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Pomegranate (*Punica Granatum* L.) Peel Hydroalcoholic Extract Supplementation Reduces Pain and Improves Clinical Symptoms of Knee Osteoarthritis: A Randomized Double-Blind Placebo Controlled Study

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Abstract

Background: Osteoarthritis (OA) is a degenerative joint disease with several pathological mechanisms. The intervention that provides for reduced pain and disability with fewer side effects may help improve OA. Pomegranate peel is known to have powerful antioxidant and anti-inflammatory properties due to its great amount of polyphenols.
Objectives: The objective of this study was to evaluate the effects of the pomegranate peel extract on clinical signs and symptoms of knee OA.
Methods: This randomized, doubleblind placebo-controlled clinical trial was conducted amongst 60 women in the age range of 38 - 60 years who were referred to the physical medicine and rehabilitation department of the Tabriz University of Medical Sciences in Tabriz, Iran (Sep 2014 - Feb 2015). The participants were allocated using the block randomization method in one of two study arms. The intervention and control groups received 500 mg of pomegranate peel, hydro alcoholic extract (n = 30) and placebo (n = 30), twice daily for 8 weeks, along with standard drug therapy. The primary outcome measure was the change in mean, Knee injury and osteoarthritis outcome score (KOOS) and the secondary outcome measure was the visual analog scale score.
Results: There were no significant differences between the two groups in the mean of age, body mass index (BMI), disease duration and parity before intervention ($P > 0.05$). The mean of KOOS increased from 46.15 ± 16.82 to 57.57 ± 15.61 after 12 weeks ($P < 0.001$) as compared to the placebo group 50.83 ± 18.83 declining to 56.10 ± 18.07 ($P < 0.001$). The VAS score declined significantly in both treatment and control groups compared with baselines ($P < 0.005$). These changes in the intervention group were significantly greater than those in the control group after adjusting for baseline values, weight changes, energy intake, physical activity, disease duration and parity ($P < 0.05$).
Conclusions: This study presented some effects of the pomegranate peel extract in reducing pain and improving clinical signs and symptoms in women with knee OA and might be considered as a complementary medicine in treating OA.

Keywords: Punicaceae, Clinical Signs, Knee Osteoarthritis

1. Background

Osteoarthritis is known as one of the main causes for disability and amongst the most common forms of musculoskeletal illnesses which cause pain, loss of range of motion, swelling and changes in joint shape (1). The knee joint is one of the most affected joints in OA especially in women. It is estimated to affect approximately 15% of the world population (2). In Iran 15.3% of urban and 19.3% of rural people have knee OA (3). OA is a heterogeneous and multifactorial disease and numerous pathological mechanisms have been associated in its progress. The genetic factors of OA are obesity, some metabolic disorders like diabetes, joint injury and bone and joint deformities. The etiology and causal mechanisms of OA are confounded; a body of evidence advocates the roll of oxidative stress in development of OA (4). Now days there are no certain cures for OA and the existing treatments objected symptom reduction, pain and inflammation, maintenance of joint mobility and limiting the loss of functional capacity. Methods of treatment are expensive and also have a lot of side-effects (5, 6). However the pub-

lic interest for the use of complementary medicine for OA is remarkable and 47% of patients use alternative prescriptions (7). Recent studies have demonstrated an association between dietary polyphenols and the prevention or management of osteoarthritis (8). Pomegranate (*Punica granatum* L, Punicaceae) is a traditional medicinal fruit that is native to Persia but grown and consumed around the world. Pomegranate pulp and peel are rich of soluble polyphenols, tannins and organic acids. Pomegranate peel is an inedible part of fruit and is characterized by an interior network of membrane comprising almost 26% - 30% of total fruit weight and contains substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid) (9). According to a study on nine different pomegranate cultivars grown in Iran, the antioxidant activity, total phenolic and flavonoid contents of pomegranate peel extract is 10 times higher than pulp extract (10). Moreover, the phytochemical concentration of pomegranate peel is high enough to be effective without further enrichment with the extracts of any other fraction of the fruit (9). Anti-inflammatory effects of pomegranate fruit extract due to protection of chondrocytes against Interleukin-1 (IL-1) -induced cartilage damage in vitro was shown recently (8). In an OA mouse model, pomegranate juice supplementation for 14 days diminished cartilage harm and proteoglycan defeat, chiefly in the groups getting the higher doses. Chondrocyte damage was prevented and there were no inflammatory cells detected in the synovial fluid of mice given pomegranate juice (11).

Several studies have demonstrated the antioxidant and anti-inflammatory potential of the active ingredients of pomegranate components. Successful in vitro and in vivo assays indicated that pomegranate and its phenolic components are a very effective treatment against inflammatory disorders (12). Hopefully there are some findings suggesting that pomegranate peel extract stimulated type I procollagen synthesis and inhibited matrix metalloproteinase 1 (MMP-1) production by dermal fibroblasts, which are very important for cartilage damage and repairmen cycle in OA (13).

2. Objectives

Existing data from prior investigations support the hypothesis that pomegranate, especially its peel, may have anti-inflammatory effects. Moreover, the intervention that provides for reduced cartilage degradation, inflammation and pain associated with OA can help improve the joint mobility of patients with OA. Up to now, no interventional studies have investigated these possible effects of pomegranate peel on humans. The current study was aimed to appraise the effects of PPE on clinical signs and symptoms in obese women with knee OA.

3. Methods

3.1. Extract Preparation

The present pomegranate peel extract is produced by the drug applied research center at the Tabriz University of Medical Science, Iran. *Punica granatum* L. (sweet Malas verity) from Shiraz, Iran was provided on December 2013 - February 2014. 300 kg peels of pomegranate were manually separated after juicing and kept at 4°C followed by a vacuum 25 mmHg oven, which dried at 40°C to a moisture content of about 5% (dry basis). Then dried peels were grounded in a mill. Grounded peels were subjected to extraction using ethanol-water 80% by the maceration method. The prepared extract was filtered using a large whatman paper NO.41. Then another filtration went through the whatman paper NO.42 in order to remove all the particles. The clear extract was poured on a tray and dried under vacuum at 50°C until a fine dry brown powder was obtained. This powder is capsulated along with rice flour (500 mg of PPE with 50 mg rice flour). The placebo capsules were filled with 550 mg of rice flour equal in color and size to the PPE capsule.

We have analyzed the ellagic acid content of extract in comparative to standard ellagic acid (Sigma, $\geq$ 95%). These analyses showed that this extract contained 90% ellagic acid on a dry basis, which was confirmed by a high performance liquid chromatography (HPLC) analysis.

The antioxidant activity of PPE was measured using 1, 1-diphenyl 2-pycrylhydrazyl (DPPH) method. A volume of 1 ml of dried extract solution (methanol and water) was enhanced to 1 mL of DPPH solution and was shaken with vortex. The control was sample deprived of PPE. The solution was incubated at room temperature for 30 min. The decreases in absorbance at 600 nm were determined with a spectrophotometer. Inhabitation percentage as a radical scavenging activity was computed with the following formula:

$$I\% = ((A_c - A_s)/A_c) \times 100$$

A_c and A_s are the absorbance of the control and the test sample respectively.

The hydro alcoholic extract showed 86.93% radical scavenging activity using the DPPH model system even after 2 minutes. This result confirmed the remarkable antioxidant property of PPE.

3.2. Subjects and Treatments

The study was a randomized, double blind, placebo-controlled trial (RCT) comparing pomegranate peel hydroalcoholic extract to placebo in patients with mild to moderate OA of the knee. This clinical trial was registered in the Iranian registry of clinical trials (www.irct.ir) with the IRCT registration number: IRCT201405183664N11. This study was conducted in line with the guidelines of the declaration of Helsinki principles and permitted by the ethics committee of Tabriz University of Medical Sciences (reference number: 9328).

All females between the ages of 38 - 60 years old with mild to moderate knee OA, based on the criteria of the American college of rheumatology (ACR) and a BMI between 30 -35 kg/m^2, were included in the study.

The exclusion criteria included the following: BMI less than 30 kg/m^2 or more than 35 kg/m^2, cardiovascular disease, diabetes mellitus, liver and kidney diseases, any history of peptic or duodenal ulcers, smoking, alcohol intake, use of multivitamin- mineral or any other supplements 4 weeks before and during the 8 weeks of the study, being allergic to pomegranate, use of non-steroidal anti-inflammatory drugs or receiving drugs that interact with inflammation.

A total of 122 women who were referred to the physical medicine and rehabilitation department of the Tabriz University of Medical Sciences in Tabriz (a referral center), Iran were screened based on a convenience sampling scheme (Figure 1). Fifty six one didn't meet all inclusion criteria or met exclusion criteria and 10 people refused to participate and all excluded.

The sample size was determined based on primary outcome of change in KOOS, which was received from a pilot study of 10 people in each group. A minimum sample size of 26 was determined for each intervention and control groups by Pocock's formula (Pocock, 1990) with a confidence level of 95% and a power of 0.80.

To cover an expected drop out of 10%, the sample size was elevated to 30 in each group.

Contributors were randomly allocated in groups by using a block randomization method, which was generated by the random allocation software (RAS). All of the people were matched for age and BMI. Subjects in the intervention group received two 500 mg of PPE capsules per day while the placebo group received two placebo capsules daily. These capsules were taken with their meal for 8 weeks. Standard drug therapy was administrated, including two acetaminophen 500 mg and one glucosamine 500 mg per day. The primary outcome measure was the change in mean KOOS (knee injury and osteoarthritis outcome score) and secondary outcome measure was visual analog scale (VAS). All of the data was kept by code until the end of the study and investigators, physician and statistician were reminded blind. The evaluating physician, patients and data analyzer were unaware of the drug packs and groups. To maintain blinding, a person who had no clinical involvement in the study performed the assignment. All patients were educated to keep their common diet and physical activity during the study. We followed up on all participants in biweekly phone calls and visits and, they were recommended to return all packs (fill or empty) to assess compliance and consumption status. Patients who consumed more than 90% of capsules were included in statistical analyses.

3.3. Anthropometric, Dietary and Physical Activity Assessments

The body weight of each subject was measured to the nearest 0.1 kg with a Seca scale (Dubai, United Arab Emirates). Each person was required to be barefoot and wear lightweight clothing. Their height was also measured while barefoot and using a measuring tape with the participant's arm hanging freely at sides and documented to the nearest 0.5 cm. The BMI was computed as weight (kg) divided by height ^{2} (m^2).

Dietary intake was assessed with three nonconsecutive day food records (two weekdays and one weekend) at baseline and at the end of the trial. All patients were instructed about food scales and recording their food consumptions before the beginning of the study. Three-day average macronutrient and energy intakes of participants were evaluated by nutritionist 4 software (First data bank Inc., Hearst Corp., San Bruno, CA).

The short international physical activity questionnaire (IPAQ) was used to assess the physical activity of participants. IPAC is an instrument written predominantly for the population investigation of adults with the age range of 15 - 69 years. This questionnaire validity and reliability has been evaluated in previous trials (9). Each patient's physical activity was calculated considering the energy requirements defined in METS (metabolic equivalent). All patients were categorized in different groups (low, moderate, high) based on their physical activity.

3.4. Clinical Examinations

KOOS and VAS tests were used for clinical assessments. The KOOS is a knee-specific tool, designed to evaluate patients' ideas about their knee and related complications. The KOOS is self-administered and is an all-inclusive instrument evaluating five subscales: symptoms (seven items); activities of daily living (ADL); function (17 items); pain (nine items); Sport and recreation function (five items); and quality of life (four items). The KOOS's five patient-related parts are independently counted. Standardized answer options are given (5 likert boxes) and each question

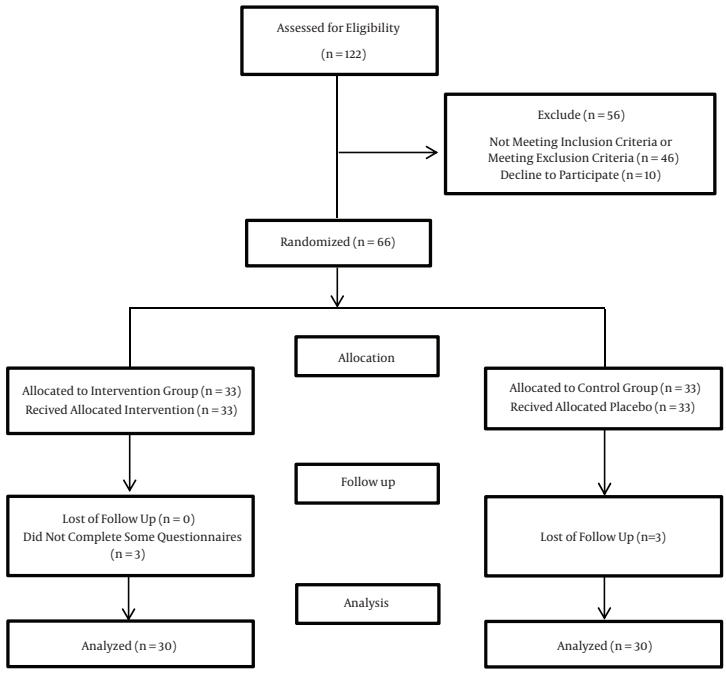

Figure 1. Consort 2010 Flow Diagram

is assigned a score from 0 (no problems) to 4 (extreme problems). The five individual KOOS subscale scores are then given as secondary outcomes to enable clinical interpretation. For calculating each subscale, we should apply the mean of the observed items within subscale, divided by 4 and multiplied by 100; when this number is subtracted from 100, we have that KOOS subscale score. KOOS subscale scores can be gathered and averaged as the primary outcome in RCTs. Final scores are presented as 0 (extreme problem) to 100 (no problem). The Persian version of the KOOS is a culturally adapted, reliable and a valid outcome measure to be used in Iranian patients with knee osteoarthritis (10). All subscales in our study exceeded the minimum cronbachs alpha level of 0.85. VAS is a scale for issues that cannot be directly calculated. Participants identify their level of agreement by showing a position along a continuous line between two end points (0 and 10). In this trial, we used VAS with the intention of assessing the greatness of the pain thus, 0 characterizes no pain and 10 characterizes the extreme bearable pain (14).

3.5. Statistical Analysis

A statistical analysis was performed using the statistical package for the social sciences (SPSS Inc., Chicago, IL, USA) version 13 based on the intention to treat (ITT) principle. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. Ordinal and non-normally distributed variables were shown as the median (interquartile range) (MED (IQR)) and numeric normally distributed ones were shown as the mean $\pm$ standard deviation (7). Pre-treatment and post-treatment differences in mean values in groups were analyzed by paired t-test and in MED (IQR) by the Mann-Whitney U-test. Independent t-test and Wilcoxon test were used to analyze differences in mean values of quantitative and ordinal variables between the two groups. In addition, ANCOVA analysis was used for the comparison of post-treatment values adjusting for baseline values, weight changes, energy intake, physical activity, disease duration and parity. $P < 0.05$ was considered statistically significant.

4. Results

Of the 66 subjects who were randomized, 60 of them completed the study. There were no missing values in the measured main outcome variables. Three subjects in the intervention group dropped out because of poor completion of questionnaires and 3 patients in the control group because of the loss to follow up. Sixty participants were followed up for 8 weeks (intervention group, n = 30; control group, n = 30; Figure 1). Patients did not report any harmful situations and supplements were consumed with high compliance (97%).

Starting point characteristics of the patients are presented in Table 1. No significant differences were found between the two groups in the mean of age, height, weight, BMI, disease duration and parity before intervention ($P > 0.05$).

Based on the results, 6 people (20%) had moderate physical activity and 24 of them (80%) were low active in the PPE group, while 22 (73.3%) of placebo group were categorized in low physical activity and 8 people (27.7%) had moderate physical activity. There were no significant differences in physical activity before or after the intervention ($P > 0.05$).

There were no significant differences in energy, macronutrients intakes and physical activity level of groups before or after the intervention ($P > 0.05$).

Table 2 shows KOOS, its subscales scores and VAS in studied subjects, at the beginning and at the end of the investigation. There were no significant differences in all of the above measurements between the two groups at baseline except sport and recreation function, which were higher in control group than that of intervention group ($P > 0.05$).

The scores of KOOS and its subscales were significantly increased and the VAS score significantly decreased compared with baseline in the supplemented group ($P < 0.05$). The placebo group also showed significant improvements for all scores but not for the score of sport and recreation function at the end of study ($P < 0.05$). These changes in the intervention group were significantly superior than in the control group ($P < 0.05$).

After adjusting the baseline values, weight changes, disease duration and parity, post-treatment KOOS, symptoms, activities of daily living and pain were significantly higher in the intervention group compared to the control group ($P < 0.05$).

5. Discussion

To the best of our knowledge, this research is the first trial that investigates the potential effects of PPE supplementation on the human knee OA. We assessed these effects by KOOS which is a self-administered and a comprehensive instrument. Our results showed a great improvement in clinical signs and symptoms of knee OA and a significant decrease in pain.

OA is characterized by loss of cartilage structure and function. A body of evidence supports this hypothesis that oxidative stress has a key role in progression of OA (4). Reactive oxygen species (ROS) production, nitric oxide (NO) and its redox products have been indicated to be responsible for cartilage injury (11, 12). Dysregulation of pro inflammatory and anti-inflammatory pathways result in raised levels of pro inflammatory cytokines such as IL-6, interleukin (IL)-1β, tumor necrosis factor (TNF)-α in cartilage extracellular matrix of OA patients. Synovial inflammation is directly linked to cartilage degradation (13). Pain is the chief sign of OA and the cause of why people look for medical interventions. Now days, nonpharmacologic treatments such as quadriceps exercise, footwear modification, life style changes and supplementation with some safe products like glucosamine help the patient relieve pain (15).

A recent study has shown an association between commonly consumed polyphenols and the prevention or treatment of OA, including curcumin, resveratrol, nobiletin, epigallocatechin gallate, green tea extract, citrus fruits, genistein, soy protein as well as pomegranate. These plants are known to have antioxidant and anti-inflammatory effects in treatment of OA (16).

Pomegranate is a therapeutic fruit and its peel is an inedible part of fruit which appears to have more beneficial effects. The total phenols, flavonoids and proanthosyanidins are higher in peel extract than in pulp extract (17). Pomegranate peel is known to contain high contents of flavonoids. Flavan-3-ol and anthocyanidins are the most flavonoids in the pomegranate peel, which are responsible for the brilliant color of the fruit. Flavones and flavonols of the peel, commonly are as glycosides with aglycons of luteolin, kaemferol and quercetin. Hydrolysable tannins (ellagitannins and gallotannins) like punicalin and punicalagin are highly found in the peel. Ellagic acid is found more than any other part of the pomegranate and takes up to 0.2% in the peel (18).

No human studies are available regarding the possible effects of PPE in OA patients. However, our findings are in accordance with results of studies about pomegranate fruit juice or its components in animal models.

In an experimental study, supplementation with flavons in OA, rabbits showed a significant decrease in the level of matrix metalloproteinase 3 (MMP-3) and caused an improvement in joint cartilage (19). Also flavones supplementation had reduced levels of prostaglandin E2 (PGE2) and NO (20). Rongkui et al. recently revealed that there is an association between polyphenols and the prevention of osteoarthritis and musculoskeletal inflammation. A clinical study demonstrated that supplementation of flavons as a traditional Chinese medicine, had lowering effects on expression levels of IL-1 and PGE2 after 12 weeks and could improve knee OA (21). An experimental study on OA mouse model showed beneficial effects of whole pomegranate extract (POMx) on matrix degradation enzyme MMPs and necrotic factor kappa b (NF-κB). POMx inhibited MAP kinase (MAPK) signal transduction pathways and NO in mouse macrophages (8). In-vitro study by

Table 1. Baseline Characteristics in Women with OA[a,b,c,d]

Variable	PPE Group (n = 30)	Placebo Group (n = 30)	MD (95% CI)	P Value
Age, yr	48.66 ± 7.77	52.23 ± 6.65	-3.56 (-7.30 to 0.17)	0.061
Weight, kg	81.44 ± 11.40	78.80 ± 7.46	2.63 (-2.34 to 7.61)	0.294
Height, cm	158.07 ± 7.21	154.90 ± 6.45	3.16 (-0.37 to 6.70)	0.078
BMI, Kg/m^2	32.41 ± 1.92	32.80 ± 1.90	-0.39 (-1.38 to 0.59)	0.431
Parity	2.36 ± 1.44	3.06 ± 1.65	-0.70 (-1.50 to 0.10)	0.087
Disease duration	4 (2 - 7.25)	5 (3 - 10)		0.690

Abbreviations: BMI, body mass index; CI, confidence interval; MD, mean difference; OA, Osteoarthritis; PPE, pomegranate peel extract.
[a] Values are expressed mean ± standard deviation.
[b] Disease duration is presented as Median (interquartile range (IQR)).
[c] P < 0.05 unpaired student t test.
[d] P < 0.05, Mann-Whitney test for disease duration.

Table 2. KOOS, its Subscales Scores and VAS in Women with OA[a,b]

Variable	Period	PPE (n = 30)	Placebo (n = 30)	MD (95% CI)	P Value Between Groups
KOOS	Initial	46. 15 ± 16.82	50.83 ± 18.83	-4.68 (-14.90 to 5.53)	0.361
	End	57.57 ± 15.61	56.10 ± 18.07	6.53 (1.72 to 11.34)	0.009[c]
	MD (95% CI) within groups	11.42 (7.24 to 15.61), < 0.001[d]	5.26 (3.62 to 6.90), < 0.001[d]		
Symptoms	Initial	53.57 ± 21.99	53.20 ± 25.40	0.36 (-12.26 to 13.00)	0.953
	End	70.66 ± 16.62	57.63 ± 24.00	13.02 (2.07 to 23.98)	0.021[c]
	MD (95% CI) within groups	17.09 (10.08 to 24.09), < 0.001[d]	4.43 (2.86 to 6.00), < 0.001[d]		
Activities of Daily Living	Initial	55.77 ± 19.31	56.79 ± 19.87	-1.01 (-11.42 to 9.39)	0.845
	End	69.17 ± 18.98	63.53 ± 18.58	5.63 (-4.34to 15.60)	0.263[c]
	MD (95% CI) within groups	13.39 (8.81 to 17.94), < 0.001[d]	6.74 (4.59 to 8.89), < 0.001[d]		
Pain	Initial	47.68 ± 21.87	45.92 ± 23.47	-3.61 (-15.77 to 8.54)	0.554
	End	60.74 ± 21.55	48.14 ± 23.99	3.38 (-8.87 to 15.55)	0.585[c]
	MD (95% CI) within groups	12.69 (5.61 to 19.78), 0.001[d]	5.74 (3.71 to 7.78), < 0.001[d]		
Sport and Recreation Function	Initial	10.0 (0.0 - 30.0)	40.0 (13.75 - 70.0)		0.001[e]
	End	20.0 (0.0 - 35.0)	40.0 (18.75 - 70.0)		0.002[e]
	(P) within groups	0.02[d]	0.40		
Quality of Life	Initial	18.75 (4.67 - 37.5)	37.5 (10.93 - 50.0)		0.076
	End	31.25 (6.25 - 50.0)	37.5 (12.5 - 56.25)		0.548
	(P) within groups	< 0.001[f]	0.020[f]		
VAS	Initial	6 (5 - 7.75)	7 (3.75 - 8)		0.090
	End	4 (2 - 5.25)	5.50 (3.75 - 8)		0.040[e]
	(P) within groups	0.001[f]	0.001[f]		

Abbreviations: CI, confidence interval; KOOS, Knee Injury and Osteoarthritis Outcome Score; MD, mean difference; OA, Osteoarthritis; PPE, pomegranate peel extract; VAS, Visual Analog Scale.
[a] Values are expressed mean ± standard deviation.
[b] Data for Sport and Recreation Function, Quality of life and VAS are presented as Median (interquartile range (IQR)).
[c] P < 0.05, analysis of covariance adjusted for baseline values, weight changes, energy intake, physical activity, disease duration and parity.
[d] P < 0.05, Paired T Test.
[e] P < 0.05, Mann-Whitney test.
[f] P < 0,05, Wilcoxon test.

Park et al. showed that the most abundant flavonoids in the pomegranate peel including kampferol and quercetin had a significant role in protecting the skin from ultra violet B (UVB)-induced damages. These flavonoids decrease and increases the expression level of MMP-1 and procollagen type I, which are important factors for bone formation (8). Newly, the effects of pomegranate juice on pro-inflammatory cytokines and MMPs were investigated in patients with knee OA. These studies indicated that the consumption of 200 mL of pomegranate juice for 6 weeks didn't change serum levels of TNF-α and IL-1ß but improved physical function and stiffness. This clinical

trials results are in accord with our study. Pomegranate juice decreased breakdown cartilage enzyme MMP-13 and increased antioxidant status in patients with knee OA (22, 23). The suppression of MMP expression in OA chondrocyte by pomegranate fruit suggests that pomegranate constituents prevent collagen degradation and may inhibit joint destruction in OA patients.

Along with the encouraging effects of pomegranate components on oxidative and inflammation status, MMPs and procollagen expression in studies mentioned above, it was possible that such conditions might be contributed in improving clinical signs and declining the pain of OA patients in our supplemented group.

This study was the first report about effects of PPE on clinical signs and symptoms of knee OA. It had some limitations, including its short duration and relatively small sample size. Results of this study are not applicable for OA patients with different BMI range, severity of disease, age group or different sex. Assessment of antioxidant and inflammatory variables would be valuable for understanding possible action mechanisms of this extract.

Larger clinical trials with different duration and dose of PPE are needed to establish our obtained results.

5.1. Conclusions

This study presented some effects of pomegranate peel extract in reducing pain and improving clinical signs and symptoms in women with knee OA and might be considered as a complementary medicine in treating OA.

5.2. Implication

This study evaluated the effects of pomegranate peel hydro alcoholic extract on improving clinical signs of knee osteoarthritis as a complementary medicine.

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