

Black seed (*Nigella sativa*) as an adjuvant therapy in the treatment of patients with rheumatoid arthritis Clinical trial

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Abstract

Aim and Objectives: This study aimed to investigate the effects of black seed oil on clinical symptoms and inflammatory biomarkers as an adjuvant therapy on rheumatoid arthritis (RA) patients taking disease-modifying anti-rheumatic drugs (DMARDs).

Materials and Methods: Twenty-five RA patients were enrolled, and each received one capsule of 1000 mg black seed oil daily as adjuvant therapy with DMARDs for eight weeks. Disease activity score for 28 joints (DAS28), visual analogue scale (VAS) for pain, serum levels of tumor necrosis factor-alpha (TNF- α) and Interleukin-10 (IL-10), white blood cells (WBCs), renal function and liver function tests were assessed at baseline and at the end of the trial.

Results: the results show a significant decrease in the DAS28 score and the VAS for pain in RA patients. Serum levels of pro-inflammatory cytokine TNF- α and a number of WBCs were decreased significantly while the increment of serum levels of anti-inflammatory cytokine IL-10 was statistically non-significant. No significant changes occurred in the levels of urea, creatinine, aspartate aminotransferase (AST) or alanine aminotransferase (ALT).

Conclusion: black seed oil as an add-on therapy induced a significant improvement in clinical symptoms and inflammation of RA.

Keywords: Rheumatoid arthritis, Black seed, DMARDs, TNF- α , DAS28.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation of synovium of the joints, leading to joint deformity or destruction and weakening of tendons and ligaments [1]. It is symmetrical in nature and consequently affects small and large joints. Progression of the disease results in involvement of other organs such as the lungs, kidneys and eyes [2].

Clinical manifestations of RA include joint pain, erythema, swelling, limited joint mobility and morning stiffness. Although the exact cause of the disease is unknown, evidence has recently revealed that genetics may be the likely cause of the disease, along with environmental factors (e.g., cigarette smoking) that play an important role [3,4].

Early diagnosis of RA is key to achieve desirable treatment outcomes and prevent disease progression and joint deformities. However, diagnosing and differentiating RA from other rheumatic diseases is challenging and based on clinical manifestations, patient history, physical examination, laboratory investigations and image analysis [5]. Treatment of RA should be initiated immediately after diagnosis to induce remission and prevent further joint destruction.

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The main goals of therapy are to reduce pain and inflammation, enhance joint mobility and overall quality of life and prevent joint deformity [6].

Treatment of RA includes both non-pharmacological and pharmacological approaches. The current pharmacotherapy of RA includes many options to prevent disease progression, relieve symptoms and reduce inflammation such as DMARDs, corticosteroids and non-steroidal anti-inflammatory drugs [7]. However, the adverse effect profile and costs may limit their use. Many herbal medicines and food components have been tried to improve the disease state [8].

Black seed or black cumin seed (*Nigella sativa*) belonging to the Ranunculaceae family is an herbal component with many important pharmacological and medicinal properties that has been used for a wide range of diseases including diabetes mellitus, cancer, hypertension, obesity, hyperlipidemia, inflammatory diseases and infectious diseases [9,10].

Black seed possesses a potent anti-inflammatory effect and is used to treat many rheumatic diseases. The main active ingredient of black seed oil, thymoquinone (TQ), is responsible for most of black seed's therapeutic and anti-inflammatory effects [11].

This clinical study is designated to evaluate the effect of black seed oil as an adjuvant with DMARDs in RA patients on pain, inflammation, hematological markers and biochemical changes. It also aims to investigate the tolerability and adverse events associated with black seed oil use and the influence of this adjuvant agent on the overall health and quality of life of RA patients. This clinical study focused on the clinical effect, anti-inflammatory effect and adverse effect profile of black seed oil as an add-on therapy based on a two-month follow up.

2. Materials and Methods

2.1. Study design

This study was a prospective clinical trial to evaluate the effects of black seed oil as an adjuvant with biologic and non-biologic DMARDs based on a two-month follow up. Anti-inflammatory effects, clinical effects and tolerability of black seed oil all had been assessed at baseline and eight weeks after intervention. The study was conducted over a period of 10 months; data collection process and patient enrollment started in November 2021, and patient follow up was completed in April 2022. All participants in the study were moderate to severe RA patients who attended the rheumatology and medical rehabilitation department at the Rizgary teaching hospital in Erbil City, Iraq. The study protocol was approved by the Research Ethics Committee of the College of Medicine at Hawler Medical University (No. 9 on November 2, 2021) in accordance with the Declaration of Helsinki and its amendments and the guidelines for good

clinical practices issued by the Committee of Propriety Medicinal Products of the European Union. The study consists of an eight-week follow-up and two visits (i.e. on day 1 and at the end of week 8) and includes blood sample collection at both visits.

2.2. Enrollment and Consent

Of 30 patients with moderate to severe RA enrolled in the study, 25 completed the study successfully. Over 80 patients were screened, and only those who met the inclusion criteria were enrolled. The participation process was voluntary; all the patients were asked to sign a consent form and all necessary information about the study was disclosed to the patients. Demographic data and information about drug regimen, allergies, medical history and medication history were gathered from the participants in face-to-face interviews and a questionnaire the researcher completed. Inclusion criteria includes moderate to severe RA patients, male or female, of adult age and with no known drug allergies. Exclusion criteria include newly diagnosed patients, severe renal or kidney disease, and use of black seed or any of its derivatives in the last six months.

2.3. Measurement of clinical effect and anti-inflammatory effect

For assessment of the clinical efficacy of black seed oil, DAS28 and VAS for pain were measured in all the patients in the study. The DAS28 score is a measure of disease activity in RA that is a composite score derived from four measures [12].

To calculate DAS28, one must count the number of tender joints and the number of swollen joints and measure the erythrocyte sedimentation rate or C-reactive protein (CRP), as well as use the VAS for assessment of overall health and quality of life [13]. A numerical rating scale version of the VAS is used in this study for assessment of pain and quality of life where patients are asked to circle the number between 0 and 10 that best describes their pain intensity [14].

For assessment of anti-inflammatory effect of black seed oil, measurements of complete blood count (CBC), IL-10, TNF- α and CRP were performed for all participants twice (i.e., at baseline and eight weeks after intervention). Blood samples were collected from patients in two tubes, one containing Ethylenediaminetetraacetic acid (EDTA) for CBC analysis to be performed immediately and the second containing a separating gel for isolation of serum to measure IL-10, TNF- α and CRP after serum samples froze at -20 °C.

2.4. Assessment of tolerability of black seed oil

For the assessment of black seed tolerability in RA patients, liver function tests (i.e., AST and ALT) and renal function tests (i.e., creatinine and urea) are performed. Additionally, continuous follow up with the patients is necessary to detect and investigate possible side effects.

2.5. Statistical analysis

Analysis of data and obtaining of results were performed

using Statistical Package for the Social Sciences (SPSS) program (IBM SPSS statistics software, Version 26). Central tendency and dispersion were used to analyze demographic data and baseline characteristics. Comparing baseline values with post-interventional values of different parameters and correlation between obtained values were analyzed by t-test chi-square test and Mann-Whitney test. A p-value of < 0.05 was considered to indicate statistical significance.

3. Results

In the present study, 30 RA patients met the inclusion criteria and were enrolled. Of these, 25 completed the study successfully, and five withdrew for various reasons.

3.1. Demographic data and baseline characteristics

The majority of the participants were female, and most were either overweight or obese. About half of the patients present with comorbidities (e.g., diabetics or hypertensives). Positive family history was recorded for 12 participants with a first- or second-degree relatives presented with history of RA. All patient

Table 1: Demographic data and characteristics of the patients at baseline.

Parameters	RA patients n = 25	
Gender n (%)	Male 5 (20)	Female 20 (80)
Age (year)	56 ± 10.4	
BMI (Kg/m²)	29.1 ± 6.1	
Family history n (%)	Negative FH 13 (52)	Positive FH 12 (48)
Cigarette smoking n (%)	Smokers 2 (8)	Non-smokers 23 (92)

Values are presented as percent or mean ± standard deviation; n: number of patients; BMI: body mass index; FH: family history

3.2. Assessment of clinical efficacy

3.2.1. Measurement of DAS28 score

Table 2 shows that the use of black seed oil as an adjuvant in RA patients caused significant decrease in tender joints, swollen joints and VAS for pain, while the change in CRP levels were insignificant. However, the overall DAS28 score decreased significantly with a p-value of 0.001 (see Figure 1).

Table 2: Comparison of DAS28 score parameters at baseline and post intervention.

DAS28 score parameters	Baseline	After 60 days	P-value
No. of tender joints	9.04 ± 7.93	6.48 ± 5.14	0.011
No. of swollen joints	3.80 ± 3.78	2.24 ± 2.60	0.002
CRP (mg/L)	7.40 ± 11.20	5.75 ± 6.70	0.301
VAS (0–10)	6.84 ± 2	5.32 ± 2	0.003

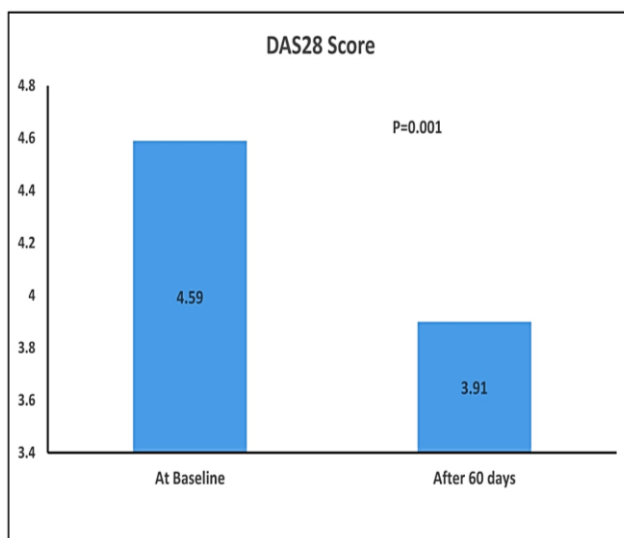


Figure 1: DAS28 score before and after the use of black seed oil as adjuvant.

3.2.2. Visual analogue scale for pain

The adjuvant use of black seed oil result in a significant change in the VAS categories. At baseline, more than half of the patients (52%) recorded a severe pain level and few (8%) had a low pain level. Records after two months of intervention indicated a significant decrease in pain severity. Sixteen percent of the patients assessed their pain as severe, while the majority (64%) had a moderate pain level (see Table 3).

Table 3: The effect of adjuvant use of black seed oil on the VAS for pain categories

Pain Level (1–10)	Baseline n (%)	After 60 days n (%)	P-value
Low (1–3)	2 (8)	5 (20)	0.024
Moderate (4–7)	10 (40)	16 (64)	
Severe (8–10)	13 (52)	4 (16)	

3.3. Assessment of anti-inflammatory effect

Black seed oil use as adjuvant caused a significant decrease in pro-inflammatory cytokine TNF-α levels in RA patients compared to baseline levels (see Table 4). By contrast, black seed oil increased the levels of anti-inflammatory cytokine IL-10, but the increment was statistically insignificant. There was also a significant decrease in the number of white blood cells (WBCs) compared to WBCs at baseline.

Table 4: Effect of adjuvant use of black seed oil on inflammatory biomarkers.

Parameter	At baseline	After 60 days	P value
TNF-α (pg/ml)	29.91 ± 11.61	24.42 ± 8.42	0.006
IL-10 (pg/ml)	21.21 ± 7.43	37.71 ± 7.16	0.14
WBC (cell×10 ⁹ /L)	8.33 ± 1.78	7.74 ± 1.23	0.005

3.4. Tolerability of black seed oil

3.4.1. Assessment of renal function

The effect of black seed oil on renal function was assessed by estimation of urea and creatinine levels, which shows insignificant change compared to baseline records (see Table 5).

Table 5: Effect of black seed oil as adjuvant on renal function in RA patients

Test	At baseline	After 60 days	P-value
Urea (mg/dl)	26.74 ± 6.67	26.59 ± 6.29	0.88

Creatinine (mg/dl)	0.68 ± 0.20	0.65 ± 0.21	0.43
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3.4.2. Assessment of liver function

Liver enzyme AST and ALT were estimated to assess the effects of black seed oil on liver function. The change in AST and ALT levels were insignificant compared to baseline estimations based on two-month follow up (see Table 6).

Table 6: Effect of black seed oil as adjuvant on liver function in RA patients

Test	At baseline	After 60 days	P-value
AST (U/L)	20.25 ± 5.98	21.90 ± 7.26	0.066
ALT (U/L)	21.35 ± 13.75	20.77 ± 12.26	0.923

3.5. Correlation between clinical effect and anti-inflammatory effect of black seed oil

Black seed oil as an adjuvant had a significant clinical effect and significant decrease in inflammation in RA patients. Figure 2 shows a significant post intervention positive correlation between DAS28 score and TNF-α biomarker with an r-value of 0.45.

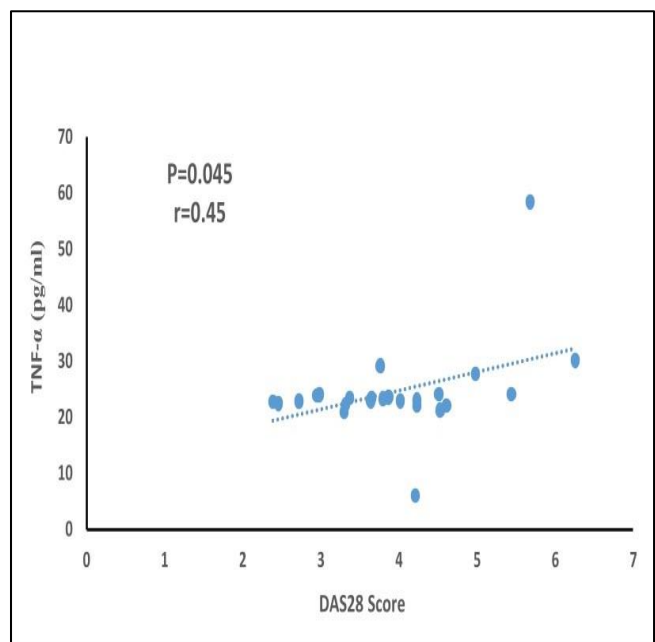


Figure 2: Correlation between the effect of black seed oil as adjuvant on DAS28 score and levels of TNF-α biomarker.

4. Discussion

Rheumatoid arthritis is a chronic, debilitating disease that causes painful joint swelling, joint synovial lining inflammation and cartilage and bone destruction. For RA treatment, a variety of anti-inflammatory and disease-modifying medications are available [15]. However, long-term use of these medications is linked to serious adverse effects. Furthermore, only a percentage of RA patients benefit from these medications. It is therefore necessary to seek promising therapeutic agents that are both efficient and harmless. A wide range of natural products, including herbs, provide a rich source for such anti-arthritis compounds [16].

Black seed is a medicinal herb that may be effective in treating and alleviating RA symptoms. The beneficial effects of black seed on RA prevention and therapy have been studied recently, and experimental research and clinical studies have yielded encouraging results. According to the data, black seed can affect the primary characteristics of RA through a variety of mechanisms [17].

In the present study, 1000 mg of black seed oil orally administered as an adjuvant therapy with disease modifying agents caused a significant decrease in DAS28 score. This effect is attributed to its anti-inflammatory effect, as black seed in the aforementioned doses leads to a significant decrease in tenderness and swelling of joints.

Black seed oil as adjuvant also caused a significant change in the VAS for pain and overall quality of life of enrolled RA patients. This finding is supported by various previous studies that have revealed the anti-nociceptive effect of black seed oil, particularly its main active ingredient, TQ. The analgesic mechanism of black seed is preventing the generation of prostaglandins by inhibition of cyclooxygenase pathway of arachidonic acid metabolism [18,19].

Administration of black seed oil as adjuvant for eight weeks significantly decreased serum levels of pro-inflammatory cytokine TNF- α and the number of WBCs and non-significantly increased the levels of anti-inflammatory cytokine IL-10. The anti-inflammatory effects of black seed and TQ in the present study were consistent with results of *in vitro* studies on the effects of black seed and specially TQ in RA patients. This demonstrates that TQ significantly decreased TNF- α induced biological and inflammatory processes [20].

Black seed oil 1000 mg increased serum levels of IL-10, but the increment was statistically insignificant. Findings of a randomized double blind clinical trial on 42 RA patients by [21] in Iran indicated that 500 mg black seed oil supplementation for two months caused a significant increase in serum levels of IL-10 compared to a placebo group in RA patients.

Correlation between clinical efficacy and anti-inflammatory effect of black seed oil was examined to determine whether

the improvement in clinical symptoms were correlate to decrease in inflammatory markers. For this purpose, analyzing the correlation between post-treatment DAS28 score and serum levels of TNF- α performed and the results indicate a significant correlation of both.

Black seed oil capsules in the present study were generally well tolerated, and no participants reported side effects. The serum creatinine and urea level results indicate that black seed oil as adjuvant does not have adverse effects on renal function. Changes in liver function tests AST and ALT were insignificant compared to baseline records. Animal studies on mice and rats to investigate acute and chronic toxicity have concluded that black seed fixed oils have a wide therapeutic range and are well tolerated in typical doses [22].

Various studies suggest that black seed oil may play a protective role for the kidney and liver. A study by [23] revealed that black seed decreased the harmful effects of chemotherapeutic agents on kidney. Black seed may protect the liver from damage by multiple mechanisms, including inhibition of lipid peroxidation and oxidative stress (i.e., an increase in antioxidant enzymes) [24]. The patients in this study were on biologic and non-biologic DMARDs, which are known for their adverse effects on liver and kidney despite that in the two-month period of taking black seed oil as an adjuvant, no increase in liver and renal function tests occurred. This finding may suggest that black seed exerts nephroprotective and hepatoprotective effects.

This study observed that black seed oil can be a beneficial adjuvant therapy for moderate and severe RA patients, as it reduces inflammation, eases symptoms and improves quality of life.

5. Conclusion

Black seed oil (i.e., 1000 mg orally administered to RA patients) caused a significant improvement in pain severity and clinical symptoms, including swelling and joint tenderness. Black seed oil in certain doses also caused a significant decrease in the serum levels of pro-inflammatory biomarker TNF- α and the number of WBCs, while non-significantly decreasing the levels of anti-inflammatory biomarker IL-10. No significant change was recorded in the levels of renal function tests (i.e. urea and creatinine) or liver function tests (i.e. AST and ALT) despite patients taking DMARDs. This may be suggestive of a protective role for black seed oil in the liver and kidneys.

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Ethics: The study protocol was approved by the Research Ethics Committee of the College of Medicine, Hawler Medical University (No. 9 in November 2, 2021)

Patient consent: The participation process in the study was voluntary; all the patients were asked to sign a consent form. All necessary information about the study was declared to the patients.

Clinical trial registration status: This article is prepared for the MSc degree program, and it is not registered due to the narrow timeline given for conducting the research.

Language and consistency: This document undergoes a professional editorial work for language, consistency and academic style. Declaration of professional editorial assistance is available upon request.

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