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Original Research Article

Comparison of *Elaeagnus angustifolia* L. extract and quercetin on mouse model of knee osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is the most commonly observed arthritic disease causing severe pain and impairing patient's quality of life. This study aimed to investigate and compare the effect of *Elaeagnus angustifolia* extract and quercetin on the mouse model of knee osteoarthritis (OA). Sixty Balb-C mice were used to establish the monosodium iodoacetate (MIA) model of OA. Then, they were randomized into untreated OA group (normal nutrition), *E. angustifolia* extract-treated group (32 mg/kg by gavage), quercetin-treated group (20 mg/kg by gavage) and ibuprofen- treated group (20 mg/kg). Fifteen mice with no MIA treatment were considered as the normal controls. The mice were treated for 28 days. The histopathological analysis was performed on knee joints. Expression levels of matrix metalloproteinase 3 and 13 (MMP-1 and MMP-13) in serum were assessed in addition. Histopathological study indicated that in the quercetin-treated group, the thickness of femur and tibia were significantly increased ($P < 0.05$). Among groups treated by *E. angustifolia* extract, quercetin and ibuprofen, the concentration of MMP-3 was 5.47 ± 1.75 ng/ml, 4.38 ± 1.78 ng/ml and 4.86 ± 1.40 ng/ml, respectively. The level of MMP-13 in sera was 3.32 ± 1.64 ng/ml, 2.67 ± 1.73 ng/ml and 5.31 ± 1.68 ng/ml in the same order ($P < 0.05$).

The results of this study suggest that the quercetin was useful in the reduction of symptoms of OA and raised the improvement of damaged cartilage. Hence, it can be a beneficial medical supplement in OA treatment. Besides, *E. angustifolia* extract and quercetin significantly reduced the serum MMP-3 and MMP-13 concentrations. It could be one of the mechanisms through that *E. angustifolia* plays a role in remission of OA.

1. Introduction

Osteoarthritis (OA) is a musculoskeletal disorders that causes degenerative changes in cartilage and impairing joint function [1]. It is estimated that over half of the population in the seventh decade of life suffers from OA [2,3]. An increasing number of the elderly population makes OA as a global medical concern in forthcoming years. Major characteristics of OA are progressive degeneration of articular cartilage and new bone formation at the center and margin of joints. The disease is not only limited to the cartilage, but also the synovial joint and all the associated tissues such as synovium, subchondral bone, meniscus and ligaments are involved. The progressive destruction of the articular cartilage is accompanied by deformation of the subchondral bone, and over time this deformation leads to formation of osteophyte around the joint and ultimately restricts the joint movement. OA can affect all joints; however, it is more common in weight-bearing joints such as knee and hip [4–6].

Matrix metalloproteinase (MMP) enzymes play an important role in the development of OA [7]. During the progression of OA, inflammatory mediators (for instance, interleukins) are secreted which stimulate the production of MMPs [8]. MMPs are subdivided in several groups according to their specificity and domain organization [9]. MMP-3 belongs to stromelysin class of MMPs. Previosuly published literature indicated that MMP-3 can serve as an OA diagnostic target based on it's crucial role in MMP cascade which leads to degradation of extracellular matrix [7,9–11]. Also,

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active form of MMP-13 or collagenase-3 plays a dominant role in cartilage destruction by hydrolyzing the principal component of cartilage matrix, type II collagen and induces the onset of OA. Pathological chondrocytes produce MMP-13, its circulating concentration elevates in parallel to the progress of the disease [8,9,12].

Although the physiological and molecular determinants and symptoms of OA are well-known and its associated risk factors have been studied in detail, no definitive treatment has yet been established to cure it [13].

Iranian traditional medicinal system introduced the Russian olive (*Elaeagnus* angustifolia L.) as a potential treatment for OA. More than 90 species of *E.* angustifolia are available worldwide. They are widely distributed in north Asia to the Himalayas and Europe. The species, *E.* angustifolia, is used in Iranian folk medicine as an anti-inflammatory and analgesic in the treatment of rheumatoid arthritis [14]. Its fruit contains minerals such as potassium, magnesium and sulfur, various fatty acids like palmitic, oleic and linoleic acids. Besides, several phenolic acids were reported in Russian olives (e.g. caffeic acid, valinic acid, protocatechuic acid, etc.) and flavonoids which exist in different variations including quercetin. It belongs to a subclass of flavonoids named flavonols. Quercetin glycosides are ubiquitously found in the plant kingdom [15] in various kind of fruits and vegetables especially in onions, apples, cherries, grapes, berries, tea, as well as some seeds [16]. Flavonoids like quercetin are antioxidants. They scavenge the reactive oxygen species (ROS) produced during the metabolic procedure in aerobic organisms. ROS in low concentration plays an influential role in signaling pathways regulating the immune system. On the other hand, excessive ROS can be harmful to biomolecules, and this may lead to inflammation-related diseases, including OA. Although organisms have their own cellular antioxidant system; sometimes to overcome or diminish the adverse effects of oxidative stress, intake of antioxidant molecules is required [17]. This strategy can be beneficial in achiebving prevention or reduction of the damage by ROS. Several studies demonstrated that Russian olives posses analgesic and antioxidants characteristics and hence are useful in treating arthritis [14,18].

The present study aims to investigate the curative effects of *E.* angustifolia extract and quercetin on knee OA and compare them with standard treatment (ibuprofen) in terms of the expression of matrix metalloproteinase-3 (MMP-3) and matrix metalloproteinase-13 (MMP-13) in serum and histopathological assessment. Hence, this study was designed to report the protective effects of *E.* angustifolia extract on knee OA and to compare effects of *E.* angustifolia extract and quercetin, as its major active ingredient.

2. Materials and methods

2.1. Plant material

In order to prepare *E. angustifolia* extract [Herbarium of National Plant Gene Bank of Iran (HNPGBI) 6868], the whole fruits (including peel, flesh and seed) were dried and powdered (150 g). Next, they were added to 1000 ml of boiling water and boiled for 20 min while shaking. The extract was filtered, evaporated and stored at −20 °C. The obtained dried extract was 20 g.

2.2. Total polyphenol and flavonoid concentration and antioxidant activity

The amount of total polyphenol content was determined by Folin- Cicalteau method using gallic acid as the standard. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, Germany), free radical scavenging method was used to evaluate antioxidant activity of the extract according based on the method reported by Cansev et al. [19]. The flavonoid concentration was assessed by Aluminum Chloride colorimetric assay based on the method reported by Lin et al. [20].

2.3. Animals

Seventy-five ten-week-old male Balb-C mice weighting 20 ± 5 g were provided by Tehran University Faculty of Veterinary Medicine. They were maintained under the standard environmental condition of temperature (20–23 °C), humidity (50 ± 5%) and photoperiod 12-h light: 12-h dark cycle and were allowed to consume food and water ad libitum. After acclimatization to the laboratory for ten days, they were randomized in five experimental groups each of 15 members. The experiment was done in accordance with the guidelines of the Publication Principle of Laboratory Animal Care (NIH publication n. 86–23, revised 1985) and certified by Central Tehran Branch of Islamic Azad University in 2016 (certificate No. 10130517942003).

2.4. OA model

Sixty mice were anaesthetized and received a single dose of 0.1 mg monosodium iodoacetate (MIA) (Sigma–Aldrich, Germany) into the left knee, as previously described by Ogbonna et al. [21]. Normal saline was injected to the right knee.

2.5. Dosage

The dried extract was mixed with double-distilled water. The powder of quercetin (Sigma–Aldrich, Germany) was mixed with normal saline. The ibuprofen (Sigma–Aldrich) was dissolved in distilled water. The interventions were introduced after 14 days from MIA-treatment, once daily for 28 consecutive days, mice were treated by following dosage: *E.* angustifolia extract-treated group, 32 mg/kg; quercetin-treated group, 20 mg/kg (the drugs were administered by gavage). Normal controls and untreated group (negative control) were fed typically by standard diet.

2.6. Biochemical analysis

After 28 days of treatment, all animals were sacrificed by narcotic overdose. The blood was collected from orbital sinus and centrifuged for 20 min with 2500 RPM at 4 °C to obtain serum for biochemical analysis. The concentrations of MMP-3 and MMP-13 were assessed by MMP kit (Bioassay Technology Laboratory, China). The present analysis was performed in triplicates.

2.7. Histological study

The soft tissue of left knee joint was removed. Samples were stored in 10% formaldehyde. Decalcification was achieved by treatment with 5% nitric acid. Serial 5-μm sagittal sections were cut. Finally, slides were stained by hematoxylin and eosin. The thickness of tibial cartilage (TTC), the thickness of femoral cartilage (TFC), number of tibial chondrocyte (NTC), number of femoral chondrocyte (NFC), along with inflammatory cells of the tibia (ICT) and femur (ICF) were assessed by microscopic observation.

2.8. Statistical analysis

Results were expressed as Mean ± SD. One-way ANOVA was used to compare groups. Tukey correction was used as the post-hoc test. $P < 0.05$ was considered as significant. All statistical analysis was calculated by SPSS version 23 (SPSS Inc, USA).

3. Results

3.1. Confirmation of OA-model formation

The injection of MIA given in a period of 14 days, caused apparent alteration in cartilage structure and its surface fibrillation. Besides, the subchondral bone was exposed and the number of chondrocytes and cartilage thickness were diminished. Therefore, the effect of MIA injection was time-dependent. The articular cartilage of both tibia and femur were uneven on the surface and in terms of fibrillation, particularly after 28 days.

3.2. Polyphenols and flavonoids content and antioxidant capacity

Average total phenols of the *E. angustifolia* extract was measured as 0.043 mg gallic acid equivalents (GAE)/g of dried extract. Average of total flavonoid content of the extract was 0.014 mg quercetin equivalents (QE)/g of dried extract. DPPH assay determined the antioxidant activity of *E. angustifolia* extract. The antioxidant capacity of the extract was 5.72 μg/ml.

3.3. Histopathological analysis

In the group of normal control, no evidence of OA was observed. The articular cartilage was intact, and the lack of damage was obvious (Fig. 1). In OA group the articular cartilage profoundly defected. In *E.* angustifolia extract-treated group, the tibial and femoral cartilage thickness was significantly higher in comparison to the OA group and also to the ibuprofen group ($P < 0.05$). It is noteworthy that the change in thickness of mentioned cartilages in this group is less than the quercetin-treated group ($P < 0.05$). The number of tibial and femoral chondrocytes were higher in this group compared to the ibuprofen-treated group ($P < 0.05$). But, the numbers were less compared to quercetin-treated group ($P < 0.05$). Counting inflammatory cells revealed that, the *E.* angustifolia extract had the highest reduction among the treated groups ($P < 0.05$). The quercetin-treated group showed significant increase of TTC, TFC, NTC and NFC compared to other groups ($P < 0.05$) indicating that quercetin is influential on OA. In addition, quercetin treatment reduced ICT and ICF significantly ($P < 0.01$); however, the effect was less significant than *E.* angustifolia extract. The change in TTC and TFC were not significantly different compared to the OA group. There was no significant change in the number of tibial chondrocytes compared to the OA group. On the other hand, NFC in both treated groups were significantly higher than OA model ($P < 0.05$). The highest NFC was observed in mice treated by quercetin in comparison to *E. angustifolia* extract-treated group ($P < 0.05$) (Fig. 2).

3.4. Concentration of MMP-3 in serum

The mean concentration of MMP-3 protein in serum of normal control, quercetin- and ibuprofen-treated groups were in the same range (Fig. 3a). The presence of MMP-3 in the OA group was significantly higher compared to normal control ($P < 0.05$). The quercetin reduced the concentration of MMP-3 significantly ($P < 0.05$). The *E. angustifolia* extract and ibuprofen groups did not show any significant decrease of MMP-3 in serum ($P > 0.05$).

3.5. Concentration of MMP-13 in serum

The *E. angustifolia* extract and quercetin sharply reduced the concentration of MMP-13 serum level ($P < 0.01$). This is probably because of their flavonoid nature, causing suppression of the enzyme (Fig. 3). Besides, the results showed that the effect of E. *angustifolia* extract and quercetin were significantly higher compared to ibuprofen ($p < 0.01$).

4. Discussion

The goal of this study was to evaluate and compare the effect of *E. angustifolia* extract and quercetin as one of its fractions on knee OA. The MIA-induced knee OA models were applied to achieve this goal. The OA manifested in mice by a reduction of synovial fluid, cellular damage and decrease in the number of chondrocytes. The results were consistent with similar studies [22,23]. The histopathological analysis revealed that after the intervention of *E. angustifolia* extract and quercetin, the degree of articular cartilage defects was significantly reduced compared to the negative control. Results also defined that *E. angustifolia* extract had anti-inflammatory effects on femur and tibia compared to the OA untreated group. In addition, the number of chondrocytes was more in

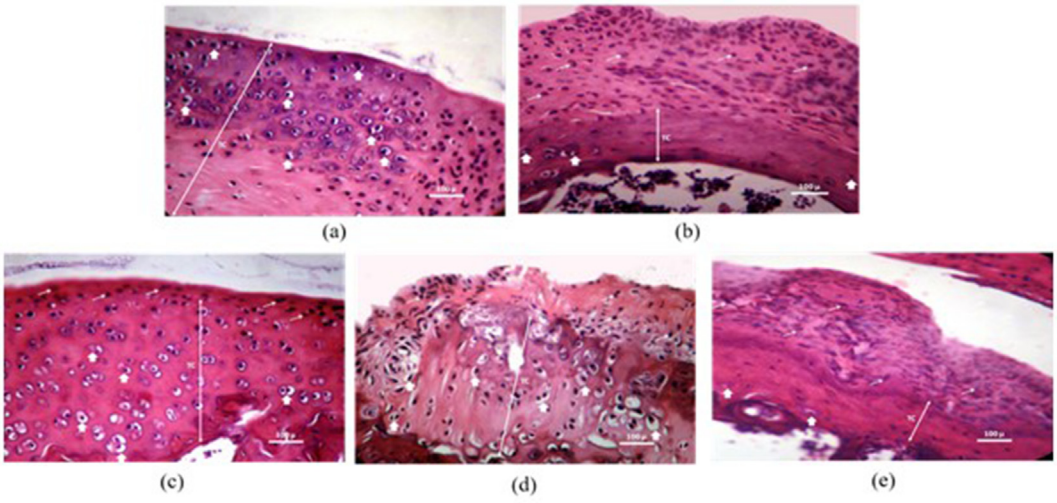

Fig. 1. Histopathological evaluation of knee joints. (a) normal, (b) OA model, (c) quercetin-treated group, (d) *E.* angustifolia extract-treated group, (e) ibuprofen-treated group (hematoxylin and eosin staining, x40).

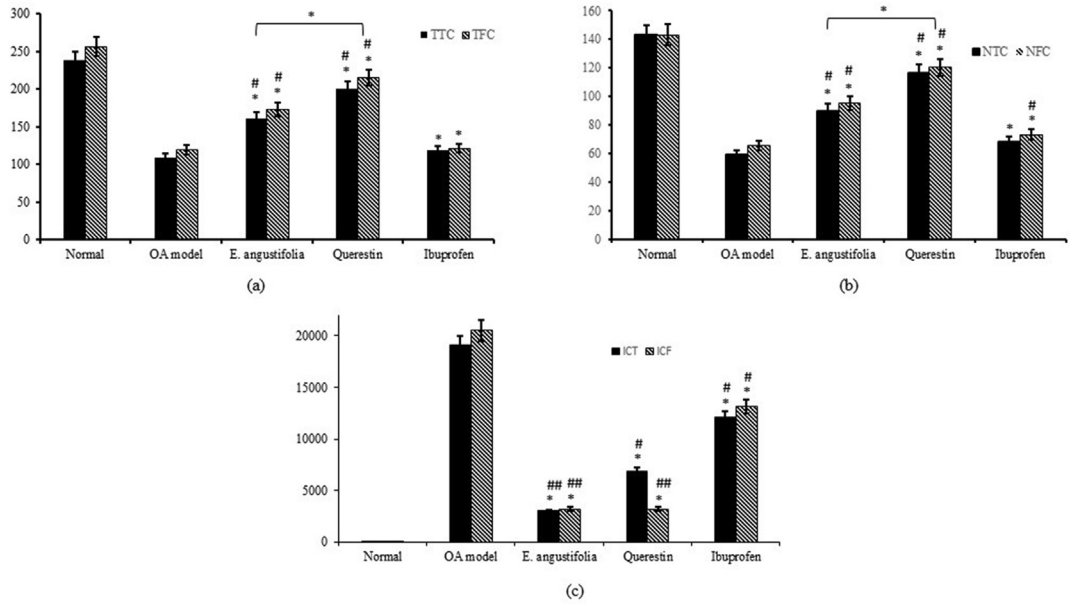

Fig. 2. (a) The thickness of tibial cartilage (TTC) and thickness of femoral cartilage (TFC), (b) Number of tibial chondrocytes (NTC) and number of femoral chondrocytes (NFC), (c) inflammatory cells of tibia (ICT) and inflammatory cells of femur (ICF) (* comparing with normal or other groups, $P < 0.05$; # comparing with OA model, $P < 0.05$; ## comparing with OA model, $P < 0.01$).

the quercetin group compared to other groups ($P < 0.05$). It was interesting that the number of chondrocytes in both tibia and femur increased in quercetin group compared to *E. angustifolia* extract-treated group, indicating that the extract has significant anti-inflammatory property. It can be suggested that the curative characteristic of *E. angustifolia* extract is probably because of anti-inflammatory agents and micronutrients existing in the whole fruit. Several studies have reported the significance of *E.* angustifolia extract in treatment of OA. In addition, some studies focused on evaluating the effect of quercetin (a major flavonoid glycoside of E. *angustifolia*) on OA. For instance, Panahi et al. demonstrated that *E. angustifolia* extract reduced the symptoms of OA patients [18].

During oxidative stress, chondrocytes undergo apoptosis and then autophagy plays an essential role in the progression of OA [24]. Although, the cellular antioxidant system of cells is activated; an imbalance appears because of the excessive ROS in damaged tissue [25]. In the knee joint, the presence of excessive ROS leads to chondrocyte matrix destruction, senescence and apoptosis of chondrocytes [26]. It appears that the damage to chondrocytes can be diminished by reducing ROS [27]. Various concentrations of flavonoids, alkaloids, saponins and terpenoids are found in Russian olives. These compounds possess antioxidant activity, and it seemed to be the reason why *E. angustifolia* extract attenuated the progress of OA in treated mice. The potential ability of this extract on the attenuation of OA pain and biochemical decrease of MMPs and inflammatory cytokines have been documented in several studies [28,29]. It is crucial to mention that the anti-inflammatory effects of the extract were significantly higher compared to quercetin; on the other hand, effect of quercetin on the chondrocytes and cartilage tissue was significantly higher compared to the extract. The positive effects of quercetin on OA have been well documented [30–32].

MMPs are considered as one of the major physiological factors in extracellular matrix degeneration [28] which is a hallmark of OA. Inflammatory and structural cells such as chondrocytes produce MMPs [33]. In osteoarthritis, the excessive degradation of extracellular matrix triggers more inflammation. To reverse the situation, natural inhibitors of MMPs should be considered [34]. Two important MMPs in OA are MMP-13 [33] and MMP-3 [35]. In the present study, the effect of quercetin and *E. angustifolia* extract on the presence of these two MMPs were assessed. The obtained results demonstrated a decrease in the levels of MMP-3 and MMP-13 among treated groups compared to the negative control. This result was consistent with the results obtained by Lee et al. [36]. The

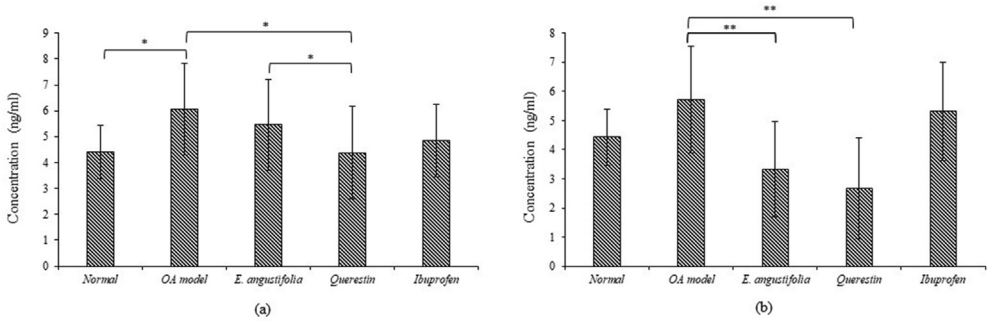

Fig. 3. (a) Level of MMP-3 in serum, (b) level of MMP-13 in serum. * Comparing with other groups, $p < 0.05$. ** Comparing with the other group, $p < 0.01$.

possible mechanism is through the AMPK/SIRT1 signaling pathway as Qiu et al. previously reported [31].

5. Conclusion

The Current study used MIA-induced OA model to evaluate the effects of *E. angustifolia* extract and quercetin on OA progression compared to ibuprofen as the positive control. After 28 days of treatment, the histology of knee joint and the level of MMP-3 and MMP-13 in serum were assessed. The results showed less damage to tibial and femoral bones and cartilage and downregulation of MMP-3 and MMP-13 in blood. The results were in accordance with ibuprofen-treated animals and in some cases, the results were better. Therefore, *E. angustifolia* extract and quercetin can be used as appropriate herbal medicines to confront the progression of OA.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Mitra Heydari Nasrabadi: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data curation, Writing- review and editing, Supervision, Project administration.

Mahtab Parsivand: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing- original draft & review and editing.

Narges Mohammadi: Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing- original draft & review and editing, Visualization.

Nastaran Asghari Moghaddam: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing- original draft & review and editing, Visualization, Supervision, Project administration.

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