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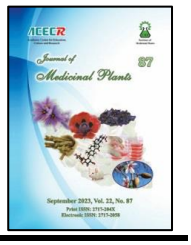

Research Article

Effects of topical Persian medicine *Amygdalus communis* L. var. Amara kernel oil on the symptoms of knee osteoarthritis: a randomized, triple-blind, active, and placebo-controlled clinical trial

Saeed Kianbakht[1,*], Farzaneh Nabati[1], Fataneh Hashem-Dabaghian[2]

[1] *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*
[2] *Department of Traditional Medicine, Institute for Studies in Medical History, Persian and Complementary Medicine, School of Persian Medicine, Iran University of Medical Sciences, Tehran, Iran*

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ABSTRACT

Background: The *Amygdalus communis* L. var. Amara (bitter almond) kernel oil (ACKO) is used topically for palliation of musculoskeletal and joint pains in the Traditional Persian Medicine. Also, it had anti-inflammatory effects in experimental studies. **Objective:** Evaluation of the efficacy and safety of ACKO in the symptomatic treatment of knee osteoarthritis. **Methods:** One hundred and fifty six patients were equally randomized to apply ACKO, diclofenac, or placebo to their knees every 8 hours for 1 month. Fifty two, 50, and 51 patients in the ACKO, diclofenac, and placebo groups, respectively, finished the trial. At the trial's start and end, the symptoms were assessed using the WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) questionnaire. Also, hematological, and liver and kidney function tests were performed. **Results:** Both ACKO and diclofenac reduced the symptoms significantly more than the placebo ($P < 0.001$). The percent changes of the WOMAC pain and stiffness scores in the ACKO group were similar to the diclofenac group while the percent changes of the WOMAC function and total scores in the ACKO group were less than the diclofenac group ($P < 0.001$). ACKO and diclofenac had no significant effect on the blood tests. Moreover, no adverse effect was identified. **Conclusions:** Topical ACKO and diclofenac are safe, and superior to placebo in reducing the symptoms of OA. While ACKO is similar to diclofenac in alleviating pain and stiffness, ACKO is less effective than diclofenac in improving the WOMAC total and function scores.

1. Introduction

Osteoarthritis (OA) is a common type of arthritis, characterized by degeneration of cartilage and other joint structures. It is a chronic and progressive disease without any cure, affecting the patients' mobility. Joint pain, stiffness and dysfunction are the cardinal symptoms of OA [1]. Treatment of OA is symptomatic. Oral and topical

Abbreviations: ANOVA, one-way analysis of variance; GC-MS, gas chromatography–mass spectrometry; N, sample size; NSAIDs, nonsteroidal anti-inflammatory drugs; OA, osteoarthritis; ACKO, *Amygdalus communis* L. var. Amara kernel oil; SD, standard deviation; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index

*Corresponding author: kianbakht@imp.ac.ir, skianbakht@yahoo.com

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nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely used for mitigating the OA symptoms. The other pharmaceutical agents for OA include acetaminophen, duloxetine, tramadol, capsaicin, and glucocorticoids. These therapies have limited efficacy and safety profiles [2]. As such, new more effective and safer therapeutics are needed for OA [1, 2]. Plant-based drugs may offer favorable options for the treatment of OA symptoms [3].

Topical use of the *Amygdalus communis* L. var. Amara (bitter almond) kernel oil (ACKO) is one of the approaches of the Traditional Persian Medicine for the treatment of musculoskeletal and joint pains [4, 5]. ACKO, like diclofenac, dose-dependently inhibited inflammation in vitro and in a mouse model [6]. However, the analgesic effect of ACKO, and its effects on the symptoms of OA have not been studied. Therefore, the effects of topical ACKO on the symptoms of knee OA vs. placebo and diclofenac were assessed using WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) questionnaire, and the safety of ACKO was examined. This study was a randomized, triple-blind, active and placebo-controlled trial. Additionally, ACKO was standardized by analysis of its fatty acids by gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

Analysis of fatty acids and preparation of the ACKO and placebo gels were performed in the Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

2.1. Analysis of fatty acids

Two hundred milligrams of ACKO was dissolved in 2 mL of iso-octane. Then, 0.1 mL of potassium hydroxide 2 M and 2 mL of 40 % sodium chloride solution were added. After separation of the mixture into two phases, the upper iso-octane organic layer was decanted into a clean vial. The iso-octane layer was dried by anhydrous sodium sulfate and injected into the GC-MS instrument. As regards GC-MS, Agilent 890 GC in conjunction with 5973N mass selective detector was used, while the electron impact ionization mode was 70 eV. The range of mass scan was from 50 to 500 atomic mass units. BPX5 column with the length of 30 m and internal diameter of 0.25 mm and the carrier gas helium with the flow rate of 0.5 mL/min were used. Two hundred and ninety degrees centigrade was the temperature of the injector. To analyze the sample, the column temperature for the first 5 minutes was 70 ºC. Then, the temperature was increased to 300 ºC with 10 ºC/min increments and kept for 3 minutes. Response time was 75 minutes. Peaks were identified by computer searches in the commercial reference libraries NIST/MS and user-generated reference library [6].

2.2. Drugs

Gel base and diclofenac (diclofenac diethylammonium salt) 1 % w/w gel (both Darupakhsh Pharmaceutical Company, Iran), and ACKO expressed without heat (Barij Essence Pharmaceutical Company, Iran) (batch number 374024) were used. The company kept the gel base composition secret. The gel bases of the ACKO, and diclofenac and placebo gels were identical. The ACKO, diclofenac and placebo gels were indistinguishable. The concentration of ACKO in the ACKO gel was 50 % w/w. The drugs' samples are kept in the Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran.

2.3. Trial

2.3.1. Ethics

The Ethics Committee of the Mashhad University of Medical Sciences approved this trial (approval code and date:

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IR.MUMS.REC.1396.142, 2017-07-15). The revised Declaration of Helsinki 2013 was observed in the conduction of the trial. All patients signed an informed consent before enrolment.

2.3.2. Registration of the trial

The trial was registered at the Iranian Registry of Clinical Trials (www.irct.ir) with the code IRCT20170821035817N1.

2.3.3. Trial design

The trial had three parallel groups including ACKO, diclofenac and placebo groups. The allocation ratio was equal (1:1:1).

2.3.4. Sample size

Considering the type I error = 0.05 and 80 % power, and the results of a pilot study, the sample size in each group was calculated at 50.

2.3.5. Randomization and sequence generation

To allocate treatments, blocked randomization with blocks each consisting of three patients was used. The random numbers were generated by computer.

2.3.6. Allocation concealment mechanism

Sequentially numbered opaque sealed envelopes were used for allocation concealment.

2.3.7. Blinding

The persons generating the random allocation sequence, enrolling the patients and assigning them to treatments, care providers, outcome assessors, data analyzer, investigators and patients were blinded to the treatments allocation.

2.3.8. Patients

Included patients had ages between 40 and 85 with grade 1 or 2 osteoarthritis of one or both knees as per the American College of Rheumatology and Kellgren-Lawrence guidelines, the minimum pain score of 9 in WOMAC at the start, and a minimum of 2 points of increase in the pain score following cessation of oral NSAID or acetaminophen.

Excluded patients were those who had diabetes mellitus and other serious systemic diseases, secondary osteoarthritis, arthroscopy, any surgery, substance use disorder, fibromyalgia and knee disease, and users of analgesics, and pregnant and breastfeeding women.

The data were collected in the Emam Reza Hospital (Mashhad, Iran) from April 2018 to April 2019.

2.3.9. Interventions

Each group applied ACKO, diclofenac or placebo around the knees every 8 hours for 1 month. The concentration of ACKO in the ACKO gel and dosage of ACKO gel were determined empirically. Drug adherence was measured by counting the returned drugs. All patients in the three groups used one 15 mg meloxicam tablet daily along with ACKO, diclofenac or placebo. But, they did not receive any other therapy for OA.

2.3.10. Implementation

Three different individuals performed generating the random allocation sequence, enrolling the patients and assigning them to interventions.

2.3.11. Practitioner

The practitioner was a general physician licensed in the Mashhad University of Medical Sciences (Mashhad, Iran) and had been practicing medicine for 5 years.

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2.3.12. Outcome variables

The WOMAC total score was the primary outcome variable. The secondary outcome variables included the WOMAC pain, stiffness and function scores; complete blood count; and the blood levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine and blood urea nitrogen. The Persian version of WOMAC questionnaire was used [7]. The blood tests were performed by an auto-analyzer (Hitachi 917, Japan). At the start and end of the trial, the primary and secondary outcome variables were assessed. The patients were asked to report any health condition.

2.3.13. Statistical methods

The SPSS version 23 and intention-to-treat approach were used for analyzing data. The distribution of data was evaluated by the Kolmogorov–Smirnov test. The Chi-squared, Mann-Whitney U, and Kruskal-Wallis tests were used. $P < 0.05$ were significant.

3. Results

3.1. Analysis of fatty acids

Oleic, stearic, palmitic, margaric and myristic acids made up, respectively, 50.0 %, 18.4 %, 17.2 %, 0.3 % and 0.2 % of ACKO.

3.2. Trial

The patients consumed more than 90 % of the administered drugs. Figure 1 shows the CONSORT flow diagram. The demographics and WOMAC scores of the groups are presented in the Tables 1 and 2, respectively. Distribution of the age, body mass index and disease duration of the groups was normal. Distribution of the WOMAC pain, stiffness, function and total scores of the groups was not normal. The WOMAC pain, stiffness, function and total scores of the placebo group increased, whereas these scores decreased in the ACKO and diclofenac groups, at the endpoint vs. baseline (Table 2). Both ACKO and diclofenac reduced the symptoms significantly more than the placebo ($P < 0.001$) (Table 2). The percent changes of the WOMAC pain and stiffness scores in the ACKO group were similar to the diclofenac group ($P > 0.05$) while the percent changes of the WOMAC function and total scores in the ACKO group were less than the diclofenac group ($P < 0.001$). ACKO and diclofenac had no significant effect on the blood tests vs. the placebo. The drugs did not cause any side effect.

Table 1. Demographic characteristics of the groups

Parameter	ACKO group (N = 52)	Diclofenac group (N = 50)	Placebo group (N = 51)	*P*-value
Age (years) (mean ± SD)	63.86 ± 13.2	62.4 ± 12.44	67.63 ± 12.03	0.222*
Gender (number)	24 males, 28 females	16 males, 14 females	19 males, 32 females	0.182**
Body mass index (kg/m^2) (mean ± SD)	31.23 ± 3.64	32.73 ± 4.1	30.4 ± 3.86	0.059*
Disease duration (years) (mean ± SD)	12.2 ± 6.24	10.96 ± 5.47	9.96 ± 5.79	0.338*

ACKO: *Amygdalus communis* L. var. Amara kernel oil. N: sample size. SD: standard deviation. *: ANOVA. **: Chi-squared test.

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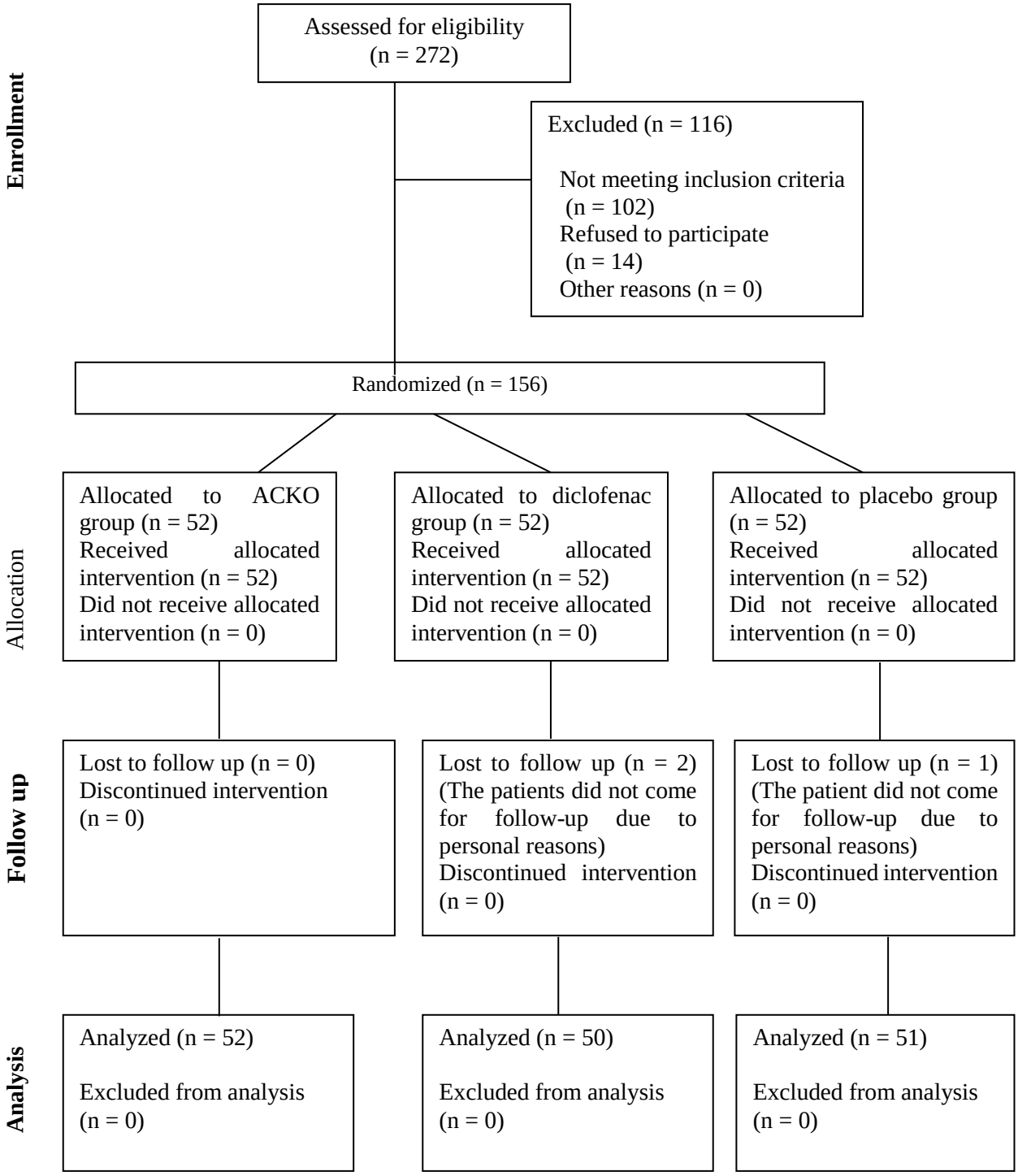

Figure 1. CONSORT flow diagram of the trial. ACKO: *Amygdalus communis* L. var. Amara kernel oil.

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Table 2. The WOMAC scores

Parameter	Time	ACKO group (N = 52) (mean ± SD)	Diclofenac group (N = 50) (mean ± SD)	Placebo group (N = 51) (mean ± SD)	*P*- value*
WOMAC pain score	Baseline	14.9 ± 2.45	15.36 ± 2.72	14.36 ± 2.38	0.258
	Endpoint	10.93 ± 2.74	10.5 ± 2.82	14.66 ± 2.15	< 0.001
	Percent change	27.46 ± 9.29	32.62 ± 8.65	-2.67 ± 8.14	< 0.001
WOMAC stiffness score	Baseline	4.96 ± 1.62	4.7 ± 1.48	5.2 ± 1.58	0.446
	Endpoint	3.7 ± 1.95	3.36 ± 1.6	5.86 ± 1.69	< 0.001
	Percent change	31.67 ± 26.27	30.25 ± 25.88	-14.5 ± 13.13	< 0.001
WOMAC function score	Baseline	49.4 ± 6.28	47.7 ± 5.13	51.6 ± 4.93	0.096
	Endpoint	37.6 ± 6.98	31.4 ± 7.2	53.5 ± 4.88	< 0.001
	Percent change	25.45 ± 5.97	34.58 ± 11.56	-4.88 ± 1.18	< 0.001
WOMAC total score	Baseline	69.6 ± 6.62	67.83 ± 6.76	71 ± 6.8	0.259
	Endpoint	51.66 ± 7.3	45.6 ± 9.17	74.06 ± 6.15	< 0.001
	Percent change	25.99 ± 4.88	33.13 ± 9.12	-4.47 ± 3.06	< 0.001

ACKO: *Amygdalus communis* L. var. Amara kernel oil. N: sample size. SD: standard deviation. *: Kruskal-Wallis test.

4. Discussion

The aim of this trial was assessment of the efficacy and safety of topical ACKO in the palliation of knee OA. The findings of this study suggest that the severity of OA was relatively high. Also, both ACKO and diclofenac are superior to placebo in palliating OA. Moreover, while ACKO is similar to diclofenac in alleviating pain and stiffness, ACKO is less effective than diclofenac in improving the WOMAC total and function scores. More than 20 % reduction of the WOMAC scores vs. baseline is taken as minimal clinically important difference [8]. Therefore, the effects of ACKO and diclofenac on the WOMAC scores are clinically significant. Additionally, topical ACKO and diclofenac demonstrate safety in OA. Notably, the effects of diclofenac align with the prior trials on the topical diclofenac in OA [9].

Chronic joint inflammation underlies the pathology and symptomatology of OA [10]. Thus, the anti-inflammatory effect of ACKO [6] may be involved in the effects of ACKO on the symptoms of OA. Fatty acid composition of the ACKO was determined in the present study. ACKO had only one unsaturated fatty acid named oleic acid. Oleic acid constituted the bulk of ACKO. Unsaturated fatty acids inhibit cyclooxygenase 2 [11]. Therefore, the oleic acid of ACKO might exert analgesic and anti-inflammatory actions similar to NSAIDs in OA. Inflammatory, nociceptive and neuropathic pains constitute the OA pain [12]. Thus, the oleic acid of ACKO may mitigate all constituents of the OA pain due to its cyclooxygenase inhibitory action.

The limitations of this study are as follows. The time needed for ACKO to take effect was not determined. Given that OA is a chronic disease and so drugs should be used for life, lack of evaluation of long-term safety of ACKO is another limitation of this study.

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5. Conclusion

Topical application of ACKO may be a safe palliative agent for knee OA. Also, oleic acid may be responsible for the effects of ACKO. Further studies to pinpoint the bioactive compounds and mechanism of action of ACKO in OA are warranted. Also, long-term safety of the topical ACKO in OA needs to be evaluated.

Conflicts of interest

None.

Author contributions

SK: Conception, design, and conduction of the trial; writing of the manuscript. FN: phytochemical analyses and writing of the manuscript. FHD: Statistical analyses and writing of the manuscript.

Acknowledgment

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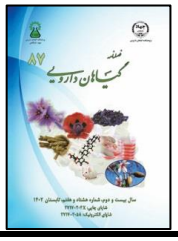

مقاله تحقیقاتی

اثرات مصرف موضعی روغن مغز بادام تلخ (*Amygdalus communis* L. var. Amara) مورد استفاده در طب ایرانی بر علایم استئوآرتریت زانو: یک کارآزمایی بالینی تصادفی، سه سو بی خبر، کنترل شده با داروی فعال و دارونما

سعید کیانبخت[۱،*]، فرزانه نباتی[۱]، فتانه هاشم دباغیان[۲]

[۱] *مرکز تحقیقات گیاهان دارویی، پژوهشکده گیاهان دارویی، جهاددانشگاهی، کرج، ایران*
[۲] *موسسه مطالعات تاریخ پزشکی، طب ایرانی و مکمل، دانشکده طب ایرانی، دانشگاه علوم پزشکی ایران، تهران، ایران*

اطلاعات مقاله

گل‌واژگان:

Amygdalus communis L. var. Amara

روغن بادام تلخ

استئوآرتریت

درد

طب مکمل و جایگزین

طب تلفیقی

چکیده

مقدمه: روغن مغز بادام تلخ (*Amygdalus communis* L. var. Amara) (ACKO) در طب سنتی ایرانی از راه موضعی برای تسکین دردهای عضلانی- استخوانی و مفصل استفاده می‌شود. همچنین، ACKO در مطالعات تجربی اثرات ضدالتهاب داشته است. **هدف:** بررسی اثربخشی و ایمنی ACKO در درمان علامتی استئوآرتریت زانو. **روش بررسی:** یکصد و پنجاه و شش بیمار به طور مساوی و تصادفی در سه گروه، ACKO، دیکلوفناک یا دارونما را به صورت موضعی هر ۸ ساعت به مدت ۱ ماه روی زانو مالیدند. پنجاه و دو، ۵۰ و ۵۱ بیمار به ترتیب در گروه‌های ACKO، دیکلوفناک و دارونما، کارآزمایی را به پایان رساندند. در شروع و خاتمه کارآزمایی، اثرات درمان بر علایم با استفاده از پرسشنامه WOMAC ارزیابی شد. همچنین، آزمایش‌های خون‌شناسی، و کار کبد و کلیه انجام شد. **نتایج:** ACKO و دیکلوفناک علایم را به طور معنی‌داری بیشتر از دارونما کاهش دادند ($P < ۰/۰۰۱$). درصد تغییرات نمرات درد و سفتی WOMAC در گروه ACKO مشابه گروه دیکلوفناک بود در حالیکه درصد تغییرات نمرات کل و عملکرد WOMAC در گروه ACKO کمتر از گروه دیکلوفناک بود ($P < ۰/۰۰۱$). ACKO و دیکلوفناک هیچ اثر معنی‌داری بر آزمایش‌های خون نداشتند. بعلاوه، هیچ عارضه جانبی شناسایی نشد. **نتیجه‌گیری:** ACKO و دیکلوفناک موضعی، ایمن هستند و از نظر کاهش علایم استئوآرتریت بر دارونما برتری دارند. در حالیکه، ACKO از نظر کاهش درد و سفتی، شبیه دیکلوفناک است، اثربخشی ACKO در بهبود نمرات کل و عملکرد WOMAC کمتر از دیکلوفناک است.

مخففها: ANOVA، آنالیز واریانس یکطرفه؛ GC-MS، کروماتوگرافی گازی- اسپکترومتری جرمی؛ N، حجم نمونه؛ NSAIDs، داروهای ضد التهاب غیر استروئیدی؛ OA، استئوآرتریت؛ ACKO، روغن مغز بادام تلخ (*Amygdalus communis* L. var. Amara)؛ SD، انحراف معیار؛ WOMAC، شاخص استئوآرتریت دانشگاه‌های اونتاریوی غربی و مک مستر؛

* نویسنده مسؤول: skianbakht@yahoo.com ،kianbakht@imp.ac.ir

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