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Effects of *Elaeagnus angustifolia* L. supplementation on serum levels of inflammatory cytokines and matrix metalloproteinases in females with knee osteoarthritis

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KEYWORDS
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Summary
Objective: In an attempt to investigate new strategies aimed at reducing inflammation in osteoarthritis, the anti-inflammatory effect of *Elaeagnus angustifolia* L. as a complementary treatment was evaluated in females with knee osteoarthritis.
Method: In this clinical trial, 90 females with mild to moderate osteoarthritis were assigned to two intervention and one placebo groups. In addition to the conventional therapy, the patients in intervention groups received 15 g/day of *E. angustifolia* L. medulla and whole fruit powders respectively for 8 weeks. The levels of tumor necrosis factor-alpha (TNF-α), interleukine-1β (IL-1β), interleukine-10 (IL-10), matrix metalloproteinase-1 (MMP-1) and -13 (MMP-13) were measured with human ELISA kits. Paired *t*-test and ANOVA were used for statistical analysis.
Results: The statistically significant decrease was observed in the mean levels of serum TNF-α in the medulla (0.004) and whole fruit (0.001) groups after 8 weeks of supplementation. In contrast to the placebo group, there was a significant rise in the mean levels of serum IL-10 in medulla (p-value = 0.01) and whole fruit groups (p-value = 0.009) at the end of study. The interventions resulted in significant decrease in the mean levels of serum MMP-1 in the medulla (0.001) and whole fruit (0.002) groups. After the interventions, no significant changes were observed in the serum IL-1β and MMP-13 levels.

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Conclusion: Daily supplementation with *E. angustifolia* L. in both forms of medulla and whole fruit powders appeared to be effective for decreasing inflammatory cytokines (TNF-α and MMP-1) and enhancing anti-inflammatory cytokines (IL-10).

Introduction

Osteoarthritis (OA) is a common age-related disabling disease. The prevalence of this disease is twice higher in women than men. It is characterized by the degradation of articular cartilage, focal cartilage loss, and osteocyte formation [1]. The underlying mechanisms of cartilage matrix degradation are not completely understood but it has been confirmed that inflammatory mediators are directly involved in the regulation of cartilage degradation [2].

The pro-inflammatory cytokines, mainly tumor necrosis factor-alpha (TNF-α) and interleukine-1-beta (IL-1β), are directly involved in the pathological processes of OA [3]. They are responsible for the inhibition of proteoglycan synthesis in chondrocytes and stimulating the production of matrix metalloproteinases (MMPs) [4]. The main role of MMPs is the remodeling and degradation of the extracellular matrix, since they are also involved in collagen degradation [5]. Collagen in its native state can be cleaved by MMP-1 (interstitial collagenase) and MMP-13 (collagenase 3), and this cleavage is thought to represent the rate-limiting step in collagen degradation [6].

Considering the pivotal role of inflammation in OA progression, it seems that application of approaches, which decrease the inflammation in these patients, could be useful in management of osteoarthritis. Although the anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) have been proven to be highly effective in controlling the symptoms and signs of osteoarthritis, they also have potential gastrointestinal (GI) adverse effects [7]. Therefore, currently a number of non-pharmacological treatments have been studied for the management of OA. One of the non-pharmacological treatments that attracts more attention nowadays is the herbal therapy. There are a growing number of OA patients who wish to use herbal anti-rheumatic medicines. Although there are several such remedies available on the market, there is a definite lack of published evidence of their efficacy as proven by clinical trials [8].

Elaeagnus angustifolia L. is one of the frequently used herbal medicines in Iranian traditional medicine and some other countries for rheumatoid disease [9]. The anti-inflammatory and anti-edema effects of this fruit have been shown in an animal study by Ahmadiani et al. [10]. In spite of its extensive use in traditional medicine, to the best of our knowledge, there is no human study about its effect on inflammation in osteoarthritis patients. In an attempt to investigate the new strategies and interventions aimed at reducing inflammation in osteoarthritis, in this study, we have evaluated the anti-inflammatory effect of *E. angustifolia* L. in two forms of whole fruit and medulla powders in females with OA.

Method

Subjects selection

Ninety volunteer patients with knee osteoarthritis who referred to the rheumatologist were recruited.

The inclusion criteria were females, aged 40—70 years, patients with mild to moderate bilateral primary osteoarthritis of knee according to American College of Rheumatologist (ACR) classification, and body mass index (BMI) of 25—34.9 kg/m^2. The patients were excluded if they had secondary osteoarthritis, active synovitis, neurological disorder that affects movement; and muscle control and balance, uncontrolled hypertension, diabetes, cardiovascular disorders, chronic kidney disorders, or functional liver disorders. The patients who used Frosemid, Probenecid, Anticoagulants, Hydantoin, Sulfonamides, Methotrexate, Lithium salts, Beta blockers and muscle relaxants, smoked, had an allergic reaction to *E. angustifolia* L, or used *E. angustifolia* L. regularly were also excluded.

Ethics approval has been received from the ethic committee of Tabriz University of medical sciences (reference number 91171) and the study was registered with the Iranian Registry of Clinical Trials (http://www.irct.ir) with the identification no. 201208241197N13. All subjects were made aware of the content of the study and informed written consent was obtained from all participants.

Study design

In this double blind randomized placebo-controlled study the sample size was calculated based on a 90% power, and a type I error rate of 1%, which necessitated at least 24 cases in each group. In this study, 90 subjects were randomly assigned to two interventions and one placebo group. The patients in the first and second intervention groups ($n = 30$ for each group) were supplemented with 15 g/day whole fruit powder and medulla powder of *E. angustifolia* L. The patients in placebo group received 15 g/day corn starch with isomaltose for 8 weeks. The patients were asked to consume the supplements in the morning and drink plenty of fluids to prevent constipation. All patients underwent routine physical examinations and conventional treatments such as Acetaminophen (Tylenol) and non-steroidal anti-inflammatory drugs (i.e., Celecoxib, Ibuprofen, Naproxen) were continued during the course of study in all groups. The use of conventional drugs and treatments was recorded at baseline, week four, and at the end of week eight.

Blood samples were taken after an overnight fasting for determination of serum TNF-α, IL-1 β, IL-10, MMP-1 and

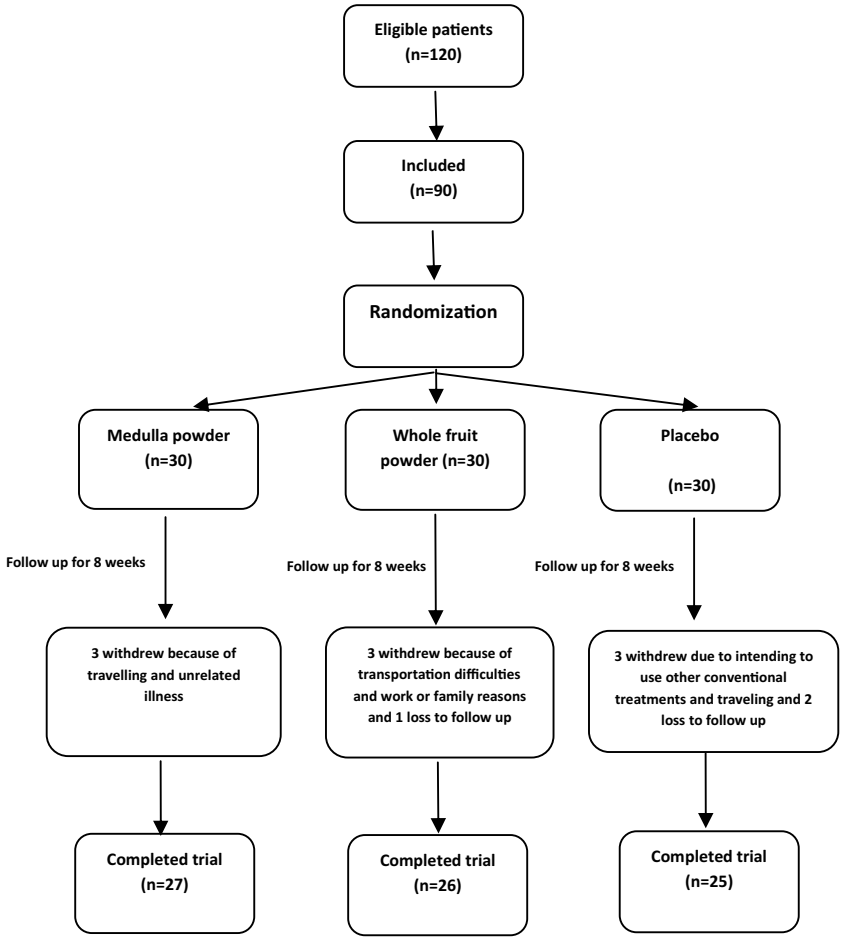

Figure 1 Flow chart for patient enrolment, randomization and retention.

MMP-13. Serum samples were separated by centrifugation and stored at −80 °C until analysis.

Serum levels of TNF-α, IL-1 β and IL-10 were measured using the Human ELISA kits from DIA Source (DIA Source, Nivelles, Belgium). Serum levels of MMP-1 and MMP-13 were determined using human ELISA kits from Boster (Boster Biosciences Co., Wuhan, China). All measurements were done following the instructions provided by the manufacturers.

BMI (weight (kg)/height (m^2)) was calculated from height and weight, which were measured using standardized protocols and calibrated equipments.

E. angustifolia L. whole fruit and medulla powder preparation

E. angustifolia L. was collected from around city of Tabriz, Iran, in autumn. Whole fruit including peel, medulla, and seed as well as the medulla of this fruit were grounded into a powder with mechanical grinder separately. Then, the powders were sealed into packs separately, each pack containing 15 g of *Elaeagnus angustifollia* L. whole fruit and medulla powder.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 and descriptive data were reported as means and SD's. Kolmogrov—Smirnov test was used to analyze the normality of the data distribution. Comparisons within groups were performed using paired sample *t*-test and analysis of variance (ANOVA) was used to assess the differences in changes in TNF-α, IL-1β, IL-10, MMP-1 and MMP-13 between groups. *P*-value <0.01 was considered significant.

Results

From ninety volunteer patients who were recruited, 12 subjects withdrew due to reasons unrelated to the study such as unrelated illnesses, family reasons, and lost to follow up (Fig. 1). As a result, the data were reported for 78 patients (27 in medulla powder group, 26 in whole fruit powder, and 25 in placebo group). Similar to the previous studies, no side effects were reported by consumption of the fruit during the study.

All the study subjects were menopause with the mean age and body mass index (BMI) of 56.31 ± 8.90 years and 32.11 ± 2.91 kg/m^2 respectively. We did not observe any significant differences between three groups at the baseline in terms of age, BMI, the duration of disease, and the levels of studied pro-inflammatory cytokines, anti-inflammatory cytokine and metalloproteinases (Table 1).

As illustrated in Fig. 2A the mean levels of serum TNF-α in different treatment groups before the study in the medulla, whole fruit and placebo groups were 28.68 ± 7.36,

Table 1 Comparison of the baseline characteristics of the osteoarthritis patients between study groups.

Variable	Medulla $N=27$	Whole fruit $N=26$	Placebo $N=25$	p-Value[a]
Age (years)	54.52 ± 11.19	57.47 ± 7.24	57.00 ± 7.77	0.49
Weight (kg)	76.37 ± 10.95	74.85 ± 10.24	75.73 ± 9.62	0.81
Height (cm)	155.87 ± 6.00	152.00 ± 5.38	154.21 ± 6.25	0.08
BMI (kg/m^2)	32.47 ± 2.67	32.50 ± 2.70	32.35 ± 2.77	0.82
Duration of disease (years)	6.35 ± 4.39	6.00 ± 4.66	6.91 ± 5.20	0.80
TNF-α (pg/ml)	28.68 ± 7.36	26.48 ± 7.11	23.50 ± 10.20	0.09
IL-1β (pg/ml)	18.90 ± 4.44	19.00 ± 5.10	19.60 ± 4.60	0.99
IL-10 (pg/ml)	48.04 ± 16.22	49.05 ± 14.67	49.40 ± 12.97	0.78
MMP-1 (ng/ml)	2.94 ± 0.79	2.14 ± 0.27	2.13 ± 0.76	0.13
MMP-13 (ng/ml)	0.49 ± 0.11	0.42 ± 0.11	0.35 ± 0.07	0.57

BMI: body mass index; TNF-α: tumor necrosis factor alpha; IL-1β: interleukine-1β; IL-10: interleukine-10; MMP-1: matrix metalloproteinase-1; MMP-13: matrix metalloproteinase-13.
[a] *P*-value of ANOVA.

26.48 ± 7.11 and 23.50 ± 10.20 pg/ml which decreased to 8.28 ± 1.19, 3.70 ± 2.69 and 15.45 ± 9.50 pg/ml respectively by the end of the study. The statistically significant decreases were observed in the mean serum levels of TNF-α in the medulla (0.004) and whole fruit (0.001) groups after the 8 weeks of supplementation.

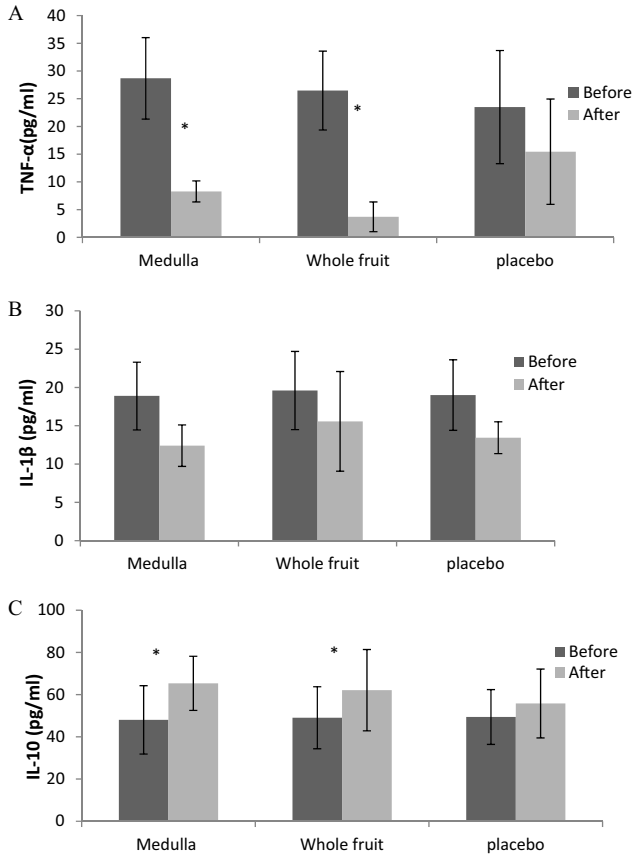

Figure 2 Comparison of serum TNF-α (A), IL-1β (B), and IL-10 (C). Levels before and after study in different groups. *Paired *t*-test; *p*-value <0.01; IL-1β: interleukine-1β; TNF-α; tumor necrosis factor-alpha; IL-10: interleukine-10

Considering the mean levels of IL-1β, in comparison with baseline, the insignificant decline was observed in all study groups at the end of the study (Fig. 2B).

At baseline, the mean ± SD serum IL-10 levels in medulla, whole fruit, and placebo groups were 48.04 ± 16.22, 49.05 ± 14.67 and 49.40 ± 12.97 pg/ml which increased to 65.35 ± 2.74, 62.11 ± 19.27 and 55.79 ± 16.30 pg/ml respectively by the end of the study. In contrast to the placebo group, significant rise was observed in the mean serum levels of IL-10 in medulla (p-value = 0.01) and whole fruit (p-value = 0.009) groups after the interventions (Fig. 2C).

As displayed in Fig. 3A, before the study, the mean levels of MMP-1 in medulla, whole fruit, and placebo groups were 2.94 ± 0.79, 2.14 ± 0.27 and 2.13 ± 0.76 ng/ml and at the end of the study these values decreased to 0.99 ± 0.04,

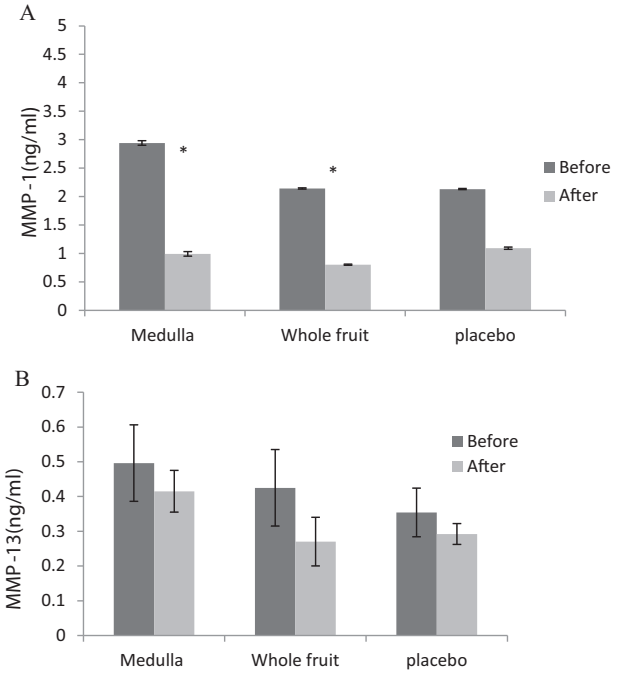

Figure 3 Comparison of serum MMP-1 (A) and MMP-13 (B) levels before and after study in different groups. *Paired *t*-test; *p*-value <0.01. MMP: matrix metalloproteinase

0.8 ± 0.01 and 1.09 ± 0.02 ng/ml, respectively. However, the decreases in MMP-1 levels were only significant in the medulla (0.001) and whole fruit (0.002) groups.

As illustrated in Fig. 3B, although the levels of serum MMP-13 decreased in all study groups, these decrements were not statistically significant.

Between groups analysis by ANOVA showed that the changes in all studied parameters were not statistically significant between three groups.

Discussion

In the last few years, numerous studies have documented the role of pro-inflammatory cytokines in the etiology and pathogenesis of OA. The results of present study showed that the mean serum TNF-α was 26.31 ± 4.96 pg/ml, which is higher than the previously reported value (11.80 ± 4.00 pg/ml) in Czech Republic study [11]. The serum levels of MMP-1 and MMP-13 in the present study were 2.40 ± 0.75 and 0.41 ± 0.05 ng/ml respectively, which were lower than values reported in Spain (MMP-1: 5.13 ± 3.4; MMP-13: 0.65 ± 0.15 ng/ml) and Belgium (MMP-1: 3.4 ± 0.2 ng/ml) [12,13]. The discrepancy in serum levels of inflammatory cytokines and also matrix metalloproteinases between the studies may be due to the stage of disease, ethnic group, and/or analyzing methods.

In the present study, supplementation with *E. angustifolia* L. (i.e., medulla powder and whole fruit) in OA patients significantly decreased the serum levels of TNF-α and MMP-1 and also significantly increased the levels of IL-10 as an anti-inflammatory marker. In the placebo group, the studied biomarkers were improved; however, these changes were not significant. It looks like these improvements in placebo group may be due to the effect of conventional treatments in these patients.

Based on the literature reviews, we believe that this is the first clinical study investigating the anti-inflammatory effects of *E. angustifolia* L. The anti-inflammatory effects of this fruit have been shown in an animal study. Farahbakhsh et al. revealed that *E. angustifolia* L. extract could suppress both cyclooxygenase enzymes type 1 and 2 (COX 1, 2), which is comparable to the effects of indomethacin in suppressing the activity of these enzymes [14]. This enzyme catalyzes the rate-limiting step in the conversion of arachidonic acid to prostaglandins and thromboxane A2 (TXA2) [15]. COX-2, prostaglandins, and their major proinflammatory product, prostaglandin E2, have been shown to be induced by IL-1 and TNF-alpha [16]. It was shown that the inhibition of COX-2 is associated with decreased prostaglandin E2 production, inflammation, and pain in OA patients [17]. Therefore, it appears that supplementation with *E. angustifolia* L. could alleviate inflammation by suppressing the TNF-alpha and COX-2 level in osteoarthritis patients.

These positive anti-inflammatory effects of the fruit may be due to the phenolic and alkaloids contents. It was shown that the peel, fruit, and seed of this fruit contains different polyphenols, flavonoids, and terpenoids compounds [10]. Analyzing the composition of this fruit by Bucur et al. indicated that kaempferol and coumaric acid are the main polyphenol compounds [18]. Besides, Ayaz and Bertoft reported the benzoic and caffeic acids as the main phenolic acids in *E. angustifolia* L. fruit [19]. In an early study, Wang et al. studied the flavonoid glycoside content of *E. angustifolia* L. pulp and identified 3 new compounds including Quercetin 3,4′-O-β-D-diglucoside, Quercetin 3-O-β-D-galactopyranoside-4′-O-β-D-glucopyranoside and Isorhamnetin 3-O-β-D-galacto-pyranoside-4′-O-β-D-glucopyranoside [20].

In an animal study, Welton et al. [21] showed that cyclooxygenase-2 is inhibited by quercetin and kaempferol in rat peritoneal macrophages. Furthermore, Nieman et al. [22], through a human study showed that quercetin could reduce the TNF-α level.

In addition to the polyphenol contents, Nikolaeva reported that *E. angustifolia* L. also contains alkaloids mainly Harmine and Harmaline (β-carbolines) [23]. The cell line studies showed that these alkaloids could strongly suppress TNF-α production [24]. Related to this, in chondrocytes cell line, Hara et al. showed the chondroprotective effect of harmine against inflammatory damage [25].

According to the present study, the supplemented fruit in both forms could significantly decrease the serum MMP-1 levels. Based on previous studies, we believed that the polyphenols of *E. angustifolia* L. not only affect MMP-1 level directly, but also, may have negative effect on TNF-α level, which is indirectly correlated with MMPs including MMP-1 [26].

Supplementation with two forms of the fruit did not suppress the serum IL-1β and MMP-13 levels significantly, which might be due to the short duration of the study or the low dose of the supplements.

In general the present findings should be interpreted taking into account the limitations of the study. The duration of the study was limited to only 8 weeks. Furthermore, the levels of cytokines and metalloproteinase enzymes were only measured in serum instead of the synovial fluids; however, some studies have shown that inflammatory events occurring within joint tissues could be reflected outside the joint in plasma and peripheral blood leukocytes (PBLs) of patients with OA.

In conclusion, daily supplementation with *E. angustifolia* L. in both forms of medulla and whole fruit powders, 15 g/day for 8 weeks, appeared to be effective in females with knee osteoarthritis for decreasing inflammation (TNF-α and MMP-1) and enhancing anti-inflammatory cytokines (IL-10). Results of this trial warrant further studies with longer duration, multiple observations considering age, sex, body composition, different disease stages and measuring other inflammatory cytokines such as IL-6.

Conflict of interest statement

There is no conflict of interest.

Acknowledgments

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