Original Article

Journal of Evidence-Based Complementary & Alternative Medicine
2015, Vol. 20(2) 126-131

DOI: 10.1177/2156587214566867
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Randomized Clinical Trial of Peganum Oil for Knee Osteoarthritis

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Abstract

Osteoarthritis affects about 50% of people aged older than 65 years. Pain is the most important symptom in this disease. Today public interest in the use of complementary medicine, especially traditional herbal medicines has increased. The present study was designed to investigate the efficacy of traditional preparation of *Peganum harmala* L oil on patients with knee osteoarthritis. The product has been analyzed and standardized by high-performance liquid chromatography. A double blind controlled randomized clinical trial consisting of 54 patients were performed. Patients rubbed the drug or control (olive oil) on the knee 4 drops 3 times a day for 4 weeks. The patients were asked to fill out the Western Ontario and McMaster Universities Arthritis Index and Visual Analogue Scale questionnaires at week 0 and 4. The adapted results from the questionnaires showed that pain and difficulty in function were significantly decreased in Peganum oil group after 4 weeks. There was no significant difference in stiffness change between 2 groups.

Keywords

Peganum harmala L, oil, knee osteoarthritis, pain, Western Ontario and McMaster Universities Arthritis Index (WOMAC)

Received September 2, 2014. Accepted for publication November 17, 2014.

Osteoarthritis is the most common form of arthritis and is characterized by degeneration of articular cartilage.[1] The main symptoms and signs are joint pain, stiffness, limitation on motion, joint deformity, and different degrees of joint inflammation.[2] Radiographic data of most people by 65 years and 80% of those older than 75 years indicate the evidence of osteoarthritis.[3] Knee osteoarthritis is of the main causes of devastated mobility in elderly people.[4] Since there is no cure for osteoarthritis, treatment focuses on reducing symptoms especially pain. Analgesics, including nonsteroidal anti-inflammatory drugs are usually used for this purpose.[2,5] Because of various side effects of these drugs such as gastrointestinal,[6] cardiovascular,[7] and renal toxicities,[4] their oral use of these medications is limited by many patients. Topical administration, which is preferred by many patients, is a good solution for this problem.[8] On the other hand, global interest in using complementary and alternative medicine, especially herbal products is increasing among people because of better tolerance and fewer well-known side effects.[9] This has led to increase of large companies' interest and investment in this area.[10] Chronic joint pains are associated with a high usage of herbal medicines.[11] In spite of long history of Iranian traditional medicine and herbal remedies, few studies have been conducted on the effect of herbal drugs on osteoarthritis in Iran.

Peganum harmala L, known as *Espand*, is a native plant of Iran from Zygophylaceae family. In many of Iranian traditional medical treatises the preparations from the plant seeds are said to be useful for joint pain. One of these preparations is a topical oily product (rubbing oil) which called *Roghan-e-Espand* (Peganum oil).[12] *Peganum harmala* seeds are known to be rich in alkaloids (β-carbolines such as harmine and harmaline), which provide a wide range of pharmacological actions, including antispasmodic, antipyretic, anticancerous, central nervous system effects, hallucinogenesis, central monoamine oxidase inhibition, binding to various receptors including 5-hydroxytryptamine and the benzodiazepine binding receptors, platelet aggregation inhibitory, immunomodulatory, and significant antinociceptive effects.[13]

There are different ways of herbal oil preparations according to Iranian traditional pharmacy, which depends on herb

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type and the herb part. Compression and distillation was used for herbs with oil-bearing part and aromatic part, respectively. Another method which can be compared to modern enfleurage, was used for fragrant part as flowers and leaves. Rigid parts and the ones without or with low concentration of oily constituent were the other group that are prepared in different way. In this method, the aqueous extraction of the herb is boiled in oily vehicle such as almond or olive oil till all the water part is evaporated.[12,14] The last method is the one mentioned for Peganum oil preparation.[12]

The present study was designed to investigate the efficacy of traditional preparation of Peganum oil, which has been claimed to be useful in joint pain according to Iranian traditional medicine, on patients with knee osteoarthritis.

Materials and Methods

Oil Preparation

The seeds of *Peganum harmala* L. (Zygophylaceae) were purchased from herb market, which were gathered from Estakhr, Fars, Iran. Samples of the plant were identified and stored in the herbarium of Shiraz Faculty of Pharmacy (voucher number PM 406). The standard olive oil was also procured from Parsay-e-Sabz-e-Shiraz Company. The product was prepared according to traditional treatise. Thus the plant material (1 kg of the seeds) was macerated in 6 times water (6 L) for 24 hours, boiled till the half of the water evaporated. The filtered extract then mixed with the same volume of olive oil (3 L) then boiled till all the aqueous part evaporated.[12]

Standardization of Peganum oil by High-Performance Liquid Chromatography

Chemicals. Harmine (Sigma, 286044) and harmaline (MP Biomedicals, 0521157601) were obtained from local. Chromatographic grade-double distilled water, high-performance liquid chromatography grade acetonitrile (Merck-100030), and isopropyl alcohol (Merck-101040), analytical grade formic acid (Merck-100264) were used.

Extraction of Alkaloids. Based on Kartal et al[15] for extracting alkaloids from *Peganum harmala* seeds, 5 g of prepared Peganum oil was macerated 4 times with 50 mL methanol at 50°C in a water bath for 1 hour. The extracts were combined and evaporated to dryness. The residue was dissolved in 50 mL hydrochloric acid (2%) and then filtered. The filtrate was extracted 2 times with 20 mL petroleum ether. The aqueous acid layer was basified (pH 10) with NH_4OH and extracted 4 times with 50 mL chloroform. The chloroform layer was combined and evaporated to dryness and subsequently the residues were dissolved in 25 mL methanol. The solution of alkaloid extract was passed through 0.45 mm filter and 5 μL extract was directly injected into the high-performance liquid chromatography column.

High-Performance Liquid Chromatography Method. The method was performed with a Cecil system consisting of CE 4100 pump and CE4200 ultraviolet/visible detector. The detector was set at 330 nm and peak areas were integrated automatically by computer using Power Stream software. The analyte separation was carried out using C18 analysis column (25× 4.6 mm; particle size 5 μm; Shimadzu, VP-OSD, Japan). Sample injection to system was performed by a loop injector (Rheodyne, P/N, 7725, USA) equipped by a 50 μL loop. HPLC analysis was performed by isocratic elution with flow rate 0.8 mL/min. The mobile phase composition was isopropyl alcohol/acetonitrile/water/formic acid (25:20:55:0.1) (vol/vol/vol/vol) and pH adjusted to 8.6 with triethylamine. All solvents were filtered through a 0.45 mm Millipore filter before use and degassed in an ultrasonic bath. Quantification was effected by measuring at the 330 nm. The chromatographic run time was 10 minutes.[15] Harmine (5 mg) and harmaline (0.5 mg) were accurately weighed into a 5 mL volumetric flask and dissolved in methanol and filled up to volume with methanol and consider as the main sample. Different concentrations were made by serial dilution of the main sample. Triplicate 5 μL injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of each drug was plotted against the concentration to obtain the calibration graph. The 6 concentrations of each compound (5, 10, 20, 30, 40, and 50 μg/mL for harmaline and 50, 100, 200, 300, 400, and 500 μg/mL for harmine) were subjected to regression analysis to calculate calibration equation and correlation coefficients. Mentioned concentrations of harmaline and haemine were freshly prepared in triplicates and analyzed by the developed high-performance liquid chromatography method for intraday validation. The coefficient of variations (CV%) of the corresponding determined concentrations were calculated. In addition, in 3 different days, the same concentrations used in interday variations test were prepared and analyzed by high-performance liquid chromatography method. Then, the corresponding CV% values were calculated accordingly. For each concentration, the absolute recovery of the method was determined as the ratio of measured concentration (based on standard curve) to the corresponding added (nominal) concentration. Limit of detection of the method was determined as the lowest concentration producing a signal-to-noise ratio of approximately 3. Limit of quantitation was determined as the lowest concentration capable of being quantitated with enough accuracy and precision for each substance.

Clinical Trial

Participants. The study was performed in Motahhari Clinic of Shiraz University of Medical Sciences from June to November 2012. Patients with diagnosis of primary knee osteoarthritis according to American College of Rheumatology (1990)[16] were enrolled. They complained of knee pain with intensity of at least 30 according to the Visual Analogue Scale of 0 to 100 and categorized as Kellgren and Lawrence[17] grade II or III based on radiography.

Exclusion Criteria. Severe reduction of joint space in radiographs, other inflammatory diseases; intra-articular injection of corticosteroids or hyaluronic acid during the past 6 months, and pregnancy.

Randomization and Blinding. This was a double blind controlled randomized clinical trial. Participants were enrolled in either control or drug groups (Figure 1) by the table of random numbers. Physician and the patient were not informed of the drug type. Neither drug nor control had any odor. To cover difference in color, both Peganum oil and control (olive oil) were administered in amber glass bottles.

Treatment Process. According to previous similar studies, and considering $p_0 = 10.7$, $p_1 = 54.7$ and power of 90%,[18] and the sample size formula for comparing 2 populations:

$$n = \frac{\left[Z_{1-\frac{\alpha}{2}}\sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{p_0(1-p_0)+p_1(1-p_1)}\right]^2}{(p_1-p_0)^2},$$

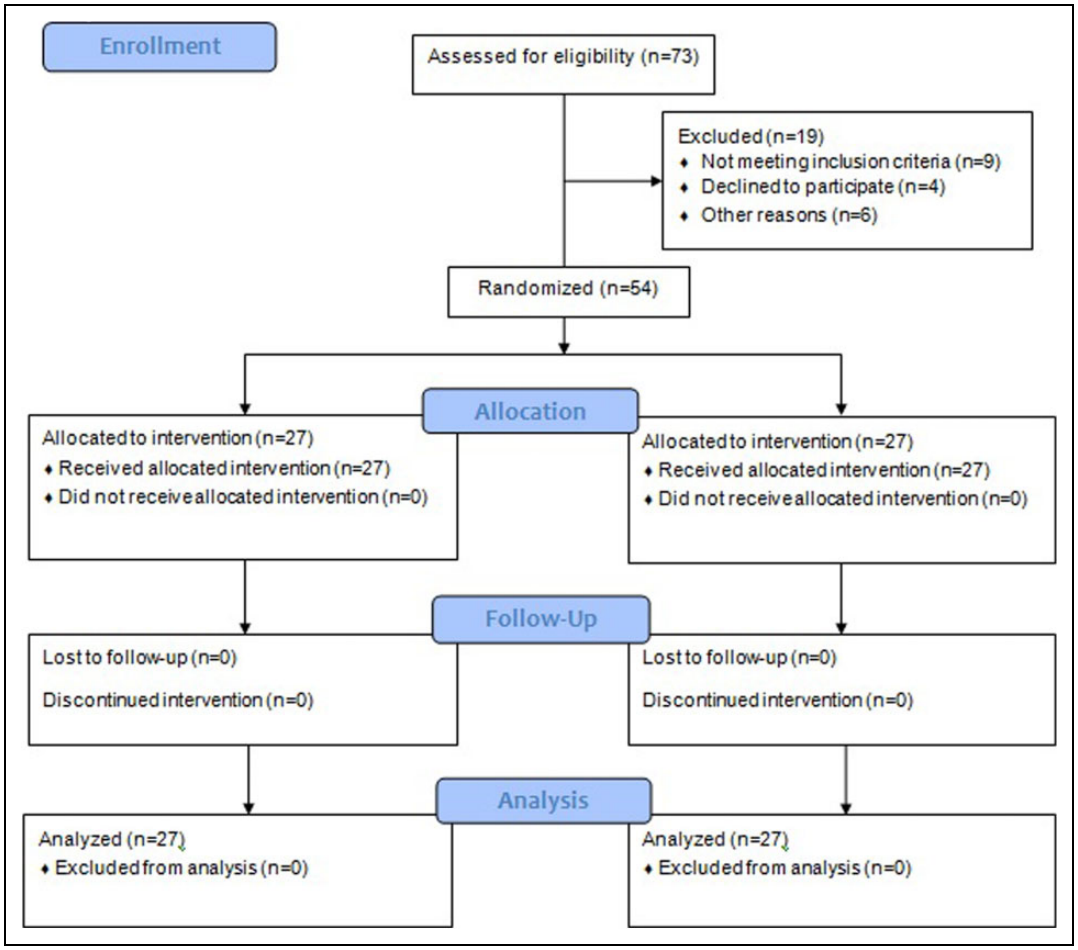

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart.

where $\alpha = 95\%$, $1 - \beta = \text{power} = 90\%$, and

$$\bar{p} = \frac{p_0 + p_1}{2}.$$

Sample size was calculated as 27 patients in each group (total of 54 patients) by using SSC software. The protocol was approved by the Research Ethic Committee of Shiraz University of Medical Sciences (CT-90-5921). It was also registered at the Iranian Registry of Clinical Trials website (IRCT201205029622N1). After explaining the procedure to patients and obtaining informed consent, 57 patients—40 to 75 years old—were randomly allocated to 1 of 2 groups by the table of random numbers. The olive oil was used as control in other group. Patients rubbed the drug or control on the knee 4 drops 3 times a day for 4 weeks. All patients were asked to stop other analgesics and herbal medicines, including nonsteroidal anti-inflammatory drugs, acetaminophen, opioids, and glucosamine chondroitin sulfate during the study.

Outcome Measures. As the primary target criteria was pain relief, the patients were asked to mark Visual Analogue Scale questionnaire at weeks 0 and 4. They were also asked to fill out the Persian version of Western Ontario and McMaster Universities Arthritis Index (WOMAC)[19] at weeks 0 and 4 to determine improvement of the pain, stiffness, and function symptoms. Nadrian et al[20] have shown reliability of the Persian version of WOMAC questionnaire by Cronbach's alpha coefficient ($\alpha = 0.63$-0.94). They also demonstrated that this version is valid and reliable in evaluating osteoarthritis hip/knee symptom and included 24 questions categorized in 3 sections of pain (5 items), stiffness (2 items), and function (17 items).[20] In addition, tenderness and pain on motion were determined and recorded by the rheumatologist in every visit at weeks 0 and 4.

Statistical Analysis

The results from questionnaires were calculated. Normality of variables was analyzed with Kolmogrov-Smirnov test. In the case of normality (parametric), *t* test and paired *t* test were used for between- and within-group analysis, respectively. For nonparametric variables Mann-Whitney and Wilcoxon analysis were performed. In this study, $P < .05$ was considered statistically significant.

Results

High-Performance Liquid Chromatography Method Validation

The method produced linear responses throughout the harmaline concentrations with r^2, slope and intercept of 0.9962, 34.097, and 11.237, respectively. These values for harmine

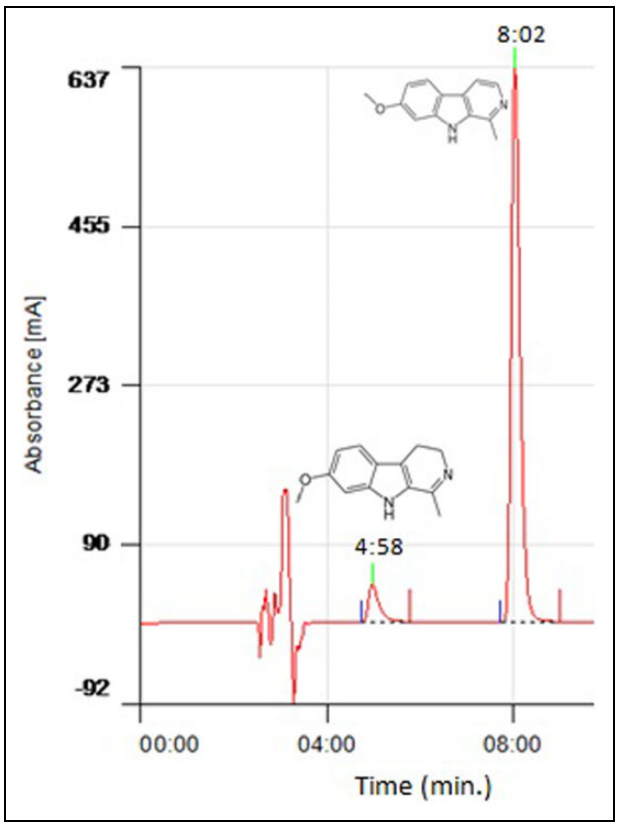

Figure 2. A typical chromatogram of the high-performance liquid chromatography method: 4:58, harmaline (20 μg/mL), 8:02, harmine (200 μg/mL).

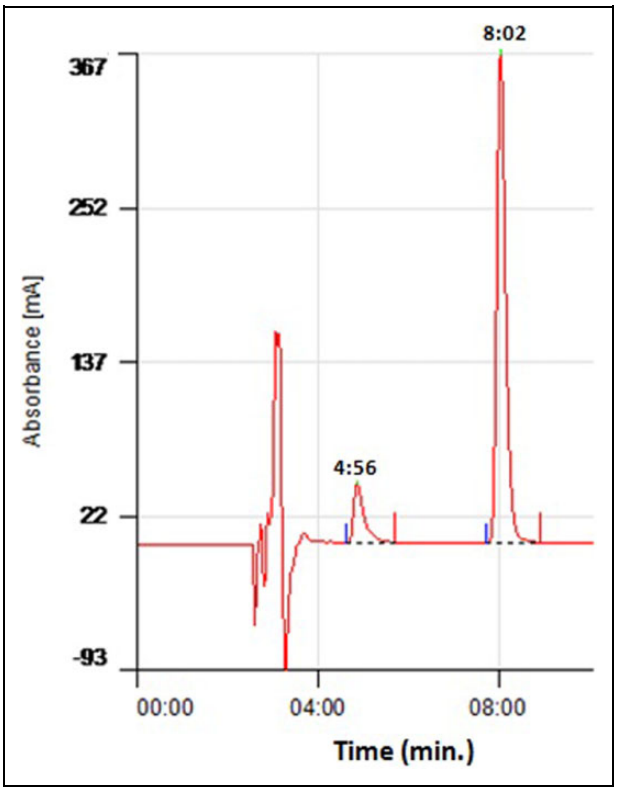

Figure 3. Chromatogram of alkaloid extraction from Peganum oil: 4:56, harmaline; 8:56, harmine.

were 0.9969, 42.416, and 155.02, respectively. A typical chromatogram of the method is shown in Figure 2. The accuracy, for all data, was in acceptable ranges (85% to 112%). This was also true for CV% (<5%). The limits of detection and quantitation for harmaline were 0. 312 and 1.25 μg/mL, respectively, and for harmine were 0.781 and 3.125 μg/mL, respectively. Based on the validated high-performance liquid chromatography method, the percentage of harmaline and harmine in Peganaum oil, after extraction, were 0.0025% and 0.057%, respectively. Chromatogram obtained by injecting the alkaloid extraction from Peganum oil, is presented in Figure 3.

Clinical Trial

A total of 54 patients (24 female and 3 male in Peganum group, 27 female in control group) randomly received trial medication or control. The patients in drug group were 42 to 70 years old (of the average age of 54.11 $\pm$ 8.08 years) and in control group were 40 to 72 years old (of the average age of 53.26 $\pm$ 8.12 years). Twenty patient (37.03%) showed right knee osteoarthritis while 14 (25.92%) suffered from left knee osteoarthritis. In 20 patients (37.03%), both knees were affected. There was no significant difference between groups. Complaints of osteoarthritis knee pain in patients were with an average duration of 5.35 years. There were no significant differences of the evaluated factors at zero time point between our subjects involved in 2 groups ($P > .05$). WOMAC variables were significantly reduced in both groups ($P < .001$) except for stiffness with $P < .05$.Comparison of Visual Analogue Scale of 2 groups at weeks 0 and 4 showed significant pain reduction in each group ($P < .05$), a decline of 34.26 mm (52.56%) in the Peganum group and 12.41 mm (17.00%) in the control group. So, the pain reduction in the Peganum group was 3 times that of the control group. For WOMAC, at the end of the trial a 37.89% reduction in the Peganum oil group and 16.41% reduction in the control group were observed. The adapted results from the questionnaires (WOMAC and Visual Analogue Scale) can be found in Table 1. Pain and difficulty in function were significantly ($P < .001$) decreased in Peganum group after 4 weeks compared with control group. It also has been shown that the drug had more effect on pain factor than the other factors (Figure 4). There was no significant difference in stiffness change between 2 groups. Physical exam of patients by the rheumatologist at week 0 clarified that almost all of them (26 of Peganum oil group and 27 of control group) showed knee tenderness. These numbers for pain on motion factor were 74.1% in the Peganum oil group and 66.7% in the control group. After 4 weeks of using medication or control, the tenderness was significantly ($P < .001$) reduced in the drug group (17 of 26 patients in the Peganum group vs 4 of 27 in the control group). It was also true for the pain on motion factor ($P < .01$), which included 16 of 20 from the Peganum group versus 4 of 18 in the control group. There were no complaints of side effects.

Table 1. Changes of Questionnaire Variables in Peganum Oil Group and Control Group.

Variables	Peganum Oil Group (Mean ± SD)	Control Group (Mean ± SD)
WOMAC		
Pain 0[a]	14.18 ± 3.51	13.48 ± 4.06
Pain 4[b]	8.70 ± 3.07	11.07 ± 3.38
Δ Pain	5.48 ± 2.37*	2.40 ± 1.50
Stiffness 0	0.52 ± 1.09	0.31 ± 0.72
Stiffness 4	0.18 ± 0.55	0.11 ± 0.42
Δ Stiffness	0.33 ± 0.62	0.18 ± 0.39
Function 0	35.15 ± 9.47	35.92 ± 11.26
Function 4	22.15 ± 7.59	30.33 ± 10.09
Δ Function	13.00 ± 4.77*	5.59 ± 2.48
Total 0	49.85 ± 12.51	49.66 ± 14.71
Total 4	30.96 ± 10.36	41.52 ± 12.78
Δ Total	18.89 ± 6.70*	8.15 ± 3.78
VAS		
VAS 0	65.18 ± 12.82	72.96 ± 7.75
VAS 4	30.92 ± 11.93	60.55 ± 10.41
Δ VAS	34.26 ± 9.48*	12.40 ± 7.77

Abbreviations: WOMAC, Western Ontario and McMaster Universities Arthritis Index; VAS, Visual Analogue Scale.
[a] At week 0.
[b] At week 4.
* Significant with $P < .001$ versus control group.

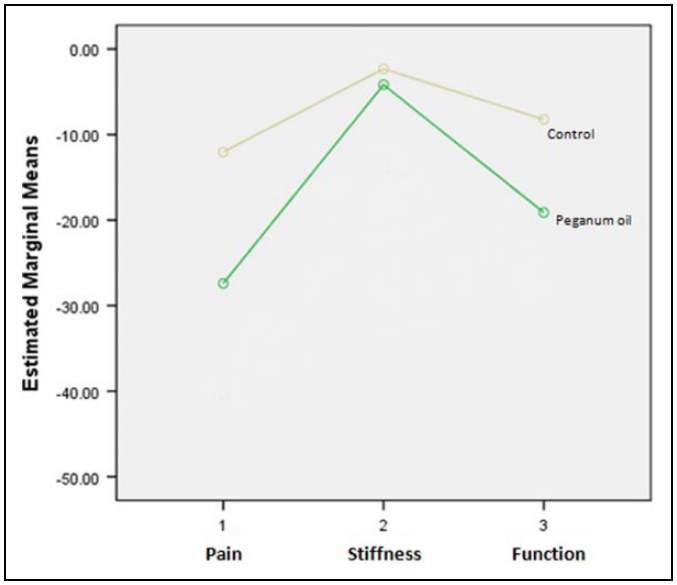

Figure 4. Schematic differences in Western Ontario and McMaster Universities Arthritis Index (WOMAC) variables in Peganum oil (drug) and olive oil (control) groups.

Discussion

Osteoarthritis affects approximately 50% of people aged older than 65 years. Pain is the most important symptom in this disease.[21] Because of the side effects of oral pain killers; public interest in the use of complementary medicine, especially traditional herbal medicines has increased.[10] Many herbal remedies are recommended for joint pain in traditional medical treatises.[4,10] Recent research has demonstrated analgesic activity of many of these plants.[10-22] Most of the present herbal remedies in global pharmaceutical market are derived from European or Chinese traditional herbal medicine like oral administration of ginger, avocado-soybean unsaponified, Devil's claw, or topical use of phytodolor and capsaicin. Several studies on these herbal remedies showed efficacy of Devil's claw, capsaicin, and phytodolor, but not ginger.[23] In case of avocado-soybean unsaponified, the results were not convincing.[24] None of the plant sources of these drugs are endemic in Iran, therefore they could not be cost beneficial. On the other hand, because of side effects of oral administrations, patients with osteoarthritis prefer topical medicines.[8] In this study, the analgesic effect of traditional preparation of *Peganum harmala*, a topical remedy from Iranian traditional pharmacy, has been well determined. At the end of the trial, pain reduction in the Peganum oil group was 3 times (according to Visual Analogue Scale) or 2 times (according to WOMAC) higher than pain reduction in control group. In addition, Peganum oil provided good effect on tenderness and pain on motion factor, which could be considerable. There are some topical herbal products in drug market that are used for chronic and acute painful conditions; among them, capsaicin products are claimed to be more effective. Because of proper effect of Peganum oil on pain and the lack of reported adverse events, though more research in this area such as systemic reactions are necessary, this remedy could become another choice for patients suffering from joint pain especially osteoarthritis and be a good replacement for topical herbal products that cause allergic reactions. On the other hand, *Peganum harmala* is a native plant of Iran, which grows in many areas. It is believed that producing an effective preparation from an indigenous plant can result in a lower priced product. The alkaloid constituents of the seed are shown to be responsible for analgesic activity. It seems that these alkaloids have both central and peripheral antinociceptive activities, may be through opioid receptors.[10]

Frequent citations of certain drugs for certain diseases in various traditional medical treatises could, somehow, confirm the effectiveness and safety of these drugs. The important thing is that if certain drug or preparation would be tested, the same traditional products that were exactly prepared in same traditional ways should be evaluated.

Standardization of traditional products is another important issue that has received little attention. This study, in addition to investigating the analgesic effect, has evaluated and standardized the traditional product by modern methods, which could be an appropriate method for some other traditional products.

It should be considered that more studies on this subject like clinical trials with more patients or comparing the product with common medications can be helpful in improving the results.

Conclusion

The fact that Peganum oil is significantly superior to the control in reducing pain and difficulty in function classifies it as an

effective traditional product and as an alternative in the management of painful conditions, especially osteoarthritis. The absence of adverse effects is another good reason for this claim; however, more studies are needed. This clinical trial has demonstrated that topical application of Peganum oil for knee osteoarthritis is an effective pain-reducing treatment. Besides, the product has been standardized in a proper way based on new methods.

Acknowledgments

This study was a part of PhD thesis that was carried out with the financial support of Shiraz University of Medical Sciences.

Author Contributions

GY, EA, and AM defined the research theme, designed methods, and experiments, ZA carried out the laboratory experiments, analyzed the data, interpreted the results, and wrote the article. All authors have contributed to, seen, and approved the article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

The protocol was approved by Research Ethic Committee of Shiraz University of Medical Sciences (CT-90-5921).

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