



Research article

Therapeutic effects of black seed oil supplementation on chronic obstructive pulmonary disease patients: A randomized controlled double blind clinical trial

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ABSTRACT

This study sought to examine whether supplementation of Black Seed Oil (BSO) can improve pulmonary function tests (PFTs), inflammation, and oxidant-antioxidant markers in COPD patients. The study involved 100 patients of mild to moderate COPD divided randomly into 2 groups who were appointed to receive standard medication only (control group) or with additional Black Seed Oil (BSO group). They were assessed initially and after 3 months, 44 patients responded in control group and 47 patients in BSO group. BSO group evidenced a significant decreasing in oxidant and inflammatory markers; thiobarbituric acid reactive-substances (TBARS), protein carbonyl (PC) content, interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), a significant increase in anti-oxidants; superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), vitamin C, and E, and a significant improvement in PFTs versus control group and baseline levels. Supplementation of Black Seed Oil may be an effective adjunct therapy to improve pulmonary functions, inflammation, and oxidant-antioxidant imbalance in COPD patients.

1. Introduction

COPD now regarded as the third major reason for death and one of the central grounds of morbidity and fatality in the world [1]. The aetiology and pathophysiology of COPD are more complicated with regard to the ecological factors (diet allergies and air pollutants), and multiple pathological mechanisms such as oxidative stress, recruitment of inflammatory cells and their activation (neutrophils, macrophages, and lymphocytes), inflammatory mediators and cytokines release (IL-1 β , IL-6, IL-8, TNF- α , and TGF- β), cell repair disorder (apoptosis, necroptosis, pyroptosis, and necrosis), and protease/anti-protease imbalance may gradually cause tissue inflammation, destruction of the airway and/or tissue alveolar, and fibrosis, which result in the development of COPD [2, 3].

COPD patients usually have serious comorbidities, particularly cardiovascular diseases and metabolic and renal insufficiency. So, the safety

profile of existing and new medicines must be neatly inspected. Probably the most attention lately was the concern about the safety of inhaled steroids, particularly at high doses [4]. The main objective of disease management is to inhibit the destructive and inflammatory processes that affect lung function, relieve symptoms severity, minimize drug adverse effects, decrease exacerbations, and probably lessen mortality [5].

Unfortunately, until now, no efficient prophylaxis measures or curing for COPD. Current treatments of COPD including bronchodilators and anti-inflammatory drugs; a corticosteroid, theophylline, and phosphodiesterase-4 (PDE4) inhibitor, either as a single therapy or together to reduce the severity of symptoms and recover the quality of life [1, 6], but there is doubt about the efficacy of these drugs [7].

Noteworthy, the current treatment of COPD neither modifies the underlying pulmonary dysfunction nor prevent the development of

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chronic inflammation and remodeling correlating to COPD [8]. On the other hand, these treatments are not without limitations, as some adverse effects appeared in some patients as a result of the excessive use of some of these treatments, such as an increased risk of developing common cardiac comorbidities and pneumonia. Besides, not all patients will show a response to present treatment options, so the need for complementary or alternative treatments for this disease increases. So, many research aims to find a multi-potential factor from a common diet to prevent and treat this disease [9].

One strategy to defeating this problem is identifying biomarkers that aid in determining patients who benefit more probably from corticosteroid therapy and less probably to experience negative effects [8]. Over the years, many efforts have been made to scout the potency and safety of plant medications and to clarify the cellular and molecular mechanisms that explain the therapeutic effect of these plants. In the field of obstructive lung diseases, several studies have contributed to unveiling of many promising botanical medicines. Finding new safe and effective treatments for asthma and COPD including plant origin medicines is an appealing and alternative therapy [10, 11].

Extensive clinical and animal researches that have been executed lately have shown that extracts of *Nigella sativa* (black seed or black cumin) (Family Ranunculaceae), have numerous medicinal and therapeutic properties which may include spasmolytic, a bronchodilator, immunomodulator, antidiabetic, antihistaminic, antimicrobial, anti-inflammatory, and antioxidant effects [12, 13, 14, 15]. Clinical studies evaluating the impact of *Nigella sativa* on chronic bronchial asthma [16, 17, 18] showed a marked amelioration in clinical signs and lung function tests.

The extraordinary biological activity of *Nigella sativa* is attributed to its bioactive compounds i.e., selenium, vitamin E (tocopherol α , β , and γ), retinol (vitamin A), vitamin C, carotenoids (β -carotene), thymoquinone, thymol, etc. Although the biological activity of *Nigella sativa* has been linked to its content of thymoquinone, which is one of the most effective substances in these seeds, black seeds also contain other compounds, such as carbohydrates, proteins, vitamins, minerals, fixed oil, volatile oil, saponins, and alkaloids, which can all contribute to its biological properties [19].

The active components of black seed (boiling extract or oil) are thymoquinone (TQ), dithymoquinone, thymol, thymohydroquinone, nigellone, nigellidine, carvacrol and fixed oils on various respiratory diseases [17, 20, 21]. TQ is the main active ingredient in volatile black seed oil (54%) and valuable studies have proven that it possesses anti-oxidant, anti-inflammatory, and anti-tumour properties [22]. In this study, we are trying to assess the impacts of black seed oil supplementation as an adjunct pharmaceutical preparation on the clinical outcome of lung function, markers of oxidant-antioxidant, and airway inflammation in COPD patients.

2. Materials and methods

2.1. Study design

This prospective randomized controlled double-blind clinical trial was performed at the chest unit, Zagazig University Hospital, Egypt. All patients submitted informed written consent before enrollment. All proceedings were in agreement with the Helsinki Declaration and authorized by the Clinical Research Authority, Ethical Committee in the Faculty of Medicine, Zagazig University. The study was registered with the Clinical Trial Registry of India (CTRI/2020/04/024927 at <http://ctri.nic.in>).

In this study, COPD (mild to moderate) outpatients were diagnosed clinically based on history, signs and symptoms (coughing, wheezing, shortness of breath, exercise intolerance), chest x-rays, and confirmed by evaluation of pulmonary function tests (PFTs). Diagnosis of COPD patients was confirmed and distinguished from asthma patients by the reversibility test to bronchodilator (β 2-agonist) in spirometry. The study

was conducted between June 2019 and December 2019 based on the inclusion and exclusion criteria.

2.2. Inclusion and exclusion criteria

Inclusion criteria: (a) Patients ages 40–66 years, regardless of gender; (b) Patients with mild to moderate COPD who have given informed written consent before starting the study; (c) ambulant and collaborative.

Exclusion criteria: (a) Patients younger than 40 years and over 66 years old; (b) Patients who had suffered from any respiratory disorder over the past four weeks; (c) Patients with chronic or acute diseases known to be associated with elevated oxidative stress; liver diseases, renal diseases, heart diseases, thyroid disorders, coagulation disorders, hematologic problems, diabetes mellitus, acute respiratory distress syndrome, any CNS disorder, etc.; (d) Patients who have exhibited any kind of allergy to any of the standard COPD drugs; (e) Patients who have taken antioxidants and vitamins supplements in the last 4 weeks; (f) pregnant/lactating females; (g) severe and very severe cases were not enrolled in this study.

2.3. Participants

2.3.1. Sample size calculation

Depending on a pilot study we performed on 10 patients of mild and moderate COPD and divided into two groups (5 patients with standard COPD treatment) as a **control group** and (5 patients receive standard COPD treatment + Black seed oil) as **BSO group** for 3 months. Pulmonary function tests as a primary outcome were assessed and revealed, FEV1 (74.33 ± 5.1 & 78.59 ± 4.33) for **control group** and **BSO group** respectively, at alpha error (0.05) and 90% power of the study and effect size (4.26%), the calculated sample size was 44 patients for each group, after adding 10% attrition rate, the number was 50 patients in each group.

After enrollment of 50 patients in each studied groups, the treatment was given as follows:

control group: treated with routine COPD medication only (standard medicine; inhaled corticosteroids with long-acting β 2-agonist (LABA), namely 50 μ g of salmeterol and 500 μ g fluticasone propionate one puff twice daily was given).

BSO group: treated with standard COPD medication in addition to an oral dose of 1 g twice daily of 100% pure cold-pressed black seed oil as soft gel capsules (contains minimum 0.95 % naturally occurring thymoquinone) (AMAZING HERBS, USA, Item model number: 13090), as a supplementary treatment.

Full medical history, full clinical examination (plain chest X-ray, pulmonary function tests, pulse rate, respiratory rate, blood pressure, body mass index) and other hematological and biochemical parameters complete blood picture, liver function tests, renal function tests, random blood sugar, oxidative stress markers [thiobarbituric acid reactive-substances and protein carbonyl content], antioxidants markers [catalase, glutathione peroxidase, superoxide dismutase, glutathione, vitamin C, and vitamin E], and inflammatory markers [tumor necrosis factor- α and interleukin-6], were done at 0 days (baseline) (pre-treated) and after 3 months (post-treated) with routine COPD medications +/- black seed oil in both groups.

2.4. Pulmonary functions

Lung function tests of all patients of both groups were evaluated by using portable MicroLab MK 8 Spirometer (Micro Medical Limited, Rochester, England). Briefly, the patient sits and holds the spirometer, a stopper is placed on the patient's nose in order to ensure that air does not enter or exit through it during the examination. The mouthpiece is inserted in the patient's mouth and at that time the patient is required to tightly close his lips around it, breathe deeply, and keep them for a few seconds and then exhale as much as possible. The proceeding was

reiterated leastwise trebled to ensure that the readings were regular while measuring the lung function and the better reading was adopted. FVC%, FEV1%, (FEV1/FVC) %, PEF%, and FEF 25–75% values were measured on 0 days and after 3 months. The expected values were computed based on the patient's age, height, and weight and compared to the corresponding measured data. These values were allocated in consonance with the Egyptian population with a correction of the ethnic group.

2.5. Patients samples

10 mL EDTA Vacutainer Tubes (dipotassium EDTA Vacutainer Tubes, Becton Dickinson) were used to collect the venous blood samples for the patients at baseline (Pre-treatment) and after 3 months from treatments (Post-treatment). Centrifuged the samples for 10 min at 3000 rpm at a temperature of 4°, after that, plasma was separated and a part of it was analyzed immediately. The remaining blood was stored at –80 °C for use at a later stage if required. The data for this study did not include any hemolyzed specimens.

2.6. Chemical procedures

Thiobarbituric acid reactive substances (TBARS) was determined by a spectrophotometric method using OXitek TBARS Kit (Cat. #: 0801192, ZeptoMetrix Corp., Buffalo, NY, USA) [23], protein carbonyl (PC) content was measured by colorimetric assay kit (Cat. #: ab126287, Abcam, Cambridge, MA, USA) [24]. Tumor necrosis factor-alpha (TNF- α) and Interleukin-6 (IL-6) were determined using Human ELISA assay kits (Cat#: DTA00D, Cat#: D6050, R&D Systems, Minneapolis, MN, USA) respectively according to the manufacturer's instructions. Catalase (CAT) [25], glutathione peroxidase (GPx) [26], superoxide dismutase (SOD) activities [27], and reduced glutathione (GSH) [28, 29] were performed with a colorimetric assay kits (Cat. #: 707002, Cat. #: 703102, Cat. #: 706002, Cat. #: 703002, Cayman Chemicals, Ann Arbor, MI, USA) respectively. Vitamin E and C were measured using vitamin E and vitamin C ELISA assay kits (Cat. #: abx150389, Cat. #: abx156668, Abnova Ltd, Cambridge, UK) respectively according to the manufacturer's instructions.

2.7. Statistical analyses

The collected data were evaluated using IBM personal computer and Statistical Package for Social Science (SPSS, Chicago, IL) version 20.0. The Quantitative, data were expressed as mean, standard deviation, and range. Student t-test and Mann Whitney U test were used to test the difference between the two studied groups according to the distribution of data. Qualitative data were expressed as frequency and percentage and compared using Chi-square test. A P value lower than (0.05) was defined as a statistical significance.

3. Results

3.1. Patients

Totally, 150 patients with COPD were considered in terms of eligibility, only 100 patients fulfilled the inclusion criteria, after enrollment, patients divided randomly into 2 groups [control group and BSO group; (n = 50/each group)]. Six patients in the control group and two patients in the BSO group were eliminated from the study for not continuing in the study protocol, also one patient in the BSO group was excluded as it consumed lower 90% of black seed oil capsules. Only 47 patients in the BSO group and 44 patients in the control group finished the study protocol. Data from these 91 patients were adopted for analysis and were reported in line with the CONSORT 2010 checklist of information [30] (Figure 1). The clinical and demographic features of 91 COPD patients are presented in Table 1.

3.2. Pulmonary function tests among the studied groups

In control group, there was insignificant difference between baseline and post-treatment values of pulmonary function test, FEV1% was (72.68 \pm 8.36 vs. 75.94 \pm 7.78), FVC (%) was (82.60 \pm 8.40 vs. 85.32 \pm 9.12), FEV1/FVC (%) was (68.70 \pm 7.22 vs. 71.20 \pm 7.61), PEF (%) was (53.32 \pm 4.7 vs. 55.52 \pm 6.64) and FEF 25–75 (%) was (27.78 \pm 3.28 vs. 29.06 \pm 3.48) in baseline and post-treatment measurements respectively (Table 2).

In BSO group, the post-treatment values of all pulmonary function test increased significantly than baseline values, FEV1% was (73.76 \pm 8.91 vs. 81.97 \pm 6.89), FVC (%) was (83.12 \pm 8.18 vs. 91.13 \pm 7.97), FEV1/FVC (%) was (67.34 \pm 0.70 vs. 75.19 \pm 0.64), PEF (%) was (54.78 \pm 6.39 vs. 65.28 \pm 5.28) and FEF 25–75 (%) was (28.72 \pm 3.21 vs. 35.04 \pm 5.20) in baseline and post-treatment measurements respectively (Table 2).

When comparing post-treatment values in control group and BSO group, it showed significantly higher values in BSO group in all pulmonary function tests with p value (<0.001, 0.002, 0.001, <0.001, and <0.001) for FEV1%, FVC%, FEV1/FVC%, PEF%, and FEF 25–75 (%) respectively.

Percentage of change between baseline values and post-treatment values was much higher in BSO group than in control group, as FEV1%, FVC%, FEV1/FVC%, PEF%, and FEF 25–75 (%) recorded percentage of change (5.5, 3.35, 6.44, 3.67, and 6.24) respectively in control group while they were (11.22, 10.4, 11.65, 20.59, and 23.29) respectively in BSO group (Table 2).

3.3. Impact of black seed oil intervention on plasma oxidative stress markers

3.3.1. Intervention impact of black seed oil on thiobarbituric acid reactive substances (TBARS)

TBARS level for control group at baseline was 6.74 \pm 1.28 nmol MDA/ml which was slightly decreased to 6.24 \pm 1.29 nmol MDA/ml (P = 0.06) after 3 months of standard treatment while for BSO group at baseline was 7.20 \pm 1.19 nmol MDA/ml which was decreased significantly to 3.76 \pm 0.70 nmol MDA/ml (P < 0.001) after 3 months of standard treatment plus intervention. The outcomes showed that TBARS values were decreased significantly only in the BSO group viz. by 46.44% comparing with 5.71 % in control group versus corresponding basic values (P < 0.001). Figure 2A.

3.3.2. Intervention impact of black seed oil on protein carbonyl (PC) content

control group showed that PC was 2.91 \pm 0.46 nmol carbonyl/mg protein at baseline which was slightly decreased to 2.74 \pm 0.48 nmol carbonyl/mg protein (P = 0.08) after 3 months of standard treatment, while BSO group showed baseline value of 2.98 \pm 0.56 nmol carbonyl/mg protein which significantly decreased to 2.04 \pm 0.52 nmol carbonyl/mg protein (P < 0.001) after 3 months of standard treatment plus intervention. The outcome showed that PC content values were decreased significantly only in the BSO group viz. by 30.27 % as against 4.14% in control group versus to that of corresponding basic values (P < 0.001). Figure 2B.

3.4. Impact of black seed oil intervention on plasma inflammatory markers in patients of COPD

3.4.1. Intervention impact of black seed oil on tumor necrosis factor-alpha (TNF- α)

The basic level of TNF- α for control group was 33.62 \pm 2.53 pg/ml which was reduced significantly to 26.29 \pm 5.03 pg/ml (P < 0.001) after 3 months of standard treatment and the basic level of BSO group was 33.28 \pm 2.98 pg/ml which was decreased remarkably to 21.52 \pm 5.09 pg/ml (P < 0.001) after 3 months of standard treatment plus intervention, but the extent of decreasing of TNF- α level was much more in BSO

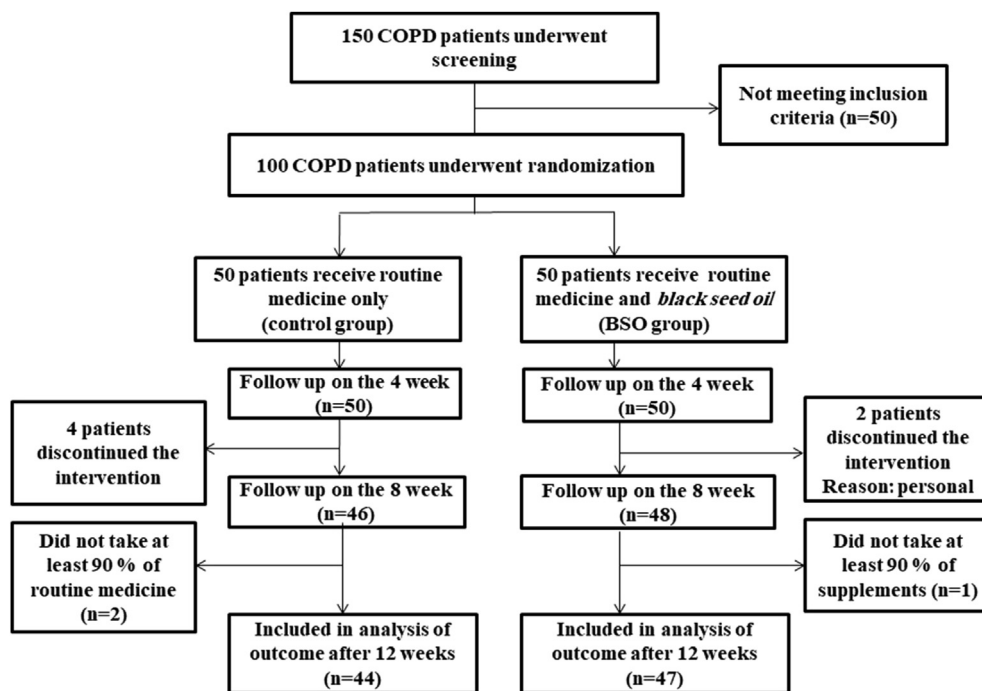


Figure 1. Participants enrolment diagram.

Table 1. Clinical and demographic characteristics of 91 COPD patients.

Characteristics	Control group (n = 44)	BSO group (n = 47)	Test (P-value)
Age (years)	55.18 ± 4.27 40–66	53.74 ± 4.68 40–66	1.61 (0.11)
Male	32 (72.7%)	31 (65.9%)	0.49 (0.48)
Females	12 (27.3%)	16 (34.1%)	
Bodyweight (Kg)	73.74 ± 8.35 55–90	71.52 ± 8.21 58–90	1.34 (0.18)
Height (Cm)	170.14 ± 7.96 155–186	169.16 ± 8.49 155–188	0.59 (0.55)
BMI (Kg/m ²)	25.46 ± 2.28 19.57–31.63	25.09 ± 3.16 16.76–32.85	0.68 (0.50)
Smokers	34 (77.3)	32 (68.1%)	0.96 (0.33)
Non smokers	10 (22.7)	15 (31.9%)	
Pack years in smokers	38.92 ± 7.82 25–51	39.61 ± 7.93 29–52	0.37 (0.72)
Heartbeats (per min)	85.08 ± 9.53 71–110	83.7 ± 8.80 72–107	0.75 (0.45)
Systolic BP (mmHg)	126.04 ± 13.26 110–145	127.62 ± 10.94 115–145	0.65 (0.52)
Diastolic BP (mmHg)	81.82 ± 8.26 65–95	83.1 ± 7.42 65–95	0.82 (0.42)

Data are expressed as mean ± SD and range; BMI: body mass index; BP: blood pressure.

group viz. by 35.17 % against 22.24 % in control group versus respective basic values (P < 0.001). Figure 2C.

3.4.2. Intervention impact of black seed oil on interleukin-6 (IL-6)

The basic value of IL-6 in control group was 4.01 ± 0.52 pg/ml which decreased significantly to 3.05 ± 0.59 pg/ml (P < 0.001) after 3 months of standard treatment and for baseline BSO group was 4.12 ± 0.47 pg/ml which significantly decreased to 2.52 ± 0.60 pg/ml (P < 0.001) after 3 months of standard treatment plus intervention. However, the degree of decrease in IL-6 level was greater in BSO group viz. by 37.52 % as against 22.79 % in control group versus relevant basic value. Figure 2 D.

3.5. Impact of black seed oil intervention on plasma antioxidant markers in patients of COPD

3.5.1. Intervention impact of black seed oil on catalase (CAT)

In control group patients, the standard treatment showed a minor increase in CAT value from 60.76 ± 5.67 at baseline to 62.74 ± 4.64 (P = 0.09), whereas in BSO group it increased from the baseline value of 57.61 ± 3.47 to 77.79 ± 6.97 (P < 0.001) after 3 months of standard treatment plus intervention. The outcomes indicated that CAT activity was improved significantly only in BSO group viz. by percentage of increase 35.49 % in comparison to 7.33 % in control group (P < 0.001). Figure 3A.

Table 2. Comparison of pulmonary function tests of COPD patients of the **control group** (standard therapy) and **BSO group** (black seed oil intervention) at the baseline and after 3 month of study.

Parameters	Control group				BSO group				
	Baseline n = 50	Post 3 months n = 44	P1-value	% increase	Baseline n = 50	Post 3 months n = 47	P2-value	% increase	P3-value
FEV1 (%)	72.68 ± 8.36	75.94 ± 7.78	0.06	5.5	73.76 ± 8.91	81.97 ± 6.89	<0.001*	11.22	<0.001*
FVC (%)	82.60 ± 8.40	85.32 ± 9.12	0.14	3.35	83.12 ± 8.18	91.13 ± 7.97	<0.001*	10.40	0.002*
FEV1/FVC (%)	68.70 ± 7.22	71.20 ± 7.61	0.08	6.44	67.34 ± 0.70	75.19 ± 0.64	0.012*	11.65	0.001*
PEF (%)	53.32 ± 4.7	55.52 ± 6.64	0.14	3.67	54.78 ± 6.39	65.28 ± 5.28	<0.001*	20.59	<0.001*
FEF 25–75 (%)	27.78 ± 3.28	29.06 ± 3.48	0.07	6.24	28.72 ± 3.21	35.04 ± 5.20	<0.001*	23.29	<0.001*

BSO group: COPD patients with standard treatment plus black seed oil; **Control group:** COPD patients with standard treatment; **FEV1:** forced expiratory volume in 1 s; **FEF 25–75%:** forced expiratory flow at 25–75% of forced vital capacity (FVC); **PEF:** peak expiratory flow; **P1 value:** between baseline and 3 months COPD with standard treatment; **P2 value:** between baseline and 3 months COPD with standard treatment plus black seed oil; **P3 value:** between COPD after 3 months of standard treatment with and without black seed oil. Data were expressed as Mean ± Standard deviation (SD), All pulmonary function tests measured as % predicted, *P < 0.05 was significant.

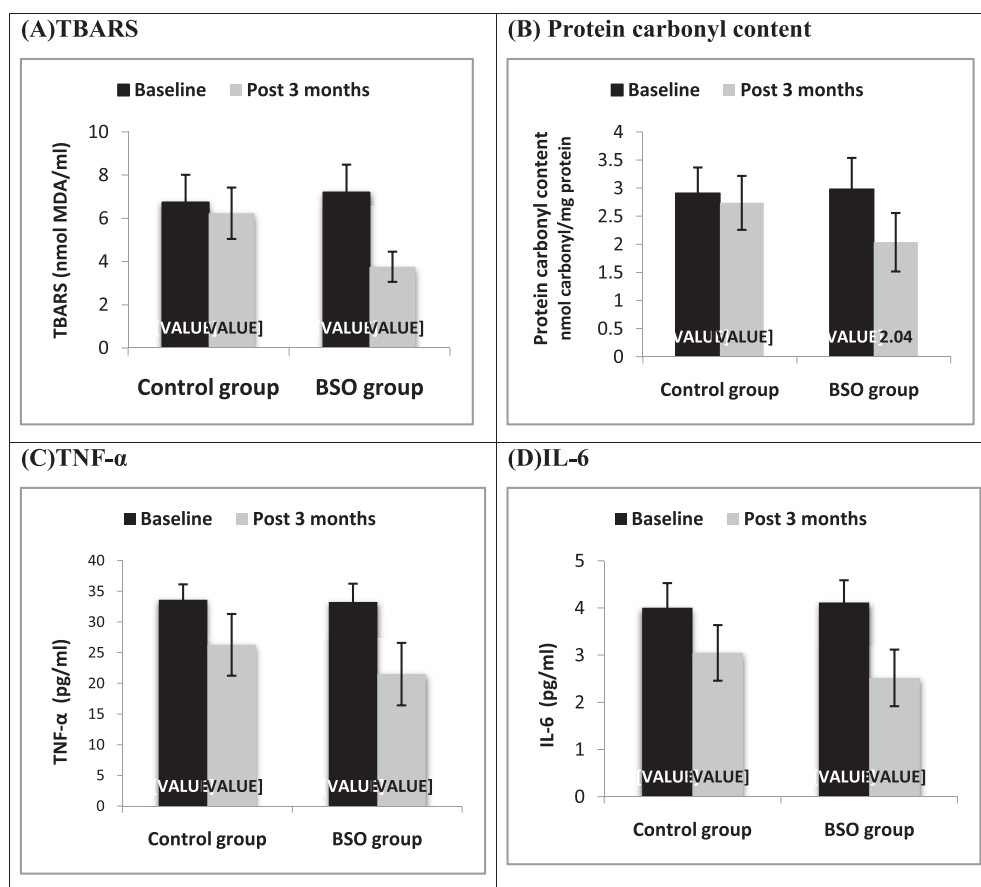


Figure 2. Changes in oxidants and inflammatory markers from baseline to the end of 3-month follow-up in the **control group** (standard therapy) and **BSO group** (black seed oil intervention). IL-6: interleukin-6; TBARS: thiobarbituric acid reactive substances; TNF-α: tumor necrosis factor-α; A: Changes in TBARS from baseline to the end of 3-month follow-up in the control group and BSO group; B: Changes in Protein Carbonyl content from baseline to the end of 3-month follow-up in the control group and BSO group; C: Changes in TNF-α from baseline to the end of 3-month follow-up in the control group and BSO group; D: Changes in IL-6 from baseline to the end of 3-month follow-up in the control group and BSO group.

3.5.2. Intervention impact of black seed oil on glutathione peroxidase (GPx)

The basic level of GPx at baseline was 52.63 ± 1.30 for **control group** which slightly increased to 55.42 ± 2.60 ($P = 0.06$) after 3 months of standard treatment and for **BSO group** at baseline was 51.19 ± 2.20 which was significantly increased to 69.46 ± 2.70 ($P < 0.001$) after 3 months of standard treatment plus intervention. The percentage of increase was remarkably higher in **BSO group** (37.62%) against only (3.47%) in **control group**. **Figure 3B**.

3.5.3. Intervention impact of black seed oil on superoxide dismutase (SOD)

The basic value of SOD in **control group** was 2.86 ± 0.87 which was slightly increased to 3.31 ± 1.16 ($P = 0.61$) after 3 months of standard treatment and the basic level in **BSO group** was 2.95 ± 0.81 which was significantly elevated to 4.02 ± 1.17 ($P < 0.001$) after 3 months of the

standard treatment plus intervention. Outcomes revealed that SOD values were improved significantly only in the **BSO group** viz. by 55.31% in comparison to 23.03% in **control group** versus concerning basic values ($P = 0.001$). **Figure 3C**.

3.5.4. Impact of black seed oil on reduced glutathione (GSH)

The results showed that plasma GSH level in **control group** at the baseline value was 13.63 ± 4.11 mg/dl which increased a little to 15.73 ± 4.38 mg/dl ($P = 0.11$) after 3 months of standard treatment, whereas for baseline **BSO group** was 14.02 ± 4.50 mg/dl which significantly increased to 20.07 ± 7.25 mg/dl ($P < 0.001$) after 3 months of standard treatment plus intervention. The GSH level heightened significantly in the **BSO group** only viz. by 56.48% in comparing to 12.98% in **control group** versus the corresponding basic value ($P = 0.001$). **Figure 3D**.

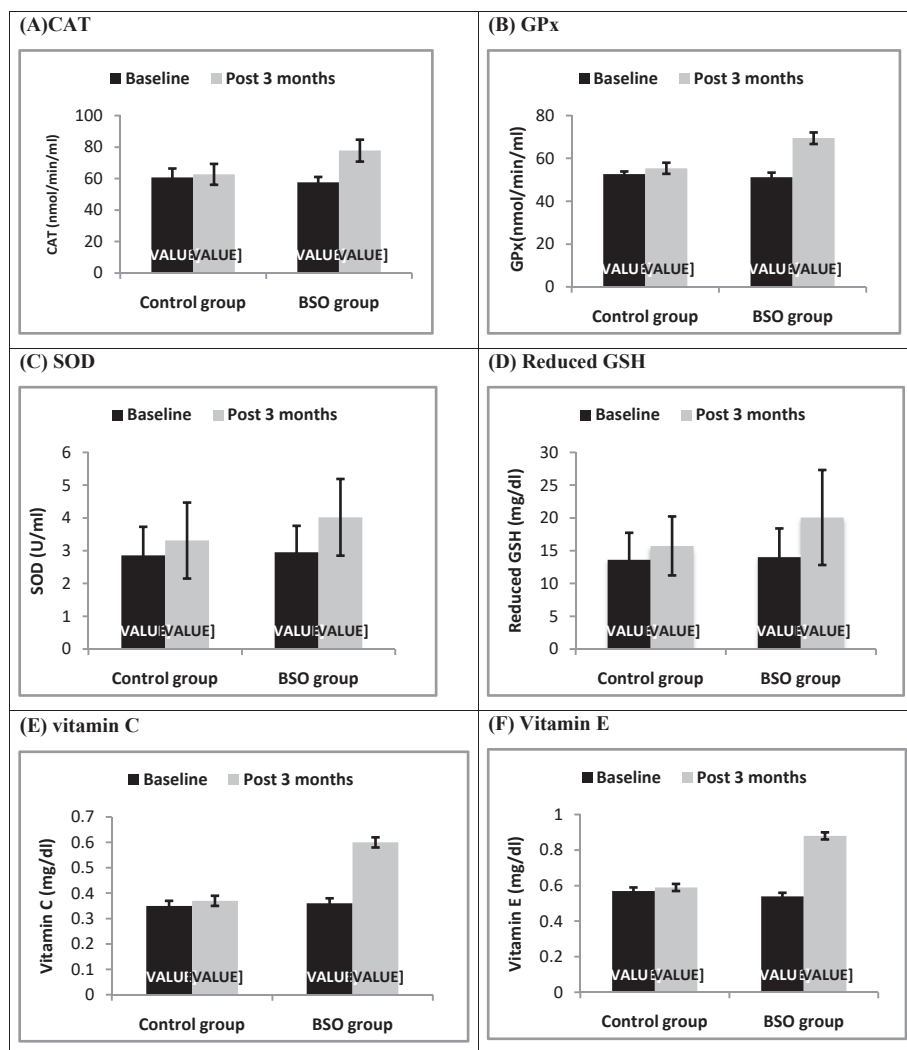


Figure 3. Changes in antioxidant markers from baseline to the end of 3-month follow-up in the **control group** (standard therapy) and **BSO group** (black seed oil intervention). BSO: black seed oil; CAT: catalase; GPx: glutathione peroxidase; GSH: glutathione; SOD: superoxide dismutase; A: Changes in CAT from baseline to the end of 3-month follow-up in the **control group** and **BSO group**; B: Changes in GPx from baseline to the end of 3-month follow-up in the **control group** and **BSO group**; C: Changes in SOD from baseline to the end of 3-month follow-up in the **control group** and **BSO group**; D: Changes in reduced GSH from baseline to the end of 3-month follow-up in the **control group** and **BSO group**; E: Changes in vitamin C from baseline to the end of 3-month follow-up in the **control group** and **BSO group**; F: Changes in vitamin E from baseline to the end of 3-month follow-up in the **control group** and **BSO group**.

3.5.5. Intervention impact of black seed oil on vitamin C

In **control group**, standard treatment showed a tenuous increasing in vitamin C level from 0.35 ± 0.02 at baseline to 0.37 ± 0.02 ($P = 0.42$) after 3 months, while the standard treatment plus intervention in **BSO group** shows a significant increase from the baseline value of 0.36 ± 0.02 to 0.60 ± 0.02 ($P < 0.001$) after 3 months. These results indicate that vitamin C levels raised significantly only in the **BSO group** viz. by 73.76 % as opposed to 16.34 % in **control group** versus corresponding basic levels ($P < 0.001$). **Figure 3E**.

3.5.6. Intervention impact of black seed oil on vitamin E

Vitamin E level for **control group** at baseline was 0.57 ± 0.02 mg/dl which insignificantly increased to 0.59 ± 0.02 mg/dl ($P = 0.72$) after 3 months of standard treatment, as for **BSO group** at baseline was 0.54 ± 0.02 mg/dl which significantly rose to 0.88 ± 0.02 ($P < 0.001$) after 3 months of the standard treatment plus intervention. These data indicate that vitamin E levels elevated significantly only in the **BSO group** viz. by 69.23 % counter to 8.26 % in **control group** versus corresponding basic levels. **Figure 3F**.

4. Discussion

The current study endeavored to appraise the influences of black seed oil intervention as an adjunct therapy to the standard medication on pulmonary functions and cellular and molecular markers of oxidant-

antioxidants and inflammation in 91 patients with mild/moderate COPD to explore the potential impacts of this intervention on this disease.

The results of this study exhibited many positive effects on various aspects of controlling COPD especially lung function, inflammation of the airways, and oxidant-antioxidant status. The peculiarity of the current study was to make the greatest possible effort to promote compliance through frequent contact with participants between visits. Therefore, we achieved a good compliance rate among our patients (>90%). Black seed oil, in general, is well tolerated and did not report any side effects by any of the participants.

4.1. Pulmonary function tests

Spirometry is the most dependable technique for appraising lung function. Our results revealed that there was a significant amelioration in lung function tests [(FVC, FEV1/FVC, PEF, and FEF 25–75 (% predicted))] in the **BSO group**. These findings recorded that black seed oil complementation particularly improved forced expiratory flow over the middle one half of the vital capacity (FEF25-75%). It is well-known that FEF25-75% is an index of small airways obstruction [31]. Besides, our findings were compatible with other clinical investigations that indicated that after 4 weeks of administration of **Nigella sativa** oil in bronchial asthma, patients had fewer asthma attacks, lower blood eosinophils and better peak expiratory flow (PEF%) and forced expiratory volume

(FEV1%) [32]. As well, the bronchodilator effect of **Nigella Sativa** (boiled extract) has been compared with theophylline on 15 asthma patients. The extract gave rise to an important increase in all pulmonary function tests measured in this study [17, 33]. In another study conducted on 77 patients with asthma, they were divided into 3 groups; the control group was given the placebo, and two groups were given 1 and 2 mg/day of **Nigella sativa** (whole ground seeds), respectively, for 3 months alongside inhalation therapy. After treatment, both groups of **Nigella sativa** showed a significant drop in asthma attacks and bestead results in lung function tests as their levels were higher than they were at baseline [34]. Also, the prophylactic impact of **Nigella sativa** (boiled extract) has been confirmed in asthma patients [16] and chemical warfare victims [33]. All symptoms and pulmonary functions were improved after 3 months of treatment. Additionally, the use of inhaled/oral β -agonists, oral/inhaled corticosteroid, oral theophylline were decreased in patients who treated with **Nigella sativa** [16, 33]. Supplements of **Nigella Sativa** oil resulted in betterment in clinical and inflammatory parameters for patients with asthma such as pulmonary function tests, asthma control test (ACT), total serum immunoglobulin E (IgE), and blood eosinophils [16, 17, 32, 34]. In addition, oil and other extracts of **Nigella Sativa** have been found to have inhibitory effects on histamine H1 receptors [33], stimulating effects on β -adrenergic receptors [35], antitussive effects [36], and anti-allergic diseases (bronchial asthma, atopic eczema, and allergic rhinitis) [18].

4.2. Inflammation and oxidative stress

There is a confirmed relevance between oxidative stress and inflammatory processes in obstructive airway diseases, especially COPD and asthma [3, 37, 38]. TNF- α is a powerful systemic proinflammatory cytokine, generated primarily by stimulatory macrophages that are believed to play a decisive part in COPD pathogenesis by promoting and maintaining expression and releasing of many proinflammatory mediators that lead to tissue damage and remodeling. Several studies have indicated elevated TNF- α levels in COPD subjects with systemic inflammation [39].

IL-6 can be produced in the lung through various sources such as macrophages, interstitial fibroblasts, epithelial cells, and different inflammatory cells. Its production is induced in answer to a set of stimulates such as exercise, respiratory viruses, allergens, environmental molecules, and inhaled toxic particles [40]. The first studies that examined the possible role of IL-6 in the pathogenesis of COPD concluded that there were increased IL-6 in induced sputum from COPD subjects, and more importantly, increased IL-6 levels are correlated with faster decline in percentage predicted FEV1 [41,42]. In addition, a longitudinal study on markers of inflammation in patients with COPD over three years indicates that high serum IL-6 levels predict increased mortality in COPD [43].

Another major cause of airway inflammation in COPD is oxidative stress. The oxidant-antioxidants imbalance may play an essential role in COPD emerging [44]. Thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) are the two most common markers applied in screening oxidative injury. MDA as a prospective biomarker for appraising the state of oxidative stress in patients with COPD has been investigated in a number of studies using the TBARS method. Most of the results of these studies revealed a remarkable increase in TBARS MDA in patients with COPD versus healthy individuals [37, 45]. Protein carbonylation (PC) is the most copious byproduct of oxidative damage in proteins. Thus, the existence of carbonyl groups in proteins is widely used as a marker of ROS-mediated protein oxidation [46]. A lot of studies have exhibited a marked increase in carbonyl protein in the plasma of COPD patients [47, 48].

The oxidative stress implicated in COPD pathogenesis is not only an outcome of increased oxidative burden but also due to decreased antioxidant capacity. Many clinical studies in COPD patients reported: a

significant reduction of blood level of antioxidants markers such as SOD, CAT, GPx, GSH, vitamin C, and E [3, 37, 49, 50, 51, 52, 53].

Different strategies have been tested to correct an oxidative/antioxidant imbalance. One technique is to give efficacious anti-inflammatory treatment to minimize lung inflammation, which in turn will reduce oxidative stress. Also, some agents can be used to inhibit releasing of oxygen radicals by the stimulated leukocytes or to suppress those oxidizing substances once they are developed by strengthening the antioxidant shield in the lungs. A further approach is to simply manage antioxidant treatment [54, 55]. The latter can be accomplished by two processes, either by enhancing the enzymatic endogenous antioxidants; superoxide dismutase, Se-dependent glutathione peroxidase, catalase, glutathione reductase or by enhancing the non-enzymatic antioxidants such as vitamins (A, C, E), tetraterpenoids, bioflavonoid, glutathione, plant polyhydroxyphenols, uric acid, melatonin, theaflavin, curcuma longa, bilirubin, and polyamines through food or drug sources [56, 57]. The different extracts and ingredients of **Nigella sativa** demonstrated antioxidant and anti-inflammatory functions in animal and human models of some obstructive airway diseases correlated with oxidative stress, inflammation, and immune system dysfunction.

Our study has revealed that the effect of the addition of black seed oil for 3 months with the routine COPD medication, significantly reduced inflammatory markers (IL-6 and TNF- α) and oxidants (TBARS and protein carbonyl content), and increases the antioxidants (SOD, CAT, GPx, GSH, vitamin C, and vitamin E) in COPD subjects. These results were consistent with other clinical studies that confirmed anti-inflammatory and antioxidant effects of black seed and its constituents on other respiratory diseases [34, 58, 59] and in other organs disease [60, 61, 62, 63].

Also, in experimental animal with lung injury and asthmatic condition with supplementation of black seed and its ingredients as preventive, antioxidative and anti-inflammatory; reduced the serum level of lipid peroxidation (LPO) and myeloperoxidase (MPO) activity and improved the GSH and SOD in rat lung sepsis model [64], decreasing lung inflammation and tracheal response with efficacy similar to vitamin C in guinea pigs subjected to cigarette smoke (COPD guinea pig model) [65], reducing the count of eosinophils in the peripheral blood and inflammatory cells in the lung tissue of the mice model of allergic asthma, with efficacy similar to the effects of dexamethasone [66], normalization of serum malondialdehyde (MDA), IL-8 levels, WBC total changes, eosinophils, neutrophils and lymphocyte ratios in a guinea pig model of COPD [67], improving WBC count, serum levels of IL-6 and SOD activity in heart and lung injury in mice induced by diesel exhaust particles (DEP) [68], mitigating changes in lung and serum biomarkers correlated with inflammatory reactions, with less lipid peroxidation (LPO) and recovery of antioxidants; decreasing TNF- α and TBARS, but increased GSH and SOD in lung oxidative injury of rats induced by Cyclophosphamide (CP) [69]. These results indicate the anti-inflammatory and antioxidant properties and consequently a relaxant (bronchodilator) and a preventive effect of black seed and their components in obstructive pulmonary diseases where both inflammation and oxidative stress function an essential role in the pathogenesis and development of these diseases; this is fully consistent with the findings of the current study in one of the most serious of these diseases, which is the chronic obstructive pulmonary disease.

There were no significant limitations in the present study. However, reaching patients on frequent visits and ensuring the drug was used was a bit challenging. This was resolved by staying in touch with patients and encouraging their regular use of the drug. Also, the number of participants is relatively small, due to strict adherence to the inclusion and exclusion criteria, in addition to the unwillingness of many patients to use medical herbal or their components. Another limitation of this study is that we applied only one dose of Black seed oil supplement, which makes it difficult to deduce its optimal effective dose.

5. Conclusion

Black seed oil as an adjunct therapy in COPD patients significantly improves lung functions and maintains the oxidant-antioxidant balance, in addition to its effect in reducing the aggravation of inflammatory processes in COPD patients by limiting the level of inflammatory markers (TNF- α and IL-6). This study has shown promising and novel results in the improvement of many parameters associated directly with the origination and development of the disease.

Declarations

Author contribution statement

M.A. Al-Azzawi, R.A.L. Ibrahim and M.A. Sakr: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M.M.N. AboZaid: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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