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ORIGINAL PAPER

Mitigation of Obesity-Promoted Diseases by *Nigella sativa* and Thymoquinone

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Abstract Obesity is closely associated with increased incidence of cardiovascular diseases, cancer, insulin resistance, and immune dysfunction, and thus obesity-mitigation strategies should take into account these secondary pathologies in addition to promoting weight loss. Recent studies indicate that black cumin (*Nigella sativa)* has cardio-protective, anti-cancer, anti-diabetic, antioxidant, and immune-modulatory properties. While black cumin and/or its major bioactive constituent, thymoquinone have demonstrated bioactivity in a variety of disease models, the mechanisms of action are largely unknown. Given the growing interest in and the use of functional foods and nutraceuticals, as well as the increase in obesity and chronic diseases worldwide, further research into the therapeutic/preventive effects of black cumin may be beneficial.

Keywords Obesity · Cancer · Type 2 diabetes · *Nigella sativa* · Thymoquinone · Bioactive compounds · Nutraceuticals

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Introduction

Epidemiological evidence indicates that certain dietary patterns are detrimental to health by contributing to the development of cardiovascular diseases, cancer, and diabetes. Conversely, the consumption of certain foods and their associated compounds has also been linked to the prevention of these diseases [1]. For example, high intake of red meat is associated with increased incidence of colon cancer, whereas adequate intake of certain fruits and vegetables has a preventive/inhibitory effect on colon cancer [2]. The health-promoting properties of fruits and vegetables have been attributed to the presence of non-nutrient phytochemicals, such as carotenoids, anthocyanins, phenolics, and flavonoids [3]. There is a growing interest in dietary bioactive compounds that protect against and/or mitigate the severity of chronic diseases without the undesirable side effects of many pharmaceutical treatments. Black cumin (*Nigella sativa*) is an annual herbaceous plant of the *Ranunculaceae* family native to the Middle East and cultivated for its seed, which is added to a variety of foods (*e.g.*, curries, pastries, honey, breads, and cheeses) to impart a slightly bitter, peppery flavor [4]. Black cumin has traditionally been used to treat a variety of chronic diseases/disorders such as hypertension, hypercholesterolemia, inflammation, asthma, bronchitis, cough, dizziness, fever, headache, and influenza [5]. It is also used to treat skin diseases such as psoriasis and eczema [4].

Black cumin has demonstrated effectiveness in the treatment of cardiovascular diseases, cancer, insulin resistance, and immunodeficiency [6–8]. Thymoquinone (TQ), a bioactive compound in black cumin has also demonstrated a variety of biological activities that warrant further investigation into its potential uses as a therapeutic agent and nutraceutical [6, 9, 10]. The current increase in obesity and its secondary pathologies (*e.g.*, cardiovascular diseases, cancer, and diabetes) poses a significant healthcare burden that warrants further investigation

into non-conventional treatments such as functional foods/nutraceuticals [11]. Black cumin has been used medicinally for millennia. However, the mechanisms of action of black cumin are still being elucidated. This review will discuss (i) black cumin bioactive constituents, (ii) obesity and its associated physiology pertaining to the activities of black cumin (iii) the activity of black cumin against cardiovascular disease, cancer, insulin resistance, reactive oxygen species, and immune function, and (iv) the toxicological properties of black cumin.

Black Cumin Constituents

Large variation exists regarding micronutrient content of black cumin in the published literature [12, 13]. This may be due to genotype, environment, genotype x environment, post-harvest processing and differences in analytical procedures (Table 1). A majority of the over 100 components present in black cumin are present in the seed, which contains approximately 36–42% fixed oils, 23% proteins, 1% volatile oils and smaller amounts of amino acids, fatty acids, alkaloids (nigellicine and nigelledine), tannins, saponins (α hederin), phytosterols, reducing sugars, vitamins (ascorbic acid, thiamine, niacin, pyridoxine, folic acid), minerals (Fe, Na, Cu, Zn, P, Ca) and other compounds [4]. The total fixed (or crude) seed oil has been found to be rich in fatty acids. Thirty-two fatty acids (99.9%) have been identified in the fixed oil and the major fatty acids were linoleic acid (50.2–60%), oleic acid (20–25%), palmitic acid (12%), margaric acid (10.3%), cis-11,14-eicosadienoic acid (7.7%), eicosenoic acid (0.5%), linolenic acid (0.3%), myristic acid (0.3%), and stearic acid (3%). Thirty-two compounds have been identified in the volatile oil, which constitute more than 86%. The major compounds of the volatile oil were trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%). The essential oil contains a variety of compounds, including TQ, thymohydroquinone, thymol, limonene nigellone, p-cymene, trans-anethole, terpinene G, terpinene α, campholenel α, thujene α, pinene β, pinene α, and carvacrol (Fig. 1) [6, 14–16].

Seed oils are typically extracted using cold-pressing technology that uses no heat treatment or solvents. However, limited information is available on how different batches of cold-pressed oils differ in their bioactive content and composition. A recent study investigated the six different batches of cold-pressed cumin seed oil on fatty acid profiles, thymoquinone contents, oxidative stability, and antioxidant properties [17]. Phenolic components ranged from 1.02 to 1.40 mg gallic acid equivalents/g oil. A two-fold change in oxidative stability index (OSI) was observed among the batches, with ~155 h, and ~76 h of the greatest and lowest OSI, respectively. Thymoquinone, a major bioactive compound in seed oil ranged from 3.48–8.73 mg/g. Furthermore, stability of edible oils is compromised during storage and marketing. In general, oxidative stabilities of crude/cold pressed oils were stronger than their stripped (removes most of the non-triacylglycerol

Table 1 Proximate composition and mineral analysis of *Nigella sativa* L. seeds

Proximate composition (%)	Nergiz & Otles [70]	Iqbal et al. [12]	Sultan et al. [13]
Moisture	6.4±0.15	–	6.5±0.17
Ash	4.0±0.29	–	4.2±0.11
Fat	32.0±0.54	–	31.2±0.82
Crude fiber	6.6±0.69	–	6.03±0.16
Crude protein	20.2±0.82	–	22.8±0.60
Carbohydrate	37.4±0.87	–	29.4±0.78
Micronutrients & vitamins (mg/100 gdw)[a]			
Potassium	1,180±10.0	0.6–1.0 (%)	808±6.6
Phosphorus	–	0.5–0.7 (%)	543±10.0
Calcium	188±1.5	7.4–10.8	570±21.5
Magnesium	–	9.4–11.6	265±4.9
Sodium	85.3±16.07	0.2–0.7	17.6±2.2
Iron	57.5±0.5	0.1–0.7	9.7±0.65
Zinc	–	0.01–0.1	6.23±0.21
Copper	–	0.02–0.03	2.60±0.03
B 1 (thiamin)	831±11.36	–	–
B 2 (riboflavin)	63±3.32	–	–
B 6 (pyridoxin)	789±8.89	–	–
PP (niacin)	6,311±16.52	–	–
Folic acid	42.0±4.58	–	–
Total tocopherols	340±8.66	–	361.7±10.2

[a]gdw: gram dry weight

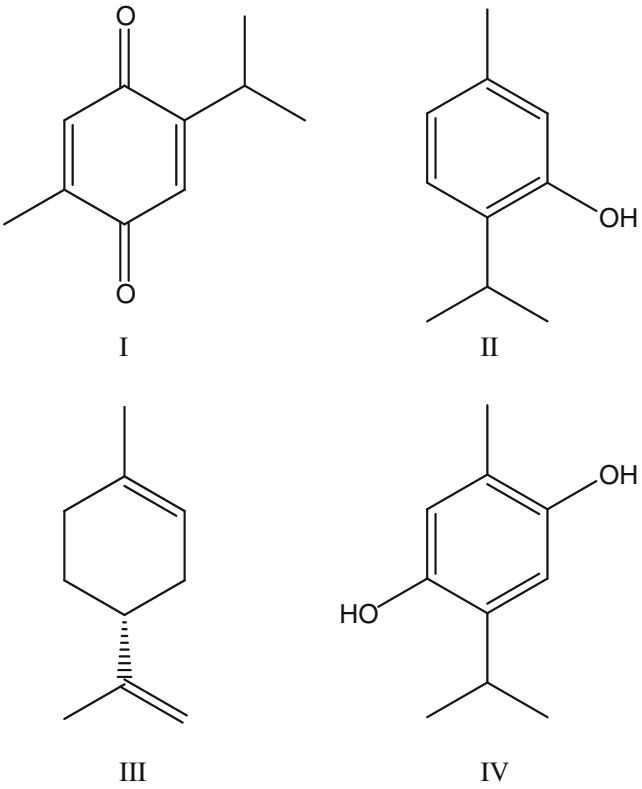

Fig. 1 Structures of thymoquinone (I), thymol (II), limonene (III), and thymohydroquinone (IV); major bioactive compounds present in *Nigella sativa*

components, including polar lipids and phenolics) counterparts [18]. Thus, these results suggest that it is critical to include details on the post-harvest storage, oil extraction methods, post-storage and marketing conditions, bioactive compound and fatty acid profiles, while reporting the health benefits of black cumin seed oil to ensure the placement of results in the proper contexts within the growing body of research on black cumin. Indeed, the entire field of dietary bioactive compounds would benefit from such context and understanding.

Obesity

Approximately 605 of Americans are obese/overweight and one-third of adults in the U.S. are obese compared to one-ninth in 1980 [19]. Obesity is generally defined as having a body mass index (BMI) greater than or equal to 30 kg/m^2, the point at which increasing body mass is significantly associated with negative health consequences. Obese individuals are at greater risk for cardiovascular diseases, certain cancers, type II diabetes, and reduced immunocompetence [20–23]. While the mechanisms underlying these associations are not fully understood, it is likely that obesity disrupts the metabolism and regulation of various biomolecules including insulin, adiponectin, ghrelin, leptin, cytokines, adipokines, and other hypothalamic neurotransmitters and peptides, which play a potential causative role in these conditions. Importantly, the physiological changes that accompany these chronic conditions are not mutually exclusive. In the case of obesity-associated diabetes, the dysregulation of insulin, glucose and free fatty acid levels promotes the development of prostate and colon cancers [24]. Accordingly, bioactive compounds that are effective in the treatment of one of these chronic conditions may also be effective in the treatment of others, as demonstrated by resveratrol [25].

Obesity is the physiological result of a complex interaction between genetic, environmental, and psychosocial factors [26]. Conventional dietary strategies to prevent/mitigate obesity often involve caloric restriction or altering the ratio of macronutrients. However, an alternative strategy would be for an individual to consume specific functional foods and bioactive compounds that, in combination with conventional treatments, may contribute to weight loss/management and reduce the risk for obesity-related diseases [27]. A recent study found a reduction in body mass index in obese males consuming1.5 g of black cumin per day for three months, whereas obese males in the control group showed an increase in their body weight and waist circumference [28].

Biological Activities

Black Cumin and Cardiovascular Disease

Black cumin mitigates hypertension and hyperlipidemia, thereby protecting against the development of cardiovascular disease. Dehkordi & Kamkhah [6] performed a randomized, double-blind placebo-controlled trial to evaluate the efficacy of two doses of black cumin seed oil (100 or 200 mg oil, twice per day) on patients with mild hypertension. The authors found that black cumin oil significantly reduced both systolic and diastolic blood pressure in a dose-dependent manner, and also reduced total and LDL-cholesterol levels. Suddek [29] examined the effect of TQ on rat pulmonary arterial rings (*ex vivo*) that were pre-contracted with phenylephrine and observed a dose-dependent decrease in the tension of rings treated with TQ. Ebru et al. [30] examined the ability of black cumin seed oil to protect against cyclosporine A-induced cardio-toxicity in rats. Rats treated with black cumin seed oil had greater levels of endogenous anti-oxidants such as superoxide dismutase, catalase, and glutathione peroxidase (enzymes involved in antioxidant defense). Furthermore, rats treated with cyclosporine A and black cumin seed oil showed reduced levels of malondialdehyde, a marker of oxidative stress, compared to rats treated only with cyclosporine A.

Pourghassem-Gargari et al. [31] and Nader et al. [32] found that black cumin seed supplementation reduced total cholesterol, LDL-cholesterol, triglyceride, and malondialdehyde in

hyperlipidemic rabbits by 43.7% ($p<0.001$), 42.8% ($p<0.001$), 34.9% ($p<0.01$) and 51.1% ($p<0.001$), respectively, after one month, and 46.7% ($p<0.001$), 48.2% ($p<0.001$), 38.5% ($p<0.001$), 49.2% ($p<0.001$), respectively, after two months of treatment compared to the control. However, no significant change in total antioxidant status, superoxide dismutase, and glutathione peroxidase levels between groups were reported, indicating that black cumin may improve cardiovascular health by other unidentified mechanisms in addition to antioxidant defense. These findings suggest that the cardio-protective effect of black cumin is partly due its ability to stimulate endogenous antioxidant defense systems.

Black Cumin and Cancer

Obesity is associated with increased incidence of certain cancers, including breast, colon, liver, pancreatic, and prostate [21]. Recent evidence suggests that these associations are mediated by hormonal imbalances that accompany excess body fat. In particular, the synthesis and signal transduction pathways of the hormones insulin and leptin are up-regulated in obesity [30]. Excess insulin induces a decrease in the production of insulin-like growth factor binding proteins (IGF-BP). These proteins bind to insulin-like growth factor (IGF), thereby inhibiting its downstream effects on cell proliferation and apoptosis. The concomitant increase in the levels of IGF and insulin stimulates anti-apoptotic pathways, increasing the likelihood of tumor growth and metastasis. Increased leptin is also associated with the promotion of neoplastic growth [33].

The oil fraction and bioactive components of black cumin (*e.g.*, TQ) have been extensively studied for anti-cancer properties. A recent rodent multi-organ carcinogenesis bioassay study reported that either 1,000 or 4,000 ppm of black cumin oil in the diet resulted in reduced tumor size and lower incidences, and multiplicities [34]. TQ, a component of black cumin oil, has been recognized as the principal component of anti-cancer activity. TQ may promote oxidative/electrophilic stress via quinine-quinol redox cycles [35], and this TQ-induced cytotoxicity is similar to that observed in cells treated with fluorouracil, a commonly used chemotherapeutic drug [9]. However, treatment with fluorouracil increases the risk of ischemia, pericarditis, congestive heart failure, and cardiogenic shock, whereas TQ does not promote these risks [4]. TQ has been tested against colon, laryngeal, breast, skin, and lung cancer cell lines *in vitro*, and in each case has been shown to induce cell cycle arrest and apoptosis [36–39]. Cancer cells treated with TQ have an increased proportion of cells in the G1-phase and fewer cells in the S-phase. Prior to DNA replication, cells in the G1-phase must pass the regulatory G1 checkpoint, which safeguards against the replication of damaged or mutated DNA. Cancer cells that have passed this checkpoint can proliferate more aggressively. Therefore, maintaining cells in the G1 phase deters tumor progression [37, 39].

TQ has shown to inhibit neoplastic growth via p53-dependent apoptotic pathways. TQ-treated colon cancer cells showed a significant increase in p53 mRNA and protein as well as the downstream target of p53, p21WAF1. When these cells were treated with pifithrin-α, a p53 inhibitor, levels of p53 and p21WAF1 returned to normal levels, and the TQ-induced cell cycle arrest was suppressed [36]. However, a study on osteosarcoma cells lacking an intact p53 pathway found that treatment with TQ still induced cell cycle arrest, indicating that TQ may also act via a p53-independent mechanism [38]. This is important since many cancers are induced and promoted by mutations in the p53 pathway, and is therefore unresponsive to drugs targeting this pathway. Importantly, TQ does not adversely affect normal cells, but selectively affects cancer cells [36]. There is a need to identify the mechanisms through which TQ exerts its anti-cancer effects, and whether these mechanisms vary depending on the type of cancer and/or mutations present.

TQ also increased the activity of c-jun N-terminal kinase (JNK), an enzyme involved in the cell differentiation, proliferation, and apoptosis. Treatment with a JNK inhibitor did not completely suppress TQ-induced cytotoxicity, indicating that TQ's anti-proliferative effects are only partly mediated by a JNK-independent pathway. The authors also reported that TQ treatment increased the levels of pro-apoptotic proteins GADD45α and AIF, and decreased the levels of anti-apoptotic Bcl2-related proteins, suggesting that it is through these pathways that TQ exerts its pro-apoptotic activity [40]. Androgen-receptor (AR)-independent and AR-naïve prostate cancer cells treated with TQ increased production of reactive oxygen species and suppressed production of glutathione. In the AR-independent cell line, TQ suppressed the levels of total and nuclear AR and AR-directed transcriptional activity in a dose-dependent manner. However, subsequent treatment with N-acetylcysteine inhibited both TQ-induced ROS generation and growth inhibition but not the TQ-induced AR suppression, indicating that TQ's anti-proliferative effects are due to increased ROS generation and decreased GSH levels and are independent of AR regulation. This is highly relevant considering that prostate cancer cells typically become AR-independent in their latter stages, rendering any treatment targeting AR-receptor regulation ineffective. Anti-cancer effects of black cumin and the mechanisms of action are extensively covered in a recent review article by Randhawa & Alghamdi [8].

TQ as a Chemotherapeutic Adjuvant

A number of common anti-cancer drugs and therapies such as anti-angiogenic drugs, COX-2 inhibitors, tyrosine kinase inhibitors, and chemotherapeutic drugs adversely affect the cardiovascular system [41]. Thus, it is critical to identify

compounds with anti-cancer activity that lack cardio-toxicity or potentially improve overall cardiovascular health. Resveratrol, a polyphenolic compound found in red and purple grape skins is one such compound that consistently demonstrates both anti-cancer and cardio-protective activities, and evidence indicates that black cumin shares these properties [42]. Alenzi et al. [43] found that both black cumin seed oil and TQ significantly decreased cyclophosmamide-induced toxicity in rats via stimulation of antioxidant defense mechanisms. Jafri et al. [44] studied the effect of TQ alone and in combination with cisplatin and found that TQ exhibited significant anti-proliferative activity, but that the combined activity of TQ and cisplatin exceeded that of either agent alone. Effenberger-Neidnicht & Schobert [45] reported that TQ enhanced the anti-proliferative activity of doxorubicin in multi-drug resistant breast carcinoma and leukemia carcinoma cell lines. The efficacy of doxorubicin treatment is compromised by rapid desensitization of cancer cells to the drug, as well as by its cardio-toxic side effects. Thus, adjuvant agents such as TQ that minimizes these undesirable effects while enhancing the tumoricidal activity of traditional anti-cancer drugs should be considered as complementary to chemotherapeutic regimens.

Black Cumin and Insulin Sensitivity

It is estimated that 2.8% of the world's population have type II diabetes, which is characterized by peripheral insulin resistance [46]. Compared to normal weight individuals, the risk for developing type II diabetes is 7 and 28 times higher in obese men and women, respectively [47, 48]. Obese individuals have higher circulating levels of non-esterified or free fatty acids, which reduce the expression of insulin receptor genes thereby lowering the ability of the liver and skeletal muscle to "sense" circulating insulin and take up glucose. In response, hepatic cells increase glucose production, resulting in temporary hyperglycemia. The pancreas responds to this increase in glucose by increasing insulin production; however, pancreatic β-cells eventually fatigue and are unable to compensate for elevated levels of glucose resulting in hyperglycemia.

The seed and seed extract of black cumin have demonstrated anti-diabetic activity *in vitro* and *in vivo*. Cultured rat pancreatic islet cells were challenged with 8.3 mmol/L glucose, and upon the addition of varying concentrations of black cumin extract (defatted or basic sub fraction), a dose-dependent increase in insulin secretion was observed. The acidic and neutral subfractions also induced insulin secretion, but only at higher concentrations [10]. Multiple studies demonstrate the effect of *N. sativa* extracts on Akt activity, and suggested that black cumin extract affects central metabolic processes and energy regulation [7, 49]. A recent *in vitro* study demonstrated enhanced Akt activity following *N. sativa* treatment [49]. The extract was also found to affect AMP kinase and act as a PPARγ mimic. Le et al. [7] reported that the addition of the petroleum ether seed extract of black cumin to the diet of Sprague-Dawley rats at 2 g/kg/day body weight for four weeks activated MAPK/Erk and protein kinase B/Akt, both of which are involved in insulin signal transduction pathways. Erk pathway is known to stimulate protein synthesis and cell proliferation, while protein kinase B/Akt activity induces several downstream effects, including glucose uptake by skeletal muscle. Both pathways are known to stimulate glycogenesis in the liver. Rats treated with the extract showed a reduction in appetite, body mass, and plasma triglycerides, and an increase in HDL-cholesterol, and normal fasting glucose levels throughout a four week treatment [7].

Treatment of diabetic hamsters with TQ (50 mg/kg body weight/day) reduced plasma glucose, and black cumin seed oil inhibited gluconeogenesis [50]. Another study [51] found that the seed oil increased the levels of circulating insulin by inducing regeneration/proliferation of pancreatic β-cells. This suggests that black cumin may reverse the events leading to insulin resistance and type II diabetes, and lower plasma glucose in the short-term.

These investigations provide evidence for the activity of black cumin to lower plasma glucose and prevent the development of insulin resistance/type II diabetes by a number of mechanisms: (i) stimulating insulin secretion, (ii) facilitating the activity of MAPK p42/44erk and protein kinase B/Akt pathways, (iii) stimulating glycogenesis, and (iv) inhibiting gluconeogenesis and glycogenolysis. These mechanisms may work synergistically to counteract the obesity-induced decrease in insulin sensitivity.

Black Cumin and Immune Function

Obesity negatively affects the innate and adaptive components of the immune system, and increases the likelihood of immunodeficiency [52]. Specifically, obesity alters antibody production, macrophage function, and lymphocyte activity, primarily mediated by the hormonal changes that accompany excess body fat [53]. Black cumin has been shown to attenuate the negative effects of obesity on the immune system by a variety of mechanisms, including the stimulation of natural killer cell activity and proliferation, monocyte function, T-cell based immunity, and macrophage activity [6, 54]. However, Haq et al. [55] found that the effect of black cumin on human blood-derived lymphocyte activity is variable. At 10 μg/ml, whole black cumin and its individual proteins had a suppressive effect on lymphocytes activated by pokeweed mitogen, but at a concentration of 1 μg/ml, black cumin demonstrated a stimulatory effect. In mixed lymphocyte cultures, black cumin (10 μg/ml) had varying effects, both stimulatory and suppressive, depending on the donor. These results suggest that the effect of black cumin on lymphocyte activity may be dose-dependent and subject-dependent. The observed suppressive effect of black cumin

(at 10 μg/ml) on lymphocyte activity is of concern, as this will result in a decrease in the output of antibodies in response to infection. Further investigation into this effect is needed if black cumin is to be prescribed for immune-related conditions, especially for individuals who are immuno-compromised.

In rodent models, black cumin has been shown to attenuate the allergic inflammatory response [56, 57]. Shahzad et al. [57] found that black cumin significantly reduced nitric oxide production and serum levels of inflammatory signals including IL-4, IL-5, IL-6, IgE, IgG1, and OVA-specific IgG1 in rats sensitized to and challenged with ovalbumin. Rats treated with black cumin experienced an attenuated T-cell response and reduced T-cell proliferation in the spleen but no histopathological changes in lung tissue. Rats not treated with black cumin showed a thickening of the alveolar wall and increased numbers of goblet cells. This study indicates that black cumin inhibits Th-2-induced T-cell proliferation and differentiation, thereby halting the inflammatory response. El Mezayen et al. [56] found that rats challenged with ovalbumin but pretreated with TQ had reduced lung inflammation mediated by a reduction in Th-2 cytokines, lung eosinophilia, and goblet cell hyperplasia. Also, TQ-treated rats showed a reduction in COX-2 expression, PGD2 production, and a slight reduction in COX-1 expression and PGE2 production. COX-2 mediates the inducible inflammatory response by converting arachidonic acid into pro-inflammatory prostaglandins, whereas COX-1 mediates constitutive or "housekeeping" inflammation. Long-term elevated COX-2 activity is recognized as an underlying cause of many chronic inflammatory disorders, thus inhibition of COX-2 is favorable in cases of chronic inflammatory conditions, for example, rheumatoid arthritis. Black cumin, specifically TQ, exerts its anti-inflammatory activity primarily via inhibition of COX-2 and PGD2 production [56]. A recent review on the effect of thymoquinone on inflammatory disorders extensively covered the anti-inflammatory effects and mechanisms of thymoquinone, an important constituent of black cumin seed oil [58].

Antioxidant Activity of Black Cumin

A number of compounds derived from black cumin have been analyzed for antioxidant (AO) activity. Four compounds (TQ, carvacrol, t-anethole, and 4-terpineol), in addition to the essential oil, have demonstrated AO activity in multiple assays [59]. Isolated TQ was found to have free radical and superoxide scavenging properties but not chelating properties [60]. Khalife et al. [61] reported that TQ reacts with glutathione to form glutathionyl-thymohydroquinone. This product and thymohydroquinone have higher free radical scavenging activities than TQ. Bourgou et al. [62] found the methanolic extract of the roots and shoots of black cumin to have 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide scavenging activities, and reducing and chelating activities due to high polyphenolic content.

In rats, black cumin oil protected against oxidative stress induced by aflatoxin [63]. Aflatoxin treatment significantly decreased erythrocyte levels of glutathione peroxidase and superoxide dismutase, whereas rats treated simultaneously with aflatoxin and black cumin seed oil showed no differences in AO levels compared to controls. Kanter et al. [64] treated rats with carbon tetrachloride (CCl_4) thereby inducing elevated liver enzymes (alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase), increased plasma malondialdehyde, weight loss, and reduced antioxidant levels (glutathione, ceruloplasmin, vitamin E, vitamin C, β-carotene, and retinol). Simultaneous treatment with black cumin oil protected rats from all aforementioned effects of CCl_4. Bourgou

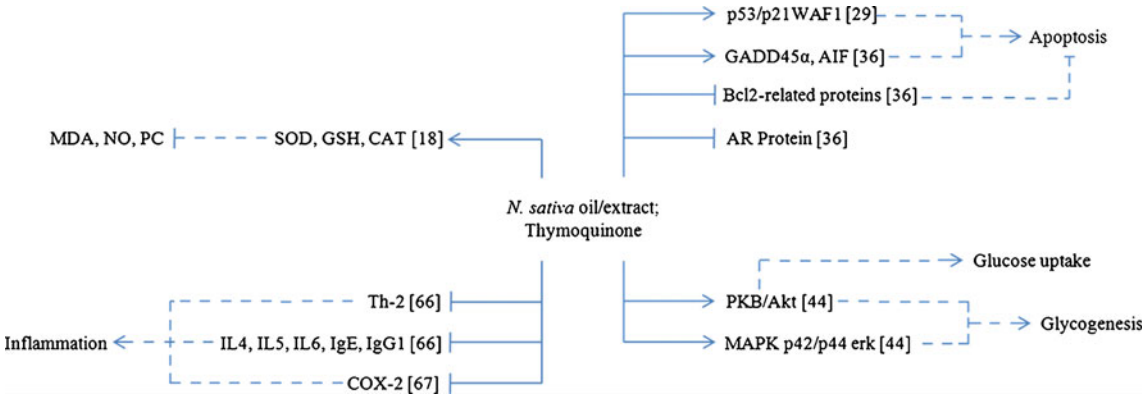

Fig. 2 Physiological effects of *N. sativa* oil/extract and/or thymoquinone. Solid lines indicate a confirmed relationship and dotted lines indicate an unconfirmed relationship (according to the references provided). Arrows indicate stimulation, and capped lines indicate inhibition. Malondialdehyde (MDA); nitric oxide (NO); protein carbonyl (PC); superoxide dismutase (SOD); glutathione peroxidase (GSH); catalase (CAT); interleukin (IL); immunoglobulin (Ig); cyclooxygenase (COX); growth arrest and DNA damage (GADD); B-cell lymphoma 2 (Bcl2); apoptosis-inducing factor (AIF); androgen receptor (AR); protein kinase B (PKB); mitogen-activated protein kinase (MAPK)

et al. [65] analyzed the ability of black cumin oil and its major bioactive constituents to inhibit *tert*-butyl peroxide-induced 2′,7′-dichlorofluorescin (DCFH) oxidation. The oil significantly inhibited the production of ROS, and TQ possessed the strongest *ex vivo* AO capacity with an IC_{50} value of 1.0 μM. The thymoquinone-rich fraction (TQRF) of black cumin increased the RNA expression of superoxide dismutase 1, catalase, and glutathione peroxidase 2 in the livers of hypocholesterolemic rats given increasing doses of TQRF (0.5–1.5 g/kg body weight) for eight weeks [66]. These enzymes serve a critical role in endogenous AO defense against oxygen-induced free radicals. Black cumin, specifically TQ, may function as a direct antioxidant in addition to stimulating endogenous antioxidant systems.

Black Cumin Toxicity

Zaoui et al. [67] investigated the acute toxicity of black cumin oil in mice. Single doses of black cumin oil ranging from 10 to 50 ml/kg body weight were administered orally (p.o.) and doses ranging from 0.25 to 6 ml/kg body weight were administered intraperitoneally (i.p.). The LD_{50} values were 28.8 ml/kg body weight and 2.06 ml/kg body weight for p.o. and i.p. administration, respectively. These high values indicate that black cumin oil is relatively non-toxic. When administered to rats at a dose of 2 ml/kg body weight orally for 12 weeks, neither any histopathological change in heart, liver, kidney, and pancreatic tissues, nor any changes in liver enzymes were observed. However, black cumin yielded significant decrease in triglycerides, cholesterol, glucose, platelet, and leukocyte counts, and significant increase in hemoglobin and hematocrit levels [67]. Given the stability of liver enzymes and lack of histopathological changes with black cumin treatment over 12 weeks, this investigation demonstrates black cumin's relatively low chronic toxicity and safety over a range of therapeutic doses. However, the black cumin-induced decrease in hemoglobin and hematocrit indicates that these markers should be closely monitored if a treatment regimen is prescribed. Interestingly, rats treated with black cumin showed significantly lower body weights after six weeks of treatment compared to control rats, suggesting that black cumin may also be an effective weight loss aid [67]. Introducing as much as 4% black cumin oil for 56 days into the diet was safe in a rodent model that measured toxicity indicators for liver, kidney, and serum [13].

Badary et al. [68] performed acute and sub-chronic toxicity studies of TQ in mice, and reported an LD_{50} value of 2.4 g/kg body weight (p.o. administration). Twenty-four hours following administration of 2 or 3 g/kg body weight, tissue levels of reduced glutathione decreased, and levels of alanine-aminotransferase, lactate dehydrogenase, and creatine phosphokinase increased. Acute toxicity was attributed to hypo-activity and difficulty in respiration, mediated by TQ-induced depletion of glutathione reserves in vital tissues. In the sub-chronic experiment, mice consumed approximately 30, 60, or 90 mg TQ/kg body weight/day for 90 days, and no toxicological effects were observed. Importantly, these doses are respectively 4, 8, and 12 times the dose previously reported to produce a cytoprotective effect suggesting that TQ, one of the major constituents of black cumin seed, confers beneficial effects at concentrations far below the toxic dose. A clinical study showed that the adult patients with solid tumors or hematological malignancies tolerated a dose range of 75 to 2,600 mg thymoquinone/day [69]. Indeed, in the United States black cumin seeds have been accorded Generally Recognized As Safe (GRAS).

Conclusion

Black cumin and one of its major active constituents, thymoquinone, have a variety of health-promoting actions (Fig. 2) and low toxicity, warranting their consideration as potential therapeutic agents and/or nutraceuticals. Given the associations between obesity and cardiovascular disease, cancer, diabetes, and immune-suppression, black cumin should be considered as a complementary treatment. Strong evidence supports the potential use of black cumin as an adjuvant in cancer therapy given its ability to prevent the adverse effects of anti-cancer drugs on the cardiovascular system and its pro-apoptotic properties selectively against cancer cells. Black cumin has been used for centuries in certain parts of the world as both food and medicine, and modern molecular methods are just beginning to provide insights into its mechanisms of action. Further studies on the activities of black cumin and its bioactive constituents should be conducted to determine or confirm (i) the compounds (or combination of compounds) responsible for its biological effects, and their mechanism(s) of action, (ii) the methods of preparation and administration that yield the maximum health benefit in specific conditions, in particular those afflicting obese individuals, and (iii) the potential for negative drug interactions. Establishing these parameters will allow the public health and medical communities to bring black cumin into the clinical setting and to test its efficacy in the free-living population.

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References

1. Paredes-Lopez O, Cervantes-Ceja ML, Vigna-Perez M, Hernandez-Perez T (2010) Berries: Improving human health and healthy aging, and promoting quality life—a review. Plant Foods Hum Nutr 65:299–308

2. Millen AE, Subar AF, Graubard BI, Peters U, Hayes RB, Weissfeld JL, Yokochi LA, Ziegler RG (2007) Fruit and vegetable intake and prevalence of colorectal adenoma in a cancer screening trial. Am J Clin Nutr 86:1754–1764
3. Szajdek A, Borowska EJ (2008) Bioactive compounds and health-promoting properties of berry fruits: A review. Plant Foods Hum Nutr 63:147–156
4. Ramadan MF (2007) Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): An overview. Int J Food Sci Tech 42:1208–1218
5. Ali BH, Blunden G (2003) Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res 17:299–305
6. Dehkordi FR, Kamkhah AF (2008) Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundam Clin Pharmacol 22:447–452
7. Le PM, Benhaddou-Andaloussi A, Elimadi A, Settaf A, Cherrah Y, Haddad PS (2004) The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. J Ethnopharmacol 94:251–259
8. Randhawa MA, Alghamdi MS (2011) Anticancer activity of *Nigella sativa* (black seed)—A review. Am J Chin Med 39:1075–1091
9. Norwood AA, Tan M, May M, Tucci M, Benghuzzi H (2006) Comparison of potential chemotherapeutic agents, 5-fluoruracil, green tea, and thymoquinone on colon cancer cells. Biomed Sci Instrum 42:350–356
10. Rchid H, Chevassus H, Nmila R, Guiral C, Petit P, Chokairi M, Sauvaire Y (2004) *Nigella sativa* seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fund Clin Pharmacol 18:525–529
11. Vaughn N, Rizzo A, Doane D, Beverly JL, Gonzalez de Mejia E (2008) Intracerebroventricular administration of soy protein hydrolysates reduces body weight without affecting food intake in rats. Plant Foods Hum Nutr 63:41–46
12. Iqbal MS, Ghafoor A, Qureshi AS, Khan MR, Chaudhary UI (2009) Quantification and diversity in the black seeds (*Nigella sativa* L.): Gene stock of Pakistan for their composition of mineral nutrients. J Chem Soc Pak 31:793–800
13. Sultan MT, Butt MS, Anjum FM, Jamil A, Akhtar S, Nasir M (2009) Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. Pak J Bot 41:1321–1330
14. Amin S, Mir SR, Kohli K, Ali B, Ali M (2010) A study of the chemical composition of black cumin oil and its effect on penetration enhancement from transdermal formulations. Nat Prod Res 24:1151–1157
15. Butt MS, Sultan MT (2010) *Nigella sativa*: Reduces the risk of various maladies. Crit Rev Food Sci Nutr 50:654–665
16. Kokoska L (2011) Chemistry and biological activity of Nigella genus: the antimicrobial and anti-inflammatory effects of seed extracts, essential oils and compounds of six *Nigella species*. Lambert Academic Publishing, Saarbrücken
17. Lutterodt H, Luther M, Slavin M, Yin JJ, Parry J, Gao JM, Yu LL (2010) Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. LWT-Food Sci Technol 43:1409–1413
18. Ramadan MF, Morsel JT (2004) Oxidative stability of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils upon stripping. Eur J Lipid Sci Tech 106:35–43
19. Ogden CL, Yanovski SZ, Carroll MD, Flegal KM (2007) The epidemiology of obesity. Gastroenterology 132:2087–2102
20. Amar S, Zhou Q, Shaik-Dasthagirisaheb Y, Leeman S (2007) Diet-induced obesity in mice causes changes in immune responses and bone loss manifested by bacterial challenge. Proc Natl Acad Sci USA 104:20466–20471
21. Bianchini F, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. Lancet Oncol 3:565–574
22. Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840–846
23. Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. Nature 444:875–880
24. Currie CJ, Poole CD, Gale EA (2009) The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 52:1766–1777
25. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: The *in vivo* evidence. Nat Rev Drug Discov 5:493–506
26. Kopelman PG (2000) Obesity as a medical problem. Nature 404:635–643
27. Rodriguez MC, Parra MD, Marques-Lopes I, De Morentin BE, Gonzalez A, Martinez JA (2005) Effects of two energy-restricted diets containing different fruit amounts on body weight loss and macronutrient oxidation. Plant Foods Hum Nutr 60:219–224
28. Datau EA, Wardhana SEE, Pandelaki K, Langi JA, Fias (2010) Efficacy of *Nigella sativa* on serum free testosterone and metabolic disturbances in central obese male. Acta Med Indones 42:130–134
29. Suddek GM (2010) Thymoquinone-induced relaxation of isolated rat pulmonary artery. J Ethnopharmacol 127:210–214
30. Ebru U, Burak U, Yusuf S, Reyhan B, Arif K, Faruk TH, Emin M, Aydin K, Atilla II, Semsettin S, Kemal E (2008) Cardioprotective effects of *Nigella sativa* oil on cyclosporine A-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol 103:574–580
31. Pourghassem-Gargari B, Ebrahimzadeh-Attary V, Rafraf M, Gorbani A (2009) Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits. J Med Plants Res 3:815–821
32. Nader MA, El-Agamy DS, Suddek GM (2010) Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. Arch Pharm Res 33:637–643
33. Jaffe T, Schwartz B (2008) Leptin promotes motility and invasiveness in human colon cancer cells by activating multiple signal-transduction pathways. Int J Cancer 123:2543–2556
34. Salim EI (2010) Cancer chemopreventive potential of volatile oil from black cumin seeds, *Nigella sativa* L., in a rat multi-organ carcinogenesis bioassay. Oncology Lett 1:913–924
35. Kohle C, Badary OA, Nill K, Bock-Hennig BS, Bock KW (2005) Serotonin glucuronidation by Ah receptor- and oxidative stress-inducible human UDP-glucuronosyltransferase (UGT) 1A6 in Caco-2 cells. Biochem Pharmacol 69:1397–1402
36. Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, Schneider-Stock R (2004) Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. Int J Oncol 25:857–866
37. Gali-Muhtasib HU, Abou Kheir WG, Kheir LA, Darwiche N, Crooks PA (2004) Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. Anti-Cancer Drug 15:389–399
38. Roepke M, Diestel A, Bajbouj K, Schonfeld P, Roessner A, Gali-Muhtasib H, Schneider-Stock R (2007) Lack of p53 augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. Pathol Res Pract 203:340
39. Shoieb AM, Elgayyar M, Dudrick PS, Bell JL, Tithof PK (2003) *In vitro* inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Int J Oncol 22:107–113
40. Koka PS, Mondal D, Schultz M, Abdel-Mageed AB, Agrawal KC (2010) Studies on molecular mechanisms of growth inhibitory effects of thymoquinone against prostate cancer cells: Role of reactive oxygen species. Exp Biol Med 235:751–760
41. Albini A, Pennesi G, Donatelli F, Cammarota R, De Flora S, Noonan DM (2010) Cardiotoxicity of anticancer drugs: The need

for cardio-oncology and cardio-oncological prevention. J Natl Cancer I 102:14–25
42. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88
43. Alenzi FQ, El-Bolkiny YES, Salem ML (2010) Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide. Brit J Biomed Sci 67:20–28
44. Jafri SH, Glass J, Shi RH, Zhang SL, Prince M, Kleiner-Hancock H (2010) Thymoquinone and cisplatin as a therapeutic combination in lung cancer: *In vitro* and *in vivo*. J Exp Clin Canc Res 29:87
45. Effenberger-Neidnicht K, Schobert R (2011) Combinatorial effects of thymoquinone on the anti-cancer activity of doxorubicin. Cancer Chemother Pharmacol 67:867–874
46. Rathmann W, Giani G (2004) Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 27:2568–2569, author reply 2569
47. Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC (1994) Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care 17:961–969
48. Colditz GA, Willett WC, Rotnitzky A, Manson JE (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med 122:481–486
49. Benhaddou-Andaloussi A, Martineau LC, Vallerand D, Haddad Y, Afshar A, Settaf A, Haddad PS (2010) Multiple molecular targets underlie the antidiabetic effect of *Nigella sativa* seed extract in skeletal muscle, adipocyte and liver cells. Diabetes Obes Metab 12:148–157
50. Fararh KM, Shimizu Y, Shiina T, Nikami H, Ghanem MM, Takewaki T (2005) Thymoquinone reduces hepatic glucose production in diabetic hamsters. Res Vet Sci 79:219–223
51. Kanter M, Meral I, Yener Z, Ozbek H, Demir H (2003) Partial regeneration/proliferation of the beta-cells in the islets of langerhans by *Nigella sativa* L. in streptozotocin-induced diabetic rats. Tohoku J Exp Med 201:213–219
52. Samartin S, Chandra RK (2001) Obesity, overnutrition and the immune system. Nutr Res 21:243–262
53. Haq A, Abdullatif M, Lobo PI, Khabar KSA, Sheth KV, Alsedairy ST (1995) *Nigella-Sativa*—effect on human-lymphocytes and polymorphonuclear leukocyte phagocytic-activity. Immunopharmacology 30:147–155
54. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T (2004) Mechanisms of the hypoglycaemic and immunopotentiating effects of *Nigella sativa* L. oil in streptozotocin-induced diabetic hamsters. Res Vet Sci 77:123–129
55. Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST (1999) Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. Int J Immunopharmacol 21:283–295
56. El Mezayen R, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, Nomiyama H (2006) Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol Lett 106:72–81
57. Shahzad M, Yang XD, Asim MBR, Sun QZ, Han Y, Zhang FJ, Cao YX, Lu SM (2009) Black seed oil ameliorates allergic airway inflammation by inhibiting T-cell proliferation in rats. Pulm Pharmacol Ther 22:37–43
58. Woo CC, Kumar AP, Sethi G, Tan KH (2012) Thymoquinone: Potential cure for inflammatory disorders and cancer. Biochem Pharmacol 83:443–451
59. Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14:323–328
60. Badary OA, Taha RA, El-Din AMG, Abdel-Wahab MH (2003) Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 26:87–98
61. Khalife KH, Lupidi G (2008) Reduction of hypervalent states of myoglobin and hemoglobin to their ferrous forms by thymoquinone: The role of GSH, NADH and NADPH. Bba-Gen Subjects 1780:627–637
62. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B (2008) Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. Cr Biol 331:48–55
63. Abdel-Wahhab MA, Aly SE (2005) Antioxidant property of *Nigella sativa* (black cumin) and Syzygium aromaticum (clove) in rats during aflatoxicosis. J Appl Toxicol 25:218–223
64. Kanter M, Meral I, Dede S, Cemek M, Ozbek H, Uygan I, Gunduz H (2003) Effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl4-treated rats. J Vet Med A 50:264–268
65. Bourgou S, Pichette A, Marzouk B, Legault J (2010) Bioactivities of black cumin essential oil and its main terpenes from Tunisia. S Afr J Bot 76:210–216
66. Ismail M, Al-Naqeep G, Chan KW (2010) *Nigella sativa* thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radical Bio Med 48:664–672
67. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M (2002) Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine 9:69–74
68. Badary OA, Al-Shabanah OA, Nagi MN, Al-Bekairi AM, Elmazar MMA (1998) Acute and subchronic toxicity of thymoquinone in mice. Drug Develop Res 44:56–61
69. Al-Amri A, Bamosa A (2009) Phase I safety and clinical activity study of thymoquinone in patients with advanced refractory malignant disease. SEMJ 10:107–111
70. Nergiz C, Otles S (1993) Chemical-composition of *Nigella-sativa* L. seeds. Food Chem 48:259–261