Int J Clin Exp Med 2019;12(12):13673-13679
www.ijcem.com /ISSN:1940-5901/IJCEM0100665

Original Article

The clinical efficacy of ligustrazine in the treatment of knee osteoarthritis

Yan Zhang, Ruixue Duo, Haili Shen

Department of Rheumatic Immunology, Lanzhou University Second Hospital, Lanzhou, Gansu Province, China

Received August 7, 2019; Accepted October 10, 2019; Epub December 15, 2019; Published December 30, 2019

Abstract: Objective: To investigate the efficacy of ligustrazine in the treatment of mild and moderate knee osteoarthritis. Methods: This prospective study recruited 69 patients with knee osteoarthritis, who were randomly divided into a control group (n=34) and a treatment group (n=35). The patients in the control group were injected with hyaluronic acid in their joint cavities for 6 weeks. Based on the treatment in the control group, the patients in the treatment group took ligustrazine phosphate tablets orally for 6 weeks. The clinical efficacy and the patients' Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores were observed before and after they were treated. The changes in the TNF-α, IL-1, IL-6, and MMP-13 levels in the joint fluid were measured. Results: After treatment, the efficacy of the treatment in the treatment group was better than the efficacy in the control group ($P<0.05$). The patients' WOMAC scores in the treatment group were better than the patients' scores in the control group after they were treated, but there was no significant difference ($P>0.05$). The patients' IL-1 and IL-6 levels in the treatment group were higher than the levels in the control group ($P<0.01$). The incidence of adverse reactions in the treatment group was lower than it was in the control group ($P<0.05$). Conclusion: Ligustrazine has a good clinical efficacy in the treatment of mild and moderate knee osteoarthritis and can improve the condition of knee joint activity and effectively reduce the levels of IL-1 and IL-6 in joint fluid as well as reduce the incidence of adverse reactions, indicating that ligustrazine can be used as a safe and effective treatment.

Keywords: Ligustrazine, knee osteoarthritis, clinical efficacy

Introduction

Knee joint osteoarthritis, also known as knee osteoarthritis or knee arthropathy, is a common joint disease in elderly people. The clinical manifestations of knee osteoarthritis are mainly articular cartilage degeneration and articular cartilage injury, joint synovium inflammation, osteoproliferation at the edge of the joint, and reactive sclerosis of the subchondral bone, etc., causing inconvenience to patients and affecting their work and life [1]. Statistical data indicate that, with the acceleration of the aging process in China, the number of patients with knee osteoarthritis is increasing year by year, and the morbidity of people over 60 years old is up by 20% [2]. Moreover, knee osteoarthritis is increasingly being diagnosed in younger patients in recent years, so it is urgent for society to pay much attention to it.

The intra-articular injection of hyaluronic acid is one of the treatments for knee joint osteoarthritis that has been widely used in clinical practice in recent years. It can relieve pain effectively, but it has some drawbacks and problems [3]. For example, when it is used to treat patients with obesity or with much intra-articular exudate, their efficacy is poor and their long-term prognosis is unclear; moreover, they will have some adverse reactions, such as intra-articular calcification and swelling. Artificial knee replacement is an effective treatment for advanced, severe knee osteoarthritis, but it also has some drawbacks. For instance, cost of the surgery is high, the patients' recovery time is long, and the surgery involves a high risk [4]. Therefore, early prevention and treatment is the key to controlling knee osteoarthritis.

In recent years, with the development of traditional Chinese medicine therapy, it has been found that the combination of traditional Chinese medicine and Western medicine has a good preventive effect on the treatment of knee osteoarthritis, and its clinical efficacy is good [5]. In traditional Chinese medicine, it is believed that knee osteoarthritis is mainly affect-

ed by the "blood stasis" in the microcirculation in the body's bones. The "blood stasis" leads to venous stasis and an increase in intraosseous pressure and exudation, as well as lesions in the bones and cartilage. Thus, improving the state of blood stasis in the bones is important for delaying the degenerative changes of the joints and cartilages [6]. Ligustrazine is a component of Chuanxiong, which can facilitate blood circulation, remove blood stasis, eliminate stroke, and relieve pain. It has been clinically used to treat ischemic cerebrovascular diseases, bruises, pain, and stroke, etc. [7].

In this study, the combination of the intra-articular injection of hyaluronic acid and the oral administration of ligustrazine were used to treat patients with knee joint osteoarthritis, and the preventative effect and efficacy of this combination therapy were evaluated in order to provide clinical support data for the application of ligustrazine in the treatment of knee osteoarthritis.

Materials and methods

Patients

This prospective study was approved by the Ethics Committee of Lanzhou University Second Hospital. All the patients signed an informed consent form. Patients admitted from July 2013 to March 2019 were recruited and randomly divided into the control group (n=34) and the treatment group (n=35) according to the odd-even sequence.

Diagnostic criteria: The classification criteria of knee osteoarthritis, hand osteoarthritis, and hip osteoarthritis issued by American College of Rheumatology in 1995 was used [8]. The content is: 1. Patients' knees are very painful most of the time over the past month; 2. A standing weight-bearing X-ray shows joint space narrowing and osteophytes in the edges of the joints; 3. Laboratory tests show that the joint fluid conforms to bone joint values; 4. Osteophytes form; 5. The duration of morning stiffness is less than 30 minutes; 6. Bone crepitus can be heard when the joints activate. If a patient conforms to 1, 2 or 1, 3, 5, 6 or 1, 4, 5, 6 he or she can be diagnosed with KOA.

Inclusion criteria: The patients were graded according to the Western Ontario and McMaster Universities (WOMAC) score of knee osteoarthritis [9]; the knee osteoarthritis was mild or moderate; the patients were between 40 and 70 years old; the patients didn't have other organ diseases; the patients didn't take other painkillers before they were treated; the patients were compliant and voluntarily joined in the experiment.

Exclusion criteria: Patients who were not between 40 and 70 years old; patients who were allergic to the drugs used in this study; pregnant and lactating women; patients with narrow knee joint spaces; patients with serious complications or mental illness.

Treatment methods

The patients in the control group were injected with hyaluronic acid in their joint cavities. The specific steps were: The patients stopped taking other drugs when they were treated. They assumed a supine position and bent their knees at 90°. Their skin was disinfected and their lateral knee eyes under the patella were punctured. Then 2 mL of medical sodium hyaluronate gel was injected into lateral knee eyes (Shandong Freda Pharmaceutical Co., Ltd.). After this, the patients would bend and stretch their joints moderately several times to make the medical sodium hyaluronate gel spread evenly on the surfaces of the cartilage in the joint cavity. The patients' knees were injected with medical sodium hyaluronate gel once a week, and they were treated for 6 weeks continuously.

Based on the treatment in the control group, the patients in the treatment group took ligustrazine phosphate tablets orally (Livzon Group Limin Pharmaceutical Factory), twice a day, with one tablet each time for 6 weeks continuously.

Outcomes

Before and after the treatment, the clinical efficacy, the WOMAC scores, and the adverse reactions of the patients in two groups were observed. Their joint fluid was collected by conventional knee paracentesis, and then the joint fluid was centrifuged to collect the supernatant kin order to determine the changes in the levels of TNF-α, IL-1, IL-6, and MMP-13 in the joint fluid using an enzyme-linked immunosorbent assay (ELISA). The experimental procedures were carried out in strict accordance with the kits' instructions (Beyotime Biotechnology Co., Ltd., China). The measurements were carried

Table 1. Comparison of the general data between the two groups

Group	Treatment group (n=35)	Control group (n=34)	$t/\chi^2/Z$	P
Gender				
Male	18	17	0.014	0.906
Female	17	17	0.013	0.91
Number of knees	49	47		
Age (years)	41-66	44-70	0.651	0.517
Average age (years)	47.3±12.8	49.1±14.3	0.551	0.583
K-L classification			-0.440	0.67
0	4	3		
I	21	20		
II	16	14		
III	8	10		

Table 2. Comparison of clinical efficacy between the two groups (n, %)

Group	Treatment group	Control group
Number of knees	49	47
Recovery	9	7
Markedly effective	25	22
Effective	14	10
Ineffective	1	8
Total effective rate	97.96%*	82.98%

Note: Compared with the control group, *P<0.05.

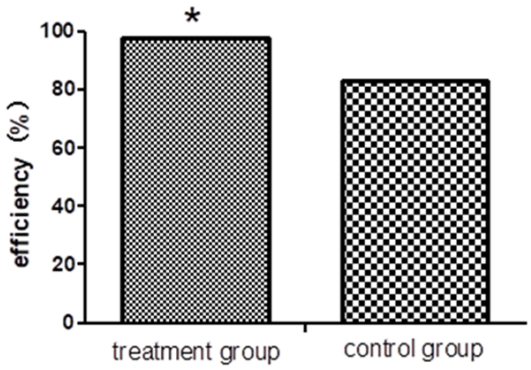

Figure 1. Comparison of clinical efficacy between the two groups. Compared with the control group, *P<0.05.

out using a multi-function microplate reader (Bio-tek, USA).

The criteria of efficacy evaluation

Curative effect index = (pre-treatment score - post-treatment score)/pre-treatment score * 100%. Recovery: Most of the patients' clinical symptoms disappeared, and they could basically work or join in activities, and their curative effect index improved by more than 90%; Markedly effective: the patients' clinical symptoms clearly improved, and their daily activities were slightly affected, 70%≤ curative effect improvement index <90%; Effective: the patients' clinical symptoms improved, but the clinical symptoms had some impacts on their daily life, 30%≤ curative effect improvement index <70%; Ineffective: the patients' main clinical symptoms didn't improve or were even aggravated, curative effect improvement index <30% [10]. Clinical effectiveness = (the number of recovery cases + markedly effective cases + effective cases)/total number of cases * 100%.

Statistical methods

All data were analyzed and processed using SPSS 17.0. The count data (n, %) were analyzed using a chi-squared test, and the ranked data were analyzed using a rank sum test. A Kolmogorov test was used to carry out a normality test for the measurement data, and the data in the normal distribution were expressed as the mean value ± standard deviation ($\bar{x}$ ± sd); the comparison between two groups was performed by an independent *t*-test. P<0.05 indicated that the differences were statistically significant.

Results

General clinical data

The patients in the two groups did not receive other drug treatments for two months before they were treated. Their disease courses were similar, and there were no patients who drank alcohol. There were no significant differences in the clinical data between the two groups (P>0.05, **Table 1**).

The combination treatment has a better clinical efficacy

The treatments' clinical efficacy in the two groups were observed and recorded for 6 weeks after the treatment. The clinical effective rate of the patients in the treatment group was sig-

Table 3. Comparison of the WOMAC scores before and after treatment between the two groups

Group	Number of knees	WOMAC score before treatment	WOMAC score after treatment
Treatment group	49	17.35±2.01	6.88±1.26##
Control group	47	17.01±3.23	7.29±1.91##
t		0.622	1.246
P		0.536	0.216

Note: Compared with before treatment, ##P<0.01. WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

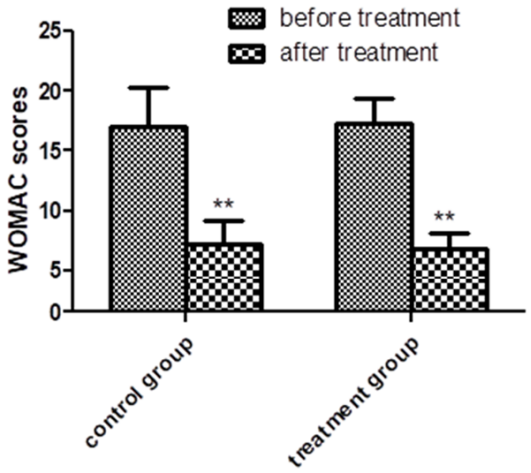

Figure 2. Comparison of the WOMAC scores before and after treatment between the two groups. Compared with before treatment, **P<0.01. WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

nificantly higher than the rate in the control group (97.96% vs. 82.98%, P=0.03, **Table 2** and **Figure 1**).

WOMAC score

Before treatment, the WOMAC scores of the patients in the treatment group were similar with those in the control group (17.35±2.01 vs. 17.01±3.23, P>0.05). After the treatment, the WOMAC scores of the patients in both groups were significantly decreased (6.88±1.26 vs. 7.29±1.91), and were clearly lower than they were before the treatment (both P<0.01). Moreover, the comparisons between the groups showed that the scores of the patients in the treatment group were lower than those of the patients in the control group after they were treated, but the differences were not statistically significant (P>0.05, **Table 3** and **Figure 2**).

The changes in the TNF-α, IL-1, IL-6, and MMP-13 levels in the joint fluid

In knee osteoarthritis, inflammatory factors are important mediators in pathogenetic processes. In this study, the levels of several important inflammatory factors in joint fluid were determined (**Tables 4**-**7**). The results showed that before the treatment, there were no significant differences in the TNF-α, IL-1, IL-6, and MMP-13 levels in the joint fluid of the patients in the two groups (all P>0.05). The levels of TNF-α, IL-1, IL-6, and MMP-13 in the joint fluid of the patients in the two groups decreased after they were treated. The improvement in the IL-1 and IL-6 levels of the patients in the treatment group was better than it was in the control group (both P<0.01). Moreover, the levels of TNF-α and MMP-13 in the treatment group were also lower than they were in the control group, but the differences were not statistically significant (P>0.05). These results suggested that the interventional treatment of ligustrazine has a good effect on controlling inflammation.

The combination treatment has less adverse reactions

Adverse reactions of the patients in two groups were observed after treatment (**Figure 3**). In the control group, 5 patients had joint stimulation, 3 patients had joint swelling, so the incidence of adverse reactions was 17.02%. There was only 1 patient who had joint stimulation in the treatment group, so the incidence of adverse reactions was 2.04%, which was significantly lower than the rate of the control group (P=0.03).

Discussion

Knee osteoarthritis is a degenerative lesion, mainly characterized by an injury of the articular cartilage, which can progress to a degenerative bone joint lesion. Chronic inflammation can be seen in diseased joints due to osteophytes. As diseased joints lack lubrication, the immune barrier of the surface cartilage is weakened. The external force induces further injury of the cartilage surface. After the cartilage is injured, it is difficult to cure and easy to relapse, which aggravates the loss of the joints' movement capacity [11]. The main clini-

Table 4. Comparison of TNF-α content in joint fluid between the two groups ($\bar{x}$ ± sd, pg/mL)

Group	Number of knees	Before treatment	After treatment
Treatment group	49	457.11±35.43	431.42±21.83##
Control group	47	460.72±32.67	433.15±27.78##
t		0.518	0.340
P		0.605	0.735

Note: Compared with before treatment, ##P<0.01.

Table 5. Comparison of the IL-1 content in the joint fluid between the two groups ($\bar{x}$ ± sd, pg/mL)

Group	Number of knees	Before treatment	After treatment
Treatment group	49	244.73±11.03	230.77±12.24#,**
Control group	47	246.22±12.56	240.28±10.65#
t		0.618	4.054
P		0.538	0.000

Note: Compared with before treatment, #P<0.05; compared with control group, **P<0.01.

Table 6. Comparison of the IL-6 content in the joint fluid between the two groups ($\bar{x}$ ± sd, pg/mL)

Group	Number of knees	Before treatment	After treatment
Treatment group	49	103.85±5.01	91.17±5.22##,**
Control group	47	102.91±4.78	95.63±5.03##
t		0.939	4.260
P		0.349	0.000

Note: Compared with before treatment, ##P<0.01; compared with control group, **P<0.01.

Table 7. Comparison of the MMP-13 content in the joint fluid between the two groups ($\bar{x}$ ± sd, pg/mL)

Group	Number of knees	Before treatment	After treatment
Treatment group	49	94.92±8.32	71.91±7.73#
Control group	47	95.03±8.55	72.32±7.01#
t		0.064	0.272
P		0.949	0.786

Note: Compared with before treatment, #P<0.05.

cal manifestations of knee osteoarthritis are knee joint pain, increased flexion pain, etc. Also, it is hard to stand and walk after being sedentary, which directly affects patients' life and work [12]. At present, it is believed that osteoarthritis and cartilage injury are caused by the abnormal expression and regulation of biological enzymes and inflammatory factors that are produced in the degenerative process of cartilage [13, 14]. Therefore, targeted treatment plans and strategies for knee osteoarthritis are verified and updated regularly.

In knee osteoarthritis, the common inflammatory factors are IL-1, IL-6, and TNF-α, which are important mediators in the pathogenetic process. The inflammatory factors can effectively inhibit the synthesis and secretion of cartilage II collagen and proteoglycans and aggravate the deterioration of osteoarthritis. IL-1 can facilitate the synthesis of collagenase and inhibit the formation of II collagen fibers, but they facilitate the synthesis of I collagen and III collagen as well as change the biological characteristics of cartilages [15]. IL-6 can induce the synthesis and secretion of immunoglobulins and facilitate the functions of IL-1 [16]. TNF-α can block the repair of injured articular cartilages. MMP-13 can degrade proteoglycan, which is an important component in cartilages, and it can facilitate the pathological degradation of collagen [17]. Therefore, indicators such as IL-1, IL-6, TNF-α, and MMP-13 in joint fluid can be the first to indicate the occurrence of the inflammation of articular cartilages and the degenerative process of the cartilage.

The injection of hyaluronic acid is one of the treatments commonly used to treat knee osteoarthritis. It can quickly relieve the pain and protect the cartilage. However, the duration of its efficacy is short, and it can't be used with patients in a deteriorated condition. At present, many studies have reported the injection of traditional Chinese medicine into the joint cavity to treat knee osteoarthritis, which has some clinical efficacy. However, this treatment has some drawbacks; for example, it easily leads to excessive ethanol residues and excessive tannin

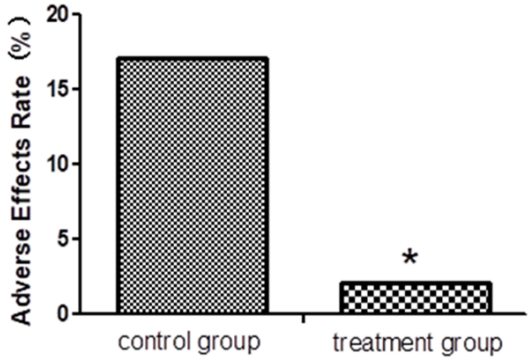

Figure 3. Comparison of the incidence of adverse reactions between the two groups. Compared with before treatment, *P<0.05.

residues. Moreover, the composition of the traditional Chinese medicine injection is complex. In some cases, patients have adverse complications such as joint stimulation, which limits the clinical application of injection medicines [18].

In traditional Chinese medicine, it is believed that knee osteoarthritis belongs to the category of blood stasis, which is mainly manifested as a meridian block and poor blood circulation. In this study, the patients were treated with an injection of hyaluronic acid in the joint cavity and the oral administration of ligustrazine. Ligustrazine is an effective component of Chuanxiong, which can facilitate blood circulation, remove blood stasis, increase energy, and relieve pain. A large number of studies show that ligustrazine can effectively expand the coronary artery and increase blood flow as well as improve the deformation ability of red blood cells. Also, it can facilitate the microcirculation in bones and reduce intraosseous pressure as well as improve the nutrient transmission in cartilages [19, 20]. The results of this study also showed that the efficacy of the injection of hyaluronic acid in the joint cavity combined with ligustrazine was remarkable, and the safety of the combination treatment is higher. Additionally, the combination therapy could alleviate the inflammatory response caused by cartilage injury. In a study by Yu et al., it was found that ligustrazine can protect chondrocytes against IL-1, and ligustrazine can be used as a therapeutic agent of osteoarthritis [21]. In a study Zhang, it was also found that prolonging the administration of ligustrazine can improve joint lesions and reduce the consumption of proteoglycans, and the efficacy is good [22]. Therefore, it can be concluded that ligustrazine is effective in the treatment of knee osteoarthritis, and that it is safe and is worthy of being used in clinical practice.

In summary, ligustrazine has a good clinical efficacy in the treatment of knee osteoarthritis with blood stasis and obstruction, and it can improve patients' clinical symptoms. However, studies documenting the drug's long-term efficacy to verify this conclusion are still needed.

Acknowledgements

This work was supported by the Gansu Natural Science Foundation (18JR3RA311) and the Cuiying Scientific and Technological Innovation Program of Lanzhou University Second Hospital (CY2017-BJ17).

Disclosure of conflict of interest

None.

Address correspondence to: Haili Shen, Department of Rheumatic Immunology, Lanzhou University Second Hospital, No. 82 Cuiyingmen, Chengguan District, Lanzhou 730030, Gansu Province, China. Tel: +86-13919329146; E-mail: shenhailid86f@163.com

References

[1] Roos EM and Arden NK. Strategies for the prevention of knee osteoarthritis. Nat Rev Rheumatol 2016; 12: 92-101.

[2] Wallace IJ, Worthington S, Felson DT, Jurmain RD, Wren KT, Maijanen H, Woods RJ and Lieberman DE. Knee osteoarthritis has doubled in prevalence since the mid-20th century. Proc Natl Acad Sci U S A 2017; 114: 9332-9336.

[3] Cooper C, Rannou F, Richette P, Bruyere O, Al-Daghri N, Altman RD, Brandi ML, Basset SC, Herrero-Beaumont G, Migliore A, Pavelka K, Uebelhart D and Reginster JY. Use of intraarticular hyaluronic acid in the management of knee osteoarthritis in clinical practice. Arthritis Care Res 2017; 69: 1287-1296.

[4] Ringdahl E and Pandit S. Treatment of knee osteoarthritis. Am Fam Physician 2011; 83: 1287-1292.

[5] Guarise C, Pavan M, Galesso D, Giordan N, Moro S, Salmaso VV, Gerli R and Gresele P. Matrix metalloprotease 3 (MMP3) inhibition effect of a viscosupplement based on a hyaluronic

acid amide derivative (HYADD4). Osteoarthritis Cartilage 2018; 26: S286-S287.
[6] Yang M, Jiang L, Wang Q, Chen H and Xu G. Traditional Chinese medicine for knee osteoarthritis: an overview of systematic review. PLoS One 2017; 12: e0189884.
[7] Zou J, Gao P, Hao X, Xu H, Zhan P and Liu X. Recent progress in the structural modification and pharmacological activities of ligustrazine derivatives. Eur J Med Chem 2018; 147: 150-162.
[8] Michael JW, Schlueter-Brust KU and Eysel P. The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. Dtsch Arztebl Int 2010; 107: 152-62.
[9] Lo GH, Song J, McAlindon TE, Hawker GA, Driban JB, Price LL, Eaton CB, Hochberg MC, Jackson RD, Kwoh CK, Nevitt MC and Dunlop DD. Validation of a new symptom outcome for knee osteoarthritis: the Ambulation Adjusted Score for Knee pain. Clin Rheumatol 2019; 38: 851-858.
[10] Zheng XY. Guiding principles for clinical research of new Chinese medicines. China Medical Science and Technology Publishing House 2002.
[11] Culvenor AG, Ruhdorfer A, Juhl C, Eckstein F and Oiestad BE. Knee extensor strength and risk of structural, symptomatic, and functional decline in knee osteoarthritis: a systematic review and meta-analysis. Arthritis Care Res (Hoboken) 2017; 69: 649-658.
[12] Li Y, Su Y, Chen S, Zhang Y, Zhang Z, Liu C, Lu M, Liu F, Li S, He Z, Wang Y, Sheng L, Wang W, Zhan Z, Wang X and Zheng N. The effects of resistance exercise in patients with knee osteoarthritis: a systematic review and meta-analysis. Clin Rehabil 2016; 30: 947-959.
[13] Zhang W, Ouyang H, Dass CR and Xu J. Current research on pharmacologic and regenerative therapies for osteoarthritis. Bone Res 2016; 4: 15040.
[14] Yun TG, Chen J, Meng ZL, Ge WY, Bian YY, Cheng SW, Xing CK, Yao JL, Fu J and Peng L. Research progress on osteoarthritis treatment mechanisms. Biomed Pharmacother 2017; 93: 1246-1252.
[15] Botha-Scheepers S, Watt I, Slagboom E, de Craen AJ, Meulenbelt I, Rosendaal FR, Breedveld FC, Huizinga TW and Kloppenburg M. Innate production of tumour necrosis factor and interleukin 10 is associated with radiological progression of knee osteoarthritis. Ann Rheum Dis 2008; 67: 1165.
[16] Latourte A, Cherifi C, Maillet J, Ea HK, Bouaziz W, Funck-Brentano T, Cohen-Solal M, Hay E and Richette P. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. Ann Rheum Dis 2017; 76: 748-755.
[17] Flannery CR, Little CB, Caterson B and Hughes CE. Effects of culture conditions and exposure to catabolic stimulators (IL-1 and retinoic acid) on the expression of matrix metalloproteinases (MMPs) and disintegrin metalloproteinases (ADAMs) by articular cartilage chondrocytes. Matrix Biol 1999; 18: 225-237.
[18] Chen WH, Liu XX, Tong PJ and Zhan HS; Orthopaedic Professional Committee, Chinese Association of Research and Advancement of Chinese Traditional Medicine, China; Joint Professional Committee, Branch of Orthopaedic of Chinese Association of Integrative Medicine, China. Diagnosis and management of knee osteoarthritis: Chinese medicine expert consensus (2015). Chin J Integr Med 2016; 22: 150-153.
[19] Xian HE, Wei CS and Cai ZGJSJoTCM. Study on pathogenesis, prevention and treatment of knee osteoarthritis in TCM. Shandong Journal of Traditional Chinese Medicine 2005.
[20] Hussein MR, Fathi NA, El-Din AM, Hassan HI, Abdullah F, Al-Hakeem E and Backer EA. Alterations of the CD4+, CD8+ T cell subsets, interleukins-1β, IL-10, IL-17, tumor necrosis factor-α and soluble intercellular adhesion molecule-1 in rheumatoid arthritis and osteoarthritis: preliminary observations. Pathol Oncol Res 2008; 14: 321-328.
[21] Yu T, Qu J, Wang Y and Jin H. Ligustrazine protects chondrocyte against IL-1β induced injury by regulation of SOX9/NF-κB signaling pathway. J Cell Biochem 2018; 119: 7419-7430.
[22] Zhang X, Shi Y, Zhang Z, Yang Z and Huang G. Intra-articular delivery of tetramethylpyrazine microspheres with enhanced articular cavity retention for treating osteoarthritis. Asian J Pharm Sci 2018; 13: 229-238.