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Review article

Review on Clinical Trials of Black Seed (*Nigella sativa*) and Its Active Constituent, Thymoquinone

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Key Words

black seed, clinical trials, diseases, *Nigella sativa*, safety, thymoquinone

Abstract

Objectives: *Nigella sativa* (black seed or black cumin), which belongs to the Ranunculacea family, is an annual herb with many pharmacological properties. Among its many active constituents, thymoquinone (TQ) is the most abundant constituent of the volatile oil of *Nigella sativa* (*N. sativa*) seeds, and it is the constituent to which most properties of this herb are attributed.

Methods: PubMed-Medline, Scopus, and Web of Science databases were searched to identify randomized control trials (RCTs) investigating the therapeutic effects of *N. sativa* and/or TQ. In this review, we investigated the clinical uses of *N. sativa* and TQ in the prevention and the treatment of different diseases and morbidity conditions in humans.

Results: Black seed and TQ are shown to possess multiple useful effects for the treatment of patients with several diseases, such as inflammatory and auto-immune disorders, as well as metabolic syndrome. Also, other advantages, including antimicrobial, anti-nociceptive and anti-epileptic properties, have been documented. The side effects of this herbal medicine appear not to be serious, so it can be applied in clinical trials because of its many advantages.

Conclusion: Some effects of *N. sativa*, such as its hypoglycemic, hypolipidemic and bronchodilatory effects, have been sufficiently studied and are sufficiently understood to allow for the next phase of clinical trials or drug developments. However, most of its other effects and applications require further clinical and animal studies.

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1. Introduction

Nigella sativa (black seed or black cumin), which belongs to the Ranunculacea family, is an annual herb with many pharmacological properties [1]. The use of *N. sativa* (NS) seeds and oil in traditional remedies goes back more than 2000 years, and the herb is described as 'the Melanthion' by Hippocrates and Discroides [2]. Black seeds and their oil have a long history of folklore usage in the Indian and the Arabian civilizations as food and medicine and have been commonly used as treatment for a variety of health conditions pertaining to the respiratory system, digestive tract, kidney and liver functions, cardiovascular system, and immune system support, as well as for general well-being [3, 4]. NS contains many active components, such as thymoquinone (TQ), alkaloids (nigellicines and nigelledine), saponins (alpha-hederin), flavonoids, proteins, fatty acids, and many others, that have positive effects in the treatment of patients with different diseases [5, 6]. TQ

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is the most abundant constituent in the volatile oil of NS seeds, and most of the herb's properties are attributed to it.

Cell culture studies and animal models have indicated several therapeutic potentials, such as anti-cancer [5, 7-9], antimicrobial [10, 11], analgesic [12, 13], antipyretic [14], contraceptive and anti-fertility, anti-oxytocic [3], anti-tussive [15], anti-inflammatory [12, 16], and anti-oxidant [17-19] potentials, for black seed and its active component TQ. NS or TQ anticancer activity has been demonstrated for blood, breast, colon, pancreatic, liver, lung, fibrosarcoma, prostate, and cervix cancer cell lines and in animal models of lung, kidney, skin, colon, and breast cancer [7]. Black seed's antimicrobial effects include those on gram-negative and gram-positive bacteria, viruses, parasites, Schistosoma, and fungi pathogens [10]. NS was also found to be able to relieve the symptoms of or cure patients with several diseases, such as hypertension, dyslipidemia, metabolic syndrome, diabetes [5, 20, 21], asthma [3], convulsion [22-24], and natural and chemical toxicities [25, 26]. Additionally, a suggestion was made that NS and TQ utilization could prevent many disorders [6], including neurobehavioral [27], kidney [28, 29], and liver [4] disorders. For these reasons, in this review, we investigated the clinical uses of NS and TQ in the prevention and treatment of different diseases and morbidity conditions in humans. Fig. 1.

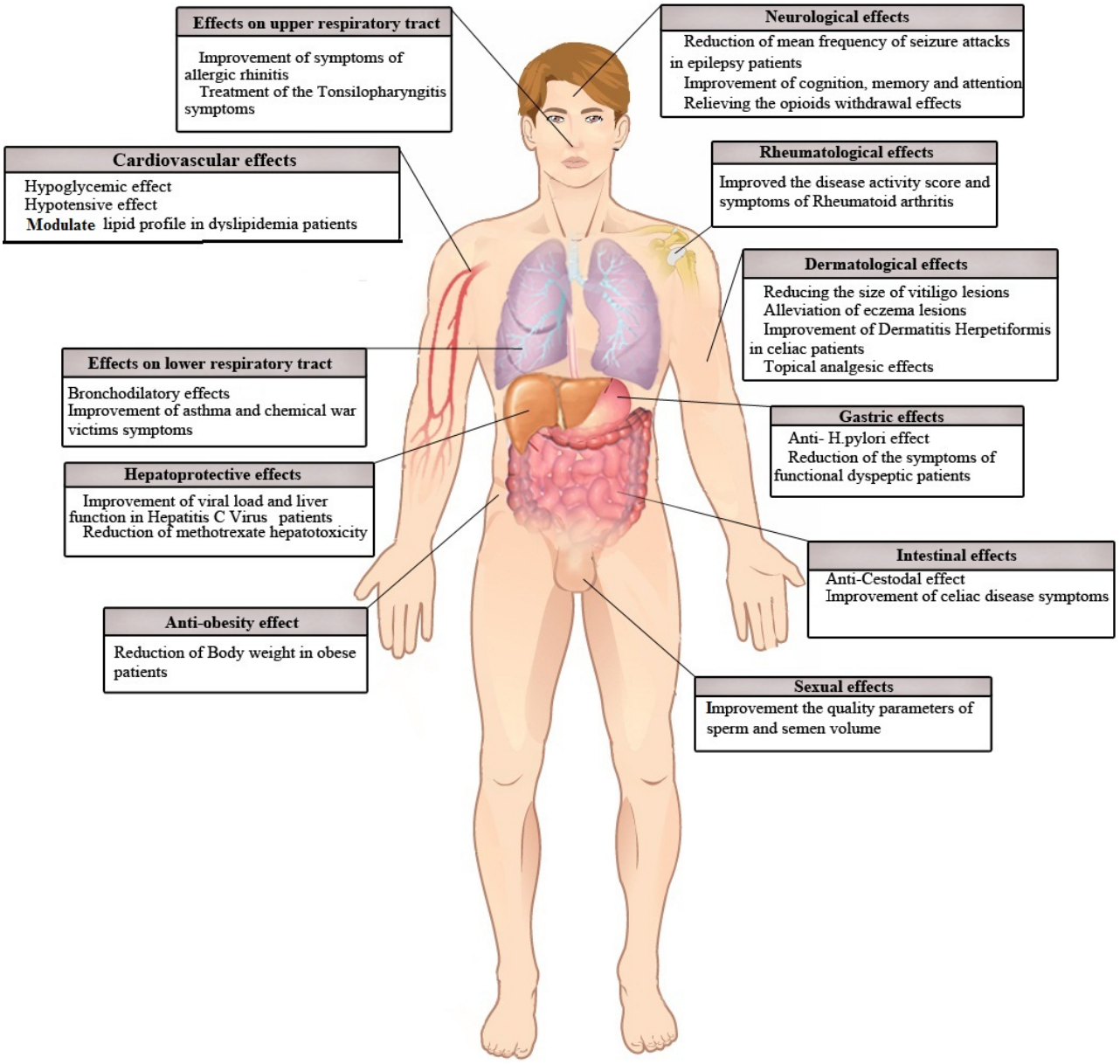

Figure 1 Schematic description for the effects of *Nigella sativa* in different parts of the human body.

2. Methods

PubMed-Medline, Scopus, and Web of Science databases were searched to identify randomized control trials (RCTs) investigating the therapeutic effects of NS and/or TQ. We reviewed the existing literature published until April 2016 by using the following keywords: "*Nigella sativa*", "black cumin", "black seeds", "thymoquinone", and "patient", "clinic" or "clinical trial". All studies assessing the effects of NS or TQ, topical use or oral intake, on human health conditions were included.

3. Results

3.1. Safety

Administration of NS oil (5 mL/day) to healthy volunteers for 8 weeks was reported not to have any notable liver, kidney, or gastrointestinal side effects [30, 31]. NS oil intake (equivalent to oil obtained from 0.7 g of seeds) for 40 days showed reasonable kidney and liver safety in patients with type 2 diabetes mellitus (DM); neither did it alter the platelet or the total leukocyte count [32]. Another study performed on 39 centrally obese men demonstrated that intake of NS seeds (3 g/day for 3 months) had no detectable side effects [33]. Qidwai *et al* reported that the administration of NS seeds (2 g/day for 6 weeks) did not affect the serum alanine aminotransferase (ALT) or the serum creatinine (Cr) levels in adults [34]. Furthermore, NS at doses of 1, 2 and 3 g/day for 3 months did not adversely affect either the renal or the hepatic functions of diabetic patients [35]. Another study revealed that NS seed powder intake for 40 days caused no significant change in total leukocyte and platelet count [36].

Treatment with NS tea (5 g/day) added to the usual oral anti-diabetic drugs, diet, and exercise for 6 months showed significant decreases in aspartate aminotransferase (AST), ALT, serum total, direct and indirect bilirubin, serum Cr, and blood urea levels in type 2 diabetic patients, as well as improved liver and kidney functions [37]. The intake of NS extract (200 or 400 mg/day) for 2 months was also reported to cause no observable complications in patients with mild hypertension [38].

No adverse effects were reported in applications of NS oil twice daily for six months to the lesions of vitiligo patients [39]. Intake of NS oil (0.05 mL/kg/day) in osteopenic postmenopausal women for three months showed no beneficial effects; however, no adverse side effects were produced, either [40]. In another study conducted on Iranian infertile men in order to assess the impacts of NS oil (5 mL/day) on abnormal semen quality, no side effects were observed [41]. Whereas no severe side effects were reported in administration of NS oil (5 mL/day) to functional dyspeptic patients, some mild adverse impacts were observed, including nausea, bloating, and burning sensation [42]. On the other hand, some cases of epigastric pain and hypoglycemia were reported as adverse effects when NS oil capsules were used to treat patients with hepatitis C virus (HCV) [43]. Dirjomuljono *et al* demonstrated the safety of NS extract (1,080 mg/day) when used to treat patients with acute tonsillopharyngitis [44]. In another study that investigated the anti-cestodal effects of NS powdered seeds (40 mg/kg body weight) on children infected with cestodes, no serious side effects were reported [45].

Treatment of seasonal allergic rhinitis patients with NS seeds (250 mg/day) for two weeks has been reported not to cause any adverse effects [46]. However, some patients with allergic rhinitis when treated using nasal drops of NS oil showed nasal dryness [47]. Dogar *et al* confirmed that side effects produced by treatment of children with acute lymphoblastic leukemia (ALL), aged between 2 - 18 years, with NS powder (40 mg/kg in two equal doses for 3 months), along with conventional therapy, were remarkably less than they were for L-asparaginase and conventional therapy (note: conventional therapy includes daunorubicin, vincristine, and prednisolone). Thus, one can conclude that NS powder, as an anti-cancer agent, is a beneficial substitute for L-asparaginase in the treatment of patients with ALL [48].

Nevertheless, in a patient suffering from DM, along with coronary artery disease and hypertension, acute renal failure due to the use of NS tablets (2,000 – 2,500 mg/day) was reported to have occurred 6 days after the start of treatment [49]. Bamosa suggested that this adverse effect could not have been related to NS because other clinical trials with many more human subjects had demonstrated the safety of NS in higher doses over longer periods of intake and that the adverse effect could probably be attributed to contamination of the tablets with other products [50]. Ibraheim also reported that only the total oil showed significant increases in the AST and the ALT levels while both the oil and the crushed seeds showed significant increases in the γ-GT and the alkaline phosphatase (ALP) activities [51]. A case of systemic and contact bullous drug eruption as erythematous plaques with vesicles and bullous lesions because of topical application and digestion of NS oil was reported [52]. In addition, another report showed that the use of NS at 5 g/day inhibited CYP2D6- and CYP3A4-mediated metabolism of dextromethorphan in healthy human volunteers, showing that it may interact with other CYP2D6 and CYP3A4 substrates [53].

Taken together, NS has been established as a safe herbal product. Nevertheless, according to the mentioned studies, some adverse effects, including nausea, bloating, and burning sensation, have been reported after administration of NS oil in functional dyspeptic patients, and a slight increase in liver and kidney enzymatic markers has been shown following consumption of NS oil and crushed seeds.

3.2. Metabolic syndrome

Metabolic syndrome is a cluster of cardio-metabolic conditions that include obesity, insulin resistance, atherogenic dyslipidemia, and high blood pressure (BP) [54] and is a major contributor to the development of diabetes [55]. A RCT done on 60 patients with metabolic syndrome showed that NS oil (5 mL/day) used in combination with atorvastatin and metformin could decrease fasting blood sugar (FBS), LDL, and TC significantly after six weeks

of use, but had no significant effect on body mass index (BMI) or waist circumference (WC) [56].

Another study by Amin *et al* evaluated the effect of black seeds (1.5 g/day) alone and concurrent with turmeric (900 mg and 1.5 g/day, respectively) on 250 patients with metabolic syndrome. The results show that NS and turmeric alone improved BMI, WC and BF% after 4 weeks, compared to baseline. Combination therapy improved all parameters including BMI, BF%, WC, hip circumference (HC), BP, total cholesterol (TC), triglyceride (TG), and C-reactive protein (CRP) except HDL-cholesterol with lower FBG and LDL-cholesterol as compared to placebo after four weeks. After 8 weeks, NS reduced lipids and FBG, whereas turmeric decreased LDL-cholesterol and CRP. Combination therapy showed an improvement in all parameters and reduced BF%, FBG, cholesterol, TG, LDL-cholesterol, CRP and increased HDL-cholesterol [57].

In another study, premenopausal women underwent the following protocol: 12-week administration of NS-powder capsules (1,600 mg/day), a 2-week washout period, and 12-week administration of a placebo, and vise versa. The authors of the study concluded that NS could help to control weight gain, the lipid profile, and the blood glucose and hormonal levels [58]. According to a clinical trial by Najmi *et al*, NS (500 mg/day) after 8 weeks was able to improve the efficacy of the therapeutic protocol (metformin + atorvastatin + aspirin) in patients with metabolic syndrome and poor glycemic control so that NS-treated patients indicated significant improvements in their FBG, postprandial blood glucose (PPBG), haemoglobin A1c (HbA1c), and LDL-C levels [59].

The effects of NS and TQ on different components of metabolic syndrome and cardiovascular disease risk factors, including high BP, obesity, dyslipidemia, and high blood glucose, have been established [21]. The clinical uses of NS and TQ in the prevention and treatment of these factors will be discussed in subsequent subsections.

3.3. Hypertension

Hypertension (HT) is a lifestyle-related disease, and dietary modifications are effective for its management and prevention. In a study performed by Dehkordi and Kamkhah, which evaluated the anti-hypertensive effect of NS, the intake of NS extract (200 or 400 mg/day) for 2 months was reported to decrease both the systolic and the diastolic BP in patients with mild HT as compared with the baseline and the placebo values [38]. Another study by Huseini *et al* on healthy volunteers who received NS oil (5 mL/day) for two months revealed the hypotensive effect of NS, with the systolic and the diastolic BP being lowered significantly as compared with both the placebo and the baseline values [31].

3.4. Obesity

Obesity, defined as excess fat mass, increases the risks for multiple metabolic diseases, such as type 2 diabetes, cardiovascular disease, and several types of cancer [60]. Some RCTs demonstrated that NS oil in combination with a low-calorie diet decreased weight in obese women in comparison to the placebo level [61, 62]. It also caused the superoxide dismutase (SOD) level to be elevated [61] and the TG and the LDL-C levels to be decreased [62].

Another study was performed on 39 centrally obese men to evaluate the effect of NS on body weight, WC, and some biochemical parameters [33]. The results of that study showed that intake of NS seeds (3 g/day for 3 months) caused a very significant reduction in body weight and WC, but insignificant reductions in the serum free testosterone level, as well as the systolic and the diastolic BP, and an insignificant increase in the adiponectin level. The reduction of serum free testosterone in the control group, who received two capsules of 750 mg flour twice daily, was more than it was in the treatment group, thus indicating that NS can inhibit the decrease in the serum free testosterone level.

3.5. Dyslipidemia

Dyslipidemia is defined as the derangements of one or more of the lipoproteins in blood, such as elevated TC, LDL-C, and/or TG, or low levels of HDL-C alone [63]. Moeen-ud-din *et al* reported that the intake of 2 teaspoons of NS seeds for 6 weeks by hyperlipidemic patients decreased their LDL-C (P-value < 0.001) and increased their HDL-C (P-value < 0.01) as significantly as niacin [64]. The results of another RCT indicated that compared to mineral oil, administration of NS oil (5 mL/day) to healthy volunteers for 8 weeks induced significant decreases in the fasting blood cholesterol, LDL, TG, glucose, and HbA1C levels [30]. Administration of NS seeds (two spoons/day) to male and female hyperlipidemic patients for 4 weeks significantly (P-value < 0.001) increased HDL-C and decreased body weight [65].

Menopausal women are one of the high risk groups for developing dyslipidemia. In a study by Ibrahim *et al*, the administration of powdered NS seeds (1 g/day) over a two-month intervention was found to decrease significantly the TG, LDL-C and TC levels, but to increase the HDL-C level [66]. However, one month after cessation of treatment, the lipid profiles in the NS-treated group tended to change towards the pretreatment levels. Another clinical trial done for a similar duration showed that a supplement of powdered NS seeds (1 g/day) could improve some biochemical parameters, including the lipid profile and blood glucose, in menopausal women, but had no significant effect on their body weight [67].

According to a World Health Organization (WHO) report, coronary heart disease is a major cause of mortality in the world [68]. The results of a study which examined the effects of NS on the lipid profiles in patients with stable coronary artery disease indicated that intake of NS powder (500 mg/day) for 6 months concurrent with statin (10 - 20 mg/day) both decreased the serum levels of TG, VLDL, LDL, and TC and elevated the HDL level significantly whereas statin alone decreased neither the TG, VLDL, LDL nor TC level [69].

Table 1 Effects of *Nigella sativa* supplementation on patients with diabetes

Study design	Population	Part of the herb, dose, and duration of treatment	Result	References
Randomized, double-blind, placebo-controlled trial	72 Type 2 DM patients (men and women aged 30 - 60 years old)	NS oil (3 g/day) 12 weeks	↓ BMI, insulin level and insulin resistance as well as HDL-C as compared with baseline , ↓ FBS, TG, LDL-C and HbA1c as compared to the placebo group	[77]
-	41 Type 2 DM patients (men and women aged 30 - 60 years old)	NS oil (equivalent to oil obtained from 0.7 g of seeds) 40 days	↓ FBS , ↑ insulin, but reversed after 40 days of placebo administration	[36]
Prospective study	60 patients with insulin resistance (men and women)	NS oil (5 mL/day) + atorvastatin (10 mg/day) and metformin (1 g/day) 6 weeks	↓ TC, LDL-C and FBS	[78]
Randomized, double blind, placebo-controlled clinical trial	43 Type 2 DM patients (men and women)	NS oil extract (1 g/day) 8 weeks	↓ TG, LDL-C, TC, and LDL-C to HDL-C ratios as compared to the placebo	[79]
-	41 type 2 DM patients (men and women)	NS tea (5 g/day) + usual oral anti-diabetic drugs, diet, and exercises 6 months	↓ FBG, PPBG and HbA1c	[37]
-	94 type 2 DM patients (men and women)	NS seeds 2 g/day or 3 g/day 12 weeks	↓ FBG, 2hPG, and HbA1c, ↓ Insulin resistance , ↑ β-cell function; no significant change in body weight	[35]
		NS seeds 1 g/day, 12 weeks	Insignificant changes	[35]
-	41 type 2 DM patients (men and women aged 30 - 60 years old)	NS seed powder, 40 days	↓ FBG, TC, LDL-C and TG, ↑ HDL-C and insulin; all mentioned values except HDL significantly reversed again after 40 days of placebo intake	[36]

DM, Diabetes mellitus; NS, *Nigella sativa*; BMI, Body mass index; HDL-C, High density lipoprotein-cholesterol; TG, Triglyceride; LDL-C, Low density lipoprotein-cholesterol; HbA1c, Haemoglobin A1c; FBS, Fasting blood sugar; TC, Total cholesterol; FBG, Fasting blood glucose; PPBG, Postprandial blood glucose; 2hPG, 2 hours postprandial glucose.

In healthy female volunteers, administration of both crushed seeds and the formulated total oil of NS caused significant reductions in the prolactin, glucose, triglyceride, and cholesterol levels. Additionally, administration of the crushed seeds caused a significant increase in the white blood cell count (WBC) and the hemoglobin level whereas administration of NS oil only increased the hemoglobin level significantly [51]. In another study, Bamosa *et al* suggested that the administration of NS at 2 g/day for 2 weeks decreased the blood levels of both glucose and cholesterol in healthy volunteers [70].

A large RCT compared the effects of simvastatin (10 mg/

Table 2 Clinical effects of *Nigella sativa* supplementation on the nervous system

Study design	Population	Part of the herb, dose, and duration of treatment	Result	References
Double-blinded crossover clinical trial	23 epileptic children	Water extract of black seed (40 mg/kg/8 h), 10 weeks	Significant reduction of mean frequency of seizures	[80]
Pilot, double-blinded crossover clinical trial	22 epileptic children	Thymoquinone (1 mg/kg), 10 weeks	Significant reduction of frequency of seizures	[81]
Prospective, randomized, single-blinded, controlled, crossover pilot study	30 intractable epileptic patients	40 - 80 mg/kg/day of black seed oil, 10 weeks	No beneficial effects in the frequency and severity of seizures	[82]
Placebo-controlled clinical trial	40 healthy elderly volunteers	500 mg NS seed capsule twice a day, 9 weeks	Improvement of cognition, memory, and attention	[83]
Placebo-controlled clinical trial	48 healthy adolescent males	500 mg NS capsule once daily, 4 weeks	Enhancements of mood, anxiety, and cognition	[84]
-	35 male opiate addicts	500 mg NS daily, 12 weeks	Significant reduction of withdrawal effects	[85]
Randomized, triple-blind, active, and placebo-controlled clinical trial	52 women with cyclic mastalgia	Topical 600 mg NS oil twice a day, 2 months	Significant improvement of the pain scores	[86]

NS, *Nigella sativa*.

day) alone and concurrent with NS seeds (500 mg/day) and garlic oil (0.625 mg/day) on the lipid profile in 258 patients with hyperlipidemia over an 8-week treatment [71]. In that study, a comparison of mean values between the two treatment groups indicated a highly significant difference (P = < 0.01) for cholesterol, TG, non-HDL, and LDL reductions and a significant difference (P = 0.03) for HDL elevation [71]. Sabzghabaee *et al* reported that the TC, LDL, and TG serum levels in hypercholesterolemic patients who took NS at 2 g/day for 4 weeks decreased significantly [72]. The uses of NS extract at 200 and 400 mg/day for 2 months were also reported to have decreased both the total and the LDL cholesterol in patients with mild HT as compared with the baseline [38].

Of course, some controversies exist in the clinical trial results. Qidwai *et al* reported that NS seeds (2 g/day) administration had not affected the BMI, waist-hip ratio, BP, FBS or serum lipids in adults after 6 weeks of use [34]. Unlike pervasive evidence for the effects of NS use on the lipid profile, the administration of powdered NS seeds was reported to have had no significant effect on BP, serum lipid levels, blood sugar, or body weight in adults [73]. According to some meta-analyses, overall, the use of NS was shown to reduce the plasma levels of TC, LDL-C and TG, but its effect on HDL-C was not significant [74, 75]. Whereas the use of NS seed oil was observed to have greater effects on the serum TC and the LDL-C levels, versus the use of seed powder, elevation of the HDL-C levels was found only after supplementation with NS seed powder [75].

3.6. Diabetes

DM is characterized by chronic elevation of blood glucose, which is a central factor in the production of reactive oxygen species (ROS) that, in turn, promote cellular damage and contribute to the development and progression of diabetic complications [76]. In a report by Heshmati *et al*, although the administration of NS oil at 3 g/day for 12 weeks was stated to have caused insignificant decreases in the BMI, insulin level, and insulin resistance, as well as an increase in the HDL-C level, in patients with type 2 diabetes, the FBS, TG, LDL-C, and HbA1c levels were observed to have been lowered significantly in the intervention group as compared to the placebo group [77].

Another clinical trial showed that NS oil intake (equiva-

lent to oil obtained from 0.7 g of seeds) for 40 days caused a significant reduction of FBS and a significant rise of insulin in type 2 DM patients [32]. In still another study, the addition of NS oil at 5 mL/day to atorvastatin at 10 mg/day and metformin at 1 g/day was shown to have induced significant reductions in the TC, LDL-C, and FBS levels after 6 weeks of use in patients with insulin resistance [78]. Furthermore, according to a study performed by Hadi *et al* on 43 type 2 diabetic patients, after an 8-week treatment, the administration of NS oil extract at 1 g/day decreased the serum levels of TG, LDL-C, and TC, as well as the LDL-C to HDL-C ratio, significantly in comparison to the placebo [79]. Moreover, treatment with NS tea at 5 g/day added to the usual oral anti-diabetic drugs, diet, and exercise for 6 months led to significant decreases in the FBG, PPBG, and HbA1c level in type 2 diabetic patients [37]. Another clinical trial showed that the administration of NS at a dose of 2 g/day over a 12-week treatment caused significant decreases in the FBG, 2hPG, and HbA1c levels without any significant change in body weight [35]. In that study, insulin resistance, calculated by using a homeostatic model assessment, was also reduced while β-cell function was increased significantly. However, a dose of 1 g/day caused insignificant changes, and no further increments in the beneficial responses were observed with a dose of 3 g/day, indicating that 2 g/day was the optimum dose. Bilal *et al* reported the highly significant decreases in the FBG, TC, LDL-C and TG levels and increases in the HDL-C and the insulin levels were observed in patients with type 2 diabetes after 40 days of NS seed powder intake [36]. However, in that study, all mentioned values, except the HDL level, significantly reversed again after 40 days of placebo intake. Table 1.

3.7. Nervous system

Akhondian *et al* demonstrated that treatment of intractable pediatric seizures with water extract of black seed (40 mg/kg/8 h) versus placebo, as an adjuvant therapy to antiepileptic drugs, led to a significant reduction in the mean frequency of seizures [80]. A similar clinical trial done by the same author had similar outcomes, although TQ (1 mg/kg) was administered as an add-on therapy instead of water extract of black seed [81]. On the other hand, another study conducted by Shawki *et al* had a different result; after administration of 40 - 80 mg/kg/day of black seed oil as an adjuvant therapy for 4 weeks, no beneficial effects on the frequency and severity of seizures in intractable epileptic children were observed [82].

In a placebo-controlled clinical trial addressing memory and cognition, healthy elderly volunteers took a 500-mg NS capsule twice a day over a period of 9 weeks [83]. At the end of that period, through special tests, the authors observed improved cognition, memory, and attention. Similarly, another CT performed on healthy adolescent males aged 14 to 17 years established the modulatory effects on cognition, mood, and anxiety of NS taken in the form of a 500-mg NS capsule once a day for four weeks [84].

Sangi *et al* introduced NS administration as a effective non-opiate treatment for opioid dependence [85]. They found that NS treatment offers some advantages in contrast with opiate treatments, such as relieving the withdrawal effects, maintaining the physiological parameters, and improving appetite. Table 2.

3.8. Analgesic effects

Huseini *et al* showed that as compared with the topical administration of diclofenac, topical administration of NS oil had significant therapeutic effects on patients with cyclic mastalgia without any adverse effects [86]. In that study, 600 mg of NS oil (in the first treatment group) and 20 mg of topical diclofenac (in the second treatment group) were applied to the painful area twice daily for two months.

3.9. Dermatology

Vitiligo is an autoimmune skin disease occurring due to the destruction of skin melanocytes that produce skin pigment. A RCT comparing the efficacy of applying NS oil and fish oil to the lesions of vitiligo twice a day for six months indicated that the former is more effective than the latter in reducing the size of the lesions [39].

Hand eczema is a pruritic papulovesicular dermatitis severely influencing the patient's quality of life. Yousefi *et al* compared the effects of NS ointment, betamethasone, and eucerin on the severity of hand eczema and the patients' quality of life [87]. In that study, for new cases of hand eczema in patients between 18 and 60 years of age the mentioned drugs were applied twice daily for 4 weeks. Through particular measures, the authors concluded that NS may be as effective as betamethasone in enhancing quality of life and alleviating the severity of eczema and that both were more effective than eucerin.

In contrast, for a comparison of the therapeutic effects of NS oil ointment and aplacebo on patients with atopic dermatitis (eczema), 20 patients were asked to apply NS oil on one arm and a placebo on the other every day for 4 weeks [88]. The authors of that study reported that no meaningful difference could be found in terms of the parameters measured, e.g., severity, pruritis, transepidermal water loss, and skin hydration, between the treatment with NS oil ointment and with a placebo.

Arsenical keratosis manifests itself in both the palms of the hands and the soles of the feet due to chronic arsenic consumption caused by drinking contaminated water. Taking capsules of NS oil (500 mg) and vitamin E (200 mg) for eight weeks has been shown to reduce the body's arsenic load, thereby contributing to an overall alleviation of the symptoms in patients with this disease [89].

3.10. Infectious diseases

Infection with HCV often leads to chronic hepatitis, which, in turn, results in liver cirrhosis and hepatocellular carcinomas. A study conducted in Egypt on HCV patients

demonstrated that ethanolic extracts of NS and *Zingiber officinale* (*Z. officinale*), alone and together, had beneficial effects on HCV patients; i.e., their liver function was improved and the viral load was decreased [90]. In that study, a mixture of these extracts was observed to be more effective than each one alone. Patients included in that study were treated with capsules containing 500 mg of NS and/or *Z. officinale* twice daily for one month. In a similar study, HCV patients received capsules of NS oil (450 mg) three times a day over a 3-month period. That treatment led to the same results reported in Ref. 90, i.e., decreased viral load and improved liver function [43].

Onifade *et al* confirmed that treatment of a sero-positive human immunodeficiency virus (HIV) infected man with NS concoction (10 mL twice/day for six months) resulted in the reduction of the viral load to an undetectable level in 3 months, an elevation of the CD4 count, an alleviation of the symptoms, and a sustained sero-reversion [91]. Similarly, another study conducted by the same author on a sero-positive HIV infected woman revealed the efficacy of NS and honey therapy (10 mL thrice/day for 1 year) for sustained sero-reversion [92]. These effects can be ascribed to the probable virucidal activity of NS [91].

The helicobacter pylori (H. pylori) bacterium can cause many diseases, such as peptic ulcers and gastric cancer. Infection with *H. pylori* has a high prevalence worldwide. Salem *et al* stated that in a four-week course, the efficacy of NS powder (2 g/day) administered together with omeprazole to eradicate an *H. pylori* infection in non-ulcer dyspeptic patients was relatively the same as that of triple therapy, although 1 g/day or 3 g/day of NS powder given together with omeprazole was not as effective, indicating that the optimal dose of NS was 2 g/day [93]. (Triple therapy includes clarithromycin, amoxicillin, and omeprazole.)

In a study conducted on children who were infected with cestodes, the efficacy of single oral administration of NS powdered seeds and ethanolic extract (40 mg/kg body weight) was proven to reduce the percentage of fecal eggs per gram, which means NS has an anti-cestodal effect [45]. Furthermore, in a RCT in which 100 infected women were included, the therapeutic effects of black seed capsules (500 mg twice daily) used together with clotrimazole vaginal cream on *C. albicans* vaginitis were compared with those of placebo capsules (500 mg twice daily) used in combination with the same vaginal cream [94]. In that study, after a 7-day treatment, the black seed capsules used with clotrimazole vaginal cream were more impressive in reducing the symptoms of the disease, such as vaginal itching, discharge, irritation, vulvovaginal redness and inflammation [94].

3.11. Reproductive system

Kolahdooz *et al* proved that treatment of infertile Iranian men with 2.5 mL of NS oil twice a day for two months, in contrast with a placebo treatment in the same manner, could enhance sperm parameters, including sperm count, motility and morphology, semen volume, pH, and its round cells [41]. Other beneficial effects of NS on Leydig cells, reproductive organs, and sexual hormones in infertile men have also been confirmed [95].

3.12. Respiratory system

As for lower respiratory tract illnesses (LRTI), Boskabady *et al* investigated the antiasthmatic effects of NS boiled extract (50 and 100 mg/kg), theophylline (5 mg/kg), and salbutamol (200 μg) [96]. In that study, 15 asthmatic patients were recruited, and each patient received one of these 4 treatments in random order at intervals of 48 hours for 2 weeks. The results of that study demonstrated that in spite of the fact that both doses of NS boiled extract showed bronchodilatory effects, their efficacies for pulmonary function test (PFT) elevation were less than those of theophylline and salbutamol. The results also revealed the prophylactic effects of NS boiled extract on adult asthmatic patients. In another study, over a 3-month period, the daily intake of 15 mL/kg of 0.1% NS boiled extract led to more impressive improvements in the PFT parameters and alleviations of the symptoms of asthma than the intake of the placebo solution did [97]. Ahmad *et al* conducted a study on 5- to15-year-old LRTI patients with wheezing and investigated the beneficial impacts of the standard treatment alone and the standard treatment combined with the use of NS oil [98]. The authors of that study concluded that the standard treatment when administered with NS oil (0.1 mg/kg for 14 days) had more beneficial effects in reducing the pulmonary index and improving the peak expiratory flow rate [98]. In another study, daily administration of 0.375 mL/kg of 50% NS boiled water extract, as compared with a placebo solution to victims of chemical warfare, as compared with a placebo solution, for two months effected meaningful improvements in the PFT measures and the respiratory symptoms, indicating that the use of NS had a prophylactic impact on victims of chemical warfare [99].

Allergic rhinitis (AR) is an inflammatory response of the nasal mucosa to natural allergens and is characterized by sneezing, rhinorrhea, nasal congestion, and itching [47, 100]. Moreover, a study comparing the therapeutic effects of NS seeds (250 mg/day) and montelukast (10 mg/day) on patients with seasonal allergic rhinitis over a course of two weeks illustrated that both improved the daytime and ophthalmic symptoms considerably, although NS was more efficient in alleviating the nighttime symptoms [46]. Similarly, Nikakhlagh *et al* showed the positive effects of NS oil capsule consumption over four weeks on the symptoms of allergic rhinitis, i.e., nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor [100]. Like the previous studies, that of Alsamarai *et al* demonstrated that nasal drops of NS oil, in comparison with nasal drops of ordinary food oil, could significantly improve the symptoms of AR patients, as well as their ability to tolerate exposure to allergens [47]. In that study, each drop comprised 15 mL of oil, and the patients applied 2 drops nasally (one in each nostril) 3 times daily for 6 weeks. In an additional study of AR patients who underwent a month of allergen-specific immunotherapy and then received treatment with NS seeds (2 g/d) during the next month, in contrast with the AR patients who only received immunotherapy for two months, the former

Table 3 Effects of *Nigella sativ*a supplementation on patients with some diseases of the respiratory system

Disease	Study design	Population	Part of the herb, dose, and duration of treatment	Result	References
Lower respiratory tract illnesses	-	15 asthmatic patients	NS boiled extract (50 and 100 mg/kg)	NS was less effective than theophylline and salbutamol, on pulmonary function tests	[96]
	Controlled clinical trial	29 asthmatic adults	15 mL/kg/day of 0.1% NS boiled extract, 3 months	Prophylactic effects of NS on asthmatic patients	[97]
	Prospective, randomized, open study	84 patients of wheeze associated LRTI	NS oil (0.1 mg/kg), 14 days	More beneficial effects in reducing the pulmonary index and improving the peak expiratory flow rate	[98]
	Randomized, double-blind, placebo-controlled trial	40 chemical war victims	0.375 mL/kg/day of 50 g NS boiled water extract, 2 months	Prophylactic effect of NS on chemical war victims	[99]
Allergic rhinitis	Single-blind comparative clinical trial	47 untreated adult patients with seasonal allergic rhinitis	NS seeds (250 mg/day), 2 weeks	Decreasing daytime, ophthalmic, and nighttime symptoms	[46]
	Double-blind clinical trial	68 patients with allergic rhinitis	Nasal drops of NS oil (each drop: 15 mL oil), 6 weeks	Notable symptomatic improvement of patients	[47]
Tonsillopharyngitis	Comparative, parallel, randomized, double-blind, placebo-controlled study	186 acute tonsillopharyngitis patients	NSPN capsule (360 mg NS and 50 mg *Phyllanthus niruri* extracts), thrice/day, 7 days	Treatment of the symptoms of the disease	[44]

NS, *Nigella sativa*.

showed more progress in their immune status, e.g., PMN functions and CD8 counts [101]. That study showed that NS administration contributed to a more effective immunotherapy. According to a study by Oysu *et al*, some of the nasal symptoms of geriatric patients, for example, nasal dryness, obstruction, and crusting, can be significantly improved by using NS oil [102].

As for patients with tonsillopharyngitis, the results of a study by Dirjomuljono *et al* indicated that NSPN capsules containing 360 mg of NS and 50 mg of Phyllanthus niruri extracts, as compared with a placebo, if given three times a day for 7 days to patients with acute tonsillopharyngitis, could significantly alleviate the symptoms of the disease due to their anti-inflammatory and immuno-modulatory effects [44]. In another study, which was undertaken on patients with inhalation allergy, the beneficial impacts of NS oil on the immune status of those patients, including enhancement of the NK cell count and percentage of lymphocytes, were confirmed [103].

3.13. Skeletal system

Rheumatoid arthritis (RA) is a chronic systemic disease characterized by inflammation of the joints, degeneration of collagen fibers in mesenchymal tissues, and atrophy of bones. Although the etiology is not well understood, autoimmunity seems to play a role in the etiology of RA. A study on 40 female RA patients who received placebo capsules twice a day in the first month and 500-mg NS oil capsules twice a day in the next month observed that the use of NS oil capsules notably improved the disease's activity

score and alleviated its symptoms, which could be attributed to the modulatory effect of NS on the immune system [104].

A clinical trial conducted on osteopenic postmenopausal women showed that the intake of NS oil (0.05 cc/kg/d) for three months had no considerable effects on bone turnover markers [40]. Furthermore, in another clinical trial, in which 15 osteoporotic postmenopausal women were included, no beneficial effects of consuming NS extract (0.05 mL/kg/d for three months) on bone turnover were observed [105].

3.14. Gastrointestinal system

Celiac disease (CD) is characterized by increased sensitivity to gluten and by inflammation and destruction of the small intestine mucosa due to an autoimmune mechanism. In a study by Osman *et al*, the prescription of a gluten free diet (GFD) together with the consumption of a NS oil capsule (450 mg) twice daily as dietary supplement for one year was more effective than a GFD alone in the treatment of patients with iron deficiency anemia associated with refractory CD; i.e., the hematological and immunological indices and the duodenal histology recovery were improved [106]. Dermatitis herpetiformis (DH) is an autoimmune skin disease caused by CD and is characterized by itchiness, a burning sensation, and chronic dermatitis. Similar to the former CT, adding NS oil capsules to a GFD for a period of 6 months was found to enhance the efficacy of a GFD in the treatment of the disease [107]. Furthermore, Mohtashami *et al*. reported that administration of a honey-based formulation of NS oil (5 mL NS oil/day), in comparison with a placebo, for 8 weeks could significantly improve the symptoms, such as dyspepsia severity, and decrease the rate of *H. pylori* infection in functional dyspeptic patients [42].

3.15. Anti-toxicity effects

Leukemia is a tumoral growth of WBCs in the bone marrow and is caused by a malignant neoplasm of hematopoietic stem cells. In ALL, which is the most common childhood malignancy [108], increased numbers of lymphoblasts are present in the circulating blood and in different tissues and organs [48]. A study by Hagag *et al* reported that NS oil (80 mg/kg/day) administered for one week after each methotrexate treatment could reduce hepatotoxicity and improve the survival rate in ALL children undergoing that treatment [108]. Table 3.

4. Discussion

This review article summarized different studies on the clinical uses of NS and TQ in the prevention and the treatment of different diseases. Results indicated that NS has beneficial effects when used in the therapies for various diseases, including cardiovascular, nervous system, skin, infectious, reproductive system, respiratory system, skeletal system, and gastrointestinal diseases. Taken together, the role of NS in the treatment of different diseases is discussed in the following sentences.

Dyslipidemia plays an important role in the genesis of cardiovascular diseases (CVDs) [71], and hypercholesterolemia is the most important risk factor for atherosclerosis [72]. Lipid abnormalities are accountable for 56% of patients with heart disease and 18% of those with an infarction; further, it is associated with one third of deaths worldwide. In this article, we reviewed 19 clinical trials that reported the ameliorative effect of NS on the lipid profile. This finding shows that the use of NS supplements can improve the lipid profile and prevent CVDs both in healthy people and hyperlipidemic patients. The exact mechanisms of the lipid-modifying effects of NS are not known, but might be associated with the inhibition of intestinal cholesterol absorption, decreased hepatic cholesterol synthesis, and up-regulation of LDL receptors [74].

On the other hand, dyslipidemia is an important risk factor responsible for cardiovascular disease in patients with diabetes [109], so alleviation/elimination of lipid abnormalities is important in the prevention of the complications of diabetes [79]. Thus, keeping the lipid profile of diabetic patients in the normal range can improve their health status, and NS intake, in combination with anti-diabetics and statins or fibrates, can help diabetic patients to control both dyslipidemia and blood sugar. We also reported the results of 13 clinical trials that presented data on the hypoglycemic effect of NS, 8 of which included patients with insulin resistance. The results showed that the use of black seed could decrease the HbA1c (5 studies) and the PPBG (2 studies) levels significantly.

Obesity is typically associated with increased risk factors of CVDs. Therefore, a therapeutic approach that aims to control body weight and the metabolic profile might be effective in preventing CVDs [62]. The results of this review demonstrate that the use of NS may have a weight-lose effect in obese men and women. Because of the lipid-modifying, hypoglycemic, and weight-lowering effects of black seed, its use in the treatment of patients with metabolic syndrome may be beneficial [21].

According to some studies reviewed, NS may be a useful herb for improving sexual function because of its inhibitory effect on prolactin [51] and excitatory effect on testosterone [33]. In addition, spermatogenesis can be stimulated by NS [95]. Consequently, it presents a good option for use in the treatment of infertile men. However, more studies are needed due to the lack of an adequate number of studies proving this effect.

TQ has an antioxidant role, improves the body's defense system, induces apoptosis, and controls the Akt pathway [7]. Immune system modulation is one of the most important properties of NS, and a number of studies have been done in order to prove this significant effect. In this article, we reviewed five studies that directly showed the immunomodulatory effect of NS [39, 44, 104, 106, 107]. In addition, other investigations have demonstrated that NS may be an optimum choice for treating patients with allergy-related diseases, such as asthma, atopic eczema and allergic rhinitis [110].

Moreover, the anti-nociceptive effect of NS has been widely investigated in animal models, and a few studies have evaluated that effect in humans. In this paper, the anti-microbial activities of NS against bacteria, viruses, and parasites have also been highlighted. The conclusion is that it can be used in the treatment of infectious diseases [111].

The existing drugs for some diseases produce adverse side effects, do not lead to complete recovery, or are sometimes expensive. Thus, herbal medicines, such as NS, can be useful alternatives; however, before such herbal medicines are used extensively, many RCTs should be conducted in order to evaluate and confirm the effects of herbal medicines [46, 48, 108]. One obvious example is sero-reversion in HIV-infected patients. Highly active anti-retroviral therapy does not cause sero-reversion in HIV-infected patients. Onifade *et al* proved that NS contributes to sustained sero-reversion in patients with HIV infection [91, 92].

Some authorities, despite achieving positive outcomes for the features of NS, suggest further investigations with larger samples and groups, diverse doses of NS, and longer periods of study [42, 83-85, 93, 97-99]. After the beneficial effects of NS have been confirmed, it can be used in treatment protocols. In contrast, some authors have reached results that conflict with the traditional beliefs about NS. Actually, the evidence for the positive effects of NS in the treatment of osteopenic postmenopausal women and intractable epileptic children is conflicting [40, 82, 105]. Therefore, more studies should be planned in these situations.

5. Conclusion

In conclusion, the use of black seeds and their active constituent TQ has been shown to have multiple useful effects in the treatments of patients with several diseases, such as inflammatory and auto-immune disorders, as well as metabolic syndrome. In this study, we also reviewed other advantages of NS, e.g., its antimicrobial properties, anti-nociceptive and anti-epileptic impacts, etc. We found that the side effects of this herbal medicine did not appear serious, so it can be applied in clinical trials because most of its major effects have been shown to be beneficial. Some properties of NS, such as its hypoglycemic, hypolipidemic, and bronchodilatory properties, are sufficiently understood so that NS can be used for subsequent phases of clinical trials or for drug development. However, most of the other effects and applications of NS require further clinical and animal studies.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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