}P < 0.05$, n = 6).

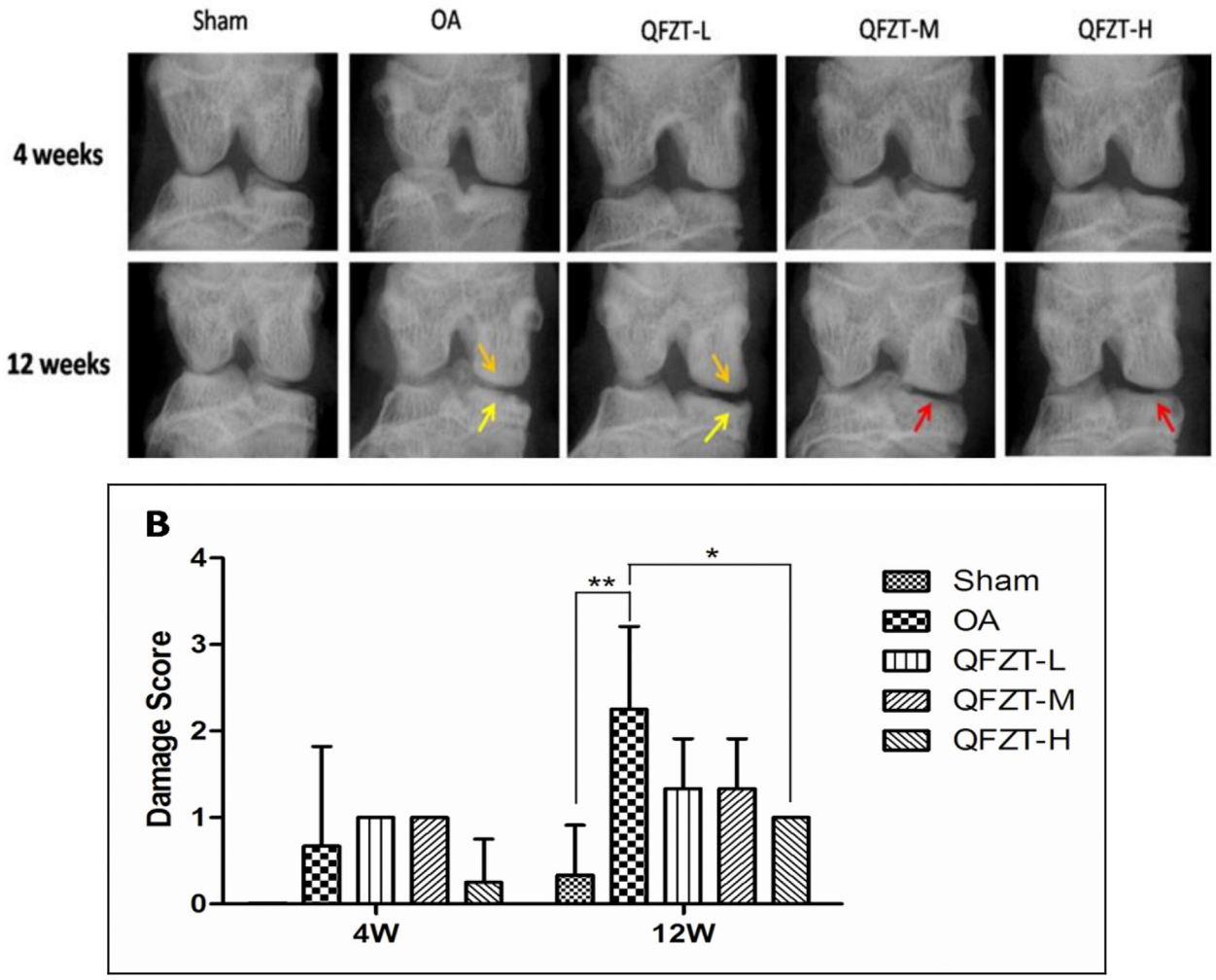


Figure 4. X-Ray image of knee joints from rats after 4 and 12 weeks treatment. A. Representative X-Ray image. Red arrow: the joint space; Yellow arrow: the calcification of articular surface; Orange arrow: the damage of articular surface; B. The joint space and joint wear damage score. No significant difference was found between the OA group and QFZT groups 4 weeks after treatment. OA group has the higher score with significant difference compared to sham control and the QFZT-H group has significant difference with OA group 12 weeks after treatment. (Note: *$P < 0.05$; **$P < 0.01$, n = 6). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

hind limb increased significantly by 22% and 19% in the QFZT-M and QFZT-H groups, respectively, compared with the OA group ($P < 0.01$) after 2 weeks treatment. In the third week after treatment, except for the OA group, the weight-bearing capacity of the right hind limbs of all animals treated with QFZT capsules had insignificant differences with the sham group. The sample size was 12 animals per group (Fig. 2A).

3.1.2. Grip strength measurement

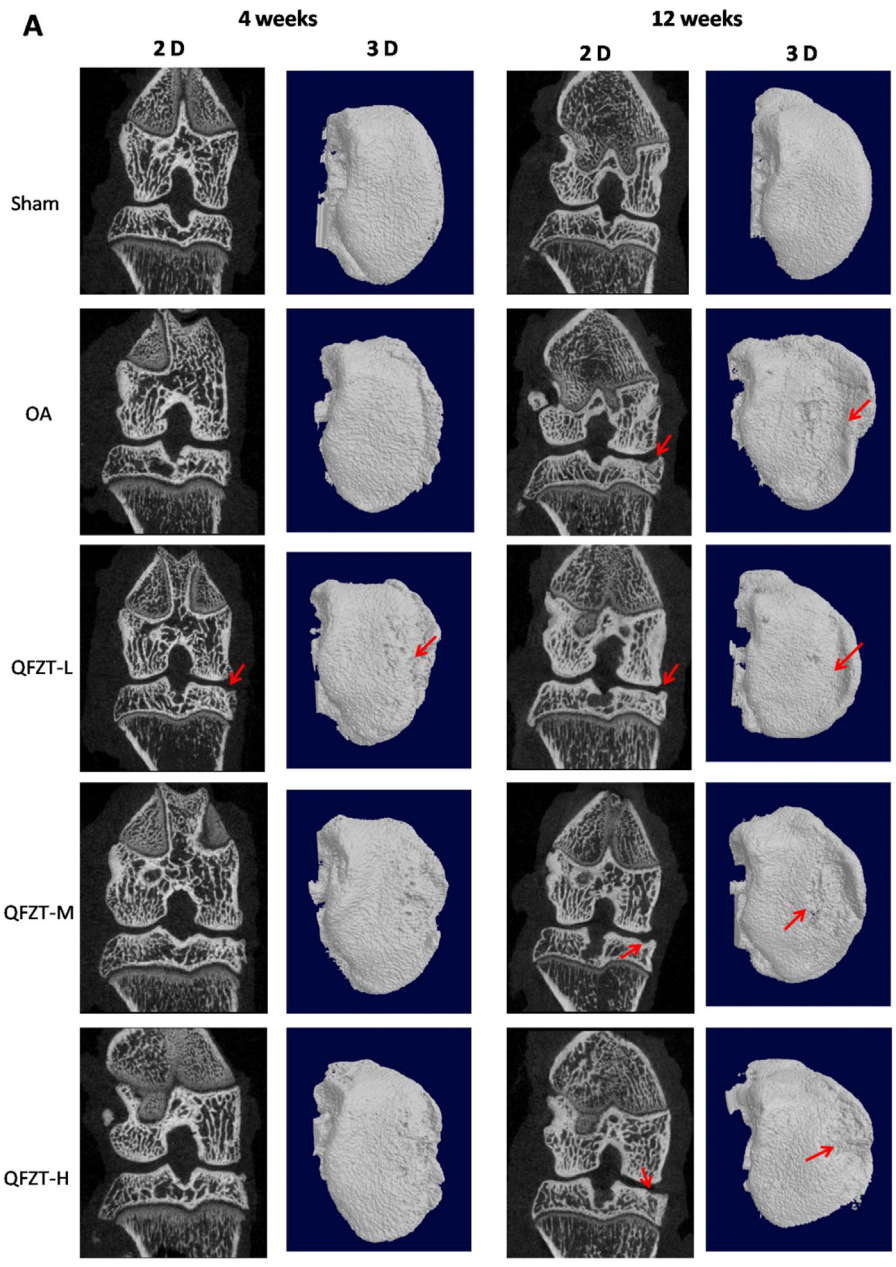
In the first week after treatment, the grip strength of the right hind limbs in the QFZT-M and QFZT-H groups increased by 14% ($P < 0.05$) and 27% ($P < 0.01$), respectively, compared with that in the OA group. The grip strength of the right hind limbs was increased significantly in all QFZT treatment groups compared with the OA group at the second week after treatment. In the third week after treatment, the grip strength was increased significantly in QFZT-M and QFZT-H group compared with the OA group ($P < 0.05$, $P < 0.01$) (Fig. 2B).

3.1.3. Joint width

The joint swelling was significant different between the sham group and the QFZT treatment groups ($P < 0.01$) in the first week after treatment. In the second week after treatment, the joint width in the QFZT-L and QFZT-H groups were 4% and 8% lower than that in the OA group ($P < 0.05$, $P < 0.01$). In the third week after treatment, significant difference was found in the OA group and QFZT groups compared with the sham group ($P < 0.01$), but there was no difference between the OA and QFZT groups (Fig. 2C).

3.2. Inflammation cytokines expression test

We harvested the six animals' knee joint at the 4 and 12 weeks after treatment to detect the mRNA expression of Inflammation cytokines. The results showed that the mRNA expression of IL-1β was significantly decreased in the QFZT-L group compared with the OA group 4 weeks after treatment ($P < 0.05$) (Fig. 3A). Moreover, the mRNA expression of IL-6 was significantly increased in the OA group compared with the sham group ($P < 0.05$),. Compared with the OA group, the mRNA expression of IL-6 in the QFZT-H group was significantly decreased ($P < 0.05$). At 12 weeks after treatment, the mRNA expression of IL-6 in the OA group was still significantly higher than that in the sham group ($P < 0.05$), but no differences were observed in other groups (Fig. 3B). At 4 weeks after treatment, the mRNA expression of TNF-α in the OA group was significantly higher than that in the sham group ($P < 0.05$), but no significant differences were observed between each group. At 12 weeks after treatment, no differences were observed in the mRNA expression of TNF-α in each group (Fig. 3C).



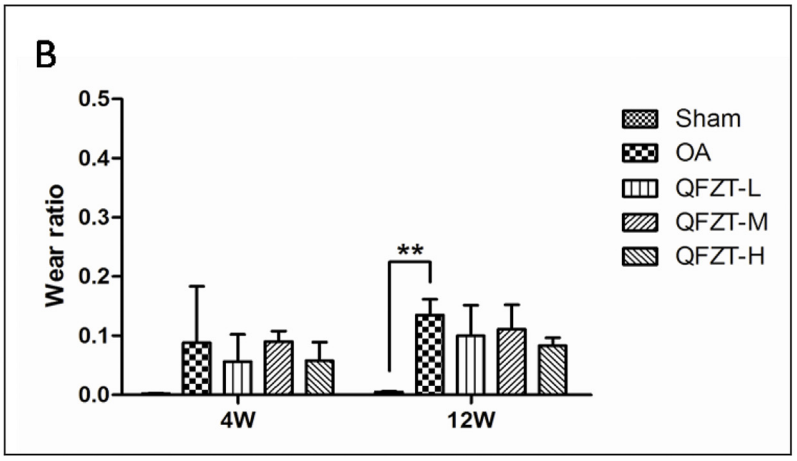


Figure 5. micro-CT images of knees after 4 and 12 weeks treatment. A. Representative 2D micro-CT images showing the coronal sectional images of the knee joints. The 3D images were taken from the top view of the 3D model of the medial tibial plateau. Red arrows indicated tibial plateau surface defects. B. No significant difference was observed at 4 weeks after treatment.12 weeks after treatment, significant difference was found between the OA group and the sham group (note: $^{**}P < 0.01$, n = 6). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

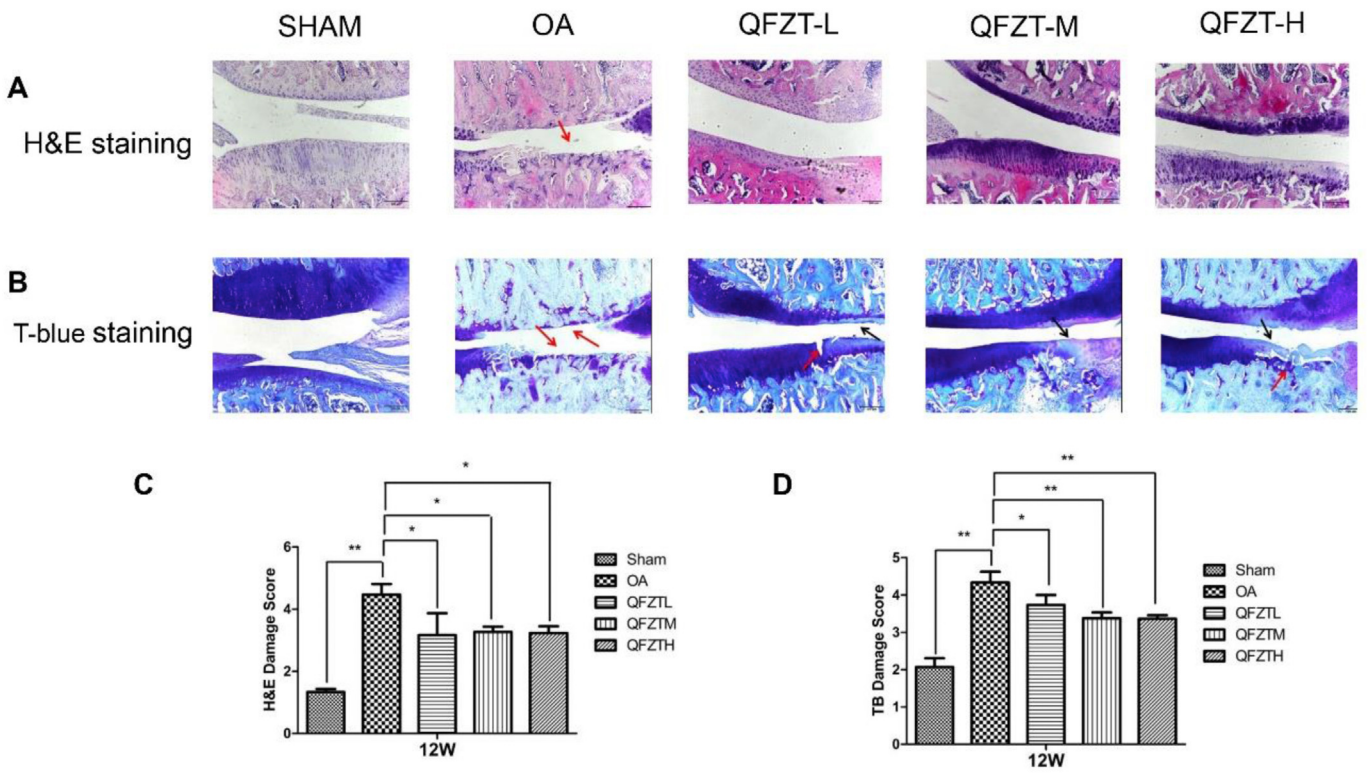


Figure 6. Histological analysis of articular cartilage and subchondral bone in experimental groups. A. H&E staining. The red arrow indicates cartilage abrasion; B. T-blue staining. The red arrow indicates the cartilage abrasion of the OA, QFZT-L, and QFZT-H groups. The black arrow shows cartilage calcification; C. H&E cartilage damage scores (note: $^{*}P < 0.05$; $^{**}P < 0.01$); D. T-blue cartilage damage scores. At 12 weeks after treatment, the sham and QFZT groups showed significant differences compared with the OA group in terms of H&E and T-blue staining (note: $^{*}P < 0.05$; $^{**}P < 0.01$, n = 6). (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Radiology assessment

According to the clinical evaluation standard for the assessment of joint space and joint wear, local changes in radiographic images in different scores reflex the narrow joint space and articular surface calcification for each group at the 4 and 12 weeks after treatment (n = 6). There were no differences among these 6 groups at 4 weeks after treatment. The OA group showed a higher score than the sham group at 12 weeks after treatment ($P < 0.01$). Moreover, the score was decreased in the QFZT-H group compared with the OA group 12 weeks later as well ($P < 0.05$) (Fig. 4).

Micro-CT assessment of the knee joints revealed bone erosions in the OA group. The results showed the defect in the subchondral bone in the OA group. Micro-CT 3-D images showed that the surfaces of tibial plateau bones were irregular, and the knee joint space was frequently obliterated 12 weeks after treatment (Fig. 5A). No significant difference was found between OA group and QFZH groups 4 week after treatment.Qualitative data showed that 12 weeks after treatment, the OA groups had noticeable abrasions compared with the sham group ($P < 0.01$), the maximum abraded area was 28.65%. Although there were no significant differences between the OA and the QFZT-H group, the maximum abraded area was only 9% in QFZT-H group which less than the OA group (Fig. 5B).

3.4. Histology assessment

3.4.1. H&E staining and cartilage damage score

Six animals of each group were harvested for histology assessment. Remarkable abrasion and damage were found in the OA groups. Compared with the OA group, the QFZT groups showed fewer lesions. Subchondral bone sclerosis, loss of hyaline cartilage, and calcification of the articular cartilage zone were also markedly reduced in OA group. The severity of cartilage degeneration and subchondral bone destruction in the QFZT-M and QFZT-H treatment group was much less than the OA group (Fig. 6A and Supplementary Fig. 1). The scores of QFZT groups were significantly lower than those of the OA group at 12 weeks after treatment ($P < 0.05$, Fig. 6C).

3.4.2. Toluidine blue staining and cartilage damage score

The toluidine blue staining results showed that remarkable abrasion and calcification was found in the OA group. The QFZT groups showed less lesions compared with the OA group. As expected, QFZT groups showed reduction of pathological disease severity than those in the OA group at 12 weeks after treatment with lower damage score (Fig. 6B and D) ($P < 0.05$ and $P < 0.01$).

4. Discussion

OA is one of the most prevalent joint diseases that may eventually lead to physical disability. Commonly, nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors are usually used in clinics to reduce the symptoms of OA [25–27]. However, these methods cannot stop disease progression and may lead to several side effects [28,29]. Therefore, to find more effective drugs with few side effects for the treatment of OA is of great importance. In this research, the *QFZT capsule* has been used in RA therapy as it can inhibit synovium hyperplasia and cartilage erosion [8,30,31]. Currently, the development and progression of OA are now considered to be related to inflammation in the early stage and the destruction of bone and cartilage in the late stage. As mention above *QFZT capsule* is mainly composed of *Geranium, Mistletoe, Dipsacus, Clematis, Angelica, Radix Aconiti, Safflower*, etc. It has been reported that *QFZT capsule* or its effective ingredients has the anti-inflammatory effect. *QFZT capsule* significantly increase the level of IL-10 anti-inflammatory

cytokines, reduce the proliferation of synovium and inflammatory cell infiltration in a Collagen induced arthritis (CIA) model [8]. *Dipsacus* extract can significantly inhibit ERK1/2 signal pathway of macrophages with the lipopolysaccharide-stimulated inflammatory response [32]. Another main component of *Geranium* is often used in the treatment of RA and gouty arthritis [33]. Based on the result of high-throughput screening platform, the anti-rheumatic effect of *Geranium* was found to inhibit the concentration of TNF-α in RA [30].Therefore, we raise our hypothesis that the *QFZT capsule* might have the potential therapeutic effect on OA.

In this study, we constructed the rats OA model and treated by the different dose of *QFZT capsule*. The results indicated that *QFZT capsule* could restore the supportability of the hind limb effectively in the early stage of OA. Furthermore, the grip strength of the treatment groups was significantly higher than that of the OA group. These data indicated that *QFZT capsule* could relieve pain in OA, especially in the early stage. With the limitations of grip strength method, gait analysis is usually used to assess the pain-related behaviour [34]. In future, we will perform a gait analysis in rodents to further confirm the anti-OA effect of QFZT capsule on the severity of OA.

Cytokines expression correlated with clinical symptoms, inflammatory indicators, disease activities, and serum biomarkers. Thus, the proinflammatory expression of TNF-α, IL-1β and IL-6 were tested after QFZT treated to assess the anti-inflammatory outcome. It has been reported that secreted inflammatory molecules such as IL-1β, IL-6 and TNF-α are the key mediators of the disturbed homeostasis in OA [35]. Interestingly, IL-1β also stimulates the production of multiple other inflammatory mediators such as IL-6 in OA as well [36,37]. In our study, TNF-α and IL-6 were significantly suppressed by *QFZT capsule*, while without statistical significance between OA group and QFZT treatment group. There may due to the sample size limitation. The results showed that mRNA level was decreased in QFZT-L group, but less 10%. Thus, we considered that IL-1β mRNA level was basically stable in most groups both in 4 and 12 weeks after treatment. Overall, it indicated that the anti-inflammation effect of *QFZT capsule* for the treatment of OA by suppressing the inflammatory cytokine TNF-α and IL-6 expression. This interesting data should be explored our future research.

The cartilage and subchondral bone has biochemical and molecular crosstalk across the affected region. Subchondral bone provides support to articular cartilage [38]. Micro-CT was able to measure significant changes in the subchondral bone. However, this approach does not effectively image uncalcified soft tissues like cartilage. Several studies have previously shown that increased subchondral bone remodelling and impaired structure adversely affect the overlying cartilage. So, the change of microstructure of subchondral bone could reflect the status of articular cartilage [39]. Our radiological and histological data further demonstrated that the *QFZT capsule* could effectively attenuate cartilage destruction and bone erosion to a certain extent, especially during the late stages of the OA disease.

5. Conclusion

We demonstrated the effects of the *QFZT capsule* for the treatment of the OA in an animal model for the first time. The *QFZT capsule* can improve the symptoms of the OA animal model by attenuating pain and retarding cartilage damage. In summary, our findings indicated that the QFZT capsule has a potential clinical application for OA therapy. To even explore the in-depth research, we will explore the effective ingredients in QFZT capsule for OA management and a more fundamental mechanism of the therapeutic effect on OA should be explored.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jot.2020.10.013.

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