Journal of Ethnopharmacology 162 (2015) 148–154

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

# *Eucommia ulmoides Oliv.* bark aqueous extract inhibits osteoarthritis in a rat model of osteoarthritis

Guo-ping Xie [a,b,1], Nan Jiang [a,1], Sheng-nan Wang [a], Rui-zhen Qi [a], Lei Wang [a], Pei-ran Zhao [b], Liang Liang [a], Bin Yu [a,b,*]

[a] Department of Orthopaedics and Traumatology, Nanfang Hospital, Southern Medical University, PR China
[b] Key laboratory of Bone and Cartilage Regenerative Medicine of Guangdong Province, Nanfang Hospital, Southern Medical University, PR China

ARTICLE INFO

*Article history:*
Received 10 June 2014
Received in revised form
23 December 2014
Accepted 28 December 2014
Available online 7 January 2015

*Keywords:*
*Eucommia ulmoides Oliv.* bark
Osteoarthritis
MMP-3
IL-1β
IL-6
PI3K/Akt signaling pathway

ABSTRACT

*Ethnopharmacological relevance:* *Eucommia ulmoides Oliv.* bark (EU) is a common traditional Chinese herbal medicine for treatment of osteoarthritis (OA), but its therapeutic effect on OA and the underlying mechanisms have not been fully clarified. Our previous study showed that *Eucommia ulmoides Oliv.* bark aqueous extract (EUE) had a protective effect on cartilage, and this study was aimed to investigate the anti-osteoarthritis effect and mechanisms of EUE in a rat model of osteoarthritis.

*Materials and methods:* Thirty-two 5-week-old specific pathogen-free Sprague–Dawley rats which were randomized into four even groups ($n=8$). Group A received sham operation while the OA model was established using the modified Hulth technique in groups B, C and D. For eight weeks after operation, in addition to routine feeding, group A received gavage with deionized water, group B with deionized water, group C with 1.35 g/kg/day EUE, and group D with 2.7 g/kg/day EUE. Eight weeks postoperatively, all of the animals were euthanized for radiological, gross and histopathological observations to evaluate the effect of EUE on OA and to determine its potential mechanisms.

*Results:* Radiological and histopathological observations showed that the articular degenerative changes were significantly more alleviated in groups C and D than in group B, while there were no obviously degenerative manifestations in group A. Mankin's scores in groups C and D were significantly lower than in group B ($P<0.01$). The severity of OA was significantly less in group D than in group C ($P<0.01$). The IL-1β and IL-6 contents in serum and MMP-3 secretion in articular cartilage were significantly lower in groups C and D than those in group B ($P<0.01$), and significantly lower in group D than those in group C ($P<0.01$). Compared with group B, phosphorylated Akt was significantly down-regulated in groups C and D.

*Conclusions:* EUE may inhibit the progression of osteoarthritis by inhibiting the PI3K/Akt pathway to delay cartilage degeneration, reduce inflammatory cytokines and prevent MMP-3 secretion. Therefore, EU is a potential therapeutic agent for OA, but its efficacy is limited.



## 1. Introduction

Osteoarthritis (OA) is a common arthritic disease and a major cause of disability in adult population. It was generally considered a whole joint disease characterized by cartilage destruction, subchondral bone sclerosis, and osteophyte formation, joint synovitis, and synovial angiogenesis (Suri and Walsh, 2012; Zhen et al., 2013; Zhang et al., 2014b). Moreover, cartilage degeneration was caused by an imbalance between anabolic and catabolic factors. Matrix metalloproteinases (MMPs) play an important role in the degenerative process of articular cartilage and may degenerate extracellular matrix (ECM) of articular cartilage, which mainly consists of collagen-α and aggrecan (Martel-Pelletier et al., 2001). Likewise, some pro-inflammatory cytokines like interleukin (IL)-1β and IL-6 might

*Abbreviations:* AAOS, American Academy of Orthopaedic Surgeons; ELISA, enzyme-linked immunosorbent assay kits; ECM, extracellular matrix; E U, *Eucommia ulmoides Oliv.* bark; EUE, *Eucommia ulmoides Oliv.* bark aqueous extract; HPLC, high-performance liquid chromatography; H & E, Hematoxylin and Eosin; IL-1β, interleukin-1β; IL-6, interleukin-6; MMPs, matrix metalloproteinases; MMP-3, matrix metalloproteinase-3; NSAIDs, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; PI3K, phosphoinositide 3-kinase; TCM, traditional Chinese medicine

* Correspondence to: Department of Orthopaedics and Traumatology, Nanfang Hospital, No. 1838, Guangzhou Ave. North, Baiyun District, Guangzhou, 510515, P.R. China. Tel.: +86 20 6164 1741; fax: +86 20 6136 0066.

*E-mail addresses:* xgp19@163.com (G.-p. Xie), hnxyjn@smu.edu.cn (N. Jiang), 151102352@qq.com (S.-n. Wang), siriusjump@hotmail.com (R.-z. Qi), 35314031@qq.com (L. Wang), 544592541@qq.com (P.-r. Zhao), palatin921@126.com (L. Liang), nanfanghot@126.com (B. Yu).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.jep.2014.12.061
0378-8741/

provoke cartilage degradation through up-regulating MMPs secretion (Sakao et al., 2009; Stannus et al., 2010; Santangelo et al., 2012). It was also reported that PI3K/Akt pathway is involved in the progression of OA and inhibition of this pathway decreases MMPs production by chondrocytes (Chen et al., 2013). However, the etiology and mechanisms of OA are still not very clear to date and there are no specific drugs especially effective for OA either. Currently, nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for OA and recommended by American Academy of Orthopaedic Surgeons (AAOS) (Jevsevar et al., 2013). However, they are associated with serious adverse events, such as gastrointestinal (Ishijima et al., 2014) and cerebrovascular diseases (Lee et al., 2007). Therefore, a safe and effective anti-osteoarthritis agent is urgently demanded. Traditional Chinese herbal medicines which contain a large number of active compounds could provide a choice for this kind of agent (Ho et al., 2013; Tong et al., 2014).

*Eucommia ulmoides Oliv*. bark (EU), also known as *Du Zhong*, is traditionally used as a tonic medicine in China, Japan, Korea and other countries (He et al., 2014). Based on theories of traditional Chinese medicine (TCM), EU is used to tonify the liver and kidneys, strengthen tendons and bones, and prevent miscarriage. Pharmacological studies have indicated that the extract of EU exhibits effects of fortifying the muscles and lungs, lowering blood pressure, preventing miscarriage and improving bone biomechanical quality (Lee et al., 2005; Zhang et al., 2009, 2014a). Since it is an indispensable ingredient of many classic prescriptions, such as Bushen Huoxue decotion and Duhuo Jisheng decotion, the aqueous extract of EU has been widely used in traditional Chinese medicine for the treatment of hypertension, osteoporosis and OA (Anon, 2010). Of note, EU is the most frequently prescribed herb and Duhuo Jisheng decotion is the most commonly prescribed Chinese formula for OA (Chen et al., 2014). Our previous study demonstrated that *Eucommia ulmoides Oliv*. bark aqueous extract (EUE) has a protective effect on the articular cartilage in rats (Lu et al., 2013), but it was unknown whether the aqueous extract of the single herb had the anti-osteoarthritis effect in OA.

Therefore, the aim of the present study was to investigate the effect of EUE for the treatment of OA in a rat model and its potential mechanisms.

## 2. Materials and methods

### 2.1. Plant materials and aqueous extract

EU was purchased from Zhixin Pharmaceutical Co., Ltd. (Guangzhou, China) and authenticated macroscopically and microscopically according to the Chinese Pharmacopoeia (Anon, 2010). A 135 g sample of EU was extracted with water (1000 ml) at 100 °C for 60 min and then centrifuged at 3000 rpm for 20 min. The extraction was repeated thrice. The extracts were then combined and filtered. The filtrates were collected, concentrated with a vacuum evaporator until the volume was 500 ml and then stored at −20 °C until use.

### 2.2. High performance liquid chromatography analysis

The qualitative chemical profile (fingerprint) of EUE was analyzed by high-performance liquid chromatography (HPLC) as described in the Chinese Pharmacopoeia. HPLC was carried on a Waters Alliance (Waters Corp, American) e2695 HPLC system consisting of a 2998 photodiode array detector, gradient pump, autosampler and C18 column (150 mm × 4.6 mm, 5 μm). Solution A comprising acetonitrile–0.1% $H_3PO_4$ (5/95, v/v) and solution B (100% acetonitrile) were used as the mobile phase with the gradient elution. The gradient program was accomplished at 25 °C by a linear gradient elution from 6% to 28% acetonitrile for 0–35 min, 28% to 35% acetonitrile for 35–60 min and 35% acetonitrile for 60–70 min at a flow rate of 0.8 ml/min. The scanned wavelength was from 210 nm to 360 nm, and the detection wavelength was 230 nm. According to the principle of HPLC, the compounds were identified by individual peak retention times compared with the standard substances.

### 2.3. Animal environment and osteoarthritis model

Thirty-two specific pathogen-free Sprague–Dawley rats (5 weeks old, 200–220 g in weight) were purchased from the Animal Laboratory Center, Guangzhou University of Traditional Chinese Medicine [SCXK (Yue) 2008-0020]. All rats were housed in a specific pathogen-free environment, which was temperature-controlled (24 ± 1 °C) and humidity-controlled (30–40%), in a 12 h light and 12 h dark cycle (lights on at 6:00 a.m.). Feed and water were provided ad libitum. All animals were disposed according to the "Guide for the Care and Use of Laboratory Animals" (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). This study was approved by the Animal Care and Use Committee at Guangzhou University of Traditional Chinese Medicine (2008C067).

The rat models of OA were established in 24 randomized rats by performing unilateral knee joint surgery according to the modified Hulth technique (Hulth et al., 1970; Hayami et al., 2003). The remaining 8 rats that received sham operation served as blank controls (group A). Specific methods were as follows: the right knee joint cavity of rat was opened via a medial parapatellar incision under general anesthesia with 10% chloral hydrate (3 ml/kg) before the anterior cruciate ligaments were transected to remove the medial meniscus. All animals were permitted to run for 1 h every day after operation.

### 2.4. EUE administration

The 24 rat models of OA were randomly and evenly divided into groups B, C and D ($n$=8). For eight weeks after operation, in addition to routine feeding, no EUE but deionized water was administered by gavage in the rats in groups A and B, but 1.35 g/kg/day EUE was administered by gavage in the rats in group C and 2.70 g/kg/day EUE in group D.

### 2.5. Radiographic evaluation, gross observation and histopathologic analysis

Radiographic evaluation was performed in all rats after anesthesia with 10% chloral hydrate at the end of 8 weeks postoperatively. Digital images of the knee joint were taken using an Axiom Multix M radiographic unit (Siemens, Germany). The right knee joint cavity was opened and soft tissues around the knee were removed after the rats were euthanatized by cervical dislocation following general anesthesia. The surface of articular cartilage was observed. Macroscopic lesions were graded according to the method described previously (Laverty et al., 2010).

The distal femur was fixed with 10% neutral buffered formalin, followed by decalcification in 4% EDTA for histological evaluation after macroscopic observation. The decalcified samples were embedded in paraffin blocks and serial sections (4 μm thick). At last, the sections were stained with Hematoxylin and Eosin (H & E). Mankin's score was used to further evaluate the cartilage damage. Semi-quantitative histopathological grading was performed according to the modified Mankin's scores system for the degradation of articular cartilage (Mankin et al., 1971; Hayami et al., 2006), covering structure, chondrocyte number, chondrocyte clustering, proteoglycan content, and subchondral bone plate and/or tidemark change.