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Effects of *Zanthoxylum piperitum* ethanol extract on osteoarthritis inflammation and pain

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ABSTRACT

Degenerative arthritis, also known as osteoarthritis (OA), is the most common type of arthritis, which is caused by degenerative damage of the cartilage, which primarily protects the joints, leading to inflammation and pain. The objective of this study was to investigate the *in vivo* and *in vitro* effects of treatment with ZPE-LR (90% EtOH extract of *Zanthoxylum piperitum*) on pain severity and inflammation. When using an *in vivo* OA model MIA (monosodiumidoacetate-induced arthritis) rats, ZPE-LR (100 mg/kg) oral-administratio significantly inhibited MIA-induced change in loaded weight ratio on the left foot, and articular cartilage thickness. To confirm the positive effects on pain relief, acetic acid, heat and formalin-induced pain were remarkably decreased by 50 and 100 mg/kg ZPE-LR oral-administration. Pain related KCNJ6 mRNA expression as well as K + current was increased after ZPE-LR treatment in BV-2 cells. To confirm the positive effects on inflammation, TPA (12-O-tetradecanoylphorbol-13-acetate) induced inflammation measured by mouse ear thickness and biopsy punch weight and TPA-induced iNOS, COX-2 mRNA and protein expression were remarkably suppressed by 50 and 100 mg/kg ZPE-LR oral-administration. In addition, TPA-induced iNOS, COX-2 mRNA level and protein expression were reduced. Acetic acid, heat and formalin-induced pain were remarkably decreased by 50 and 100 mg/kg ZPE-LR oral-administration. We examined *in vitro* ZPE-LR effects in LPS-induced RAW 264.7 cells. LPS-induced p65 translocation to the nucleus was prohibited by ZPE-LR 100 μg/ml oral administration. Moreover, ROS generation by LPS was significantly inhibited by ZPE-LR 50 and 100 μg/ml treatment. To investigate new ZPE-LR activating mechanisms, the gene fishing method (not a typical term, should probably use PCR based genetic screening) was used. LPS-induced HPRT1 (hypoxanthine phosphoribosyltransferase 1) was decreased by ZPE-LR. However, RPL8 (Ribosomal protein L8) which showed no change in mRNA expression due to LPS, did show increased mRNA levels after ZPE-LR treatment. Our data elucidate mechanisms underlying ZPE-LR and suggest ZPE-LR may be a potential therapeutic agent to modulate osteoarthritis inflammation and pain.

1. Introduction

Osteoarthritis (OA) is a disease caused by aging and bone density decrease etc. And as a secondary change, decrease cartilage surrounding joints [1]. In the past, this was believed to be a just from aging phenomenon. But, in recently study, OA reported as a joint cartilage disease caused by varios causes such as inflict morphological damage, osteoporosis, inflammation and reumatoid arithris etc.[2].

Arthritis is a degenerative disease caused by combined cartilage elasticity loss, reduction in water quantity, and cartilage and muscle loss [3–5]. This disease does not only damage vasculature or the nervous system but also metabolism which prevents surrounding tissue damage were slowed [6]. When cartilage is acutely destroyed, damage can occur without subjective symptom presentation. Interventions for cartilage damage include chemical inhibitors, antibodies, and gene therapy. These treatments ameliorate damage by suppressing inflammation inducing factors such as TNF-α or IL-6 [7–10]. However, this therapy has a disadvantage, it is in re-division after first division in

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mesenchymal stem cells, that maintain cartilage cell traits [11,12].

There are many studies on natural substance effects on inflammation and pain amelioration for the benefits of low side-effects. These receive much attention due to their low side-effects. *Zanthoxylum piperitum* is a summergreen shrub from the *Ructaceae* and *Zanthoxylum* species which grows widely throughout Eastern Asia: South Korea, China, and Japan. *Z. piperitum* has been widely used as a traditional spice, medicine, and for oil refinement because it containing many kinds of spice and active oil ingredient [13–16]. *Zanthoxylum periperitum* has been used as a natural medicine, spice and preservation agent due to detoxification effects, hypertension reduction, stroke, anti-bacterial and anti-oxidant, tyrosinase along with osteosarcoma proliferation-control [17–20]. But, *Zanthoxylum piperitum's* ability to ameliorate and treat osteoarthritis is unknown, and there are few structured studies on its biological activity.

In this study, we investigated ameliorating effects on pain and inflammation of. *Z. piperitum* extract (ZPE-LR) through tissue observation, microbiological change, and biological marker such as p65, COX-2 and iNOS.

2. Materials and methods

2.1. Experimental design

2.1.1. Fractionation scheme

Zanthoxylum piperitum DC was collected at Jeju of Korea. *Z. piperitum* DC dried leaves and ramulus were ground into a powder and subsequently extracted with 90% EtOH at room temperature (ZPE). Solutions were filtered and evaporated in a vacuum to give 90% EtOH extract. *Z. piperitum* dried material (10 kg) was ground into a powder and extracted with 90% EtOH for 6 h, 3 times. The 90% EtOH extracts (500 g) were evaporated in a vacuum and suspended in distilled water (800 mL). The suspension was successively extracted with *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-BuOH.

2.1.2. Active component isolation from Z. piperitum

The EtOAc fraction 9.8 g was subjected to open column chromatography (elution from 40% MeOH) using a glass column (5 × 50 cm) packed, to separate 7 fractions. Fraction 6 was again subjected to open column chromatography (elution from 100% MeOH). Fraction 6 was divided into 3 different samples. If 6b fractions were detected as a spot identical to tannic acid on a TLC plate, they were concentrated and completely dried, followed by medium-pressure liquid chromatography (MPLC, TELEDYNE ISCO, USA) using a RediSep (130 g vol.) flash column (elution from 50%MeOH). The obtained 100 mg subfraction was purified with preparative HPLC (Agilent, USA) using a reverse phase column packed with octadecylated silica (gradient elution from 20:80 to 70:30 of MeOH:H_2O), to separate active fractions at 10–20 minutes in which compound 1 and 2 were detected. These were combined and dried under reduced pressure. When analyzed using afzelin (Sigma, USA) as a standard, the active fractions separated above included 97.0% (g/g) afzelin based on the fraction weight.

2.1.3. Z. piperitum active compound determination

Purified compounds 1 and 2 were dissolved in a 1.5 N hydrochloric acid solution and subjected to hydrolysis in a 100°C water bath for 30 min to obtain quercetin and afzelin, which were analyzed compared to standard (Sigma, USA) by HPLC (Fig. 1B, C and D).

2.1.4. Animals

Twenty 8-week-old male Sprague-Dawley rats, weighing 180–220 g, were purchased from Raon Bio Inc, Korea. Rats were housed in solid-bottomed plastic cages designed to allow easy access to standard laboratory food and water and kept in sanitary ventilated animal rooms with a controlled temperature (25 ± 1 °C) and regular light cycle (12 h light: 12 h dark). Animal experiments were conducted in accordance

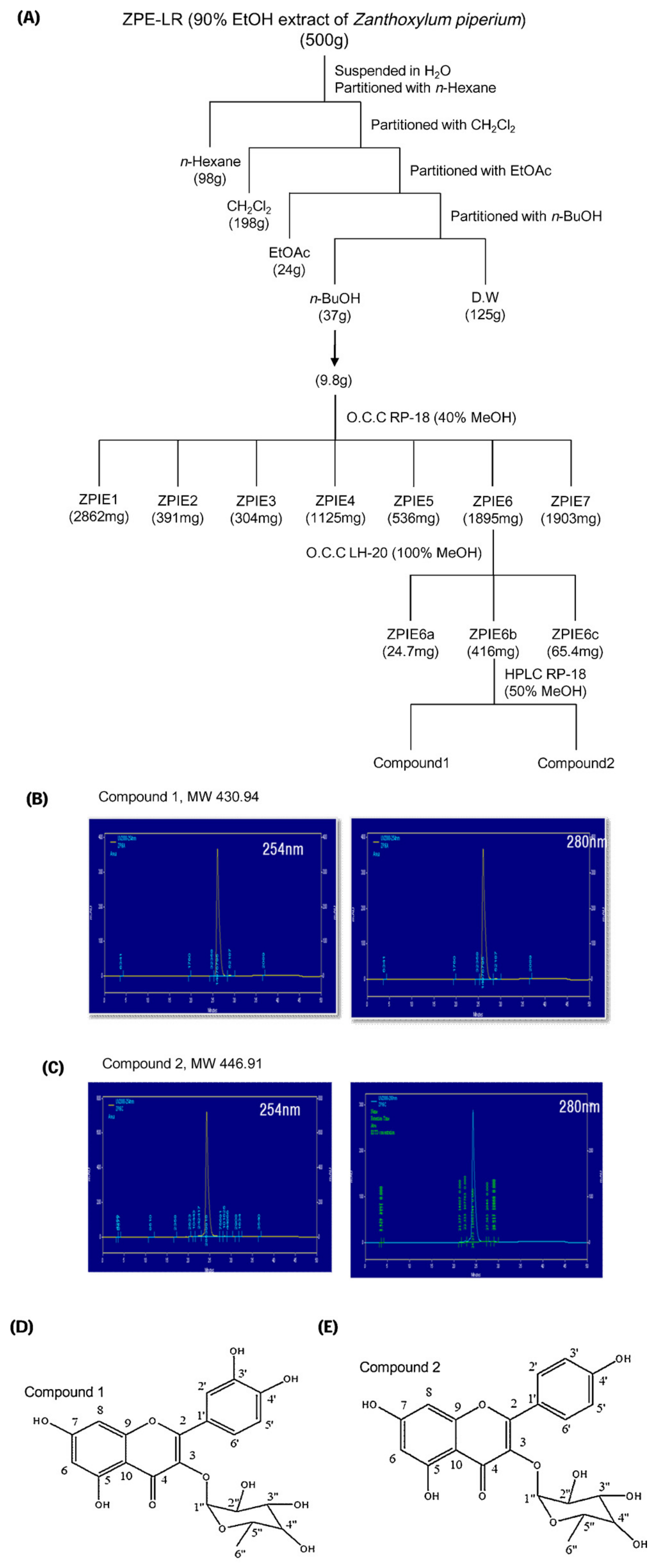

Fig. 1. Fraction and HPLC chromatogram scheme for the active fraction obtained from *Z. piperitum* extract (ZPE-LR). (A) Extraction and solvent partitions from *Z. piperitum*. (B) and (C) Compound 1 and 2 separation were conducted on a Symmetry C18 column, 10 μL injection at 37°C, detection at 254 and 280 nm by HPLC. (D) and (E) Compound 1 and 2 structure.

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