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Research Article
Protective Effect of Pueraria Flower in the Treatment of Osteoarthritis Rat by Attenuating Inflammatory Pathway

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Abstract

Background and Objective: Osteoarthritis is a chronic joint disorder and the conventional drug used for the management of it has several limitations. This study used a rat model of osteoarthritis (OA) to investigate the protective effect of *Pueraria lobata* Ohwi (PF), a plant commonly referred to as kudzu in the treatment of this disease. **Materials and Methods:** The OA was induced by injecting 50 μL of monosodium iodoacetate into the intra-articular space through the patellar ligament. The rats were then treated with 250 and 500 PF mg kg^{-1} for the next 21 days. The weight-bearing distribution ratio and serum cytokine concentrations were assessed at 7, 14 and 21 days. The expression of inflammatory mediators including inducible nitric oxide synthase, cyclooxygenase-2 and matrix metalloproteinases-3 and 9, in the knee tissues of the OA rats was also evaluated, together with the histopathological changes as seen on haematoxylin and eosin-stained sections. **Results:** The results showed that the weight-bearing distribution ratio was significantly higher in the PF groups than in the OA group. The expression of inflammatory mediators was significantly lower in the knee tissues of the PF group than in those of the OA group, a result that was confirmed histopathologically. **Conclusion:** Based on these findings, PF by inhibiting the expression of pro-inflammatory mediators and cytokines, merits further examination for its efficacy in the treatment of OA in humans.

Key words: Pueraria flower, osteoarthritis, cytokines, pro-inflammatory mediators, monosodium iodoacetate, joint disorder, knee tissues

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Osteoarthritis is a chronic disorder of joint characterized by inflammation of joint, degeneration of cartilages[1]. Pathogenesis of OA mainly includes the activation of proteolytic enzyme and production of cytokine enhances that contributes in the degeneration of cartilage[2]. Expressions of cytokines such as IL-1β, IL-6 and TNF-α found to be enhanced in the knee joint which result in the degeneration of cartilaginous extracellular matrix (ECM) and also causes inflammation of joint[3]. Reported study also reveals that damage of cartilage also occurs due to increase in the production of reactive oxygen species[4]. Drugs that used for the management of OA mainly reduce the pain such as NSAIDs[5]. However, a study reveals that these drugs shows only slight beneficial effect than placebo and some serious adverse drug reactions are associated with these drugs as in OA patient has to take the drug chronically[6].

In the recent era use of alternative medicine for the management of chronic disorders like OA gained spotlight. Pueraria lobata Ohwi from Leguminosae family commonly called as Kudzu in Asian countries[7]. Flowers of Kudzu regularly used in China for the management of obesity and hangover[8]. In addition, traditionally it is used for the treatment of complications associated with menopause, liver injury and intoxication of alcohol by many Asian countries[9-11]. Flowers of pueraria reported to have many pharmacological activities such as the anti-inflammatory, antioxidant, hormone regulating and anti-mutagenic activity[12-15]. In osteoarthritis expression of MMP-2 and 9 reported to be altered which cause cartilage damage[16]. Pueraria flower attenuates the expression of MMP-2 and 9 and thereby regulating the ERK1/2 signalling, thereby shows wound healing activity[17]. Thus present investigation evaluated the protective effect of pueraria flower in osteoarthritis in rat model.

MATERIALS AND METHODS

Preparation of extract: Pueraria flowers were collected in the autumn by a botanist and the plant was authenticated by a second botanist, Dr. Wu Yang of The Second Affiliated Hospital of Heilongjiang University of Chinese Medicine, China. The collected flowers were dried in the shade and ground to a coarse powder, which was then boiled in distilled water for 2 h. The resulting solution was then concentrated under low pressure, filtered through filter paper, lyophilised and kept at 4°C until further use. Prior to its use for treatment, the Pueraria flower extract (PF) was dissolved in distilled water.

Animals: Male Wistar rats (180–200 mg kg^{-1}) 6 weeks of age were procured from the SLAC Animal Laboratory, Shanghai. All rats were housed under the controlled conditions described in the guidelines. The use of the animals in the experiments was approved by the Institutional Animal Care and Use Committee (IACUC) of Shandong Provincial Hospital Affiliated to Shandong First Medical University, China (IACUC/SPH-SU/2017/19).

Acute toxicity study: The acute toxicity of the PF extract was tested as per OECD guidelines (423). All rats were fasted overnight before those in the treatment group were administered a dose of 2000 mg kg^{-1}, which is below the lethal dose (LD_{50}). The effective dose of PF is 1/10th and 1/20th of the LD_{50}.

Experimental protocol: The animals were anaesthetised using isoflurane. The OA was induced by the administration of 50 µL of monosodium iodoacetate, injected into the intra-articular space through the patellar ligament using a 26.5-G needle[18]. The animals were divided into four groups: a control group in which the articular space was injected with saline, an OA group and two PF-treated groups, orally administered 250 or 500 mg kg^{-1} PF for 3 weeks. At the end of the study, serum biochemical, weight-bearing and histological parameters were assessed.

Assessment of the weight-bearing distribution: Alteration of the weight-bearing distribution ratio of rats in the 4 groups was estimated using an incapacitance tester. The weight-bearing force on the hind limb during a 3 sec period was determined and the weight-distribution ratio was then calculated according to the following equation:

$$\text{Weight distribution ratio} = \frac{\text{Weight on right hind limb}}{\text{Weight on left hind limb+weight on right hind limb}} \times 100$$

Serum cytokines: Serum was separated from the collected blood by 10-min centrifugation at 1500×. The serum levels of the cytokines prostaglandin (PG)E_2, IL-6, TNF-α and IL-1β were measured using ELISA kits.

Real-time PCR: Total RNA was extracted from the joint tissues using the RTI reagent and cDNA was then prepared by reverse transcription. The cDNA, together with GAPDH as the control was amplified and quantified using SYBR green and a real-time polymerase chain reaction (RT-PCR) kit (*Taq*Man® Universal PCR master mix containing DNA polymerase), in accordance with the manufacturer's instructions. The reaction conditions were as follows: 2 min at 50°C, 10 min at 94°C, 15 sec at 95°C and 1 min at 60°C for 40 cycles. The Ct method was

used in accordance with the manufacturer's instructions to estimate the target gene concentration.

Histopathological evaluation: The rats were euthanised by cervical dislocation. The isolated knee joints were fixed in 10% formalin, paraffin-embedded and sectioned using a microtome. The sections were stained with haematoxylin and eosin (H and E) and evaluated histologically by light microscopy.

Statistical analyses: All data are expressed as means±SD (n = 10). One-way ANOVA was performed using GraphPad Prism software (ver. 6.1, GraphPad Software Inc., San Diego, CA, USA). *Post hoc* comparisons of means were carried out using Dunnett's *post hoc* test. The level of statistical significance was set at $p<0.05$.

RESULTS

Effect of PF on the weight-bearing distribution ratio: The results in Fig. 1 show the effect of PF on the weight-bearing distribution of the four groups of rats. In the OA group, the weight-bearing distribution ratio was significantly lower than in the saline control group. In the PF groups, the weight-bearing distribution ratio was significantly higher than in the OA group.

Effect of PF on serum cytokine levels: The data in Fig. 2a-d shows the serum cytokine levels of the 4 groups of rats and

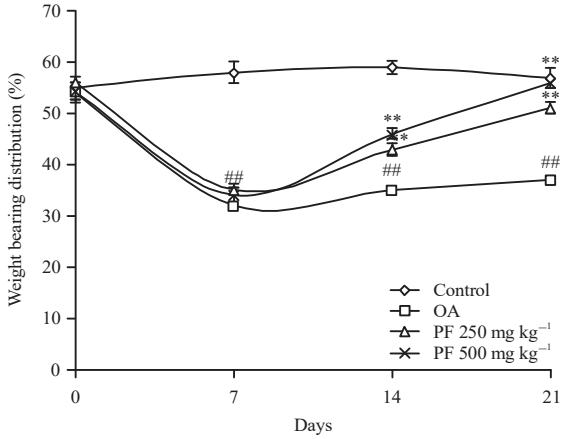

Fig. 1: Effect of *Pueraria* flower (PF) extract on the weight-bearing distribution in rats with osteoarthritis (OA)
Mean±SD (n = 10), ##p<0.01 compared to the saline-treated control group, **p<0.01 compared to the untreated OA group

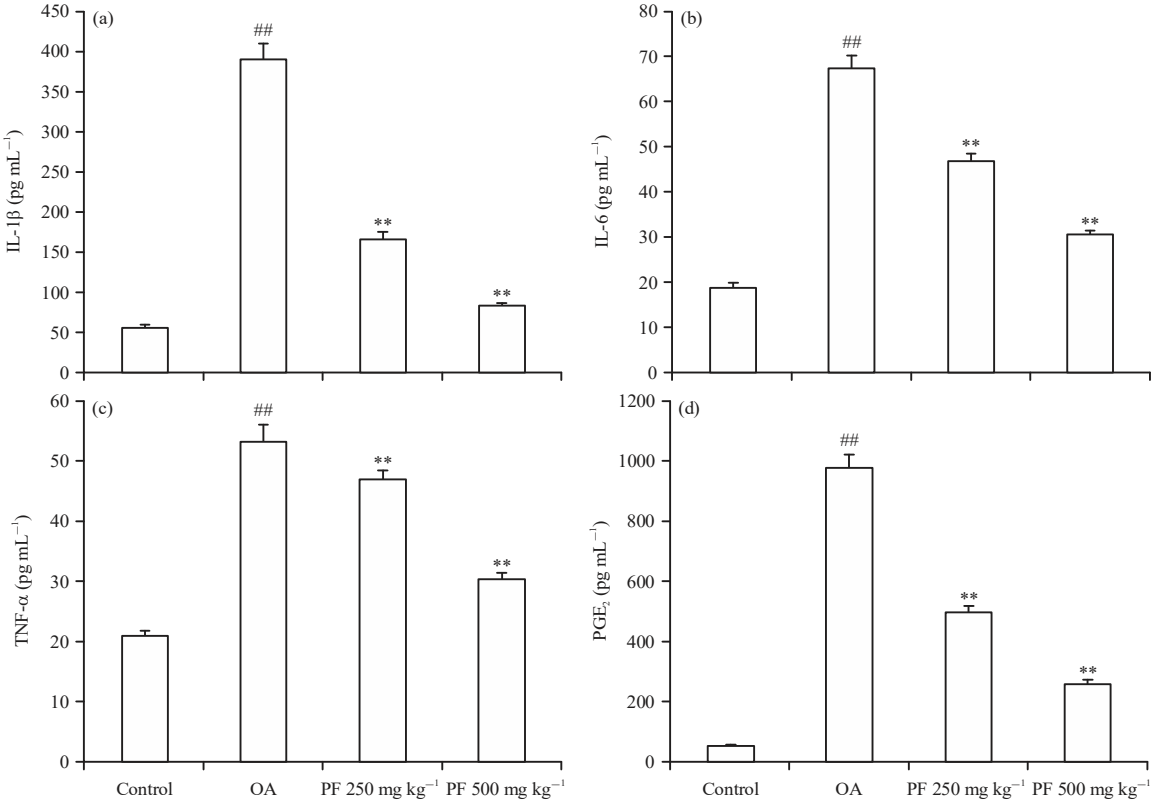

Fig. 2(a-d): Effect of PF on the serum cytokine levels in OA and PF-treated OA rats (a) IL-1β, (b) IL-6, (c) TNF-α and (d) PGE_2
Mean±SD (n = 10), ##p<0.01 compared to the saline-treated control group, **p<0.01 compared to the untreated OA group

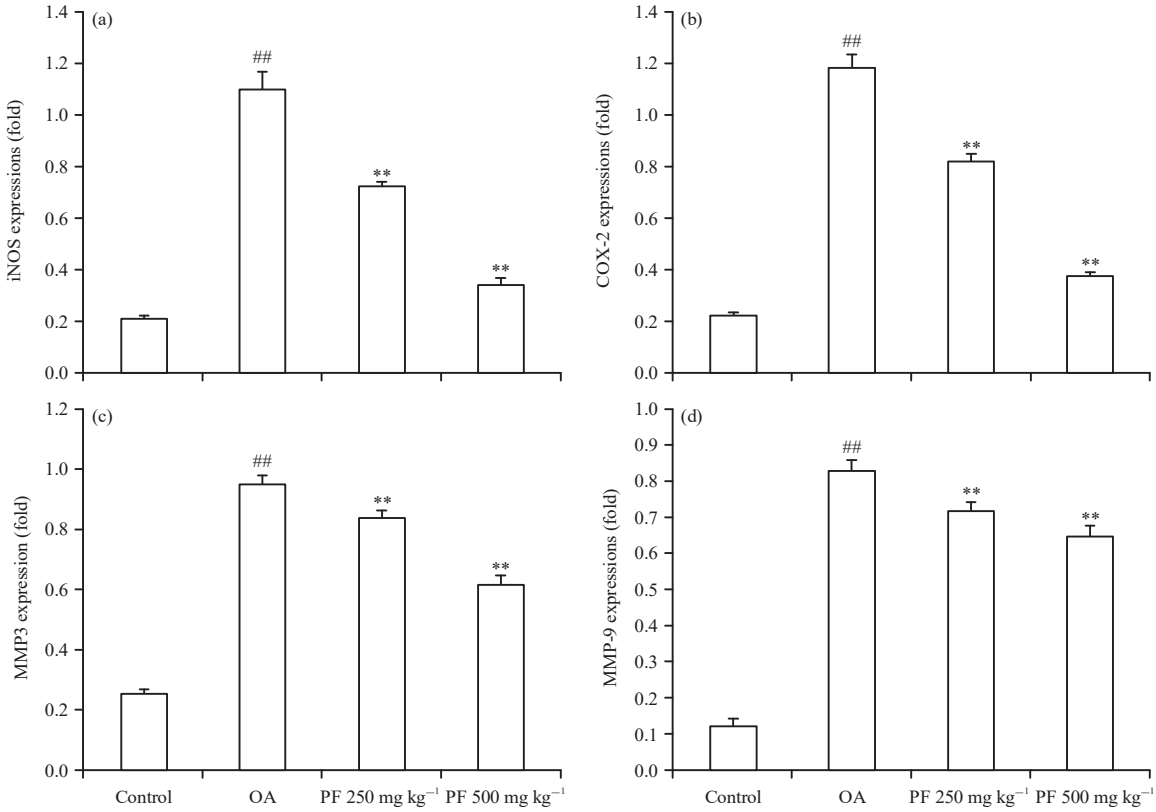

Fig. 3(a-d): Effect of PF on the expression of inflammatory mediators in the knee tissues of OA rats (a) iNOS, (b) COX-2, (c) MMP-3 and (d) MMP-9

Mean±SD (n = 10), ##p<0.01 compared to the saline-treated control group, **p<0.01 compared to the untreated OA group

the effects of PF. Serum levels of PGE2, IL-6, TNF-α and IL-1β were significantly higher in the OA group than in the control rats. The PF treatment, however, dose-dependently attenuated the enhancement in OA rats.

Effect of PF on inflammatory mediators: The effect of PF on the expression of the inflammatory mediators cyclo-oxygenase (COX)-2, iNOS, MMP-9 and MMP-3 in the knee tissues of OA and PF-treated rats is shown in Fig. 3a-d. The levels of these mediators were significantly higher in the knee tissues of OA rats than in those of the saline control rats. Compared to those in the OA rats, the levels in the PF-treated rats were significantly lower.

Effect of PF on the histopathology of knee joint tissues: Figure 4a-d shows the effect of PF on the histopathology of the knee joint tissues of the 4 groups of rats, evaluated on H and E-stained sections. Severe symptoms of arthritis, including cartilage erosion, synovial hyperplasia, exudation into the synovial space and the infiltration of inflammatory cells were clearly seen in the OA rats. In the PF-treated rats, however, both the synovial hyperplasia and the cartilage damage were attenuated.

DISCUSSION

Present investigation determined the osteoprotective effect of methanolic extract of pueraria flower in osteoarthritis rat model. Effect of PF estimated by weight bearing distribution and level of proinflammatory mediators and cytokines were estimated in the serum and knee tissues of OA rats. It was observed that weight bearing distribution was reduced in PF treated group in a dose dependent manner in OA rats. In the recent time herbal/alternative medicine showed potential to manage chronic disorders such as osteoarthritis[19].

This study evaluated the effect of pueraria flower in the management of osteoarthritis. Phytochemical investigation on pueraria flower reveals that it contains several pharmacologically active isoflavone such as genistein, glycitin, tectoridin, diazine viz[20]. These polyphenolic compounds showed the similar action as that of estrogen and thus these are called as phytoestrogens[15]. Phytoestrogen has shown well documented potency in the management of OA[21]. Pain in the osteoarthritis decreases the movement of joint and the drug that relives the pain enhances the mobility of joints in osteoarthritis. Here, PF effectively protected the weight

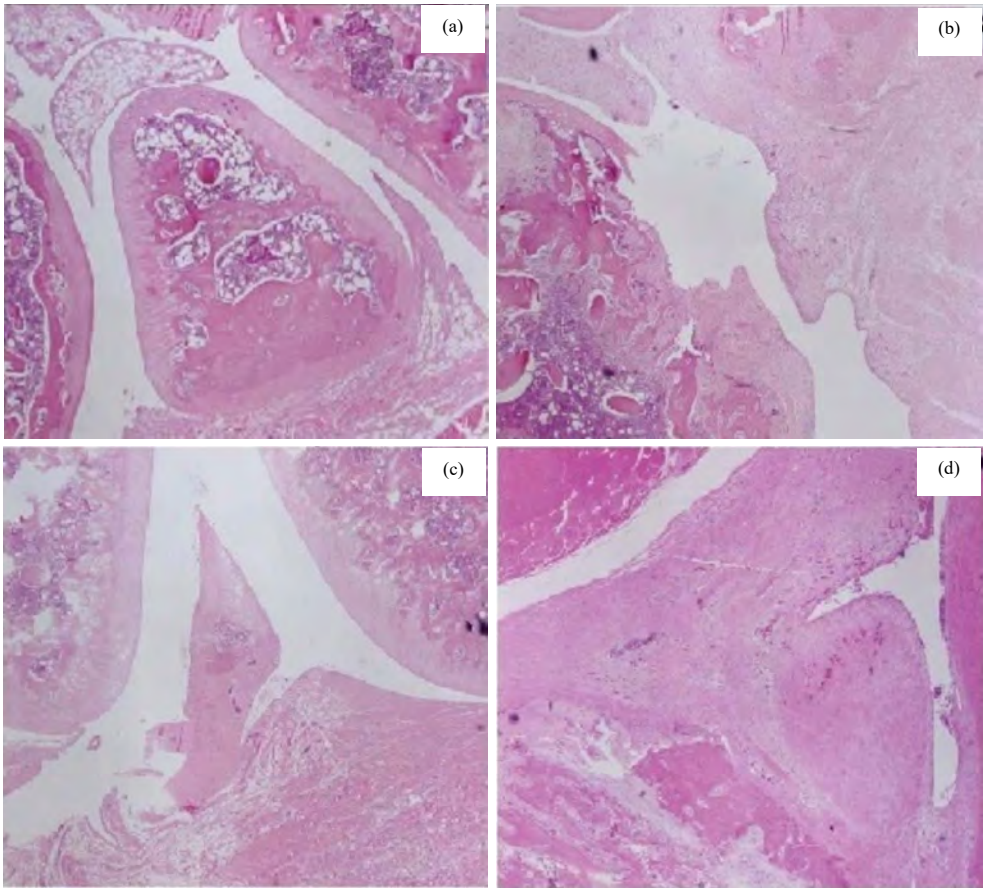

Fig. 4(a-d): Effect of PF on the histopathology of knee joint tissues of OA rats (a) Saline control, (b) OA, (c) PF 250 mg kg^{-1} and (d) PF 500 mg kg^{-1}

bearing in OA rats and that suggested it reduces the pain. In OA destruction of cartilage results due to inflammatory mediators as these mediators are catabolic in nature and that influence the pathophysiology of osteoarthritis[22].

Data of the study suggested that the treatment with PF significantly decreases the level of cytokine in the serum of OA rats than OA group. Data of the study is supported by the previously given reports. Literature revealed that dysregulation of anti-inflammatory and pro inflammatory pathway results in OA[22]. Balance of cartilage repair and damage was disturbed by pro-inflammatory mediators such as PGE2 and NO[23]. This results in degradation of joint in OA and thus for the management of OA important approach is to inhibit the pro-inflammatory cytokines. Result of the study suggested that the PF attenuated the OA by inhibiting the inflammatory mediators and cytokines.

CONCLUSION

Data of the investigation revealed the protective effect of methanolic extract of pueraria flower against osteoarthritis by inhibiting the pro-inflammatory mediators and cytokines. Result of the study suggested that PF could be use clinically for the management of osteoarthritis.

SIGNIFICANCE STATEMENT

This investigation discovers the protective effect of pueraria flower against osteoarthritis rat model. Moreover, methanolic extract of pueraria flower also reported to reduces the level of pro-inflammatory mediators and cytokine in the serum and knee tissues of osteoarthritis rats. It also decreases iNOS, MMP-9 and MMP-3 in the knee tissues of osteoarthritis rats. This study provides an alternative therapy for the management of osteoarthritis.

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