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Original Article

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Impact of Oral Black Seed Oil Capsules on Serum Tenascin-C Levels in Patients with Knee Osteoarthritis: A Triple-Blind Randomized Clinical Trial

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Abstract

Background: This study evaluated the influence of oral black seed oil capsules on serum Tenascin-C (TNC) levels in knee Osteoarthritis (OA) patients.

Methods: This randomized, triple-blind clinical trial was carried out with patients suffering from knee OA who were referred to Qaem Hospital, or rheumatology clinic. Knee OA patients (grades 2 and 3) were randomly allocated to two groups: one group received a placebo, while the other was administered two capsules of black seed oil (1000 *mg* each) daily for a duration of three months, with each capsule containing 5.3 *mg* of thymoquinone. Demographics and serum TNC levels were recorded. The Western Ontario and Mcmaster Universities Arthritis Index (WOMAC) questionnaire and Visual Analog Scale (VAS) were used to evaluate results before and four months after treatment initiation. The data analysis was performed at a significance level of $p<0.05$.

Results: Forty patients were randomly allocated to intervention and placebo groups, with similar TNC levels. Four months after the intervention, the WOMAC score decreased significantly in both groups (placebo: 57.6±11.8 to 54.13±13; intervention: 56.2±8.9 to 50.7±10.1, both $p<0.001$). The reduction in WOMAC scores was significantly greater in the intervention group compared to the placebo group (5.5±5.6 *vs.* 3.5±5.5, $p=0.044$). Similarly, VAS scores dropped significantly in both groups (placebo: 6.3±0.8 to 5.6±1.2; intervention: 6.7±0.8 to 5.4±1.5, both $p<0.001$), with a more substantial reduction in the intervention group (1.3±1.2 *vs.* 0.7±1, $p=0.018$). However, no significant differences were observed in TNC levels in groups.

Conclusion: The study suggests that black seed oil compounds can effectively alleviate symptoms and pain severity in knee OA patients.

Keywords: Knee osteoarthritis, Plant oils, Tenascin-C, Thymoquinone

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Introduction

Osteoarthritis (OA) is a degenerative joint condition marked by the gradual breakdown of cartilage, the protective tissue at the ends of bones within joints (1,2). Among major joints, the knee is the most frequently affected site (3). Studies have shown that approximately one-third of the population over the age of 45 in the United States are affected by OA. By 2022, due to decreased life expectancy, sedentary lifestyles, and rising obesity rates, OA is anticipated to emerge as the fourth predominant cause of disability globally (4). Treatment for OA focuses on alleviating symptoms and improving joint function. Current options include medications, physical therapy, injections, and surgery. Nonsteroidal anti-inflammatory medications (NSAIDs) are frequently used for analgesia. Also, recent studies have shown promise for methotrexate, traditionally used for autoimmune diseases, to reduce pain and stiffness in OA (5-8). Topical agents, including capsaicin and topical NSAIDs, offer localized pain relief with minimal systemic effects (9). Physical therapy involving tailored exercise programs can improve strength and flexibility in affected joints (10). Intra-articular injections, including corticosteroids and hyaluronic acid, provide temporary relief by reducing joint inflammation or restoring lubrication. Platelet-Rich Plasma (PRP) therapy, an experimental treatment, may promote tissue repair and decrease inflammation (11,12). For advanced cases where conservative measures fail, surgical options such as arthroscopy to remove damaged tissue, osteotomy for bone realignment, or total joint replacement with an artificial implant are considered (13).

Despite ongoing research, no definitive cure for OA exists. Current investigations into disease-modifying agents, stem cell therapies, and biological treatments targeting inflammation and cartilage repair hold promise for future advancements in OA management. Tenascin-C (TNC) is an extracellular matrix protein that exists in various molecular forms due to alternative splicing and post-translational modifications (14). It interacts with numerous binding partners, including fibronectin, collagen, and proteoglycans, and plays a crucial role in cell proliferation and migration. While TNC is widely expressed in embryonic tissues, its presence is more limited in adults. However, elevated levels of TNC are observed in conditions such as inflammation, wound healing, cancer, and other pathological states. TNC modulates both pro-inflammatory and anti-inflammatory signaling pathways and is acknowledged as a crucial element in tissue injury and repair (15,16). Additionally, it influences immune responses by regulating cytokine production and is implicated in the progression of neurological diseases through various signaling mechanisms (17).

Evidence suggests that TNC contributes to the pathogenesis of OA and rheumatoid arthritis. During the development of articular cartilage, TNC gene expression is high, but decreases during cartilage maturation, nearly disappearing in healthy adult cartilage. However, its expression is elevated in the cartilage and synovial tissue in OA. Elevated levels of TNC have also been identified in the blood of OA patients, making it a potential biomarker for these conditions (18,19).

Recent evidence supports the role of black seed as an effective complementary and alternative treatment with anti-inflammatory effects for patients with knee OA. Studies suggest that black seed, due to its thymoquinone content, can alleviate pain in these patients. On the other hand, elevated levels of TNC can induce inflammatory mediators and lead to cartilage matrix degradation in OA. This, highlights the critical role of this glycoprotein in driving inflammation and pain in knee OA (20). Since no studies, domestically or internationally, have investigated the impact of black seed on TNC levels, this study examined to evaluate the impact of oral black seed oil capsules on serum TNC levels in patients with knee OA (21). Plants have served as the foundation of traditional medicine throughout human history and continue to be a source for modern pharmaceuticals (22). Among various medicinal plants, *Nigella sativa* or black seed (Ranunculaceae) is considered one of the most valuable nutrient-rich plants worldwide, with numerous scientific studies underway to confirm the efficacy of this species (23,24). The therapeutic effects of black seed are primarily attributed to its bioactive components, particularly thymoquinone. This molecule exhibits potent anti-inflammatory and antioxidant properties, which are essential in mitigating the inflammatory

processes associated with OA (20,25). Research has demonstrated that thymoquinone can inhibit the production of pro-inflammatory cytokines such as Interleukin-1 beta (IL-1β) and Tumor Necrosis Factor-alpha (TNF-α), both of which play critical roles in the pathophysiology of OA (26,27).

This study aims to investigate the effects of oral capsules containing black seed oil on serum levels of TNC in individuals suffering from knee osteoarthritis. Elevated levels of TNC are indicative of joint damage and inflammation, making it a relevant biomarker for assessing the progression of OA. By exploring the relationship between anti-inflammatory effects of thymoquinone and TNC levels, this research seeks to contribute to the understanding of the therapeutic efficacy of black seed oil in knee OA.

Materials and Methods

Inclusion and exclusion criteria

Inclusion criteria were: (1) Patients diagnosed with knee OA classified as grade 2 or 3 based on radiographic findings according to the Kellgren and Lawrence criteria. A grade of 2 indicates the presence of osteophytes and narrowing of the joint space, while a grade of 3 signifies more extensive osteophyte formation and sclerosis (2). No restrictions on age or gender (3). Patients in the initial stages of diagnosis who have not previously received any medication for knee OA.

Exclusion criteria were: 1) Diagnosis of secondary OA, 2) Presence of infection or primary inflammatory disease in the knee joint, 3) Arthritis caused by crystal deposition, 4) Intra-articular tumors, 5) Instability of knee joint ligaments, 6) History of previous intra-articular fractures, 7) Knee joint surgery (arthroscopic or otherwise) within the past 12 months, 8) Intra-articular injection of any medication within the past 3 months, 9) Presence of severe comorbid conditions (*e.g.,* metabolic diseases or a history of stroke), 10) Uncontrolled hypertension, 11) Diabetes, 12) Use of systemic corticosteroids or intra-articular corticosteroids or hyaluronic acid within the past 3 months, 13) Use of systemic or topical NSAIDs within the past week, 14) Exclusion from the study in cases of adverse drug reactions caused by black seed consumption, 15) Patients with concomitant neuropathy requiring medications like gabapentin or similar drugs, 16) Patients with psychological disorders or fibromyalgia, 17) Use of omega-3 supplements or glucosamine in the past 3 months, 18) Concurrent use of heparin or warfarin.

Study design

This randomized, triple-blind clinical trial was carried out with patients suffering from knee OA who were referred to Qaem Hospital, or rheumatology clinic. Patients with knee OA defined as grades 2 and 3 according to the Kellgren and Lawrence criteria, as verified by the radiologist, were randomly allocated to intervention and control groups. Results of the study conducted by Page and colleagues (28) was used to calculate the sample size. Sampling was performed conveniently from patients referred to the recruitment centers. Random allocation to the two groups was conducted using a block randomization method. Considering an estimated sample size of 80 patients, 20 blocks of 4 were randomly created from the following combinations: AABB, ABAB, ABBA, BBAA, BABA, and BAAB. Patients were assigned to the treatment groups according to the generated sequence. In the control group, patients received a placebo, while in the intervention group, patients were instructed to take two black seed oil capsules daily (one every 12 *hr*) for 3 months. The patients, researchers and the analyzer were blind to the groups which patients were assigned.

Capsules were made by Barij company and purchased from a pharmacy without financial or material support from the manufacturer. Each black seed oil capsule contained 5.3 *mg* of thymoquinone, a dose considered effective for its anti-inflammatory properties and its potential for the reduction of cartilage degradation. Placebo was prepared in a method which matched the active capsule in color, odor, and appearance. Both groups were advised to document the type and number of NSAIDs that were being consumed during the study. Patients were informed that they would be under the care of the study investigator throughout the trial and were instructed to avoid self-administration of other medications, physical therapy, acupuncture, or alternative treatments. During the prescription of black seed oil, potential drug-related side effects were considered. Black seed oil acts as an antioxidant, so its use alongside other chemical medications required

careful monitoring by the attending physician to avoid harmful drug interactions. In patients with food allergies, black seed oil might cause symptoms such as nausea, headache, and dizziness. Additionally, in some cases, patients undergoing cardiovascular treatments experienced significant drops in blood pressure after consuming black seed oil. Such patients were advised to refrain from using this herbal remedy to prevent adverse outcomes.

The Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1401.319--Mashhad, Iran) approved the study protocol. Patients gave written informed consent and were informed about the possible adverse reaction of the treatment prior to the participation. The study protocol has been registered in the Iranian Registry of Clinical Trials (IRCT.ir) under the identification code: IRCT20210923052559N1

Demographic data for each patient were first collected, including age, gender, Body Mass Index (BMI), occupation, marital status, and other pertinent information. Subsequently, 4 *ml* of blood was collected from each patient to assess TNC levels. Serum TNC levels were measured using the ELISA method with a TNC kit from Zellbio GmbH, both before the start of treatment and three months after treatment. Patients were monitored monthly for knee pain and examined, during which the Western Ontario and Mcmaster Universities Arthritis Index (WOMAC) questionnaire and pain intensity assessments were completed.

To evaluate treatment outcomes in patients with knee OA, the WOMAC questionnaire—an internationally standardized tool was utilized. This tool statistically evaluates three domains: pain (5 questions), joint stiffness (2 items), and physical function (17 things). Each question has five response options, scored from 0 to 4. The overall score varies from 0 to 100, with 0 representing least difficulty and 100 denoting utmost difficulty. A reduction in the WOMAC score indicates improvement. The results for each domain were expressed quantitatively in five subcategories of WOMAC.

This questionnaire has been translated into Persian and localized for use in Iran, and the validity and reliability have been proven in several investigations. Pain severity was evaluated using the Visual Analog Scale (VAS), in which patients rated their pain on a scale from 1 to 10. Additionally, patients recorded the number of NSAIDs they consumed each month, if applicable. The WOMAC questionnaire and VAS pain scores were completed for each patient before treatment and four months after initiating treatment. The collected data were then categorized and analyzed using SPSS software.

Statistical analysis

SPSS version 28 was used for statistical analysis. Descriptive and demographic information was presented through frequency distribution tables and appropriate charts. Chi-square tests were utilized for result analysis to investigate the relationship between qualitative variables and study groups. For quantitative variables, normality was first assessed using the Shapiro-Wilk test, and the results are shown in the table 1. Independent t-tests were employed to compare the mean values of normally distributed quantitative variables between the two groups. The Mann-Whitney test or other comparable non-parametric tests were utilized for quantitative data exhibiting a non-normal distribution. Spearman's correlation test was utilized to evaluate the relationship between the scores of various factors, with rho values provided. A significance threshold of 0.05 was utilized for all tests.

Results

Population study

Initially, 100 patients were screened based on the inclusion and exclusion criteria upon their referral to recruitment centers. Ten patients were excluded, among which five were excluded due to a history of intra-articular injection within the past three months, three due to knee joint instability, one due to a previous intra-articular fracture, and one due to knee surgery within the past 12 months. Ultimately, 90 patients were randomly assigned to either the intervention or placebo group. However, five participants from each group withdrew their consent to continue the study due to insufficient compliance, primarily caused by reluctance to take oral medication. Consequently, 40 patients in each group completed the study (Figure 1). In the normality assessment using the Shapiro-Wilk test, all variables except TNC levels were not normally distributed, likely due to the small sample size.

Table 1. Baseline characteristics of patients in the two groups at the start of the study

Variable	Placebo group (n=40)	Intervention group (n=40)	p-value
Age (Median [IQR])	52.5 [46.0, 56.0]	51.5 [48.25, 56]	0.828*
Body Mass Index (BMI) (Median [IQR])	23 [21.25, 24.0]	23 [21.0, 23.0]	0.380*
Gender (Frequency [%])			
Male	7(17.5)	3 (7.5)	†0.176
Female	33(82.5)	37 (92.5)	
Osteoarthritis Grade (Frequency [%])			
Grade 2	19(47.5)	22 (55)	†0.502
Grade 3	21(52.5)	18 (45)	

* Mann-Whitney U test. † Chi-square test.

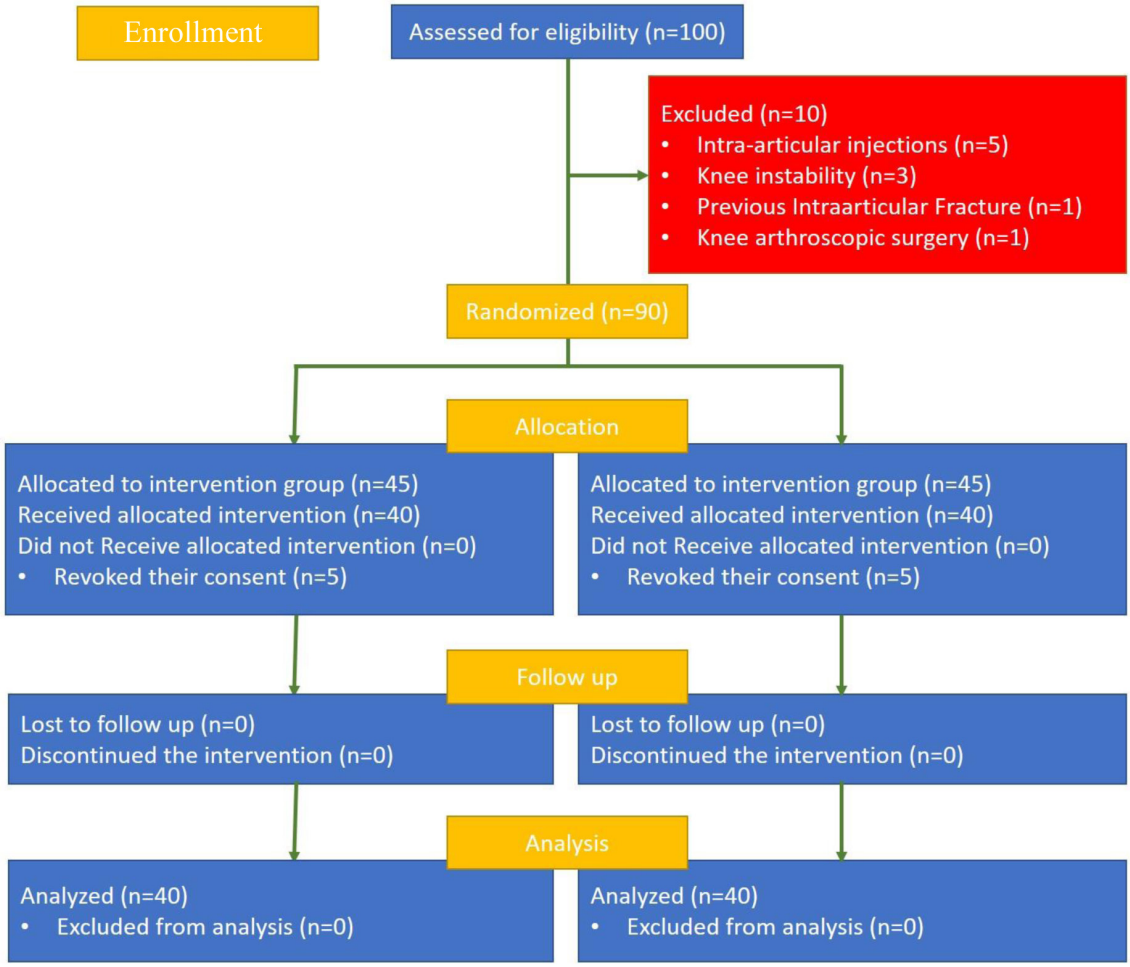


Figure 1. Diagram the patient recruitment process for the study.

There was no statistically significant difference in the median age of patients in the intervention group [51.5 (48.25, 56) years] compared to the placebo group [52.5 (46.0, 56.0) years] (p=0.828). The BMI of the intervention group was 23 [21.0, 23.0], whereas the BMI of the placebo group was 23 [21.25, 24.0]. No statistically significant difference was found (p=0.380). The predominant demographic in both groups were female, accounting for 82.5% in the placebo group and 92.5% in the intervention

group (p=0.176). The majority of patients in the placebo group demonstrated grade 3 OA (52.5%, 21 patients), whereas the majority in the intervention group displayed grade 2 OA (55%, 22 patients); this difference was not statistically significant (p=0.502) (Table 1).

Pre-intervention and post-intervention WOMAC Scores in placebo and intervention groups

The pre-intervention median [IQR] WOMAC scores for the placebo and intervention groups were 56 [48, 70] and 56 [48, 64], respectively, showing no statistically significant change (p=0.521). Likewise, the pre-intervention median [IQR] VAS ratings were 6 [6, 7] in the placebo group and 7 [6, 7.75] in the intervention group, revealing no statistically significant difference (p=0.414). The pre-intervention mean TNC levels were similar between the two groups (p=0.557) (Figures 2-4). The median [IQR] WOMAC score in the placebo group diminished from 56 to 51 post-intervention, a change that was statistically significant according to the Wilcoxon test (p<0.001). In the intervention group, the WOMAC score fell from 56 to 50, a change that was statistically significant (p<0.001). Analysis of the alterations in WOMAC scores across the two cohorts indicated that the decrease was much more pronounced in the intervention group compared to the placebo group (6 *vs.* 5, p=0.048) (Figure 2).

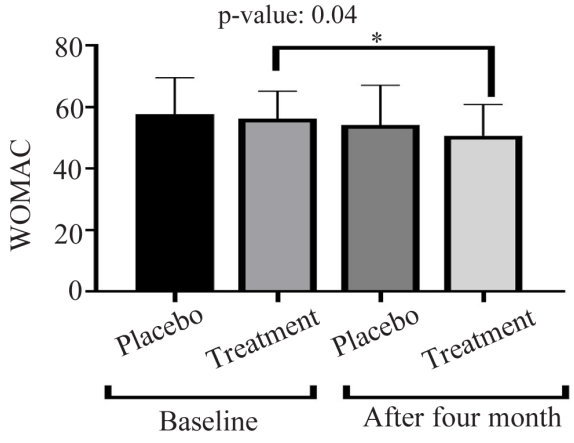


Figure 2. Comparison of WOMAC and its changes between the two study groups (Wilcoxon test, median of WOMAC scores are reported).

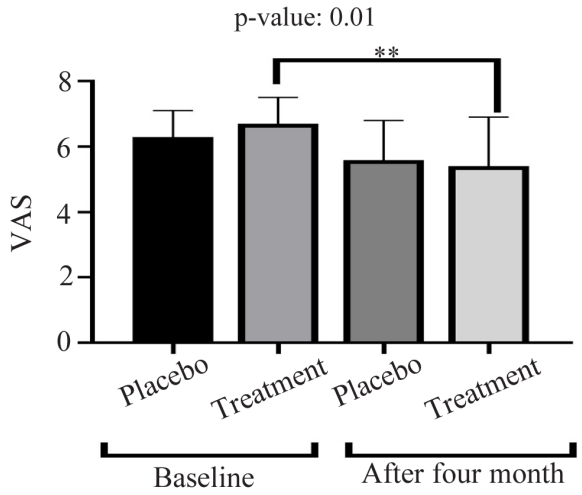


Figure 3. Comparison of VAS and its changes between the two study groups (Wilcoxon test, mean of VAS scores are reported).

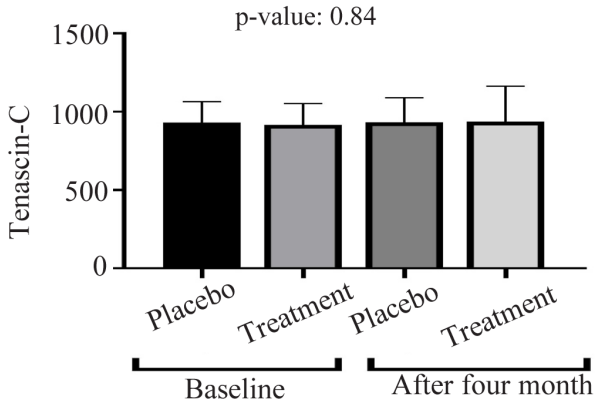


Figure 4. Comparison of serum TNC levels and their changes two study groups (Wilcoxon test, mean Tenascin-C is reported).

Changes in VAS and TNC levels in placebo and intervention groups

The VAS score in the placebo group diminished from 6.3±0.8 to 5.6±1.2 post-intervention, a change that was statistically significant according to the Wilcoxon test (p<0.001). In the intervention group, the VAS score fell from 6.7±0.8 to 5.4±1.5, a change that was statistically significant (p<0.001). Analysis of the alterations in VAS ratings across the two cohorts indicated that the decrease was markedly more pronounced in the intervention group compared to the placebo group (1.3±1.2 *vs.* 0.7±1, p=0.018) (Figure 3). The findings of the study concerning TNC levels and their variations revealed no statistically significant differences between the two groups

(p=0.847) (Figure 4).

The correlation analysis indicated a strong positive association between changes in WOMAC scores and VAS scores in the placebo group (r=0.84, p<0.001). No substantial association was seen between alterations in serum TNC levels and WOMAC scores (r=0.27, p=0.082) or VAS ratings (r=0.176, p=0.277). A substantial positive association was identified in the intervention group between alterations in WOMAC scores and VAS scores (r=0.95, p<0.001). No substantial association was seen between alterations in serum TNC levels and WOMAC scores (r=0.09, p=0.551) or VAS ratings (r=0.13, p=0.421). Concerning treatment-related side effects, patients in both cohorts did not report any particular adverse events.

Discussion

This triple-blind, randomized clinical trial with a parallel-group design was conducted to evaluate the effects of oral black seed oil capsules on the severity of symptoms and serum levels of the biomarker TNC in patients with knee OA. The findings of the study demonstrated that although both groups experienced significant reductions in WOMAC and VAS scores following the intervention, the extent of reduction was notably greater in patients receiving oral black seed oil capsules. However, the study results showed no significant difference in TNC levels or changes between the two groups during the study. Additionally, a secondary analysis revealed no correlation between serum TNC levels and WOMAC or VAS scores, although a significant positive correlation was observed between WOMAC and VAS scores. Based on these findings, formulations containing black seed oil may effectively reduce symptom severity and pain in patients with knee OA. The significant reduction in WOMAC and VAS scores in the intervention group aligns with existing evidence supporting the anti-inflammatory and analgesic properties of thymoquinone, the active compound in black seed oil. Previous studies have demonstrated that thymoquinone can inhibit key pro-inflammatory mediators, such as IL-1β and TNF-α, which are central to OA pathogenesis (29). This mechanism likely contributed to the observed improvements in pain, stiffness, and physical function. Notably, the more substantial reductions in WOMAC and VAS scores in the intervention group compared to the placebo group suggest that black seed oil may provide an adjunctive benefit over standard care (30). Despite these promising clinical outcomes, the absence of significant changes in TNC levels raises questions about the role of TNC as a biomarker in OA treatment. Although elevated TNC levels have been reported in OA patients, our findings suggest that short-term intervention targeting inflammation may not sufficiently impact systemic TNC concentrations. This could be due to several factors, including the persistence of local joint pathology, the complexity of TNC regulation, or the potential need for longer intervention durations to observe measurable changes. On the other hand, maybe black seed oil is not an efficient substance for regulating TNC. Interestingly, previous studies have proposed that TNC expression is more localized to affected tissues rather than reflected consistently in systemic circulation. This could explain why black seed oil, despite its anti-inflammatory effects, did not alter serum TNC levels (14,31). Additionally, the baseline variability in TNC levels and the relatively small sample size may have limited the ability of our study to detect subtle changes. Future research should consider exploring the effects of black seed oil on synovial fluid TNC levels, which may provide a more localized and sensitive measure of therapeutic impact (14,32). Another noteworthy finding is the significant reduction in NSAID use reported by patients in the intervention group. This indicates that black seed oil may be used as an alternative treatment for managing pain in OA. It may help decrease the dependence on NSAID and their related side effects. These findings are particularly important considering the common use of NSAIDs in managing OA and the increasing interest in safer, plant-based treatment options. The research conducted by Amir Taheri Afshar *et al* examined the efficacy of black seed oil on serum indicators of inflammation and oxidative stress, alongside quality of life in individuals with knee OA. They utilized a double-blind, randomized, parallel-group trial design. Forty-five patients were randomly assigned to three groups in a 1:1:1 ratio. The first group received 2.5 *ml* of oral black seed oil twice daily along with topical placebo oil, the second

group received 2.5 *ml* of topical black seed oil three times daily along with oral placebo oil, and the third group received both oral and topical placebo oils. The results indicated that during a six-week intervention, oral black seed oil significantly enhanced blood levels of high-sensitivity C-reactive protein (hs-CRP) (p=0.003), Malondialdehyde (MDA) (p=0.003), and Total Antioxidant Capacity (TAC) (p=0.003). Oral black seed oil markedly decreased blood hs-CRP in comparison to both the placebo and topical black seed oil [mean difference (95%CI): −0.81 (−1.45 to −0.19); p=0.012 and −0.76 (−1.38 to −0.15); p= 0.016, respectively].

Furthermore, significant improvements in the subscales of general health, physical, and mental health were observed in both oral and topical black seed oil groups compared to the placebo group (p<0.05). The study concluded that oral black seed oil reduced serum hs-CRP and MDA levels and increased serum TAC levels. However, this effect was only significant for hs-CRP compared to placebo. Both topical and oral black seed oil significantly improved quality of life scores and subscales compared to placebo. Therefore, it can be concluded that oral black seed oil is an effective adjunctive treatment for OA, with no major side effects (33). These findings are consistent with the present study, supporting systemic effects of black seed oil in reducing serum biomarkers. In the study conducted by Huseini *et al*, the effects of black seed oil administration on the WOMAC score, VAS score, and the daily consumption of acetaminophen in patients with knee OA were evaluated in a double-blind clinical trial. Consistent with the findings of the present study, this research also demonstrated that the administration of black seed oil was associated with significant improvements in the WOMAC score, reductions in the VAS score, and decreased acetaminophen consumption (34). Similarly, Salimzadeh *et al* assessed the effects of processed oral black seed oil on relieving symptoms of knee OA in a randomized, double-blind clinical trial. In alignment with the present findings, their results showed statistically significant improvements in symptoms of both groups post-intervention, with more pronounced therapeutic effects in the intervention group. However, not all evaluated outcomes showed significant improvements in the intervention group; parameters such as knee injury and OA outcome scores and the average number of acetaminophen tablets used by patients did not exhibit statistically significant differences between the two groups (35). *In vitro* and animal studies also support the observed findings. Turhan *et al* demonstrated that intra-articular administration of black seed oil improved joint surface repair in mice (36).

In the present study, oral black seed oil administration was not associated with any specific adverse effects, a finding consistent with previous studies (33,34). Based on the findings of this study, which highlight the benefits of oral administration of black seed oil in reducing symptoms and pain in patients with knee OA, its inclusion in the treatment protocol for these patients can be considered. On the other hand, given the lack of correlation between serum levels of TNC and established disease activity factors such as WOMAC and VAS scores in OA patients, researchers do not recommend measuring serum levels of this biomarker as a routine practice.

Challenges and future directions

The most significant strength of the present study lies in its precise design and its triple-blind, randomized approach. This methodology effectively mitigates the risk of bias, particularly considering the high likelihood of placebo effects in patients with OA. However, the generalizability of the results could have been enhanced with a larger number of patients in each group. Unfortunately, this was not feasible due to the considerable costs associated with assessing serum levels of TNC. Also, the study focused exclusively on grades 2 and 3 knee OA, and the findings may not be applicable to more advanced stages of the disease. Future studies should explore the synergistic effects of black seed oil in combination with other pharmacological approaches for managing knee OA. Furthermore, it is essential to evaluate the impact of black seed oil-containing formulations on patients' quality of life and other inflammatory biomarkers, such as hs-CRP, and follistatin-like protein 1, in subsequent research.

Conclusion

This triple-blind, randomized, parallel-group clinical trial was conducted to investigate the effects of

oral black seed oil capsules on symptom severity and serum levels of the biomarker TNC in patients with knee osteoarthritis. The results demonstrated a significant reduction in WOMAC and VAS scores in both groups following the intervention. However, the reduction in these scores was substantially greater in patients treated with oral black seed oil capsules. Notably, the study found no significant differences in TNC levels or changes between the two groups during the trial. Additionally, secondary analyses revealed no correlation between serum TNC levels and WOMAC or VAS scores, although a significant positive correlation was observed between WOMAC and VAS scores.

Ethical approval and consent to participate

The Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1401.319 Mashhad, Iran) approved the study protocol. Patients gave written informed consent and were informed about the possible adverse reaction of the treatment prior to the participation. The study protocol has been registered under the identification code: IRCT20210923052559N1.

Consent for publication

All authors consent to publish the article.

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Conflicts of Interest

Authors declare no conflict of interest.

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