

The Effect of *Elaeagnus Angustifolia* Extract on the Joint Friction and Antioxidant Activity in Knee Non-Traumatic Osteoarthritis Model in Rat

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SUMMARY

Object. The objective was to investigate the effect of *Elaeagnus angustifolia* (*E. angustifolia*) extract on the joint lubrication and the activity of antioxidant in non-traumatic knee osteoarthritis model in rat.

Methods. In this study, 28 male albino Wistar rats were randomly assigned into four groups, each comprised of seven rats: Control (healthy animals); Saline (intra-articular injection of 50 µL Saline); monoiodoacetate, MIA (intra-articular injection of 50 µL MIA); and MIA+extract (intra-articular injection of MIA and 500mg/kg daily of *E. angustifolia* extract intraperitoneally for four weeks). Osteoarthritis was induced by injection of 50 µL solution of 3 mg MIA in rats through infrapatellar ligament. Six weeks after intra-articular injection, Joint friction parameters consisting of cycle number, maximum peak, exponential and linear slope of cycle decay, and coefficient of friction (COF) were measured in knee joint. Activity of the superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx) were determined in blood plasma.

Results. MIA injection reduced the cycle number of joint oscillation compared to the Control group. In the MIA+extract group, cycle number and maximum peak increased significantly compared to the MIA group (P=0.032 and P=0.016, respectively). *E. angustifolia* extract resulted in the increase of SOD and GPx activity (P=0.011 and P=0.05, respectively) and the decrease of MDA activity (P=0.000) compared to MIA group.

Conclusions. The results showed that *E. angustifolia* extract is effective to improve the antioxidant enzyme activity and subsequently to decrease the joint friction in the non-traumatic knee osteoarthritis model.

KEY WORDS

Elaeagnus angustifolia extract; knee osteoarthritis; joint friction; Superoxide dismutase (SOD); Malondialdehyde (MDA); Glutathione peroxidase (GPx); rat.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disease in adults, and its prevalence increases with age, causing disability in the elderly population (1). OA is a multifactorial disorder of synovial joints, which is characterized by escalated degeneration and loss of articular cartilage(2). Hyaline cartilage in the diarthrodial joints is serving as a low friction,

wear resistant surface for load support, load transfer and joint motion (3).

Proposed mechanisms including hydrodynamic lubrication, boundary lubrication, elastic deformation, and fluid pressurization are responsible for maintaining the low-friction and good lubrication in the diarthrodial joint (4). The excessive friction accelerates cartilage wear after failure of carti-

lage lubrication. Damage of cartilage may be a key factor in the onset of osteoarthritis (5).

The joint coefficient of friction (COF) is thought to increase with osteoarthritis progression, and this increase may occur due to a decrease of lubricants concentration such as lubricin (6).

Lubricin as a glycoprotein, thought to be a boundary lubricant in synovial fluid and articular cartilage (7) is deficient in aspirates of acute post-traumatic knee effusions, which is likely to be result of inflammatory destruction (7).

Friction measurements are important to determine the relative tribological contributions made by synovial fluid or cartilage (8) and/or to assess the efficacy of therapeutic modalities for preventing the development of osteoarthritis (9).

Evidence from both experimental and clinical studies suggests that oxidative stress plays an important role in the pathogenesis of OA (10, 11). It is indicated that the oxidant parameters including total peroxide (TP), lipid hydroperoxide and Oxidative stress index (OSI) increased and antioxidant parameters such as plasma total antioxidant capacity (TAC), thiol level, catalase activity and prolidase activity decreased in patients with osteoarthritis; therefore, these patients may be exposed to a potent oxidative stress (12). Malondialdehyde (MDA) and superoxide dismutase (SOD) have often been treated as matching indicators, of which the SOD level is associated with free radical scavenging ability, whereas MDA reflects the degree of damage caused by free radicals to the cells. When the level of free radicals was high, it was found that SOD failed to effectively remove them, leading to lipid peroxidation and cell damage (13).

The extracts of leaves and flowers of *E. angustifolia* leaves contain phenolic and flavonoid compounds which have antioxidant properties and protect cells from oxidative damages and delays or reduces the risk of many degenerative diseases. It has shown that the amounts of phenolic and flavonoid compounds are higher in leaves than flower of the plant. The explanation might be due to the process of photosynthesis which occurs in the leaves and also the existence of high amount of flavonoid biosynthetic pathway precursors in the leaves compared to any other organs (14). *E. angustifolia* leaves contain various chemical compounds including amino acids, flavonoids, phenolic compounds, polysaccharides and some other essential elements (15). The polysaccharides are important component of *E. angustifolia* leaves with anti-radiation, antioxidant and immune regulatory activities (16). The anti-inflammatory effects of the fruits of this plant on rheumatoid arthritis and osteoarthritis have been reported (17).

Hence, the aim of this study was to investigate the effect of *E. angustifolia* extract on the joint friction and the activity of antioxidants in the non-traumatic OA model in rats.

MATERIALS AND METHODS

This research was carried out according to the internationally valid guidelines and the institutional animal ethics committee in Baqiyatallah University of Medical Sciences. In this study, 28 adult male albino Wistar rats (12-14 weeks age and weighing 200-250 g) were purchased from the laboratory animal center of the university. The rats were kept under standard housing laboratory conditions (room temperature of 23 ± 2 °C with relative humidity of $60\pm 5\%$, and 12 hr/12 hr light/dark cycles) and were fed with a standard laboratory pellets rat chow and water *ad libitum*. One week after arrival and adaptation, rats were randomly divided into four groups. Each group comprised of seven rats and were observed regularly. Control group (consisted of healthy animals that underwent no intervention); Saline group (50 μ L of normal Saline 0.9%, injected into the right knee joint as a placebo group); Monoiodoacetate group (MIA group, received 50 μ L solution containing 3 mg MIA into their right knee joint); and MIA+ *E. angustifolia* extract group (MIA+ extract group, MIA injection and 500mg/kg body weight daily *E. angustifolia* extract injection intraperitoneally for four weeks (12).

Method of osteoarthritis induction

Osteoarthritis was induced by intra-articular injection of MIA. MIA injection is considered to induce osteoarthritis in joint by damaging glycolysis in the joint, resulting in the eventual death of chondrocytes by inhibiting glyceraldehyde-3-phosphate dehydrogenase activity and increasing inflammation (18). First, the rats of MIA and MIA+extract groups were anesthetized by using Ketamine (50 mg/kg) and Xylazine (5 mg/kg). After shaving the knee skin and bending the knee joint, a single dose of 3 mg MIA (Sigma-Aldrich, St. Louis, MO) dissolved in 50 μ L physiological Saline (0.9%) was injected through infrapatellar ligament of the right knee using a 26-gauge needle. In the Saline group, by using a 26-gauge needle, 50 μ L Saline 0.9% solution was injected into the right knee joint capsule through infrapatellar ligament. At 43th day, all animals in four groups were euthanized by overdose of the anesthetic drugs and were sacrificed to measure the joint friction. For blood plasma analyses, blood samples obtained from anaesthetized mice were centrifuged immediately (Megafuge 1.0 Heraeus Sepatech GmbH) at 3000 rpm for 15 min at +4 °C to determine the activity of

superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx).

Method of preparation and injection of *E. angustifolia* extract

The fruits of *E. angustifolia* were collected from mountain area of Niavaran, Tehran, Iran and was identified and approved by Herbarium Department of Pharmacognosy of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher specimen [No. 1257] is preserved in the herbarium of this department for reference.

The aqueous extract was prepared by adding 1000 ml of distilled water to 100 g of fruit powder (seed, flesh, and peel) and the resulting solution was boiled for 8-10 minutes. Then, the mixture was filtered and the solution was completely dehydrated for 8-10 hours in water bath to provide a crude extract with 20% yield. This extract was then weighed and after being dissolved in physiological Saline, and consequently 14 days after induced OA, 500 mg/kg was injected intraperitoneally in animals in the MIA+extract group, daily for four weeks.

Joint friction (biotribological) measurement

The knee joint was resected following transection of the middle of tibia/fibula and femoral shafts. Friction testing was performed immediately after the joint resection by a pendulum friction tester. The knee joint was placed in the free pendulum tester. Pendulum rotation was calculated for flexion–extension in the sagittal plane and other motion out of this plane was ignored (**figure 1**). Through an automated gated mechanism, movement was started from a fix point and free oscillation around the knee joint recorded. Ravanbod *et al.*, 2011, report more technical details on the friction tester(19).

The pendulum load was approximately half of the body weight of animal. Angular changes were recorded to the equilibrium point at a sampling frequency of 30 Hz. Cycle number to reach equilibrium, maximum peak oscillation value, exponential and linear slope of cycles decay were calculated with Labview 7.1 Software (National instrument Labview 7.1 software, Austin, TX, USA). Coefficient of friction (COF) was calculated according to Stanton's equation (19).

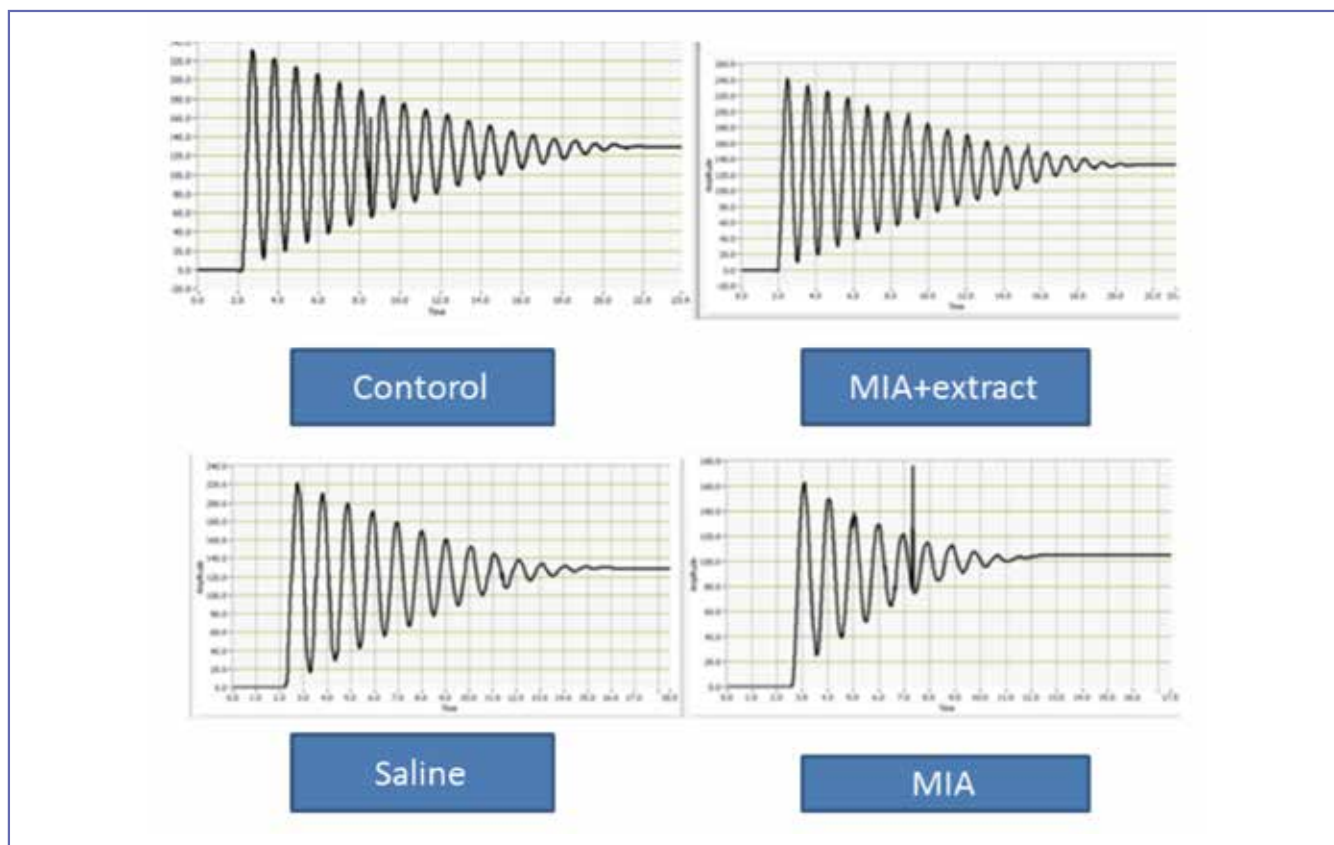


Figure 1. A sample of free oscillation in Control, Saline, MIA, and MIA+extract groups.

Determination of biochemical parameters

SOD activity was determined according to Light spectroscopy method by Winterbourn *et al.* (20).

Glutathione peroxidase activity was determined by glutathione reductase enzyme coupling reaction according to method by Lushchak *et al.*(21). Reduction of glutathione oxide occurs by Gpx reaction with NADPH in the presence of glutathione reductase (GR). In this reaction, NADPH oxidation to NADP⁺ reduces absorption at 340 nm, which is proportional to GPx activity ($H_2O + GS-SG \rightarrow 2GSH + H_2O_2$). Malondialdehyde is the ultimate oxidizing peroxidase product in the body's lipid membrane cells. Thiobarbituric acid assay (TBA) (22) was used to measure MDA level.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to determine the normal distribution of data. Whereas data did not follow a normal distribution $P < 0.05$, the non-parametric Kruskal-Wallis test and Mann-Whitney u test was used to analyze and compare the group differences. The statistical significance was defined as $P \leq 0.05$. These statistical analyses were carried out using the SPSS for Windows Statistical Software Package.

RESULTS

The joint friction parameters

The measurements of joint friction parameters are shown in **table I**.

Cycle number

The cycle number to reach equilibrium showed a significant difference through the study groups ($P=0.005$). Cycle number in Saline, MIA, and MIA+extract groups decreased compared to Control group ($P < 0.05$). After using the *E. angustifolia* extract, the cycle number in the MIA+extract

group was significantly higher than MIA group ($P=0.032$). An increase in the cycle number reflects the reduction of joint friction.

Maximum peak

The maximum peak oscillation showed a significant difference among the groups ($P=0.002$). The maximum Peak oscillation decreased significantly in Saline, MIA, and MIA+extract compared to Control group ($P<0.05$). After using the *E. angustifolia* extract, the maximum peak oscillation increased significantly compared to Saline group ($P=0.016$) and MIA group ($P=0.016$). Increasing the maximum peak reflects the facilitated movement in the joint and the reduction of joint friction.

Exponential slope of cycle decay

The exponential slope of cycle decay showed a significant difference among the groups ($P=0.010$). The exponential slope of oscillation showed an increase in all groups compared to Control group, but this increase was only significant in Saline group ($P= 0.008$), and MIA group ($P= 0.008$). After administration of the *E. angustifolia* extract, the exponential slope of the oscillation decreased and its value showed no significant difference compared to Control group. The decrease in the exponential slope of cycle decay indicates that the joint movement is performed with low friction.

Linear slope of cycle decay

The slope of cycle decay showed no significant difference among the groups ($P= 0.062$).

Joint friction coefficient

The friction coefficient calculated by the Stanton formula showed no significant difference among the groups ($P = 0.131$).

Table I. The value of joint friction parameters (Mean±SD)

Variables	Control	Saline	MIA	MIA+extract
Cycle Number	20.60±1.51	12.60±2.88 ^a	12.20±1.48 ^a	15.40±2.40 ^{ab}
Maximum Peak	28.14±1.76	21.38±1.03 ^a	21.35±1.34 ^a	24.07±0.85 ^{abc}
Exponential Slope	0.04±0.007	0.12±0.08 ^a	0.11±0.03 ^a	0.08±0.3
Linear slope	0.008±0.000	0.011±0.002	0.012±0.001	0.010±0.001
Friction coefficient	0.24±0.01	0.31±0.05	0.26±0.15	0.28±0.04

a: Significant decrease compared to Control group; b: Significant increase in the MIA+extract group compared to MIA group; c: Significant increase in the MIA+extract group compared to Saline group.

Biochemical parameters

The measured biochemical variables including the activity of antioxidant enzymes: SOD, GPx and MDA as a lipid peroxidation index are shown in **table II**.

Superoxide dismutase (SOD) activity

The amount of SOD activity was significantly different in the groups ($P=0.001$). The induction of osteoarthritis using MIA reduced the amount of SOD activity. This decrease was significant in both MIA ($P=0.000$) and MIA+extract ($P=0.025$) groups compared to Control group. Using *E. angustifolia* extract increased the amount of SOD activity compared to MIA group ($P=0.011$).

Glutathione peroxidase (GPx) activity

The level of GPx activity revealed significant differences in the groups ($P=0.041$). The GPx activity in the MIA+extract group was significantly higher than Control ($P=0.018$), Saline ($P=0.021$), and MIA ($P=0.05$) groups.

Malondialdehyde (MDA) activity

The level of MDA activity showed significant differences in the groups ($P=0.002$). In MIA group, the level of MDA activity increased compared to Control and Saline groups; this increase was significant in Saline group ($P=0.031$). The level of MDA activity in the MIA+extract group decreased compared to Control ($P=0.002$), Saline ($P=0.005$) and MIA ($P=0.000$) groups.

DISCUSSION

In this study, the effect of *E. angustifolia* extract was investigated on the SOD, GPx, and MDA activity and also the decrease of joint friction in the non-traumatic OA model. Although the measuring of friction is very important to determine of joint function, but it is an aggressive method that is not feasible in human studies. So, in this study we used MIA rat model to investigate the effect of *E. angustifolia* extract on the joint friction. The MIA model in rat, is

well established and resembles the histological and pain-related behavior of human degenerative OA (23). Injection of MIA leads to a decreased number of chondrocytes and subsequent histological and morphological articular alterations similar to human osteoarthritic changes (23).

The results of this study revealed that induction of osteoarthritis by MIA injection reduced the cycle number of joint oscillation compared to Control group. In the MIA+extract group, after four weeks administration of *E. angustifolia* extract, the number cycle increased significantly compared to MIA group. The use of Saline also reduced the cycle number of oscillation, which was previously reported by Caligaris *et al.* (24). After using the *E. angustifolia* extract, the maximum peak oscillation increased compared to the Saline and MIA groups. Administration of *E. angustifolia* extract displayed the decrease of exponential slope of cycle decay, and after treatment, no significant difference observed between MIA+extract group and Control group. Both linear slope of cycle decay and the friction coefficient showed no significant decrease in the groups. Crisco *et al.*, (2007) showed that exponential slope of cycle decay is more accurate than the linear slope of cycle decay for predicting the joint friction coefficient (25). They suggest that the exponential model is capable to predict the friction coefficient accurately, when the viscous damping is quite small. In our study, exponential slope of cycle decay determined the difference of joint coefficient of friction among groups; whereas, both linear slope of cycle decay and the friction coefficient prediction by Stanton formula (based on the linear decay) could not determine any difference among groups.

Dunham *et al.*, (1993) suggested that, "the intra-articular injection of MIA leads to inhibition of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes, which causes interruption of glycolysis, increased cartilage destruction, as well as a decrease of proteoglycan synthesis and ultimately cell death" (18). It is noted that MIA injection causes cartilage destruction (9). Naveen *et al.*, (2014) showed that, two weeks after induced OA by MIA, the ratio of glycosaminoglycan/total protein content and the cartilage stiffness were decreased (26).

The joint friction can be significantly changed in articular diseases even before appearance of clinical or laboratory

Table II. The measured biochemical parameters (Mean±SD)

Variables	Control	Saline	MIA	MIA+extract
SOD(um/l)	0.74±0.05	0.73±0.03 ^b	0.61±0.04 ^a	0.65±0.07 ^{ab}
GPx(u/l)	195.5±62.19	190.3±36.31	235.7±130.4	401.7±186.9 ^{abc}
MDA(umol/l)	4.49±1.19	4.24±1.38	5.71±0.72 ^{ab}	2.36±1.08 ^{abc}

a: Significant difference with Control group, b: significant difference with MIA group, c: significant difference with Saline group

symptoms (27). The interaction of articular cartilage and synovial fluid through the two important mechanisms of boundary lubrication and fluid film produces smooth and low friction movements in the normal synovial joints and causes performing of daily functional activities with minimal friction and good lubrication to prevent the joint cartilage wearing. Some studies have shown that there is a direct correlation between the increase of friction coefficient and the intensity of OA (28-30).

The experimental and clinical studies suggest that oxidative stress may be considered as one of the main etiological factors in the pathogenesis of OA (10, 11, 31). SOD expression is significantly down regulated in OA chondrocytes *in vivo*, but this precedes the development of OA lesions, raising the possibility that alterations in SOD expression are associated with the earliest stages of OA pathogenesis (32). *E. angustifolia* is consisting of various chemical compounds including amino acids, flavonoids, phenolic compounds, polysaccharides and some other essential elements (15). Most studies on *E. angustifolia* is related to its antioxidant capacity of phenolic compounds and antocyanosides. Polyphenols can suppress the oxidative stress produced by reactive oxygen species (ROS) with their hydroxyl groups (33, 34).

Our results showed that the intra-articular injection of MIA caused the decrease of SOD activity level. The level of SOD and GPx activities increased in MIA+extract group. The amount of GPx activity in the MIA+ extract group was significantly higher than Control, Saline, and MIA groups. Moreover, the amount of MDA activity increased in the MIA group compared to the Control and Saline groups. The level of MDA activity decreased significantly after administration of *E. angustifolia* extract in MIA+extract group compared to other groups. It was found that the intra-peritoneal injection of *E. angustifolia* extract was effective in increasing the level of activity of antioxidant enzymes (SOD and GPx) and decreasing the level of MDA activity. It is reported that antioxidants have been effective in altering the oxidative enzymes levels in osteoarthritis (35). Increased MDA and decreased glutathione (GSH), and catalase activities (CAT) were evident in OA patients (11). MDA or lipid peroxidation increased in plasma and synovial fluid of osteoarthritis and rheumatoid arthritis patients (11). Anti-

oxidant enzymes, such as SOD, CAT, GPx are decreased in OA patients, confirming the role of oxidative stress in OA pathogenesis (10, 12).

In the non-traumatic OA model, Maghzi *et al.*, (2015) showed that the oral treatment with *E. angustifolia* extract decreased the Mankin score (glycosaminoglycan index) and cellular degradation in articular cartilage (36).

Based on the effectiveness of *E. angustifolia* extract on the prevention of chondroblast cell death, it may be facilitated the expression of joint lubricants such as glycoproteins and hyaluronic acid and subsequently decrease of joint friction. We could not measure the level of these lubricants in this study and it suggests to consider in future studies. It seems that the prevention of cartilage degradation and the synthesis of lubricant agents after intraperitoneal injection of *E. angustifolia* extract, may be effective for decreasing the joint friction and better lubrication during functional activity, but special histological and biochemical studies should be considered in the future studies.

CONCLUSIONS

Induction of osteoarthritis by MIA injection reduced the cycle number of joint oscillation. Administration of *E. angustifolia* extract decreased the exponential slope of cycle decay, and after treatment this parameter showed no significant difference compared to Control group. *E. angustifolia* extract was effective in increasing the level of activity of antioxidant enzymes (SOD and GPx) and decreasing the level of MDA activity. It seems that *E. angustifolia* extract through its antioxidant effect be able to increase the joint lubrication in the osteoarthritis.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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