A phase I clinical trial to evaluate the safety of thymoquinone-rich black cumin oil (BlaQmax®) on healthy subjects: Randomized, double-blinded, placebo-controlled prospective study

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Abstract

Black cumin or black seed (Nigella sativa L.) is a popular medicinal herb and culinary spice belonging to the Ranunculaceae family. Thymoquinone (TQ) is the major active phytoconstituent in black cumin and is abundant in the volatile oil fraction. Though black cumin oil containing low TQ content (less than 1%) has been clinically investigated, clinical efficacy and safety data of TQ-rich oil is limited. A recent study with black cumin oil formulation containing 5% TQ (BCO-5) exhibited significant clinical efficacy to alleviate sleep disorders and stress. So, the present phase 1 randomized, double-blinded, placebo-controlled trial evaluated the safety of BCO-5 at a dose of 200 mg/adult/day for 90 days on healthy subjects (n = 70). Both the biochemical and hematological parameters were analysed along with the adverse events or side effects to establish the clinical safety of BCO-5. The study reported neither serious adverse side effects nor any significant alterations in the hematological parameters. The absence of significant changes in the biochemical parameters related to liver function (ALT, AST, ALP), renal function (serum creatinine and urea) were also observed. However, analysis of lipid profile showed a significant (P < 0.05) reduction in total cholesterol, LDL, VLDL and triglycerides, but within the normal range. In conclusion, BCO-5 is safe at 200 mg/adult/day for human consumption and may be clinically evaluated for various health beneficial pharmacological activities where black cumin oil has been shown to have positive effects.

1. Introduction

Black cumin or black seed (Nigella sativa L.) is an annual flowering herb belonging to the family Ranunculaceae. The herb possesses a wide range of medicinal properties in addition to its application as a culinary spice. It has been known in commerce for the past 2000 years and very often regarded as a "miracle cure" and "seed of blessing" owing to its long history of usage to treat various diseases [1]. Both the seeds and its oil have been used extensively in Indian and Arab traditional systems of medicine such as Ayurveda and Unani [2]. Black cumin is considered to be rich in proteins, mucilage, dietary fibre, and non-volatile phytochemicals such as alkaloids, saponins, tannins, minerals, and vitamins [3,4]. Despite its high phytochemical constituents, most of the therapeutic properties of black cumin has been attributed to its oil part obtained by cold-pressing or supercritical extraction methods [5]. Black cumin oil (BCO) contains both the fixed and essential oil fractions with thymoquinone (TQ) being the major molecule in the essential oil part along with other molecules including p-cymene, carvacrol, thymohydroquinone (THQ), dihydrothymoquinone (DHTQ), a-thujene, thymol, t-anethole, a-pinene, p-pinene and p-terpinene [6]. The fixed oil fraction accounts for 32–40% (w/w) and contains linolenic acid as the major essential fatty acid along with arachidonic, eicosadinoic, oleic, palmitic, stearic and myristic acids. It was also found to contain β-sitosterol, cycloexuenol, cycloartenol, and sterol esters in relatively low amounts [7–9]. The use of black cumin seeds and its oil in food is Generally Recognized as Safe (GRAS) by United States Food and Drug Administration (USFDA) (Code of federal regulation: 21CFR182.10) [10].

A number of extraction methods such as solvent extraction, cold-
pressing, microwave-assisted extraction, Soxhlet extraction and supercritical fluid extraction techniques have been described for the black cumin oil preparation [11,12]. Though cold pressing was one of the oldest methods for the commercial scale production, supercritical extraction is now gaining importance as the green technology of preference [12]. Cold-pressed oil is widely available as black cumin oil and was found to contain only less than 0.5% TQ, when a validated high performance liquid chromatography (HPLC) method was used for quantification [13]. As per the current understanding, TQ has been identified as the major bioactive molecule responsible for the wide range of therapeutic effects and safety [1,14–18]. Hossen et al., and Ratheesh et al., demonstrated that the anti-inflammatory effect of black cumin oil was influenced by its TQ content [16,19]. Ramalingam et al., established the influence of TQ content on the acetylcholine esterase inhibition activity and also reported the dependence of TQ content on the acute toxicity of black cumin oil [17,18].

A number of pharmacological effects have been reported for black cumin and its oil including antioxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, anti-cancer, hypoglycaemic, anti-hypertensive, hypolipidemic, cardioprotective, hepatoprotective, nephroprotective, neuroprotective, gastroprotective, immunomodulatory, anti-allergic and anti-obesity effects in various animals and human models (Fig. 1) [20–27]. An internet search using PubMed-Medline, Science Direct and Scopus search engines revealed about 76 clinical trials on either black cumin seed or oil; out of which, 31 were randomized double-blinded placebo-controlled trials and 17 were randomized control trials. Most of the clinical trials have used high dosage (3–10 mL/day) of cold-pressed oil due to the low TQ content. Therefore, a validated HPLC-method for TQ quantification and optimum dosage of TQ are important for both safety and efficacy.

In this regard, we developed a black cumin oil formulation containing 5% TQ (BCO-5; Patented and registered as BlaQmax®) (Patent No: US 10485837 B2; dated 26 November 2019). Preclinical toxicity studies as per Organization for Economic Co-operation and Development (OECD) guidelines could establish the possible safe dosage of BCO-5 in humans as not more than 900 mg/adult/day or not more than 50 mg of TQ/adult/day [17]. When supplemented at 200 mg/day, BCO-5 has been shown to improve the sleep quality by reducing sleep latency, sleep disturbance and deep sleep (REM sleep) duration with a significant reduction in anxiety and stress [28]. Thus, the present study was aimed to investigate the long-time safety of BCO-5 on healthy subjects at 200 mg/day for 90 days. Haematological and biochemical parameters were examined along with anthropometric measurements to monitor safety. All adverse events and side effects including food/water consumption were also recorded.

2. Materials and methods

2.1. Chemicals and Raw materials

Pharmacognosist identified dried seeds of black cumin were collected directly from the farmers and a voucher specimen (AK-NS-018) was stored at the herbarium of Akay Natural Ingredients, Cochin, India. The oil was produced at the Good Manufacturing Practices (GMP)-certified manufacturing plant of Akay Natural Ingredients, Cochin, India, (BlaQmax® Batch No: BCOQ 32/21 dated 12/04/2021). All solvents used for the analysis was of HPLC-grade and were purchased from M/s Sigma Aldrich, Bangalore, India. Analytical standard of TQ (CAS No: 490–91–5) was also obtained from M/s Sigma Aldrich, Bangalore, India.

High performance thin layer chromatography (HPTLC) in comparison with the botanical reference material was performed to confirm the identity of the raw material used for producing BCO-5. HPTLC densitometric analysis was performed on 10 cm × 20 cm aluminium-backed

Fig. 1. Reported pharmacological effects of *Nigella sativa* and its oil.
plates coated with 0.2 mm layers of silica gel 60 F254 (E-Merck, Germany) employing CAMAG HPTLC system, Switzerland. Samples were applied to the TLC plates as 6 mm bands using an automatic TLC sampler fitted with a microlitre syringe. A constant application rate of 150 nL/s 80 mm was performed with Toluene: ethyl acetate (7:3 v/v) as mobile phase in an automatic developing chamber previously saturated with mobile phase vapour for 30 min at 22°C. After development, the plates were treated with Vanillin/Sulphuric acid solution, heated to 110 °C and was scanned at 366 nm.

Thymoquinine content was estimated using a standardized HPLC method [29], employing SPD Shimadzu model instrument fitted with M20A photo-diode array detector (Shimadzu Analytical India Private Limited, Mumbai, India) and a reverse-phase C18 column (150 × 4.6 mm, 5 μm) (Phenomenex, Hyderabad, India). BCO-5 oil used in the study was found to contain, 5.2% (w/w) of TQ content.

2.2. Study materials

Identical soft gelatin capsules containing either BCO-5 or placebo (200 mg/capsule) were obtained from Akay Natural Ingredients, Cochin, India in tightly closed and sealed HDPE bottles (95 capsules/bottle). Detailed certificate of analysis and declaration of its suitability for human consumption was also received from the manufacturer. Identical capsules of flax seed oil were used as the placebo.

2.3. Participants and study design

The study was designed as a randomized, double-blinded, placebo-controlled trial to evaluate the safety of BCO-5 in healthy volunteers. During visit 1, 120 participants were screened as per the eligibility criteria and prior medical history. The selected seventy participants (both males and females) were randomized using computer generated randomization technique into two groups (n = 35/group) so as to receive either placebo (Group I) or BCO-5 (Group II) at a dose of 200 mg/day, 10–20 min before bed time.

The study duration was 90 days. Inclusion and exclusion criteria followed in the study are given in Table 1. A written informed consent was obtained from all study participants, prior to the study. Demographic and haematological parameters as well as vitals were recorded on Day 1 (Visit 2) and Day 30 (Visit 3) along with safety parameters. A cohort diagram displaying the study design is depicted in Fig. 2. The study was conducted at the Department of General Medicine, Balangadharanath Swami Global Institute of Medical sciences, BGS Health and Education city, Bangalore, India under the guidance of a qualified medical practitioner. The study was in strict compliance with the clinical research guidelines established by the Government of India and the declaration of Helsinki. The study was registered in clinical trial registry of India at http://ctri.nic.in/ [CTRI/2021/05/033780].

2.4. Safety outcomes

The adverse reactions were monitored using a questionnaire describing the earlier adverse effects reported upon exposure to black cumin, its oil or the active phychochemical thymoquinone. To judge the tolerance, the participants were informed to record any changes from the regular pattern of food or water intake, changes in sleep pattern, gastrointestinal disturbances, nausea or headache. Safety was further evaluated by recording the changes in anthropometric parameters, vital signs, haematological and biochemical parameters. The primary safety endpoints included the changes in liver and kidney function markers from the baseline till the end of study. Abnormality in physical examination, changes in vital signs, significant changes in clinical laboratory parameters and any other incidence of adverse events were considered for overall safety evaluation.

| Table 1 |
| --- |
| **Inclusion and exclusion criteria.** |
| **Inclusion criteria** |
| 1. Healthy male and females aged 18 – 50 years (both inclusive). |
| 2. Participants having PSQI scores ≥ 5. |
| 3. Healthy subjects with body weight ≥ 50 kg |
| 4. Female participants of childbearing age agreeing to use approved birth control methods during the study and should have negative urine pregnancy test at the screening. |
| 5. Participants ready to abstain from alcohol consumption, smoking and caffeinated beverages. |
| 6. Participants who understand the study procedure and willing to provide signed informed consent to participate in the study. |
| **Exclusion criteria** |
| 1. Subjects suffering from any chronic health conditions like diabetes, hypertension, chronic renal failure, heart, thyroid and liver disease and requiring medical treatment. |
| 2. Participants with hepatic impairment (Alanine transaminase/Aspartate transaminase levels ≥ 3 upper limit of normal) or renal impairment (serum creatinine ≥ 2.0 mg/dl). |
| 3. Participants with history of chronic metabolic disease, psychiatric illness, drug abuse, smoking, addiction to alcohol, endocrine abnormalities including thyroid disease. |
| 4. Participants who have undergone cardiovascular surgery or any other major surgery. |
| 5. Subjects with immunodeficiency disease, like, HIV or Hepatitis B / any other immuno-compromised state participants. |
| 6. Subjects who may be allergic to any of the natural constituents of the investigational product. |
| 7. Pregnant and lactating women. |
| 8. History of clinically significant illness or any other medical disorder that may interfere with subject treatment, assessment or compliance with the protocol. |
| 9. Currently participating or having participated in another clinical trial during the last 1 months prior to the beginning of this study. |
| Any additional condition(s) that in the Investigators opinion would warrant exclusion from the study or prevent the subject from completing the study. |

2.5. Blood collection

Blood samples for the analysis of haematological and biochemical parameters were obtained from antecubital vein, after an overnight fasting (10 h). Blood samples were collected (10 mL) into plain vacutainer™ tubes for various analysis. Serum was separated after centrifugation (3500 rpm for 10 min at 4 °C) of the clotted blood samples and stored at – 80 °C for biochemical analysis [30].

2.6. Analysis of serum biochemical parameters

The activities of liver function parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated by the method of Huang et al., and Schumann et al., the lipid profile - Total cholesterol (TC), Low density lipoprotein (LDL), High density lipoprotein (HDL), Very low-density lipoprotein (VLDL) and Triglycerides (TG) were estimated by the method described by Rader and Hobbs; renal function markers, creatinine was estimated by the method of Peake and Whitam and urea by Rock et al., [31-35]. All tests were measured using an automated biochemical analyzer (Cobas 501, Roche diagnostics India Private Limited, Maharashtra, India).

2.7. Estimation of haematological parameters

Haematological parameters were measured employing Mindray auto haematology analyzer, BC-6000 (Abomed Biosystems Private Limited, Odisha, India).

2.8. Statistical analysis

IBM SPSS version 28 software was used for statistical analysis. Mean and standard deviation for continuous variables and percentages for categorical variables were reported accordingly. A 2 × 2 Repeated Measures ANOVA was employed to check the significance. ‘P’ values
< 0.05 were considered as statistically significant [36].

3. Results

3.1. Analysis of study material

Physical, chemical and microbial analysis of the black cumin oil formulation (BCO-5) used in the current study, in comparison with the widely used cold-pressed oil, is depicted in Table 2. HPTLC analysis was employed to authenticate BCO-5 as Nigella sativa oil (Fig. 3). HPLC analysis showed 5.12% of TQ content which is almost 10 times higher than the TQ content in the normal cold-pressed oil.

3.2. Vital signs, anthropometric and demographic characteristics

Seventy participants including both males and females \((n = 70)\) were enrolled in the study. BMI of males were \(23.96 \pm 0.78\) and females were \(23.44 \pm 0.73\), which was maintained throughout the study duration. It was observed that none of the subjects exhibited significant difference in pulse, systolic and diastolic blood pressure from the baseline until the end of study. The vital signs, anthropometric and demographic parameters at baseline and end of study of both males and females are listed in Table 3.

![Fig. 2. Cohort diagram showing study design.](image)

![Fig. 3. HPTLC analysis of Black cumin seed and BCO-5; BCRM-01 - authentic reference of black cumin; BCRM-02 - raw material used for the extraction of BCO-5.](image)

3.3. Safety parameters

The recruited participants did not exhibit any signs of clinical toxicity, serious adverse or side effects. The details of haematological and biochemical assays in males and females are listed in Tables (4a, 4b). The hematological parameters were within normal range and revealed no significant difference \((P > 0.05)\) at baseline versus end of study. Activities of liver toxicity markers (ALT, AST, ALP) and the markers of kidney function (serum creatinine, serum urea) exhibited no
no significant variations from the normal serum levels were observed. Mild diarrhea during the study. A total of six subjects reported the water intake, gastrointestinal irritation due to prolonged intake of baseline (placebo group showed 2.41%, 2.86%, 3.31%, 5.93% and 1.61% in TC, BCO-5 for 90-days duration.

The initial five days. Other two participants from BCO-5 group developed subjects from BCO-5 group and one from placebo reported bloating at baseline to end of study of males and females in placebo and BCO-5 treated groups.

There were no significant differences in the vital signs, demographic and anthropometric parameters from baseline to end of study in placebo and BCO-5 treated groups.

The significant percentage change observed in female participants were 9.78%, 12.82%, 14.52%, 16.33%, 19.66%, 13.27%, 17.81% and 21.12% in TC, TG, LDL, VLDL and HDL respectively in BCO-5 group compared to placebo. However, a significant decrease in male participants in TC, TG, LDL, VLDL and HDL were 12.1%, 19.66%, 8.21%, 15.27% and 12.12% in BCO-5 group compared to placebo. Therefore, the present ran significant difference (P < 0.05) from the baseline to the end of study. Intergroup comparison also showed no significant difference (P > 0.05) in BCO-5 group compared to placebo. However, a significant difference in the lipid profile was observed from the baseline (P < 0.05) in BCO-5 group compared to placebo. Lipid profile of the individuals were also within the safe limit. The significant percentage change observed in male participants in TC, TG, LDL, VLDL and HDL were 12.1%, 19.66%, 13.27%, 17.81% and 21.12% in BCO-5 group compared to placebo which was statistically significant (P < 0.05), whereas in placebo the percent change noted were 5.23%, 8.88%, 3.64%, 1.56% and 0.21% respectively (Table 4a). The percentage difference observed in female participants were 9.78%, 12.82%, 14.52%, 16.33%, 19.66%, 13.27%, 17.81% and 21.12% in TC, TG, LDL, VLDL and HDL respectively in BCO-5 treated group which was statistically significant (P < 0.05) whereas placebo group showed 2.41%, 2.86%, 3.31%, 5.93% and 1.61% in TC, TG, LDL, VLDL and HDL respectively (Table 4b). Intergroup comparison also exhibited a significant decrease in lipid concentration from the baseline (P < 0.05). There were no reports regarding changes in food-water intake, gastrointestinal irritation due to prolonged intake of BCO-5 for 90-days duration.

### 3.4. Adverse events

No severe adverse effects were reported during the study. Three subjects from BCO-5 group and one from placebo reported bloating at the initial five days. Other two participants from BCO-5 group developed mild diarrhea during the study. A total of six subjects reported the borborigum and burping with a taste of black cumin oil in mouth at different instances during the 90-day trial period. Majority of the reported adverse effects were mild or moderate in black cumin oil treated group. Incidences of adverse effects are represented in Table 5. Further, all the biochemical parameters were analysed for adverse reactions and no significant variations from the normal serum levels were observed. There were no significant differences in the vital signs (pulse rate, systolic and diastolic blood pressure) of the participants at the two study points.

### 4. Discussion

The study demonstrated the safety and tolerance of the proprietary formulation of thymoquinoine-rich black cumin oil (BCO-5) when supplemented at a dosage of 200 mg/day for 90 days in healthy volunteers. Use of herbal medicines and supplements to maintain health and well-being has witnessed a great demand in the past couple of decades, and today it is reported that more than 80% of American population rely over them for their primary healthcare [37,38]. Poor quality of sleep and moderate stress has been reported as a major cause for the reduced quality of life, especially among the post-COVID patients around the world. While there are no specific medicines for such treatments in a safe manner, natural remedies, especially those derived from edible plants is of great significance. The previous data that BCO-5 helps to improve the sleep quality and modulate stress is a significant property which required to be exploited further [28]. In this regard, the present randomized controlled toxicity study is of great importance.

Biological activity of black cumin is mainly attributed to its essential oil part, particularly to TQ and carvacrol, the major components in the oil part, particularly to TQ and carvacrol, the major components in the...
The bioactivity of black cumin has been studied extensively, with preclinical studies confirming its strong anti-carcinogenic, anti-neoplastic, anti-mutagenic and anti-proliferative activities [38,40].

Despite the natural status and favourable pharmacological effects, safety is an important element to be considered for the herbal remedies. Our previous studies have shown that TQ content is important for the safety and efficacy of black cumin oil [17,28]. In the single-dose acute toxicity study on rats as per OECD guidelines, black cumin oil with 0.6% TQ showed an LD₅₀ in the range of 300–2000 mg/kg b. wt., while the LD₅₀ for the one with 5% TQ was 50–300 mg/kg b. wt. [17]. Further subchronic studies suggested 5 mg TQ/kg b. wt. as a NOAEL of BCO-5, which translated to about 900 mg/day safe dosage for humans. In the current study, only 200 mg/day was used for 3 months since BCO-5 at this dosage was found to significantly improve the quality of life and reduce the stress [28].

The current study did not demonstrate any significant toxic effects, adverse effects or deviations in clinical parameters. Inter and intra-group comparison at the end of the study period did not reveal any significant deviation in the hematological and biochemical parameters of liver and kidney. However, the observed decrease in lipid profile and hematology were not in the toxic level, but in a positive manner indicating the modulation of lipid profile and immunity. The absence of significant changes on the relative activities of liver and kidney toxicity markers observed in the present study were in agreement with the previous findings. In a randomized, double-blinded, placebo controlled trial involving healthy volunteers, supplementation of black cumin oil at 5 mL/day for eight weeks was demonstrated to cause no adverse/toxic effects [45]. No deviations were noted in the activities of serum ALT, AST, ALP and in the levels of kidney markers (creatinine, urea) from the baseline till the end of study [45]. These findings were further in accordance with the randomized, double-blinded, placebo-controlled clinical trial of Amini et al., who employed the oil at 5 mL/day for 8 weeks [46]. In another study, supplementation of black cumin oil at different doses of 1.5, 3 and 4.5 mL/day for 20 days did not cause any adverse impacts or any significant deviations in the activities of serum ALT, AST, ALP and in the levels of renal function markers [47]. However, the supplementation of BCO-5 was found to offer a significant reduction in the levels of TC, TG and LDL cholesterol, with an atherogenic LDL and ATP binding transporter A1). Moreover, TQ exhibits hypolipidemic effect of black cumin may be via the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) gene, which once activated leads to elevated expression of CD-36 (a receptor for atherogenic LDL and ATP binding transporter A1). Moreover, TQ up-regulates hepatic LDL-receptor, inhibits 3-hydroxy 3-methyl glutaryl CoA reductase gene and downregulates Apo B100 gene leading to the reduction in the levels of TC, TG and LDL cholesterol, with an atherogenic LDL and ATP binding transporter A1). Moreover, TQ up-regulates hepatic LDL-receptor, inhibits 3-hydroxy 3-methyl glutaryl CoA reductase gene and downregulates Apo B100 gene leading to the reduced synthesis and increased clearance of LDL-cholesterol [49].

A brief report on the important clinical trials of black cumin oil and its beneficial effects are summarized in Table 6. In a recent randomized, double-blinded, placebo-controlled study, supplementation of cold-pressed oil at 500 mg twice daily for 8 weeks was effective in reducing clinical parameters associated with cardiac health viz, fasting blood sugar, total cholesterol, triglycerides, BMI, and blood pressure indicating its effectiveness in maintaining the cardiometabolic risk factors in Type 2 diabetes [40]. Hochubi et al., reported that the oil was found to provide the protective effect against inflammation [50], oxidative stress, fasting blood glucose and lipid profiles in type II diabetes mellitus participants, when supplemented at 500 mg × 2/day for 8 weeks [50]. In another randomized study using a TQ-rich oil (14.5%, evaluated using GC-MS), a dosage of 3 g/day for 12 weeks was found to offer significant reduction in body weight, BMI, fasting blood glucose, glycerated hemoglobin, triglycerides, LDL-cholesterol and insulin resistance [51]. However, the analysis of TQ content in that study seems to be lower than expected.

Table 5b

| Parameters | Group | Baseline | End of study | Normal range |
|------------|-------|----------|--------------|-------------|
| Hb (g/dL)  | Placebo | 13.41 ± 0.49 | 13.39 ± 0.43 | 11.6 – 15 |
|            | BCO-5  | 13.45 ± 0.69 | 13.28 ± 0.46 |             |
| RBC count (million/μL) | Placebo | 4.87 ± 0.13 | 4.90 ± 0.19 | 4.2 – 5.4 |
|            | BCO-5  | 5.18 ± 0.47 | 4.94 ± 0.46 |             |
| MCV (FL/red cell) | Placebo | 81.24 ± 3.15 | 81.93 ± 3.43 | 80 – 100 |
|            | BCO-5  | 78.67 ± 6.17 | 82.57 ± 2.65 |             |
| MCH (pg/cell) | Placebo | 27.47 ± 0.77 | 27.06 ± 1.22 | 27.5 – 33.2 |
|            | BCO-5  | 25.63 ± 1.77 | 26.96 ± 1.06 |             |
| ALT (U/L)  | Placebo | 22.50 ± 2.46 | 22.28 ± 2.43 | 19 – 25 |
|            | BCO-5  | 22.73 ± 2.70 | 22.10 ± 3.04 |             |
| AST (U/L)  | Placebo | 20.87 ± 2.33 | 22.25 ± 3.99 | 9 – 32 |
|            | BCO-5  | 25.15 ± 3.12 | 23.55 ± 4.8 |             |
| ALP (U/L)  | Placebo | 77.88 | 85.09 | 44 – 147 |
|            | BCO-5  | 90.61 | 103.80 |             |
| Creatinine (mg/dL) | Placebo | 0.97 ± 0.11 | 1.00 ± 0.18 | 0.59 – 1.04 |
|            | BCO-5  | 0.97 ± 0.12 | 0.98 ± 0.11 |             |
| Urea (mg/dL) | Placebo | 11.83 ± 1.28 | 13.57 ± 1.70 | 9.8 – 20 |
|            | BCO-5  | 14.32 ± 2.56 | 18.75 ± 1.18 |             |
| TC (mg/dL) | Placebo | 158.84 | 162.68 | 125 – 200 |
|            | BCO-5  | 17 ± 16.1 | 23.57 |             |
| TG (mg/dL) | Placebo | 110.48 | 107.31 | 35 – 135 |
|            | BCO-5  | ± 30.62 | ± 24.84 |             |
| LDL cholesterol (mg/dL) | Placebo | 90.15 | 102.43 | < 100 |
|            | BCO-5  | ± 20.20 | ± 20.25 |             |
| HDL cholesterol (mg/dL) | Placebo | 106.70 | 91.20 |             |
|            | BCO-5  | ± 23.55 | ± 21.27a |             |
| VLDL cholesterol (mg/dL) | Placebo | 44.08 ± 2.80 | 43.37 ± 1.46 | > 50 |
|            | BCO-5  | 41.98 ± 0.97 | 46.01 ± 1.96 |             |
| a          |         | ± 26.16a | ± 26.16a |             |

* P < 0.05 for 90th day versus baseline performed using paired sample t test; AST- aspartate aminotransferase; ALT- alanine aminotransferase; ALP- alkaline phosphatase; TC-total cholesterol; TG-triglycerides; LDL-low-density lipoprotein; HDL-high-density lipoprotein; VLDL-very low-density lipoprotein; TGL-total leucocyte count; Hb-haemoglobin; MCV-mean corpuscular volume; MCH-mean corpuscular haemoglobin; RBC-red blood cell

Table 6

| Parameter | Placebo | BCO-5 |
|-----------|---------|-------|
| Number of participants with AEs | 29 | 31 |
| Number of AE's reported/participant (mean ± SD) | 0.65 ± 0.23 | 0.85 ± 0.74 |
| Number of participants with serious AEs (%) | 0 | 0 |
| Number of participants with severe AEs (%) | 0 | 0 |
| Number of participants with mild to moderate AEs (%) | 3.44 | 12.90 |

AEs- Adverse events; BCO-5- Black cumin oil

Essential oil part [39]. Strong antioxidant, free radical scavenging and anti-inflammatory effects of TQ have been believed to be the reason for the bioactivity of black cumin [39,40]. It has been found to elicit strong free radical scavenging effects and has shown to upregulate the transcription genes responsible for the production of endogenous anti-oxidant defenses, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and shown to upregulate the cytoprotective genes [41,42]. Preclinical studies have also confirmed the anti-convulsant, anti-microbial, anti-viral, anti-histaminic, immune-modulatory, anti-diabetic anti-hypertensive, anti-lipidemic and neuroprotective activities of TQ [1]. TQ also exhibited anti-carcinogenic, anti-neoplastic, anti-mutagenic and anti-proliferative activities [43,44].
have issues since the recent safety studies did not support such a high dosage of an oil having 14.5% TQ. Further, Najimi et al., have also indicated the beneficial effect of steam distilled black cumin oil against insulin resistance, when supplemented at a dose of 5 mL/day for 6 weeks along with atorvastatin and metformin [52].

A couple of human studies have indicated the beneficial effect of cold-pressed BCO containing 0.7% TQ in asthma patients. A randomized, controlled trial reported significant improvement in mean asthma control score by normalization of the blood eosinophilia, and thus improving pulmonary function, when supplemented at 500 mg twice daily for 8 weeks [53]. In another study employing 15 mL/kg of 0.1% Nigella sativa boiled extract with 2% thymoquinone improved pulmonary function test and alleviated the symptoms of asthma [54].

Nephroprotection is another health function studied clinically. Alam et al., reported that black cumin oil at a dose of 2.5 mL/day for 12 weeks when supplemented along with alpha keto analogue of essential amino acid was found to offer significant improvement in blood urea, serum creatinine and total urine protein on patients with chronic kidney disease [55]. These findings were further supported by Ansari et al., who reported that the same dosage supplementation for 12 weeks to diabetic nephropathy patients with chronic kidney disease (stage 3 and 4) effectively reduced the respective hematological and biochemical parameters with improved glomerular filtration rate compared to control group [56].

Another area where black cumin oil has shown promising effect was in the management of non-alcoholic fatty liver disease (NAFLD). Khonche et al., reported that the supplementation of cold-pressed BCO with a thymoquinone fraction (0.98 mg/mL) at 2.5 mL when provided every 12 h for 3 months, could effectively decrease liver steatosis and injury, levels of triglycerides, LDL and increased HDL cholesterol among NAFLD patients [57].

Yet another area of pharmacological relevance is the overweight or obesity management with black cumin oil. Cold pressed BCO with 12.5% TQ by GC-MS analysis at 3 g/day was shown to significantly decrease the body weight with a significant elevation in SOD when subjected to obese population [58]. Another study has also reported a similar effect in reducing body weight, waist circumference, and other biochemical parameters [59]. Recently, Razmpoosh et al., reported that BCO with 0.01 mg TQ content/1000 mg was effective in managing obesity [60].

In addition to the above studies, black cumin oil has also shown some promising results in various other end points. In a double-blind crossover clinical trial in children with refractory epilepsy, supplementation of TQ (1 mg/kg for four weeks) significantly reduced the frequency of seizure (anti-epileptic) [61]. Supplementation of cold-pressed BCO at 500 mg twice daily for one month was effective in managing rheumatoid arthritis by decreasing the disease activity score, swollen joints and morning stiffness [62]. The efficacy of topical application of BCO hydrogel on sixty participants with acne vulgaris was also reported. It was observed that the application of hydrogel twice
daily for sixty days effectively reduced comedones, papules and pustules [63]. However, some studies have also reported no significant beneficial effect for black cumin oil. For instance, Shawki et al., reported that the administration of BCO at 40–80 mg/kg/day for 10 weeks did not exhibit any significant improvement in the episodes of seizure or on the oxidative stress markers [64]. But, no information on the type of oil or its TQ content was provided. It has to be noted that none of the fore-mentioned clinical trials have not reported any serious adverse events or toxicity; even with the study which claimed 3 g/day supplementation of an oil having 14.5% TQ for 12 weeks [51]. Nausea, bloating, gastritis, fatigue, nasal dryness, and burning sensation were some of the side effects reported in various studies [40,65,66]. However, some of these patients were also diagnosed with other diseases and were under polypharmacy. Supplementation of BCO at 5 g/day has been shown to inhibit the activities of drug metabolizing enzymes like CYP2D6 and CYP4A [67]. In summary, black cumin seed powder and its oil has not shown significant side effects or adverse events in various studies reported so far. Some of the side effects reported can be correlated to prior medical conditions and/or high dose and extended duration of the drug. In the present study, BCO-5 was also found to be safe when supplemented at a dose of 200 mg daily for 3 months.

5. Conclusion
Black cumin oil possesses significant pharmacological and therapeutic potential. The positive effects of black cumin and its oil have been delineated with about 76 clinical trials so far. High TQ content in BCO-5 would be a potential nutraceutical ingredient for a number of health issues.

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CRediT authorship contribution statement
J.V.T - principal investigator who supervised the study and was involved in the protocol designing, data collection; M.M.E - recruitment of study participants, conduct of clinical trial; P.P - data analysis and drafting of original article; S.D.S - review & editing of original article; B. M - Resources and approval of study; K.I.M- Conceptualisation, approval of protocol, review & editing of original article.

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Declaration of competing interest
The authors disclose the following conflict of interests. BlaQmax® is a patented and registered product of Akay Natural Ingredients, Cochin, India. J.V.T & M.M.E belongs to non-profit research organizations who conducted the study and critically evaluated the data. P.P, S.D.S, B.M and K.I.M belong to the company Akay Natural Ingredients (Cochin, India), who prepared the study drugs, conceived the idea, and approved the protocol.
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