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Original Article

The effect of a multi-herbal product, *C. autumnale, W. somnifera,* and *P. lentiscus*, on knee osteoarthritis: a triple-blind randomized clinical trial

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Abstract

Background and purpose: Osteoarthritis (OA) is a common joint disease that affects millions of people worldwide and is characterized by cartilage degeneration, stiffness, and limited mobility.
This clinical trial aimed to investigate the efficacy and safety of a herbal combination of *Colchicum autumnale* root, *Withania somnifera* root, and *Pistacia lentiscus* gum in the relief of knee OA symptoms.
Experimental approach: Seventy patients diagnosed with knee OA were randomized to receive the herbal product or a placebo for 6 weeks. Pain and functional outcomes were measured using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index and Visual Analogue Scale (VAS). The heel-to-thigh distance parameter was also assessed by measuring the distance between the thigh and heel at maximum knee flexion in the prone position.
Findings/Results: Significant improvements were observed in WOMAC scores for pain, stiffness, and physical function in the herbal product group compared to placebo at 3 and 6 weeks. VAS scores confirmed these results and showed lower pain intensity in the herbal product group. Heel-to-thigh distance decreased significantly within all groups during the study.
Conclusion and implications: This study provided evidence for the efficacy of the herbal combination in the management of knee OA symptoms and was well tolerated by all patients with no severe adverse effects. The observed benefits emphasized the potential of herbal medicines as a complementary approach in the management of knee OA. Further research is needed to fully elucidate the therapeutic mechanisms and optimize the clinical application of this herbal combination.

Keywords: Ashwagandha; Autumn crocus; Knee osteoarthritis; Mastic; Pain; Persian medicine.

INTRODUCTION

Osteoarthritis (OA) is a common joint disease that affects a large number of people worldwide, and its prevalence has increased over the past few decades to 14.8% of the global population older than 30 years. Also, the review of the global burden has shown that knee OA is the most common form of OA, with the prevalence being higher in females than males, even after accounting for demographics (1).

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Knee OA often affects the knee, as it plays an important role in weight-bearing and mobility. As the cartilage wears away, the bones rub against each other, causing pain, swelling, and restricted movement, leading to severe functional limitations. Factors such as age, obesity, and trauma contribute to OA. Knee OA symptoms include pain during activity, stiffness, and inflammation. Over time, that can lead to muscle weakness, gait disturbances, and psychological effects. Effective treatment is therefore essential to improve function and prevent further deterioration (2,3).

Treatment for knee OA aims to relieve pain, improve joint function, and slow the progression of the disease through lifestyle changes, medication, physiotherapy, and sometimes surgery. Common medications such as nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, corticosteroids, and hyaluronic acid injections provide relief but can cause side effects such as stomach problems, liver damage, or infection. Although there is no cure, effective treatments help maintain mobility and improve quality of life (4,5).

Alternative medicine can offer valuable benefits in managing knee OA and potentially reduce dependence on medications that have significant side effects (6,7). Traditional medicine offers promising options for managing knee OA, supported by its historical use and emerging scientific evidence. Herbal remedies such as turmeric, ginger, hemp, zedoary, black pepper, and Boswellia have been shown to have anti-inflammatory and analgesic properties, potentially relieving pain and improving joint function (8-10).

In addition, the combination of *Colchicum autumnale* root, *Withania somnifera* root, and *Pistacia lentiscus* gum is recommended in Persian medicine for the management of joint pain (11). Also, several studies have reported the beneficial effects of *C. autumnale* (12), *W. somnifera* (13), and *P. lentiscus* (14) in improving the symptoms of knee OA. In an innovative approach, researchers have used the synergistic effects of medicinal plants in combination. According to Heidari's study, the symptoms of OA patients improved when a combination of turmeric, ginger, and pepper was used (15). Also, a previous study found that *C. autumnale* combined with *P. lentiscus* gum and 7 other herbs in equal portions was safe and effective for low back pain (16).

Several studies have shown that plant extracts and secondary metabolites can affect the diagnostic and prognostic biomarkers of knee OA. They can affect cartilage dysfunction, subchondral bone lesions, synovium, and muscle weakness through different molecular mechanisms (17,18). The main secondary metabolite of *C. autumnale* is alkaloids, including colchicine, colchicerine, colchamine, colchicoside, demethyl-3-colchicine, cornigerine, and 2-demethylcolchifoline, which are responsible for the antitumor and anti-inflammatory effects (19). *P. lentiscus* is rich in phenolic compounds and essential oils, including monoterpenes and sesquiterpenes, which are responsible for the therapeutic effects (20). Also, the main components of *W. somnifera* include withaferin-A, 12-deoxywithastramonolide, and withanolide-A, which are responsible for anti-microbial, anti-inflammatory, and anticancer properties in this root (21).

According to beneficial effects reported by many traditional healers who used a combination of *C. autumnale*, *W. somnifera*, and *P. lentiscus*, as well as reports of the effectiveness of each plant in improving the symptoms of knee OA in experimental studies and clinical trials, we conducted a study to investigate the efficacy and safety of the herbal combination in improving knee OA symptoms.

MATERIALS AND METHODS

Plant collection and herbal product preparation

Plant collection and authentication

Plants used in this study included the roots of *C. autumnale* and *W. somnifera* and the gum of *P. lentiscus* purchased from a local herbal store in Kerman, Iran. A botanist examined and confirmed the genus and species of plants. After that, the plants were assigned herbarium numbers of KF-1559 for *W. somnifera*, KF-1364 for *C. autumnale*, and KF-1136 for *P. lentiscus*, and a sample of them was kept in the herbarium of the Faculty of Pharmacy of Kerman University of Medical Sciences. To evaluate the quality of purchased plants, total ash, loss on drying, and extractive value percent were determined.

Phenolic content analysis

The total phenolic content of *C. autumnale* and *W. somnifera* ethanolic extracts was evaluated spectrophotometrically using the Folin-Ciocalteu reagent method (22). In this assay, 100 µL extract or a standard solution of gallic acid (100-1000 µg/mL) was mixed with 500 µL of diluted Folin-Ciocalteu reagent (1:10 v/v) and was slightly shaken for 1 min. Afterward, 400 µL of an aqueous solution of Na_2CO_3 (7.5% w/v) was added, and the obtained mixture was incubated for 30 min at room temperature in the dark. After incubation, 3000 µL distilled water was added, and the optical density was measured at 765 nm against a blank (50% ethanol) using a multi-mode microplate reader (BioTek®, USA). The total phenolic content was calculated from the calibration curve of gallic acid ($Y = 0.0052X + 0.1031$, $R^2 = 0.995$). The total phenols were expressed as mg of gallic acid per gram of extract.

Preparation of the herbal and placebo products

To produce the final product, the plants were first carefully checked for impurities. Next, all plants were ground separately and passed through a 50-mesh sieve. Then, the powder of 3 plants was mixed in equal proportions and filled into 750 mg capsules. The capsules containing corn starch, which were completely similar to the herbal product in terms of weight, shape, and appearance, were used as a placebo.

Packaging and coding

The herbal combination and the placebo capsules were packaged in similar-colored bottles and were given special codes by the pharmacist.

Participants

The study enrolled patients aged 45 to 70 years who were diagnosed with knee OA according to the American College of Rheumatology criteria, confirmed by a rheumatology specialist, and who had moderate (visual analogue scale (VAS) score of 3.5 to 7.4) knee pain within the last 24 h, and who gave informed consent. Exclusion criteria were pregnancy or breastfeeding, inability to answer questions, debilitating illness during treatment, severe allergies to herbal medicines, illicit drug or alcohol use, systemic illness (e.g., heart failure, uncontrolled kidney problems, and hypertension), routine analgesic use (except 500 mg acetaminophen daily), regular dietary supplements affecting OA symptoms, intra-articular corticosteroid injection in the last 6 months, and unwillingness to participate further.

Ethics approval and consent to participate

The present study was performed in accordance with the Declaration of Helsinki and CONSORT guidelines and approved by the Local Medical Ethics Committee of Kerman University of Medical Sciences (ethical code: IR.KMU.REC.1402.018). This study was also registered at the Iranian Registry of Clinical Trials Database (IRCT No: IRCT20230628058606N1). Written informed consent was obtained from all participants before their enrollment, and their information was kept confidential.

Randomization and blinding

Before the start of the study, participants were assigned to groups using a randomization list generated through an online system (23) (available from https://www.sealedenvelope.com/simple-randomiser/v1/lists) employing random blocks of two. Participants were randomly assigned to two treatment groups according to this randomization list. This list was provided before the start of the study. A trained secretary then randomly assigned eligible patients to each group. The herbal product and the placebo (corn starch) were packaged in identically shaped and colored bottles that were uniformly coded by a pharmacist. The coder was not involved in the study until after the intervention was completed. Neither the participants nor the rheumatologist, interviewer, or data analyst knew the codes or the type of medication assigned to each patient.

Study design

The current study was conducted on patients referred to the rheumatology clinic of the Rafsanjan University of Medical Sciences (Kerman, Iran) in 2023-2024. After the diagnosis

of knee OA in the referred patients based on diagnostic criteria, if they met the inclusion criteria and after signing the informed consent form, the patients were divided into herbal product and placebo groups in a 1:1 ratio.

Intervention

At the start of the study, participants filled out a demographic questionnaire that gathered information on their age, gender, occupational status, and activity (h/day). Also, the body mass index (BMI) was calculated by recording their weight (kg) and height (m). Clinical assessments and disease confirmations were conducted by a rheumatology specialist. All procedures, including interviews, questionnaires, and anthropometric measurements, were carried out by a qualified expert who did not know the participants' group assignments.

Each patient took 2 capsules daily for 6 weeks. Patients were examined at the beginning of the study as well as 3 and 6 weeks after receiving the intervention. Patients were monitored for possible side effects such as gastrointestinal complications, body itching, sleep disorders, etc., by referring to the clinic every 3 weeks and also receiving a weekly phone call. Also, to ensure full patient adherence to medication, daily reminder messages were sent to the patients' personal phones or to their caregivers. It was important to note that all patients took one 500-mg acetaminophen tablet daily.

Outcome measures

Pain VAS

The intensity of knee pain was assessed using the VAS, a 10 cm scale on which patients could rate their pain from 0 to 10. The measurements were taken at the beginning, after 3 weeks of treatment, and after 6 weeks. Pain intensity was categorized as follows: 0-3.4 means mild pain, 3.5-7.4 means moderate pain, and 7.5-10 means severe pain (24).

Western Ontario and McMaster Universities Osteoarthritis Index

The Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index is a validated questionnaire to assess patient performance (25). It consists of 24 items: 5 on pain, 17 on physical function, and 2 on joint stiffness. Each item is scored on a 5-point scale from 0 (no pain, difficulty, or stiffness) to 4 (extreme pain, severe difficulty, or worst stiffness). The minimum and maximum scores of the WOMAC questionnaire were 0 and 96, respectively. Changes in scores were assessed after 3 and 6 weeks compared with baseline scores.

Heel-to-thigh distance measurement

To assess physical performance, the distance between the thigh and heel was measured at maximum knee flexion in the prone position. The range of knee motion testing helps to assess joint integrity, to monitor the efficacy of treatment regimens, and to determine the knee flexion (26).

Sample size

The sample size was determined using Ramakanth's study (13). Considering a type I error probability (α) of 0.05 and a test power of 0.95, the mean (standard deviation) of the mWOMAC score was 47.40 (1.91) in the treatment group and 49.31 (2.11) in the placebo group. The sample size was calculated using the following equation.

$$n_0 = \frac{(1.96 + 1.65)^2[(1.91)^2 + (2.11)^2]}{(47.40 - 49.31)^2} \cong 29$$

Taking into account an expected dropout rate of 20%, the final sample size for the pilot study was adjusted to 35 participants per group.

Statistical analysis

All phenolic content analysis tests were done in triplicate, and the data were expressed as mean ± standard deviation (SD). Other data were presented as mean ± SD or frequency and percentage. The Shapiro-Wilk test was used to check the normality of the distribution of quantitative variables. To compare the variables at the beginning of the study (baseline) in 2 groups, the Chi-square test was used for qualitative variables, and the independent samples t-test or Mann-Whitney U-test was used for quantitative variables. The paired samples t-test or Wilcoxon signed-rank test was employed to compare the biochemical indices before and after the intervention within each group. Repeated measurement analysis of variance was utilized to examine the investigated variables over time in 2 groups. If

the hypothesis of sphericity was not established, the results were reported based on the Greenhouse-Geisser adjustment. Bonferroni adjustment was used for pairwise multiple comparisons. The baseline measurements for WOMAC subscales and total were included as a covariate in the model to account for differences at baseline. To compare different dimensions over time within each group separately, the Friedman two-way analysis of variance by ranks was utilized. For the post-hoc tests, a Bonferroni adjustment was applied. To investigate the changes in VAS score (moderate and mild) over time in 2 groups, a binary logistic regression model with the estimation method of generalized estimating equations (GEE) with an exchangeable working correlation matrix was applied. SPSS software version 27 was used for all statistical analyses. *P*-values < 0.05 were considered significant.

RESULTS

Plant material analysis

The amount of total ash, loss on drying, hydroalcoholic extract yield, and total phenolic content were 2.54 ± 0.12%, 4.34 ± 0.32%, 16.56 ± 1.58%, and 32.99 ± 0.29 mg/g for *C. autumnale* and 5.35 ± 0.16%, 4.52 ± 0.41%, 11.21 ± 1.16%, and 27.29 ± 0.58 mg/g for *W. somnifera*, respectively.

Flowchart of the study

This study included 89 patients suffering from knee OA. Nineteen patients were excluded because they did not fulfill the inclusion criteria (n = 13) or refused to participate (n = 6). A total of 70 patients were randomly assigned to the herbal product group or the placebo group. Finally, 35 patients in each group completed the follow-up, and their results were analyzed (Fig. 1).

Demographic and baseline clinical characteristics

Most of the participants in the treatment group were female (82.9%). The mean age of the participants in the herbal product and placebo groups was 60.37 ± 8.10 and 59.30 ± 8.30 years, respectively. Unemployed people were the most common occupational subgroup in the treatment groups. The average activity in the herbal product and the placebo groups was 5.49 ± 1.70 and 4.80 ± 1.71 h/day, respectively. The average BMI was also 29.58 for the herbal product group and 28.62 for the placebo group. There were no significant differences between the groups in terms of demographic characteristics (Table 1).

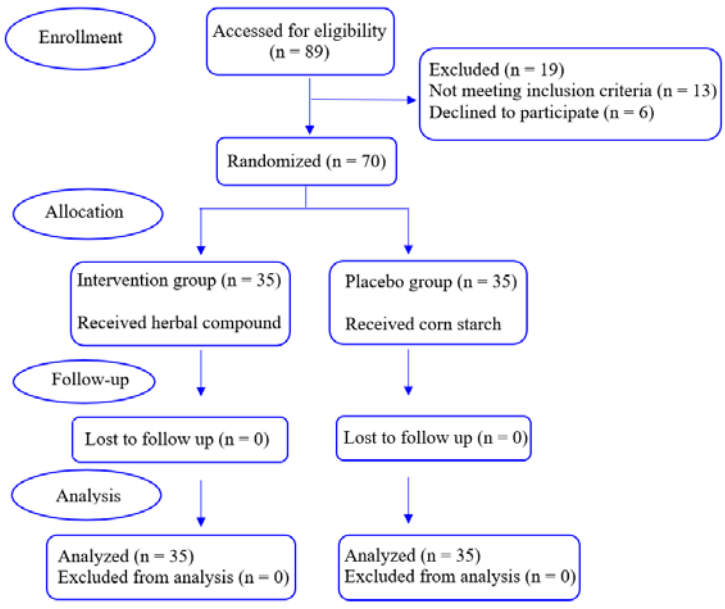

Fig. 1. Consort flow diagram of clinical trial.

Table 1. Basic characteristics of patients by treatment group at the beginning of the study. Data were expressed as mean ± SD and (range) or n (%). *Chi-square test; **Mann-Whitney U *test;* #independent samples t-test.

Variables	Sub-groups	Herbal product group (n = 30)	Placebo group (n = 30)	*P*-value
Sex	**Male**	6 (17.1%)	7 (20.0%)	0.759*
	Female	29 (82.9%)	28 (80.0%)	
Age		60.37 ± 8.10	59.30 ± 8.30	0.552**
Occupational status	**Unemployed**	19 (54.3%)	20 (57.1%)	0.659*
	Government employee or freelancer	11 (31.4%)	8 (22.9%)	
	Retired	5 (14.3 %)	7 (20.0 %)	
Activity (h/day)		5.49 ± 1.70	4.80 ± 1.71	0.097**
BMI (kg/m²)		29.58 ± 3.08	28.62 ± 3.82	0.253#

BMI, Body mass index.

Baseline clinical manifestation

At baseline, there were significant differences between the 2 groups in WOMAC pain scores ($P = 0.001$), WOMAC stiffness ($P = 0.039$), WOMAC physical function ($P = 0.024$), and WOMAC total scores ($P = 0.003$) (Table 2). However, there was no significant difference in the heel-thigh distance parameter at baseline ($P = 0.067$) (Table 2).

The comparison of the VAS scale

As shown in Table 3, all participants suffered from moderate knee pain at the beginning of the intervention. After 3 weeks, the number of patients complaining of moderate knee pain was 16 in the herbal product group and 30 in the placebo group. Even after 6 weeks of the intervention, this number had decreased to 9 patients in the herbal product group, while there was no change in the placebo group (Table 3).

The odds of having a moderate VAS score were lower in the herbal product group than in the placebo group. In addition, the odds of having moderate pain decreased over time in the herbal product group compared to the placebo group, meaning that more patients in the herbal product group experienced a decrease in their pain intensity over time (Table 4).

The comparison of the WOMAC score

In the group receiving the herbal product, the values for all WOMAC subscales and the total WOMAC decreased significantly after 3 and 6 weeks compared to the initial value (baseline). The data comparison in the herbal product group between 3 and 6 weeks also showed significant changes in the mentioned parameters, including significant decreases for WOMAC pain ($P = 0.036$), WOMAC physical function ($P < 0.001$), and WOMAC total ($P < 0.001$) observed at 6 weeks in comparison to 3 weeks. The data comparison of WOMAC stiffness ($P = 0.069$) between 3 and 6 weeks was not significant (Table 2).

In the placebo group, there were significant decreases in WOMAC pain, WOMAC stiffness, WOMAC physical function, and WOMAC total in weeks 3 and 6 compared to baseline, but the data comparison of week 6 with week 3 showed no considerable decrease in these parameters (Table 2).

Although there were significant differences in the WOMAC pain, WOMAC physical function, and total WOMAC parameters in each group, the comparison of the two groups at 3 and 6 weeks after the intervention showed a significant difference ($P < 0.001$), and the mean score reduction in the herbal product group was greater than that in the placebo group (Table 2). The difference of WOMAC stiffness score between the 2 groups at 3 and 6 weeks after the intervention was not significant (Table 2).

The comparison of the heel to thigh distance

There were no significant changes in the distance between the heel and thigh in the herbal product group compared to the placebo group at the end of 3 weeks and 6 weeks of intervention. This distance decreased significantly in the group receiving the herbal product during the study. This reduction was significant in the herbal product group when compared to the start of the intervention at 3 weeks and 6 weeks, but no significant difference was found when comparing the results of 3 weeks with 6 weeks (Table 2).

Table 2. The results of WOMAC and heel-to-thigh distance variables in treatment groups during the study and the intra-group and interaction effects. a, Comparison between times for each group separately using Friedman's two-way ANOVA by ranks; b, Mann Whitney U test for baseline comparison; c, comparison between groups (*P*-value derived from repeated measurements ANOVA); d, comparison between times (*P*-value derived from repeated measurements ANOVA); e, interaction time × group. Comparison was conducted between groups within time (*P*-value derived from repeated measurements ANOVA); +, group comparison during time (*P*-value derived from repeated measurements ANOVA and adjusted by Bonferroni adjustment). The heel-to-thigh distance results were obtained by Greenhouse-Geisser analysis. Data were expressed as mean ± SD or 95% CI for mean. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ demonstrate significant differences compared with baseline; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ versus week 3.

Time	Parameter	Treatment				*P*-value	*P*-value
		Herbal product group		Placebo group			
		Mean ± SD	95% CI for mean	Mean ± SD	95% CI for mean		
Baseline	**WOMAC pain**	9.5 ± 1.7	(8.9, 10.1)	8.1 ± 1.5	(7.6, 8.7)	0.061[d]	0.001[b]
Week 3		5.6 ± 1.5***	(5.1, 6.1)	7.3 ± 1.4**	(6.8, 7.8)		< 0.001[+]
Week 6		4.5 ± 1.4$^{***,\#}$	(4.0, 5.0)	7.3 ± 1.4*	(6.8, 7.8)		< 0.001[+]
***P*-value[a]**		< 0.001		< 0.001			
***P*-value**		< 0.001[c]				0.008[e]	
Baseline	**WOMAC stiffness**	2.6 ± 0.8	(2.3, 2.8)	2.1 ± 1.0	(1.8, 2.5)	0.362[d]	0.039[b]
Week 3		1.1 ± 0.7***	(0.9, 1.3)	2.0 ± 0.7	(1.7, 2.2)		< 0.001[+]
Week 6		0.6 ± 0.7***	(0.4, 0.8)	1.7 ± 0.8	(1.5, 2.0)		< 0.001[+]
***P*-value[a]**		< 0.001		0.033			
***P*-value**		<0.001[c]				0.211[e]	
Baseline	**WOMAC physical function**	31.1 ± 4.6	(29.5, 32.7)	28.8 ± 4.8	(27.2, 30.4)	0.660[d]	0.024[b]
Week 3		17.8 ± 5.2***	(16.0, 19.6)	24.4 ± 3.9***	(23.1, 25.8)		< 0.001[+]
Week 6		13.8 ± 5.8$^{***,\#\#\#}$	(11.8, 15.8)	23.5 ± 4.8***	(21.9, 25.2)		< 0.001[+]
***P*-value[a]**		< 0.001		< 0.001			
***P*-value**		< 0.001				0.002[e]	
Baseline	**Total WOMAC**	43.2 ± 6.3	(41.1, 45.4)	39.1 ± 6.5	(36.8, 41.3)	0.797[d]	0.003[b]
Week 3		24.5 ± 6.9***	(22.2, 26.9)	33.7 ± 5.0***	(32.0, 35.4)		< 0.001[+]
Week 6		18.9 ± 7.5$^{***,\#\#\#}$	(16.3, 21.5)	32.5 ± 6.0***	(30.5, 34.6)		< 0.001[+]
***P*-value[a]**		< 0.001		< 0.001			
***P*-value**		< 0.001[c]				0.001[e]	
Baseline	**Heel-to-thigh distance (cm)**	5.7 ± 4.3	(4.2, 7.2)	3.8 ± 3.7	(2.6, 5.1)	< 0.001[d]	0.067[b]
Week 3		3.5 ± 3.3***	(2.4, 4.7)	3.0 ± 3.0*	(2.0, 4.0)		0.501[+]
Week 6		2.8 ± 2.8***	(1.9, 3.8)	2.9 ± 2.9**	(1.9, 3.9)		0.934[+]
***P*-value[a]**		< 0.001		< 0.001			
***P*-value**		0.324*				< 0.001[e]	

Table 3. The frequency of the VAS score by treatment groups during the study. *At baseline, the two groups were the same; therefore, no statistics were computed. Note: The *P*-values were obtained using the Chi-square test.

Time	VAS score	Treatment		*P*-value
		Herbal product group	Placebo group	
		Frequency (%)		
Baseline	Mild (0-3.4)	0 (0.0)	0 (0.0)	-*
	Moderate (3.5-7.4)	35 (100.0)	35 (100.0)	
Week 3	Mild (0-3.4)	19 (54.3)	5 (14.3)	< 0.001
	Moderate (3.5-7.4)	16 (45.7)	30 (85.7)	
Week 6	Mild (0-3.4)	26 (74.3)	5 (14.3)	< 0.001
	Moderate (3.5-7.4)	9 (25.7)	30 (85.7)	

Table 4. The results of VAS intensity by treatment group during the study period, based on logistic regression using GEE.

Parameter	Coefficients	Standard deviation	OR	95% CI for OR	*P*-value
Herbal product group	-1.08	0.51	0.34	(0.13, 0.92)	0.034
Placebo group	-	-	-	-	-
Time	-0.91	0.16	0.40	(0.29, 0.56)	< 0.001
Herbal product group × time	-1.06	0.33	0.35	(0.18, 0.66)	0.001
Placebo group × time	-	-	-	-	-

OR, Odds ratio; GEE, generalized estimating equations.

Similarly, the reduction was significant in the placebo group at 3 weeks ($P = 0.005$) and 6 weeks ($P = 0.014$) when compared with baseline. Yet, no significant difference was found when comparing the results of 3 weeks with the ones of 6 weeks ($P = 1.00$) (Table 2).

As shown in Table 2, the comparison of the effect of time and the interaction of time and group showed that there was a significant difference in heel to thigh distance between the herbal product and placebo groups over time.

Safety and tolerability

The herbal product was well tolerated by all patients, and none of the patients complained of a serious complication that led to discontinuation of the intervention. Two patients in the placebo group had mild abdominal bloating in the second week after the intervention and continued the treatment. In addition, 2 patients in the herbal group complained of slight itching of the skin, and one patient had slight abdominal bloating, which subsided after a few days.

DISCUSSION

This study examined the effectiveness of a combination of *C. autumnale*, *W. somnifera*, and *P. lentiscus* in alleviating symptoms of knee OA. The herbal product showed promising efficacy in relieving knee OA symptoms compared to a placebo. Significant reductions in WOMAC scores for pain, stiffness, physical function, and total score were observed in the herbal product group after 3 and 6 weeks. These improvements were significantly greater than ones in the placebo group, indicating a positive effect of the herbal combination in relieving knee OA-related discomfort and functional limitations.

The selection of this herbal combination was based on traditional medicinal practices and increasing scientific evidence that supports its distinct anti-inflammatory and analgesic capabilities, which may exhibit synergistic benefits when combined. The herbal concentrations were established by preceding the Persian medicine references, information, as well as the experiences of traditional healers and folk medicine.

The mechanisms underlying the therapeutic effects of *C. autumnale*, *W. somnifera*, and *P. lentiscus* should be further investigated. While each herb possesses different bioactive compounds with anti-inflammatory and antioxidant properties, their synergistic interactions could enhance the therapeutic results. *C. autumnale* has been reported to contain alkaloid compounds, in particular

colchicine, which has antitumor and anti-inflammatory effects (27). It has been reported that the main alkaloid of the *C. autumnale* plant is colchicine and that it also contains other compounds, including colchicerine, colchamine, colchicoside, demethyl-3-colchicine, cornigerine, and 2-demethylcolchifoline (19).

Also, it has been shown that the essential oil of *P. lentiscus* contains terpenes and terpenoids, mainly monoterpenes and sesquiterpenes, which are responsible for the smell and taste of the plant. The most important secondary metabolites in the plant extract include phenolic compounds such as gallic acid, flavonol glycosides such as quercetin and myricetin and anthocyanins (20). Literature review has shown that terpene compounds can have a good anti-inflammatory effect against IL-1β, IL-6, TNF-α, and COX-2. In addition, the presence of polyphenols in the plant reduces the release of arachidonic acid, prostaglandin, and leukotrienes and ultimately leads to the anti-inflammatory effects of the plant (28). Furthermore, numerous investigations have linked the anti-microbial, anti-inflammatory, and anticancer properties of the *W. somnifera* plant to the presence of potent compounds such as withaferin-A, 12-deoxywithastramonolide, and withanolide-A (21,29,30). Lim *et al*. demonstrated that treatment with *W. somnifera* root extract significantly decreases hyperalgesia in response to von Frey stimulation of the hind paw in rat model. Moreover, *W. somnifera* root extract significantly alleviated pro-inflammatory cytokine levels associated with pain, including IFN-γ and IL-10 (31).

In accordance with the current study, several clinical studies have been carried out in which the plants mentioned were used individually or in combination in patients with OA. Almost all of these studies have shown the positive effect of these 3 herbs in the management and control of OA symptoms (13,14,16,27,32).

In the study by Mohtshami *et al*., 50 patients with knee OA received capsules of *P. atlantica* 3 times/day for one month, while 50 other patients received a placebo. The results showed that the *P. atlantica* group improved knee function, reduced pain, and decreased stiffness compared to the placebo group (14). Similarly, Mohammad's clinical study indicated that taking a special herbal composition containing various plant extracts such as *C. autumnale* and *W. somnifera* improved the symptoms of knee OA. This herbal composition could be used as an alternative treatment for the management of knee OA symptoms (27). In a double-blind study, 60 patients suffering from knee joint pain and discomfort were randomized to receive either *W. somnifera* or a placebo. Assessments using the WOMAC, Knee Swelling Index (KSI), and VAS were performed at the beginning and end of weeks 4, 8, and 12. The results showed that twice-daily treatment with *W. somnifera* over 12 weeks significantly reduced mean WOMAC, KSI, and VAS scores as well as stiffness and disability in the *W. somnifera* group compared to baseline and the placebo group (13). Another study evaluated the effectiveness of Rymanyl tablets, which contain 30 mg of *W. somnifera* along with *Alpinia galanga*, *Tinospora cordifolia*, and *Ricinus communis*, on improving symptoms of knee OA. Results showed that Rymanyl tablets could significantly reduce knee swelling, WOMAC-pain, stiffness, and physical function score, and VAS pain scale (33). The results of a systematic review and meta-analysis of intervention trials on the efficacy of colchicine for the treatment of OA showed that colchicine did not lead to a reduction in clinically important pain or an improvement in patients' function compared with placebo. This conclusion could be due to the lack of large group studies and the moderate quality of the evidence (32).

Future studies elucidating the molecular mechanisms of action could provide valuable insights into the pharmacological basis of the efficacy of the herbal combination. The clinical implications of current findings are significant, particularly in the context of the management of knee OA. Given the limitations and potential adverse effects associated with conventional pharmacotherapy, alternative treatments such as herbal medicine offer a promising avenue for patients seeking safer and more holistic approaches to relieve their symptoms (34). Incorporating herbal remedies into multimodal treatment regimens can improve overall therapeutic outcomes and patient satisfaction (35).

Altogether, the present study recognized the existence of numerous effective and cost-effective interventions for the treatment of knee OA pain, but specifically examined the efficacy of a combination of herbal remedies-

C. autumnale, *W. somnifera*, and *P. lentiscus*-known for their promising anti-inflammatory and analgesic properties. While conventional drugs play an important role in the treatment of knee OA, this research aimed to provide an alternative or complementary approach, especially for patients seeking natural therapies or suffering from the side effects of conventional treatments. Given the growing interest in integrative and holistic approaches to health, these herbal remedies could alleviate symptoms, reduce inflammation, and improve quality of life.

Despite the promising results, several limitations must be noted. The relatively small sample size and the short duration of the study may limit the generalizability of current results. Long-term efficacy and safety evaluations and larger randomized controlled trials are needed to confirm these results and determine the optimal dosage and duration of treatment. Factors such as medical history, drug history in recent months, and dietary habits of patients can also affect the results, which were not considered in the current study. Moreover, the use of other diagnostic parameters, such as knee joint radiography, can help to accurately diagnose the grade of knee OA.

Further research is needed to clarify the mechanisms of action and potential interactions of the herbal combination with conventional knee OA therapies. In addition, these findings could stimulate further research into the mechanisms of these herbal agents and improve understanding of their effects on cartilage maintenance.

CONCLUSION

The present study provided evidence for the efficacy and safety of a combination of *C. autumnale*, *W. somnifera*, and *P. lentiscus* in improving the symptoms of knee OA. The observed benefits emphasized the potential of herbal medicine as a complementary approach to conventional knee OA management. Further research is needed to fully elucidate the therapeutic mechanisms and optimize the clinical application of this herbal combination.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

H. Yousefi-Mohammadabad was responsible for investigation, methodology, and writing-original draft; M. Abbasifard participated in investigation, methodology, and project administration; S. Haji-Maghsoudi contributed to the formal analysis, data curation, software, and validation; H. Karegar-Borzi participated in conceptualization, funding acquisition, supervision, and review and editing; M. Raeiszadeh was responsible for supervision, resources, investigation, and writing-original draft, review, and editing. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

AI declaration

The authors did not use any AI-assisted technologies in the preparation of this manuscript.

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